A thesis entitled

THERMAL DEGRADATION OF ORGANOPHOSPHORUS COMPOUNDS

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S. Chady.

As menazon [O,O - dimethyl-S-(4,6-diamino-1,3,5-triazin-2-ylmethyl) phosphorodithioate] is an industrial product, the general information on its chemistry and chromatographic behaviour is contained in research reports of limited circulation. The relevant information has been abstracted from these reports and is summarised. The literature relating to the ultraviolet, infrared, ^{1}H nuclear magnetic resonance spectra and mass spectra of 1,3,5-triazine and phosphorus compounds of relevance to the topic is briefly discussed.

The behaviour of heated samples of menazon on paper (one and two dimensional) and thin layer (analytical and preparative) chromatography is described. Details are given of the chromatographic systems adopted for the separation and isolation of the products of thermal degradation. Infrared, ultraviolet, ^{1}H nuclear magnetic resonance and mass spectra of the isolated compounds are recorded and evaluated.

Structures are proposed for the isolated compounds and the spectroscopic properties of authentic samples compared with those of the isolated compounds. The validity of the proposals is discussed and areas requiring further study indicated.

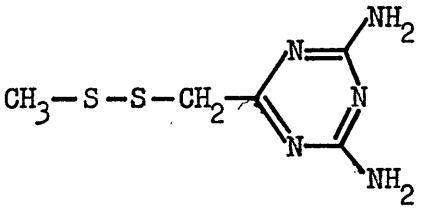
A discussion of some of the mass spectra which have been obtained in this work is presented.

SUMMARY

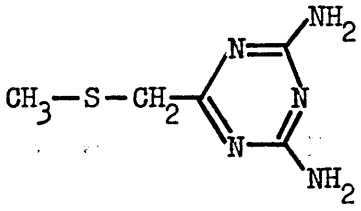
Menazon is thermally degraded to a complex mixture of products. These have been separated by preparative thin layer chromatography and four of the major ones identified, mainly by the application of physical methods and comparison with synthesised compounds. The structures of the four compounds are given below (A - D).

Most of the degradation products appeared almost simultaneously, even after a short period of heating and it was consequently impossible to construct a degradation pathway.

During the course of this work the physical data of a number of related triazines and phosphorylated triazines have been recorded and structural correlations made.



A



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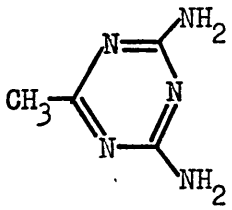

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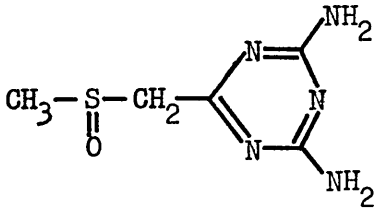
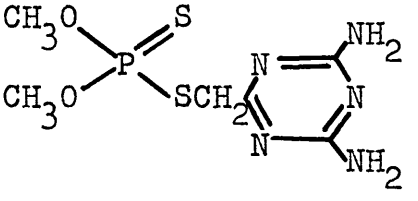


D

INTRODUCTION

1 Menazon as an organophosphorus aphicide

Menazon [0,0'-dimethyl-S-(4,6-diamino-1,3,5-triazin-2-ylmethyl) phosphorodithioate] is produced on a tonnage scale by Imperial Chemical Industries Limited. It combines outstanding aphicidal properties with low toxicity to most other insect species and to mammals. Aphids are a major pest of many important food crops including potatoes, brassica, sugar beet, broad beans, apples and certain soft fruits. Only slight cholinesterase inhibition is shown by menazon *in vitro* and its mammalian toxicity is very low; the LD50's are 1950 mg/Kg orally and 615 mg/Kg intraperitoneally in female rats[1].



Menazon

From the study of the absorption, translocation, and metabolism of menazon in plants[2], it has been found that the aphicide is extremely persistent in plants under greenhouse conditions when applied as a foliage spray. This is because it is only poorly absorbed through the leaves, most of the chemical remaining as a stable deposit on the surface.

Absorption occurs readily through the roots, but no evidence has been obtained for activation to a more toxic metabolite. Oxidation of the P = S bond to P = O to give the thiolate occurs to a limited extent in plants. Hydrolysis of menazon, or its initially formed thiolate, appears to be the main detoxifying mechanism in the plant; ultimately the triazine ring is degraded to carbon dioxide.

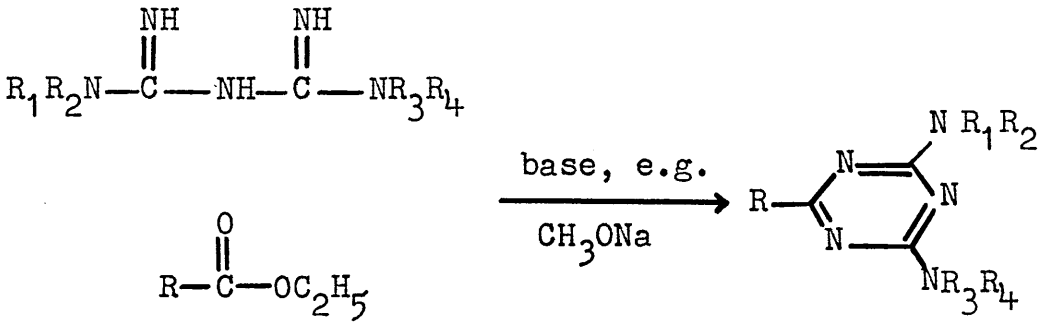
Menazon differs from most of the commercial organophosphorus pesticides in being comparatively insoluble. The low solubility of menazon is attributed to the diaminotriazine structure with the possibility of a high degree of conjugation and hydrogen bond formation. The phosphorus containing moiety confers solubility in organic solvents. Thus the solubilities of menazon in water and chloroform are 0.024 and 0.16% (g/100 ml) or 240 and 1600 ppm respectively at room temperature[3].

Small alterations to the menazon structure markedly affect solubility and general properties. Alkyl substitution of the amino group results in greatly enhanced solubility particularly in organic solvents. Change from P = S to P = O results in increased polarity and water solubility. The presence of substituents with bondable hydrogen atoms (NH_2,OH) on the 1,3,5-triazine ring causes a decrease in solubility relative to the unsubstituted heterocyclic compound. This decrease in solubility is due to intramolecular hydrogen bonding from O or N of the substituent to the negatively charged N in the ring; this takes place in preference to hydrogen bonding with water.

Aphicidal activity is quite sensitive to changes in molecular structure. Modification of the menazon structure either by replacement of the methoxy groups by higher alkoxy groups or by further substitution of the methylene bridge reduces activity. Substitution of the amino groups by more than one methyl group or by higher alkyl groups also reduces activity[4].

2 Synthesis of 2,4-diamino-1,3,5-triazines and their phosphorylated derivatives

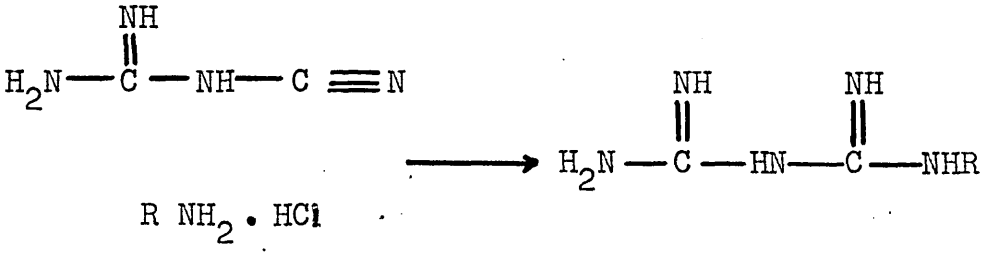
An outline of the various routes available for the synthesis of 2,4-diamino-1,3,5-triazines has been given by Smolin and Rapoport[5]. Probably the most convenient route is by the reaction of a biguanide base with an ester in the presence of a full equivalent of a strong base such as NaOH, CH_3ONa.



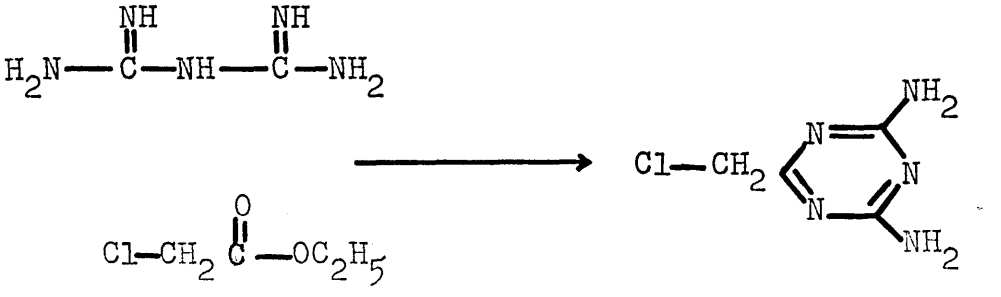
(R, R_1, R_2, R_3, R_4 = alkyl or H)

Biguanides substituted on only one nitrogen atom may be prepared from dicyandiamide and alkylamine hydrochloride either by fusion at 140 - 150° or under reflux in

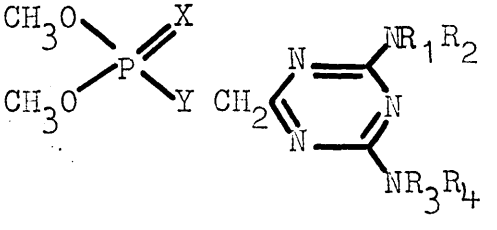
2-ethoxy-ethanol.[7]



A very important intermediate in the synthesis of phosphorylated 2,4-diamino-1,3,5-triazines is 2-chloro (or bromo)methyl-2,4-diamino-1,3,5-triazine which is obtained by reacting ethyl chloro (or bromo)acetate and biguanide.[8]

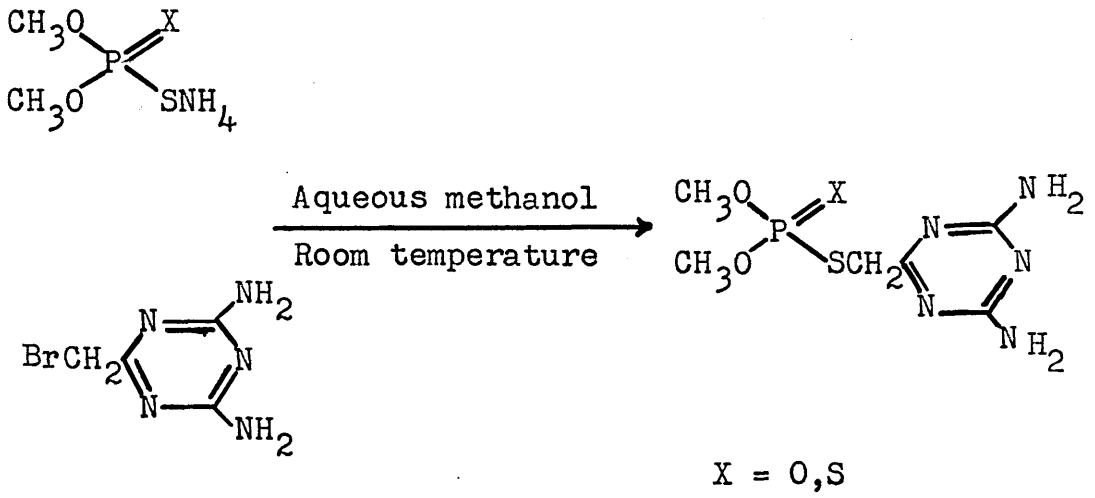


Several triazinyl phosphate esters including menazon have been synthesised during exploratory work at Imperial Chemical Industries Limited[4] with a view to studying the structural requirements conferring high aphicidal activity within the group



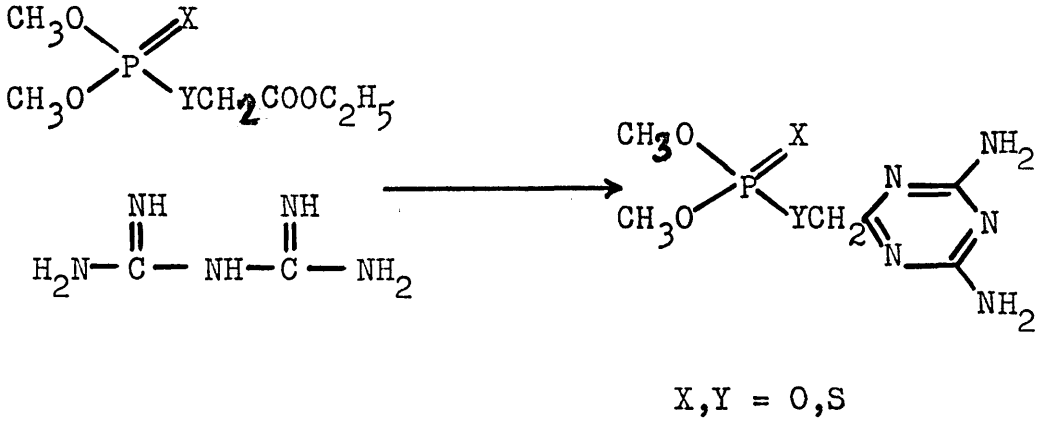
R = alkyl R_1, R_2, R_3, R_4 = alkyl or H X,Y = O,S

The most versatile route for their synthesis was by reaction of ammonium dialkyldithiophosphate or ammonium dialkylphosphorothioate with the appropriate halomethyl-1,3,5-triazine, e.g.,



This method gives good yields of menazon (about 50%).

The main alternative to the above route is the reaction of biguanides with phosphorylated acetic esters, e.g.,



3 Separation of 1,3,5-triazine compounds by paper chromatography

An ion-exchange method for separating menazon in plant extract solutions has been described[9], which involves applying the acidified solutions to a column containing the strongly acid cation-exchange resin Zeo-Karb 225 containing one per cent divinylbenzene, followed by elution with ammonium acetate. The separation of mixtures of 1,3,5-triazine compounds by ion-exchange has not been adequately studied, but fairly extensive data is available for achieving their separation by paper chromatography[3] on sheets of Whatman No. 1 paper.

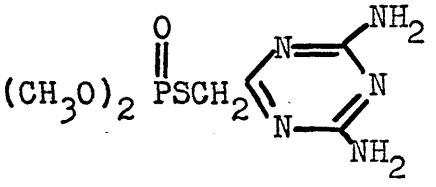
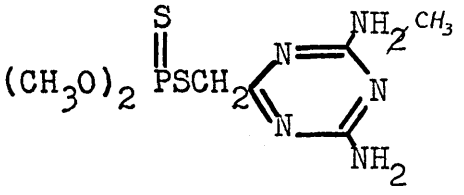
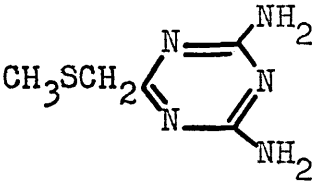
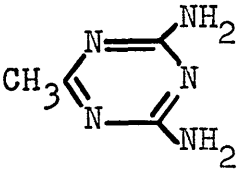
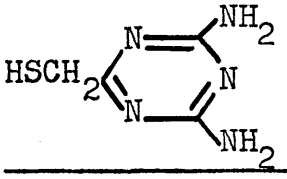
Of the large number of solvent systems tried, n-butanol: acetic acid : water (12:3:5) (henceforth this solvent mixture will be referred to as BAW) has been found to be the most useful solvent possessing the singular property of compacting spots so that little diffusion or elongation occurs during chromatography. For confirming the identity of 1,3,5-triazine compounds, two dimensional paper chromatography has been recommended by developing (descending-D) overnight with BAW solvent, and then at right angles (D) with n-hexane : chloroform : methanol : water (5:5:10:2) (henceforth this solvent mixture will be referred to as HCMW) for seven hours. The R_F values of some typical 1,3,5-triazine compounds are given in Table 1.

Two main methods of detection on paper chromatograms have been found satisfactory and in many cases are complimentary.

Table 1 *

Paper chromatography of some 1,3,5-triazine compounds

(R_F values)

Compound	1	2	3	4
Menazon	0.85	0.69	Q	Pink
	0.62	0.60	Q	Brown pink
	0.95	0.87	Q	Pink
	0.61 - 0.75	0.53	Q	Yellow
	0.59		Q	
	0.32		Q	Brown

* Taken from ref. 3.
1 BAW solvent (D)
2 HCMW solvent (D)
3 uv light quenching at 254 nm (Q)
4 DBQ spray

(a) Quenching under uv light Compounds containing the 1,3,5-triazine ring are visible on the paper as quenching areas against the background fluorescence of the paper when viewed under uv light of approximately 254 nm.

(b) 2,6-dibromoquinone-N-chloroimide[10] (henceforth this will be referred to as DBQ) in chloroform solution (0.5% w/v). Many compounds containing P=S, P-S-C, C-S-C and C-S-H groups give typical colours with DBQ. The groups P=S, P-S-C give a reddish brown or pink colour with the reagent, the C-S-C group gives a yellow colour, while the C-S-H group gives a brown colour.

4 Microanalysis of phosphorus

A. Calderbank and J.B. Turner have described an accurate and reproducible method for the microanalysis of phosphorus, and have applied it to estimate quantitatively microgram quantities of menazon[9]. It is essentially an adaptation to residue analysis of a method described by Chen, Toribara and Warner[11], and is widely applicable for estimating quantitatively microgram quantities of many types of organophosphorus compounds.

The method consists in breaking down a known amount of organic phosphate quantitatively by wet digestion with concentrated sulphuric-perchloric acid to orthophosphoric acid, and the solution is neutralised with alkali. A mixed reagent of ammonium molybdate, ascorbic acid and sulphuric acid, called reagent C, is added which gives a deep blue molybdophosphate complex, and the absorbance of the solution

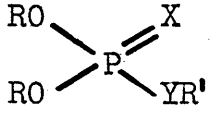
measured spectrophotometrically at 820 nm.

The phosphorus microanalysis has also been rendered specific for menazon by paper chromatographic separation, and locating the area of the paper which contains the menazon as a quenching area under uv light of approximately 254 nm, and ashing it with concentrated sulphuric-perchloric acid prior to the phosphorus microanalysis. This procedure is also applicable to many organophosphorus compounds provided they can be adequately separated by paper chromatography and located by physical methods.

5 Infrared spectra of organophosphorus compounds

5.1 General

The literature on the infrared absorption spectra of organophosphorus compounds is quite extensive. Of interest in the present work are the infrared absorption spectra of compounds of the type



, R,R' = alkyl
X,Y = O,S.

The P=O, P=S, P-O-C, P-S-C stretching frequencies have been most studied and the data will be briefly reviewed.

5.2 The P=O stretching frequency

For some compounds two apparent phosphoryl absorption bands appear in the spectrum, and the reasons for this have

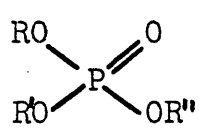
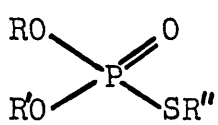
been discussed by several workers[12,13,14]. On some cases such as triaryl phosphates, there is little doubt that rotational isomerism is involved, and the doublet peaks are often not fully resolved. In other cases the doublet may be spaced apart by up to 50 cm^{-1}, and it is usually assumed that it arises from Fermi resonance interaction between the phosphoryl vibration and another vibration of approximately the same frequency.

Thomas and Crittenden have recently summarised the data available on the phosphoryl frequency[15], and the constancy of this band for two particular families of compounds is illustrated in Table 2.

Table 2

Frequency limits for P=O valence vibrations

(after Thomas[15])

Structure	Limits (cm^{-1})	Comments
	1258 - 1286	R,R',R" = alkyl
	1261 - 1297	R,R',R" = mixed alkyl and aryl
	1247 - 1269	R,R',R" = alkyl

It has also been found that when an unsaturated alkyl

group is directly attached to the phosphorus atom, there is no lowering of the phosphoryl frequency. This implies that there is little or no conjugation between the π electrons of the phosphoryl group and those of substituent groups. Since the P=O bond cannot therefore lie in the same plane as the multiple bonds of any substituents, the inductive effects of the substituents are the dominant factors in determining the phosphoryl frequency. For predicting accurately the phosphoryl frequency, Thomas and Crittenden[15] have proposed the linear relationship,

$$\nu_{cm^{-1}} \quad (P=O) = 930 + 40 \sum \pi \ ,$$

where π is called the phosphorus inductive substituent constant, and its values are chosen so as to give the best fit over the greatest number of compounds. A selection of the π constants for a few substituents are given in Table 3.

Table 3

Some π constants for P-X groups (after Thomas[15])

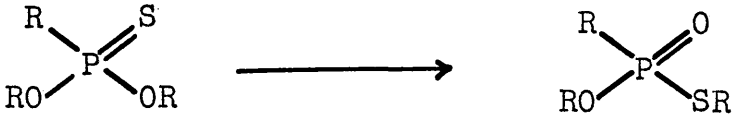
X	π	X	π	X	π
CH_3	2.1	$-C_6H_5$	2.4	$-OCH_2-C_6H_5$	2.7
$-CH_2-$	2.0	$-OCH_3$	2.9	$-OC_6H_5$	3.0
-CH=	1.8	$-OCH_2$	2.85	-SR	2.4

However, it should be noted that no π constants are

included for OH groups, or for substituents containing OH groups, and this is due to the unpredictable magnitude of the perturbation to the phosphoryl frequency resulting from hydrogen bonding.

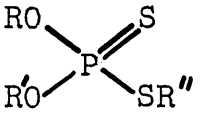
5.3 The P=S vibration

This band is not very strong and is difficult to identify, as it falls in a region where many other bands appear. A careful comparison of pairs of compounds with P=O and P=S groups has enabled two bands to be identified which seem connected in some way with the P=S bond. Both are absent in the corresponding P=O compounds, and both vanish during the isomerisation of phosphorothionates to phosphorothiolates,



There is as yet no satisfactory explanation for the origin of these two characteristic bands.

Crittenden and Thomas[16] have extracted all the data available on both bands, and have tabulated the frequencies for various structures. For phosphorothionates of the type



R,R',R" = alkyl,

the range quoted[16] for the higher frequency band is 790 - 833 cm^{-1}, and for the lower frequency band is 645 - 663 cm^{-1}.

It has also been found that the two bands do not show any relative change in intensity with temperature[16], thus eliminating one possible explanation in terms of rotational isomerism. Bellamy[17] has recently reviewed the subject, and suggests that it is more likely that the higher frequency band is due to the P=S mode. However, this point is not of great importance for the characterisation of unknowns when both bands may be used.

The positions of both bands is sensitive to the nature of the substituents, but neither of them follows the type of relationship shown by the P=O bond. Thomas discusses this[16], and suggests that the reduced ionic character of the P=S bond will make it less responsive to inductive effects, and will throw the impact of other factors such as field effects across space rather than through formal bonds and geometry into greater prominence.

5.4 The P-O-(C) and C-O-(P) groups

There is general agreement that the presence of a P-O-C group in a molecule is characterised by a strong band near 1000 cm^{-1}, but there is little agreement whether this band is due to P-O-(C) or C-O-(P) group. Thomas and Crittenden[18] have assigned this band to the P-O-(C) vibration, and have divided the overall range into two groups corresponding to the esters of primary and secondary alcohols, and which generally fall within the limits

$$P \overset{\overline{\nu}}{-} O - (CH_3) \qquad 1015 - 1060\ cm^{-1}$$

$\bar{\nu}$
P - O - (CH_2-) 987 - 1042 cm^{-1}

$\bar{\nu}$
P - O - (CH) 950 - 1018 cm^{-1}

In the case of quinquevalent esters, ethyl and upwards containing unbranched carbon chains, a second absorption band is found in the region 939 - 982 cm^{-1}.

The absorption band corresponding to the aliphatic C-O-(P) vibration is usually medium to weak in intensity and found in the regions [18].

CH_3 - O (P)	1168 - 1200 cm^{-1}
-CH_2 - O -(P)	1105 - 1170 cm^{-1}
-CH - O -(P)	1087 - 1190 cm^{-1}

In the case of ethyl esters, this band occurs in the much more restricted region 1152 - 1170 cm^{-1}.

5.5 The P-S-(C) and C-S-(P) groups

In many cases two absorption bands ascribed to the P-S-(C) bond are found, but as in the case of P=S vibrations, it has not yet proved possible to advance any satisfactory explanation for the origin of the two bands. But two general observations have been made [16].

(i) The P-S-(C) vibration frequencies are lower in P=S compounds than in corresponding P=O compounds.

(ii) When two absorption bands occur, they are of medium intensity and separated by 30 - 40 cm^{-1}.

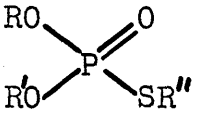
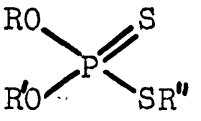
The frequency ranges of the higher and lower frequency bands for two particular families of compounds are given in

Table 4.

Table 4

The P-S-(C) vibration frequency limits

(after Thomas[16])

Structure	Limits	Comments
	600 - 610 563 - 574	R,R',R" = alkyl
	527 - 540 500 - 520	R,R',R" = alkyl

It has not yet proved possible to identify conclusively the (P)-S-C vibration, but there is a weak absorption band[19] in the range 600 - 700 cm^{-1}, and until further studied will not be of diagnostic aid for structure determination.

6 Infrared spectra of 1,3,5-triazine compounds

A short review of the infrared spectra of 1,3,5-triazines has been provided by Katritzky and Ambler[20]. The vibrational assignment of the 1,3,5-triazine parent heterocycle has been much discussed[21-25] and a complete assignment has been made by Lancaster, Stamm, Colthup[25]. Various 1,3,5-triazine derivatives such as chlorotriazines[26,27], N-substituted triamino-1,3,5-triazines[28] have been studied. Complete

assignments are also available for 2,4,6-triamino-1,3,5-triazine[29], and since the results are pertinent to the present work they are given in Table 5.

Table 5

Vibrational assignment for 2,4,6-triamino-1,3,5-triazine*

Compound Film (cm^{-1})	Description of mode
3464 3116 3320	NH_2 stretching
3178 2674 2193	Combination tones
1660 1643 1626	NH_2 bending
1583 1565	Ring stretching
1549 1531	Side-chain asymmetric CN stretching
1469	Side-chain CN breathing
1434	Ring stretching
1190 1175	NH_2 rocking
1062	Ring breathing
813	Ring bending
760	Side-chain out-of-plane CN bending
730	Ring bending
619	NH_2 wagging
581	Ring bending
518	Side-chain in-plane CN bending
458	Combination tone

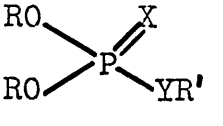
* Taken from ref. 29.

The ring bending mode near 800 cm^{-1} in 1,3,5-triazine spectra is very strong and sharp, and is useful in characterising 1,3,5-triazine derivatives. It has been fairly extensively studied[27]. Its position is influenced by the nature of the substituents on the 1,3,5-triazine ring and is a reasonably good function of the resonance component, σ_R, of the Hammett σ constant of the substituent group[27], and it has been suggested that the variation in position of this band is determined primarily by the resonance interaction between the substituents and the triazine ring. This band is shifted towards longer wavelengths relative to the unsubstituted 1,3,5-triazine as the electron density in the triazine ring is increased through resonance, practically independent of the inductive effects of the substituents.

7 The proton nuclear magnetic resonance (^{1}H nmr) spectra of organophosphorus compounds

7.1 General

A comprehensive review of the ^{1}H nmr spectra of organophosphorus compounds has recently been given by G. Mavel[30]. In this section the main features of the ^{1}H nmr spectra of organophosphorus compounds related to menazon will be outlined. These are of the type



R = Me, Et

R' = Alkyl, aryl

X,Y = O,S

7.2 Proton chemical shifts

The proton chemical shifts observed in organophosphorus compounds are quite sensitive to changes in molecular structure. They are on the whole very difficult to explain in detail because of the number of intervening intra-molecular contributions, such as those arising from localised electronic charges (especially lone pair electrons on P, O, S) from magnetically anisotropic heteroatoms or bonds (especially phosphoryl, thiophosphoryl groups); and inter-molecular contributions such as hydrogen bonding, solvent-solvent interaction. However, apart from PH groups and other exchangeable groups such as P(OH), chemical shifts are not too sensitive to solvent effects[30]. More detailed studies show that significant solvent effects occur only for chlorinated molecules, possibly due to greater polarisibility of the chlorine atoms[31].

It is also possible to explain some observed trends in chemical shifts on the basis that electronegative substituents make the phosphorus more positive and causes the proton resonance signals to be shifted downfield[32-34]. For example the resonance signals corresponding to the Me group in $CH_3SP(O)Cl_n$ are shifted downfield as the inductive effect of the chlorine atoms is increased through successive degrees of chlorination[35].

Intra-molecular shielding contributions are greatly reduced by the interposition of a heteroatom (N,O,S) The chemical shift of the Me group in CH_3OP- (τ = 5.8 - 6.8) is consistently lower than that in CH_3SP- (τ = 7.0 - 8.0)

due to the greater electronegativity of oxygen[30]. When allowance has been made for the major influence of the heteroatom, sensitivity to other substituents on the phosphorus atom is again observed[35].

7.3 Coupling between hydrogen and phosphorus

P O C H, P S C H couplings are noticeable, but are too small to be measured with accuracy from ^{31}P resonance spectra. Proton resonance spectra are more suitable for measuring these small coupling constants. Little is known as regards the relative signs of these coupling constants, and in this discussion only the moduli will be quoted.

It is possible to correlate the coupling constant J with bond type. The nature of the Y heteroatom is of primary importance in the magnitude of P Y C H couplings. The magnitude of J is governed mainly by inductive effects, and is found to decrease with the electronegativity of Y, for example,[35,36]

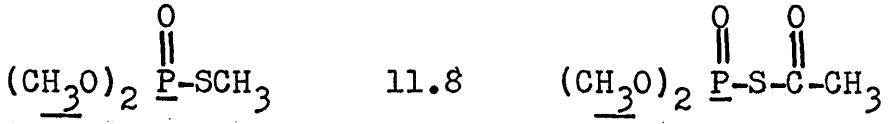
$$\underline{P}\,O\,C\,\underline{H} \;<\; \underline{P}\,N\,C\,\underline{H} \;<\; \underline{P}\,S\,C\,\underline{H}$$

$$CH_3C\underline{H}_2Y\underline{P} \;\cdots\cdots\cdots\; <\; C\underline{H}_3\;Y\underline{P}$$

The important decrease from $C\underline{H}_3$ to $CH_3C\underline{H}_2$ follows the decrease in the inductive effect. Some typical coupling constants in Hz are[30] :

$(C\underline{H}_3O)_3\underline{P}(O)$	11.0	$(C\underline{H}_3S)_3\underline{P}(O)$	15.1
$(C\underline{H}_3O)\underline{P}(S)$	13.4	$(C\underline{H}_3S)_3\underline{P}(S)$	17.5

In relatively few cases, conjugative effects seem to be important, for example,



7.4 ^{1}H nmr of some organophosphorus pesticides

In two recent publications[37,38], the ^{1}H nmr spectra of some 80 organophosphorus compounds have been studied. In Table 6, the data available[30-39] for two groups of organophosphorus pesticides are summarised, and the limits within which the chemical shifts and coupling constants lie are quoted.

Table 6

Chemical shifts and coupling constants in some organophosphorus pesticides

$(CH_3O)_2\underline{P}(X)-YR'$ R' = Alkyl, aryl X,Y = O,S		$(CH_3CH_2O)_2\underline{P}(X)-YR'$ R' = Alkyl, aryl X,Y = O,S	
$\tau(CH_3)$	6.0 - 6.3	$\tau(CH_3)$	8.5 - 8.7
		$\tau(CH_2)$	5.6 - 6.0
J(P-H)	10 - 16 Hz	J(P-CH_3)	0.5 - 1.5 Hz
		J(P-CH_2)	7 - 10 Hz
		J(H - H)	6 - 8 Hz

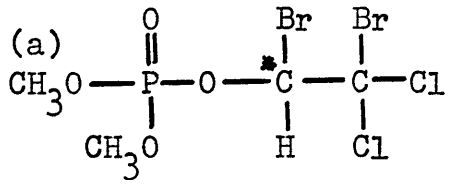
The ^{1}H nmr spectra of Dibrom, Dimethoxon, Cygon, Ronnel,

Table 7

'H nmr of some organophosphorus pesticides

(after ref. 37)

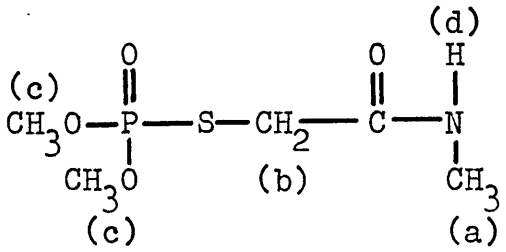
Dibrom



(a') (b)

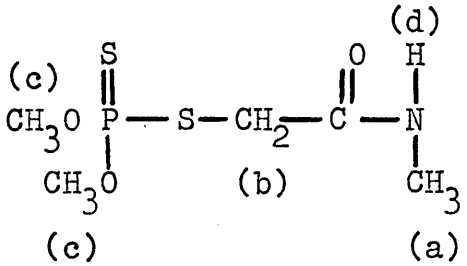
			J(P-H) Hz
(a)	doublet	6.08	12
(a')	doublet	6.09	12
(b)	doublet	3.30	9

Dimethoxon



			J(P-H) Hz
(a)	doublet	7.17	5
(b)	doublet	6.49	17
(c)	doublet	6.17	12.5
(d)	not resolved	2.55	

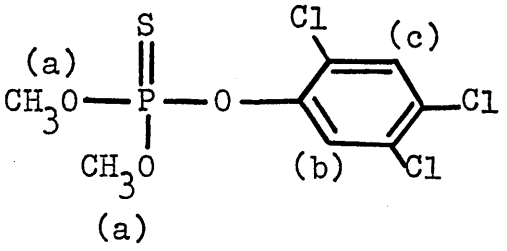
Cygon



			J(P-H) Hz
(a)	doublet	7.15	4.5
(b)	doublet	6.44	18
(c)	doublet	6.20	15
(d)	not resolved	3.13	

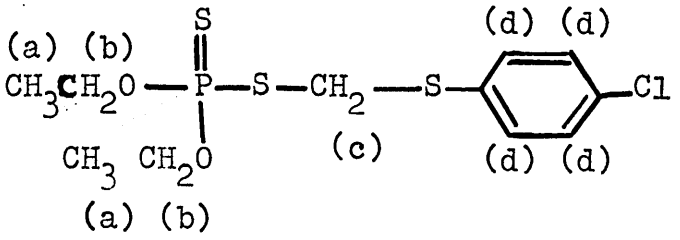
Table 7 (continued)

Ronnel



			J(P-H) Hz
(a)	doublet	6.10	14
(b)	doublet	2.55	1.8
(c)	doublet	2.47	1.0

Trithion



			J Hz
(a)	triplet further split	8.67	7 (H-H) 0.8 (P-H)
(b)	pair of quartets	5.88	10 (P-H) 7 (H-H)
(b')	pair of quartets	5.91	10 (P-H) 7 (H-H)
(c)	doublet	5.72	13.5 (P-H)
(d)	multiplet	2.68	

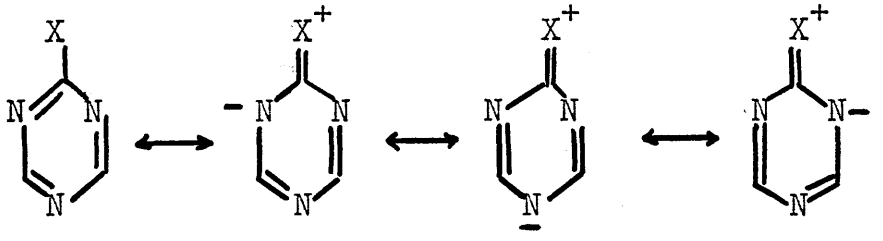
and Trithion[37] are given as examples in Table 7. It is interesting to observe that the Me of the two methoxy groups in Dibrom give rise to a pair of very close doublets a,a', and this is due to the presence of an asymmetric carbon atom (C^*) in the molecule four bonds away from the Me groups. In Dimethoxon, Cygon and Ronnel the Me of the methoxy groups give rise to only one doublet. In Trithion, 16 peaks are observed from the methylene signal instead of the expected 8 peaks. This is probably due to nonequivalence of the two methylene groups, each group being in a slightly different environment due either to steric effects[40,41] or to a lack of symmetry of the molecule as a whole[42,43]. In Ronnel long range coupling occurs between ^{31}P and the aromatic protons[37], and may take place through the π electrons of the aromatic system.

8 The ^1H nmr spectra of 1,3,5-triazines

The ^1H nmr spectra of only a few 1,3,5-triazine derivatives have been reported[44]. The spectrum of the parent 1,3,5-triazine heterocycle shows a very sharp singlet at $\tau = 0.82$ (in $CDCl_3$), and this low field signal is obviously expected from its aromatic nature. The aromatic protons in pyrimidines and pyrazines give a broadened resonance signal, a phenomenon usually attributed to quadrupole relaxation. It is difficult to explain simply why a sharpened signal is obtained in the case of 1,3,5-triazine, for although the molecule is more symmetrical than pyrimidine or pyrazine local asymmetries may still be present.

The proton chemical shifts of a few 1,3,5-triazine derivatives[44] are recorded in Table 8.

The aromatic protons in 1,3,5-triazines also give a sharp singlet. It is clear from the data in Table 8 that electron donating substituents in 1,3,5-triazine derivatives displace the triazinyl proton signal relative to that of the unsubstituted heterocycle. The aromatic proton in 2,4-dimethoxy-1,3,5-triazine is shielded by 0.67 ppm (1.49 - 0.82) with respect to the parent heterocycle. In anisole, however, the proton situated in the meta position is shielded by only 0.04 ppm. Again in dimethylaniline, the meta position is shielded by 0.10 ppm, while the two diethylamino groups shield the triazinyl proton by 1.20 ppm (2.02 - 0.82). Electron donating groups have thus an enhanced effect in the triazinyl system. This may be simply explained by considering the mesomeric forms:

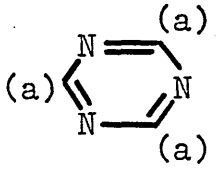


It is clear that the mesomeric forms can carry a negative charge on the nitrogen heteroatom thus transmitting to the meta positions the effect of the perturbing group X.[44]

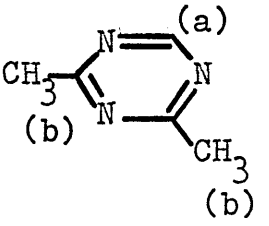
But there is no simple general rule for predicting quantitatively the shielding caused by two substituents on the 1,3,5-triazine ring, since the shielding caused by a single

Table 8

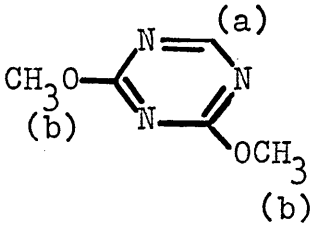
The proton chemical shifts (τ values) of some 1,3,5-triazines (in $CDCl_3$)*



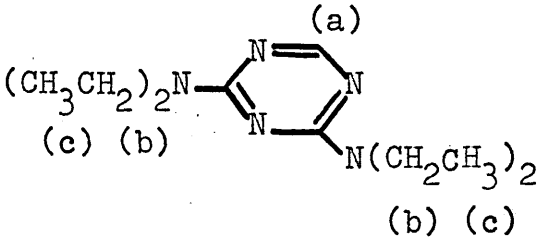
a	0.82



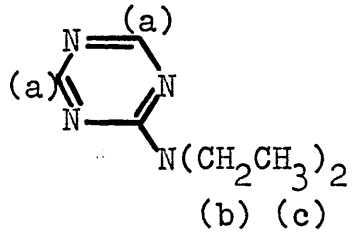
a	1.20
b	7.46



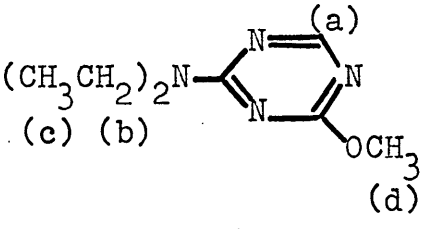
a	1.49
b	5.99



a	2.02
b	6.46
c	8.85



a	1.60
b	6.37
c	8.80



a	1.81
b	6.39
c	8.81
d	6.04

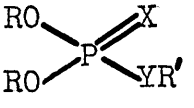
* Taken from ref. 44.

diethylamino group is 0.78 ppm (1.60 - 0.82) while the introduction of a second diethylamino group causes a further shielding of only 0.42 ppm (2.02 - 1.60).

9 The mass spectra of organophosphorus compounds

9.1 General

The main features of the mass spectra of various organophosphorus pesticides related to menazon will be outlined. As before these are of the general structural type



R = Me, Et

R' = alkyl, aryl

X,Y = O,S.

The mass spectrometric identification of these compounds is in general difficult, due largely to the fact that isomerisations, saponifications, and oxidations occur relatively easily. Also the formation of fragments is sometimes sensitive to the kinds of substituents present, so that the general features of the fragmentation may not always be readily recognizable. An example of each main type will be given, but generalisations should be made with care.

9.2 Dichlorphos

The mass spectrum of dichlorphos[45] 4, Fig. 1 (page 27), illustrates the general features commonly encountered in the mass spectra of phosphoric esters of structural type 1. It shows a characteristic ion 2 of m/e 109 which decomposes

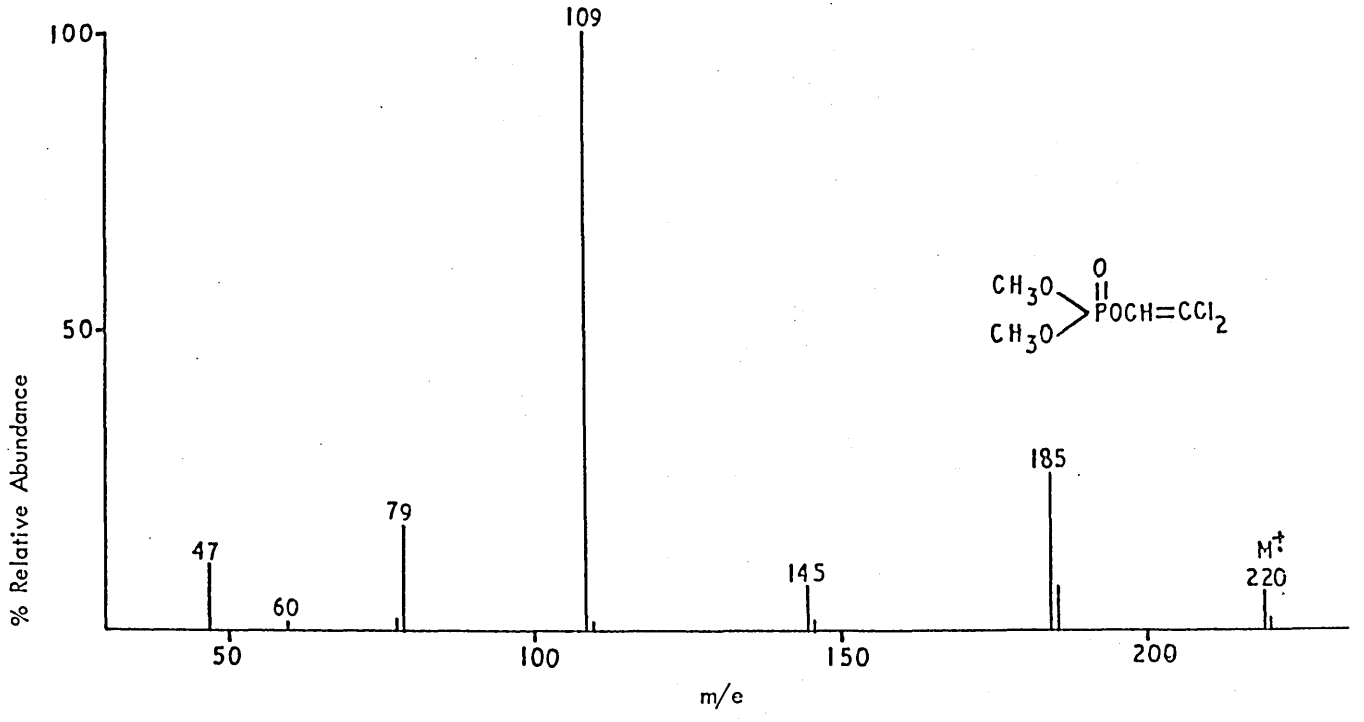
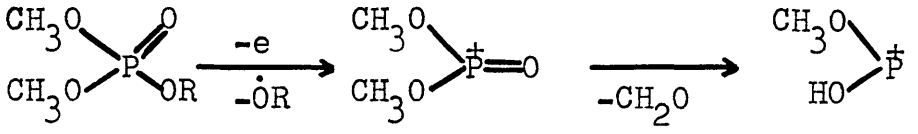
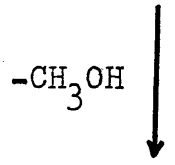


Fig. 1 Mass spectrum of dichlorphos

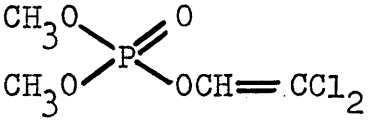
with elimination of formaldehyde yielding the fragment 3 of m/e 79. There appears also a fragment of m/e 47 to which formula PO^+ can be attributed.



1 2 (m/e 109) 3 (m/e 79)



PO^+
(m / e 47)



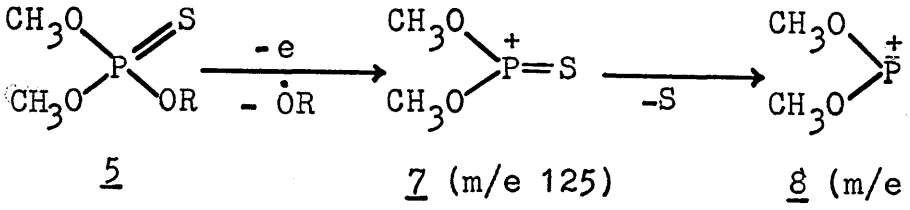
Dichlorphos 4

The molecular ions of phosphoric esters of type 1 are of varying intensity depending on the nature of the radical R. In compounds where R is of aromatic nature, they are almost always very strongly pronounced, whereas when R is predominantly aliphatic they attain often only a low intensity. The fragments characteristic of the phosphorus ester part of the molecule seem to be formed in greater frequency when R is an aliphatic group containing several halogen atoms, as well as when R is an aromatic group substituted with nitro groups[45].

9.3 Methyl parathion

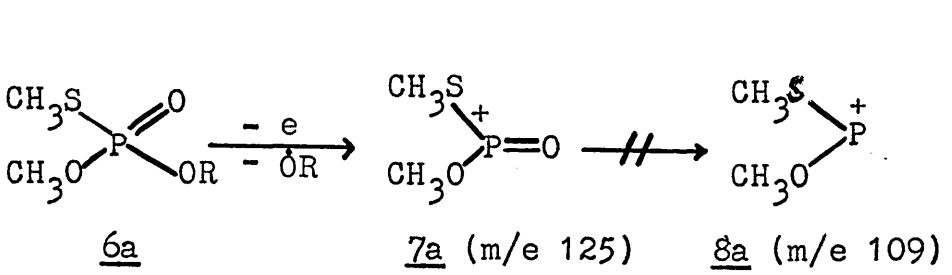
The mass spectrum of methyl parathion[46] 11, Fig. 2

(page 30), shows the general features often present in the mass spectra of phosphorothionic esters of structural type 5. It is known that these esters can undergo an isomerisation, especially at elevated temperatures, leading to the formation of a thiol ester (6 or 6a) of lower energy[47]. Therefore, besides the expected structure 7, the fragment of m/e 125 can also be attributed the structure 7a as a result of the previous isomerisation (5 - 6a). Similarly the fragment of m/e 109 can have structure 9 as a result of such isomerisation (5 - 6).

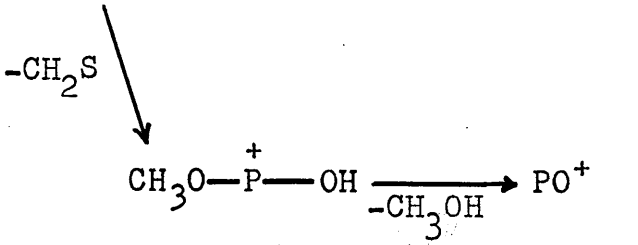


5 7 (m/e 125) 8 (m/e 93)

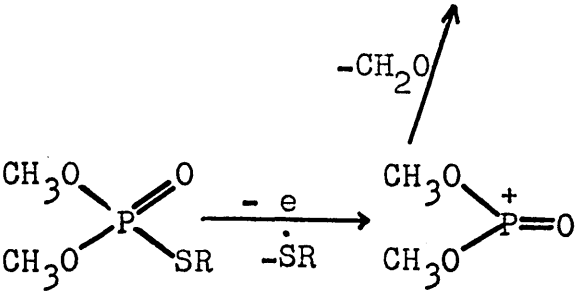
Heat



6a 7a (m/e 125) 8a (m/e 109)



10 (m/e 79) (m/e 47)



6 9 (m/e 109)

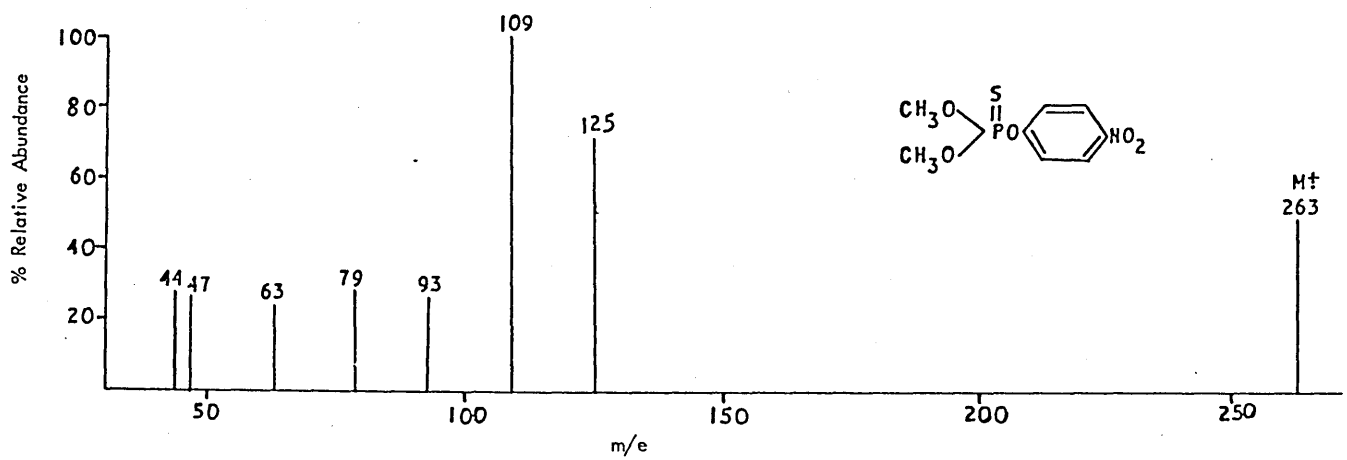
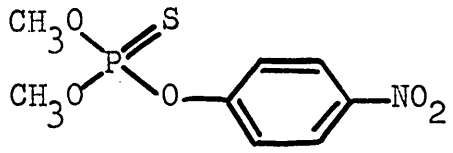


Fig. 2 Mass spectrum of methyl parathion



Methyl parathion 11

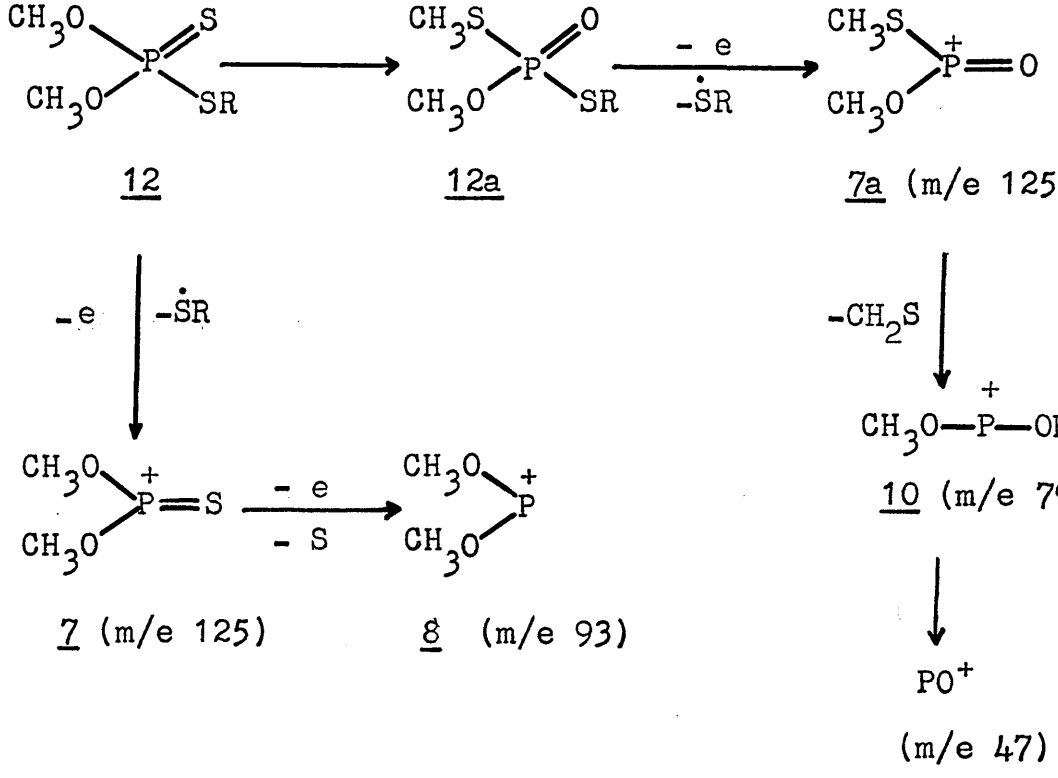
Although the elimination of formaldehyde from 7 (m/e 125) is possible by analogy with the previously discussed degradation of fragment 2, it recedes considerably compared with the loss of sulphur[45] to give ion 8 of m/e 93. Additionally is formed a fragment PS^+ analogous to PO^+ of m/e 63.

The degradation products 10 of m/e 79 and m/e 47 can be derived from both 9 and 7a [45], and it is not known which of the two isomerisation paths is preferred. The elimination of oxygen from 7a, analogous to the elimination of sulphur from 7, is formally conceivable, but such processes have been so far observed[48,49] only during the degradation of N-oxides and aromatic compounds, where other degradation paths are impossible.

The mass spectrum of methyl parathion (Fig. 2, page 30) shows intense peaks at m/e 125, 104, 93, 79, 63, 47. These peaks as discussed above are due to phosphorus fragments, and are typical of the mass spectra of esters of type 5. The loss of NO from the molecular ion of methyl parathion to produce an intense peak at M - NO would be expected since the loss of NO from aromatic nitro-compounds is well known[50], but surprisingly was not observed.

9.4 Methyl trithion

The mass spectrum of methyl trithion[46] 13, Fig. 3 (page33), shows many of the features commonly encountered in the mass spectra of phosphorodithioates of structural type 12. For the most part beta-cleavage occurs to produce a relatively intense peak with the charge residing on the R moiety[46]. They can also yield fragments 7 (m/e 125), 8 (m/e 93) and a fragment of m/e 63. The isomerisation reaction which leads to 12a permits us to expect the already known fragment 7a (m/e 125) and in the further course, its degradation products 10 (m/e 79) and m/e 47.



The mass spectrum of methyl trithion (Fig. 3, page 33) shows, like the mass spectra of many phosphorodithioates of type 12, typical phosphorus fragments of m/e 125, 93, 79, 63

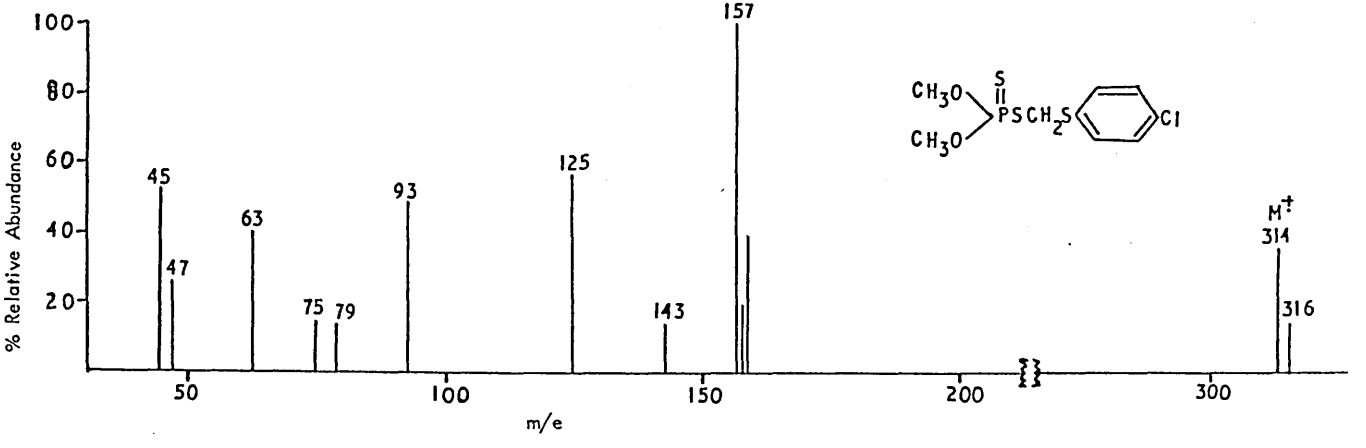
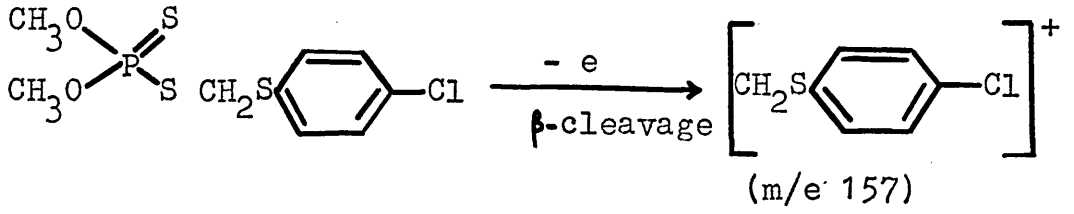


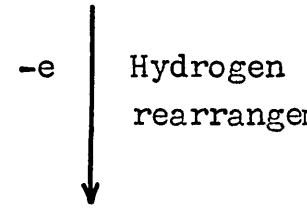
Fig. 3 Mass spectrum of methyl trithion.

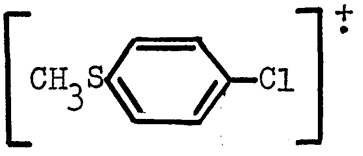
and 47. It also shows as expected a fragment of m/e 157 which is the base peak of the spectrum due to beta-cleavage of the molecule with the charge residing on the R fragment. The peak at m/e 158 is possibly due to a rearrangement with a hydrogen migrating to the methylene group of the R moiety.



(m/e 157)

Methyl trithion 13



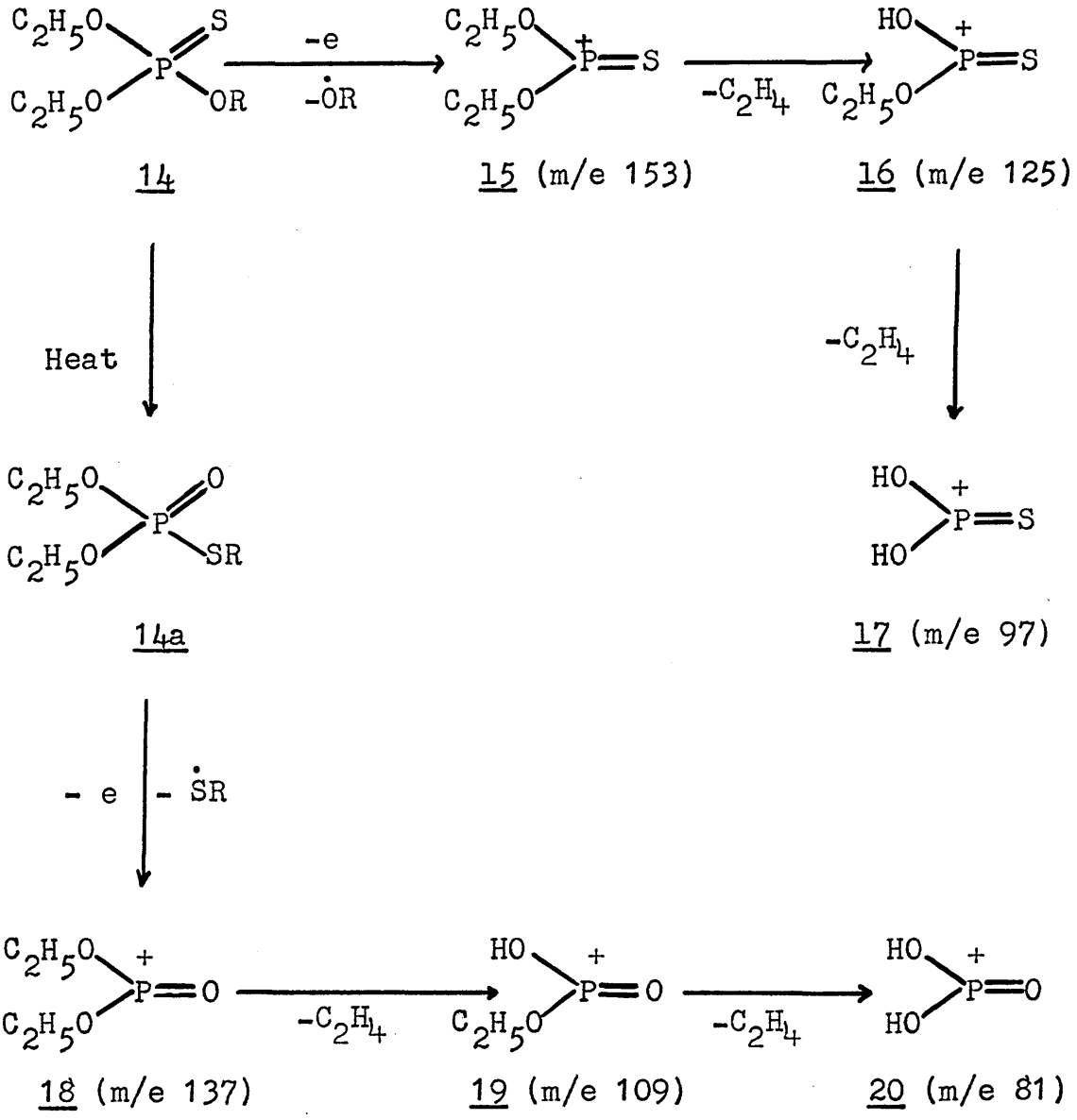


(m/e 158)

9.5 Parathion

The mass spectra of ethyl or higher alkyl phosphate esters are generally more complicated than those of the corresponding methyl esters. The case of the mass spectrum of triethyl phosphate has been much discussed[51-54]. The mass spectrum of parathion, Fig. 4 (page 36), contains many more peaks than that of methyl parathion (Fig. 2, page 30). It illustrates many of the features present in the mass

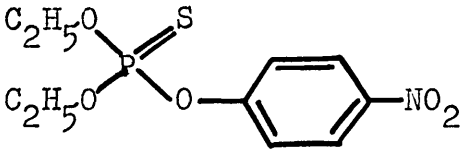
spectra of ethyl esters of structural type 14. Fragments appear in the spectrum by gradual elimination of ethylene[45].



14 15 (m/e 153) 16 (m/e 125)

14a 17 (m/e 97)

18 (m/e 137) 19 (m/e 109) 20 (m/e 81)



Parathion 21

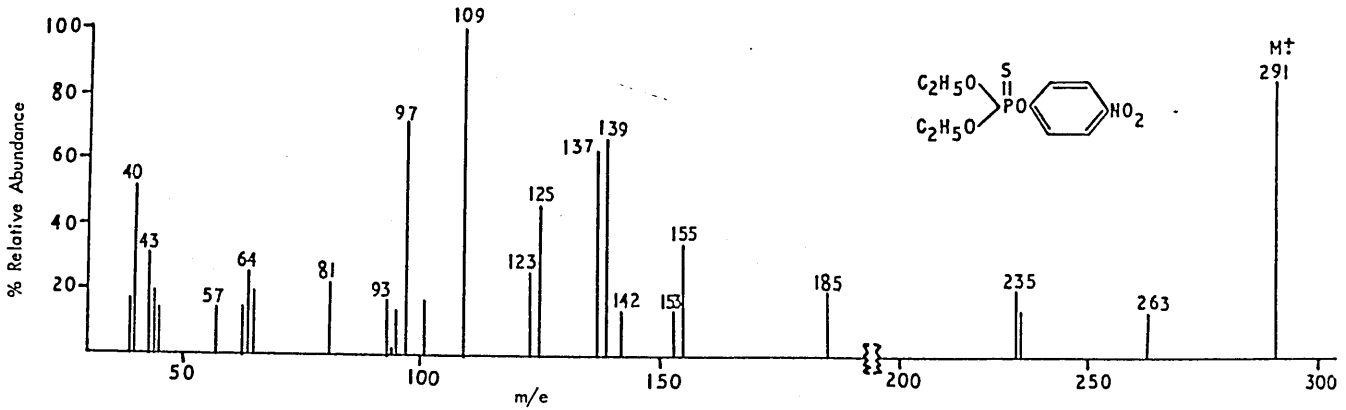


Fig. 4 Mass spectrum of parathion

Isomerisation (14 – 14a) may also occur leading to the formation of fragments 18, 19, 20. Besides the typical phosphorus fragments of m/e 153, 137, 125, 109, 97, 81, the mass spectrum of parathion also shows an intense fragment ion peak at m/e 139. This may be due to the p-nitrophenol ion, since an abundant phenol ion has been observed in the spectra of dialkyl phenyl phosphates[51].

10 The ultraviolet spectra of 1,3,5-triazines

10.1 General

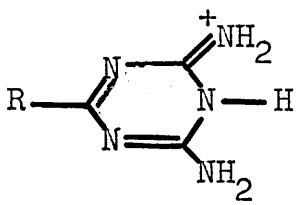
The most extensively investigated and best understood absorption bands of heterocyclic compounds are the $n - \pi^*$ bands of the azines, which have no counterpart in the spectrum of benzene. In the vapour phase, these long wavelength bands have a sharp vibrational structure which is blurred in non polar solvents and lost in hydroxylic solvents[55,56]. In contrast the shorter wavelength $\pi - \pi^*$ bands of the azines have a diffuse vibrational structure in the vapour phase, and they retain in aqueous solution the structure given in non polar solvents. On changing from a non polar to a hydroxylic solvent the long wavelength, the $n - \pi^*$, bands of the azines shift to the blue, and in acid solution they often disappear. On the basis of these characteristics, Kasha[57] assigned the long wavelength absorption band to transitions arising from the promotion of a non bonding lone-pair nitrogen electron to an antibonding π-orbital. In hydroxylic solvents the lone-pair electrons of the azines are hydrogen bonded, and the promotion of such an electron to a π^*-orbital

requires the provision of additional energy to weaken or break the hydrogen bonds, thus accounting for the blue shift. Also in hydroxylic solvents the energies of the nitrogen lone-pair electrons are not sharp, as hydrogen bonds have a range of energy values, and the vibrational structure of the azine n – π^* bands in these solvents is effectively blurred.

10.2 Diamino-, hydroxyamino-, dihydroxy-1,3,5-triazines

The position of the long wavelength n – π^* absorption band of a large number of substituted 1,3,5-triazines has been studied by Overberger and Chapiro[58-61]. Diamino-, hydroxyamino-, dihydroxy-1,3,5-triazines can be readily differentiated by their characteristic uv absorption, due to the n – π^* transition, at different pH values[3]. Other substituents often have a marked effect on the spectra, for example N-oxide formation[3]. Ultraviolet absorption can be a valuable tool in identifying 1,3,5-triazine compounds provided they can be isolated in an adequate amount and in a pure state.

Menazon like other diamino-1,3,5-triazines shows an absorption maximum in neutral solvents or aqueous buffer (pH 5-8) in the region of 260nm . Below pH 3 this maximum disappears, due to the proton adding to one of the ring nitrogen atoms, and the spectra in acid solution are probably produced by the species[3],



Hydroxyamino-1,3,5-triazines show an increased uv absorption in acid solution which suggests retention of ring conjugation under these conditions, and it is likely that addition of the proton to the $-NH_2$ groups occurs. Dihydroxy-1,3,5-triazines are much more soluble in water than the diamino- or hydroxyamino-1,3,5-triazines, and do not have this characteristic absorption maximum in acid or neutral solutions. This is because they exist mainly in the Keto form under these conditions, giving rise to a C=O band in the infrared region near $1740cm^{-1}$. In alkaline solution, however, hydroxy structures predominate, and this is reflected in the appearance of a strong uv absorption band at approximately 250 nm .[62]

11 Proposed programme of work

One of the routine tests applied to formulated pesticides is an accelerated storage test. This consists of holding the sample at 80^{o} for three days followed by estimation of any degradation. Under these conditions it was found by chromatography that menazon partially degraded into several products. It was felt that, if more was known about the identity of the degradation products and their method of formation, it might prove possible to modify the formulation to reduce the extent of the degradation.

It was decided that actual separation and isolation of the degradation products followed by identification would be the most satisfactory method of tackling the problem. Previous experience suggested that gas liquid chromatography

would not be suitable as a means of separation. Reproducible separation of the degradation products had been obtained on thin layer chromatography plates, and it was thought that isolation of the separated compounds for complete identification by infrared, ultraviolet, nmr spectroscopy and mass spectrometry would be feasible.

RESULTS

1 Chromatography of samples of heated menazon

Menazon was recrystallised, dried, and the purity examined by two dimensional paper chromatography, developing with BAW solvent in one direction and at right angles with HCMW solvent. The chromatogram was examined under ultraviolet light (254 nm) (henceforth referred to as uv) and then sprayed with 2,6-dibromoquinone-N-chloroimide (henceforth referred to as DBQ). A single uv quenching area was detected. This area also gave a reddish brown colour with DBQ; no other areas gave a colour reaction with this reagent. This sample of menazon was regarded as being chromatographically pure, and was heated in an oven, set at 80°, for 18 days. Portions (about 200 mg) were removed after 2, 3, 6, 10 and 18 days. These were stirred with dioxan-water (1:1 v/v; 100 mg/2 ml) at 45°, any insoluble material being filtered and weighed. The 2 day sample of heated menazon gave a clear solution. The 3 day sample gave a slightly turbid solution. The sample of menazon heated for 6, 10 and 18 days gave, respectively, 6.5 mg, 22 mg and 40 mg of insoluble pale yellow residue. An indication of the extent of thermal decomposition can thus be obtained by weighing the amount of insoluble residue left when the heated sample is digested with dioxan-water.

The dioxan-water solutions were chromatographed on paper (one and two dimensions) and on thin layer chromatography (henceforth referred to as tlc) plates (as spots and bands) to assess the extent of thermal decomposition and to

evaluate the most appropriate conditions for isolation of the products of the degradation.

1.1 One dimensional paper chromatography of the dioxan-water solutions of heated menazon

The dioxan-water solutions of the 2, 3, 6, 10 and 18 day samples of heated menazon were applied to sheets of Watman No. 1 chromatography paper as spots containing 50 µg, 100 µg, 200 µg, 250 µg, 300 µg, 350 µg, and 400 µg of material by means of an Agla syringe alongside a menazon "marker" containing 50 µg. The paper was developed (ascending) for 12 hours in a tank containing BAW solvent. The chromatograms were viewed under uv light and then sprayed with DBQ. The 2, 3, 6, 10 and 18 day samples all gave:

(i) eight uv quenching areas which had various colours after spraying with DBQ

and (ii) three areas which gave a colour reaction with DBQ but did not quench uv light.

The amount of material in each located area varied with the duration of heating. This meant that examination of the chromatograms of the higher loadings (300 - 400 µg) was necessary with the samples that had been heated for 2 and 3 days. However it was clear that the uv quenching area at relative front (hereafter referred to as R_F) values of 0.85, corresponding to menazon, was more intense in the 2 and 3 day samples than in the 6, 10 and 18 day samples. For samples which had been heated for 10 days the maximum loading which

could be applied as one spot and still be separated into eleven distinct areas was 150 µg.

A tracing of a typical one dimensional paper chromatogram, at a loading of 150 µg as a single spot, of a sample of menazon heated for 10 days at 80° C is shown in Fig. 7 (App., page 175). The R_F values, the intensity of the uv quenching areas, and the DBQ colour of the separated thermal breakdown products are given in Table 9 (page 44).

1.2 Two dimensional paper chromatography of the dioxan-water solutions of heated menazon

In view of the large number of areas located on the one dimensional paper chromatogram, it was thought advisable to examine the separation of the breakdown products by two dimensional paper chromatography. This would in any case produce wider separations of the relevant areas and may also produce additional separation of areas which appeared to be homogeneous on the one dimensional chromatogram. Accordingly, varied amounts of the heated menazon samples were applied as single spots to Whatman No.1 chromatography paper. The papers were developed in one direction with BAW solvent (descending) for 12 hours and at right angles with HCMW solvent (ascending) for 6 hours. The chromatograms were examined under uv light and also sprayed with DBQ.

It was found that the maximum amount of heated menazon that could be applied as a single spot, and still result in complete separation of the components was 350 µg.

A tracing of a two dimensional chromatogram of a sample

Table 9

One dimensional paper chromatography of menazon heated for 10 days at 80°

Area No.	R_F	uv quenching	DBQ colour
1	0.85	medium strong	reddish brown
2	0.79	strong	yellow
3	0.66	strong	yellow
4	0.49	nil	yellow
5	0.45	strong	no colour
6	0.38	nil	pale orange
7	0.35	medium strong	orange
8	0.30	weak	brown
9	0.25	weak	yellow
10	0.20	nil	yellow
11	0.06	very weak	yellow

Solution of heated menazon obtained in dioxan-water (1:1 v/v). 150 µg applied to Whatman No.1 paper as a single spot, and developed (ascending) with BAW solvent for 12 hours. Chromatogram examined under uv light and also sprayed with DBQ.

of menazon heated for 10 days at 80° C is shown in Fig. 8 (App. page 176); the R_F values, the intensity of the uv quenching areas and the DBQ colour of the separated thermal breakdown products are given in Table 10 (page 46). In addition to eight uv quenching areas, there were six non-quenching areas which gave a colour with DBQ. The one dimensional paper chromatograms showed eight uv quenching spots and only three non-quenching spots which were located by their colour after spraying with DBQ. The two dimensional system of separation did seem therefore, to achieve a more complete separation of the thermal breakdown products of menazon. Moreover these results indicated that a clearly defined area on a developed chromatogram could, in fact, consist of more than one component.

It was now necessary to find out if the separations obtained by paper chromatography of heated menazon could be achieved by thin layer chromatography.

1.3 Thin layer chromatography of heated menazon, applied as spots

Dioxan-water solutions of the samples of menazon heated at 80° for 2, 3, 6, 10 and 18 days were applied to the plates (silica gel HF_{254}; 0.25 mm thick) as spots containing 100 µg 200 µg, 300 µg, 400 µg and 500 µg. The developed (BAW) plates were examined under uv light and also sprayed with DBQ. Ten distinct areas were located at the 100 µg, 200 µg, 300 µg and 400 µg levels. At the 500 µg level overlapping of these areas took place. Table 11 (page 47) summarises the results

Table 10

Two dimensional paper chromatography of menazon heated for 10 days at 80°

Area No.	R_F Values		uv quenching	DBQ colour
	BAW	HCMW		
1	0.84	0.70	medium strong	strong reddish brown
2	0.79	0.61	strong	strong yellow
3	0.68	0.85	nil	pale yellow
4	0.66	0.54	strong	strong yellow
5	0.48	0.78	nil	pale yellow
6	0.47	0.48	very strong	no colour
7	0.39	0.40	nil	pale orange
8	0.36	0.77	nil	strong yellow
9	0.35	0.12	weak	pale brown
10	0.31	0.32	medium	strong brown
11	0.26	0.24	weak	no colour
12	0.21	0.60	nil	strong yellow
13	0.07	0.11	very weak	no colour
14	0.03	0.43	nil	strong yellow

Solution of heated menazon obtained in dioxan-water (1:1 v/v). 350 µg applied to Whatman No.1 paper as a single spot, and developed (descending) with BAW solvent for 12 hours, and at right angles (ascending) with HCMW for 6 hours. Chromatogram examined under uv light and also sprayed with DBQ.

Table 11

Thin layer chromatography of menazon heated for 10 days at 80°, applied as a spot

Area No.	R_F	uv quenching	DBQ colour
1	0.76	very strong	reddish brown
2	0.72	very strong	strong yellow
3	0.61	very strong	yellow
4	0.45	nil	orange
5	0.42	very strong	yellow
6	0.35	strong	orange
7	0.27	weak	pale orange
8	0.20	strong	pale brown
9	0.12	very weak	pale yellow
10	0.05	very weak	pale yellow

Solution of heated menazon obtained in dioxan-water (1:1 v/v). 400 µg applied as a single spot to tlc plate (silica gel HF_{254}; 0.25 mm thick), and developed with BAW solvent for 3 hours. Plate examined under uv light and also sprayed with DBQ.

of the tlc of menazon heated at 80° for 10 days, applied at the 400 µg level.

There were nine uv quenching areas and one area which did not quench but gave a colour reaction with DBQ.

As the ultimate aim was to use preparative tlc it was now necessary to investigate whether the separations already achieved with thin layer chromatography could be repeated when the heated menazon was applied as a band. It was also of vital importance to know to what extent increasing the amount of applied material affected the separations.

1.4 Thin layer chromatography of heated menazon applied as a band

The dioxan-water solutions of the samples of menazon heated at 80° for 2, 3, 6, 10 and 18 days, were applied to the thin layer chromatography plates (20 x 20 cm silica gel (HF_{254}; 0.25 mm thick) as narrow bands, using an Agla syringe mounted on a trolley (Fig. 5, page 49). Each of the heated samples was applied to plates at the 2 mg, 4 mg, 6 mg and 8 mg levels. The plates were developed in BAW solvent examined under uv light and sprayed with DBQ. All the heated samples gave essentially the same overall pattern of uv quenching bands although there were variations in intensity. The maximum amount of heated menazon that could be applied to the plate as a band, and still result in complete separation was 6 mg.

A photograph of a typical thin layer chromatogram is shown in Fig. 9 (page 50). The R_F values, the intensity of

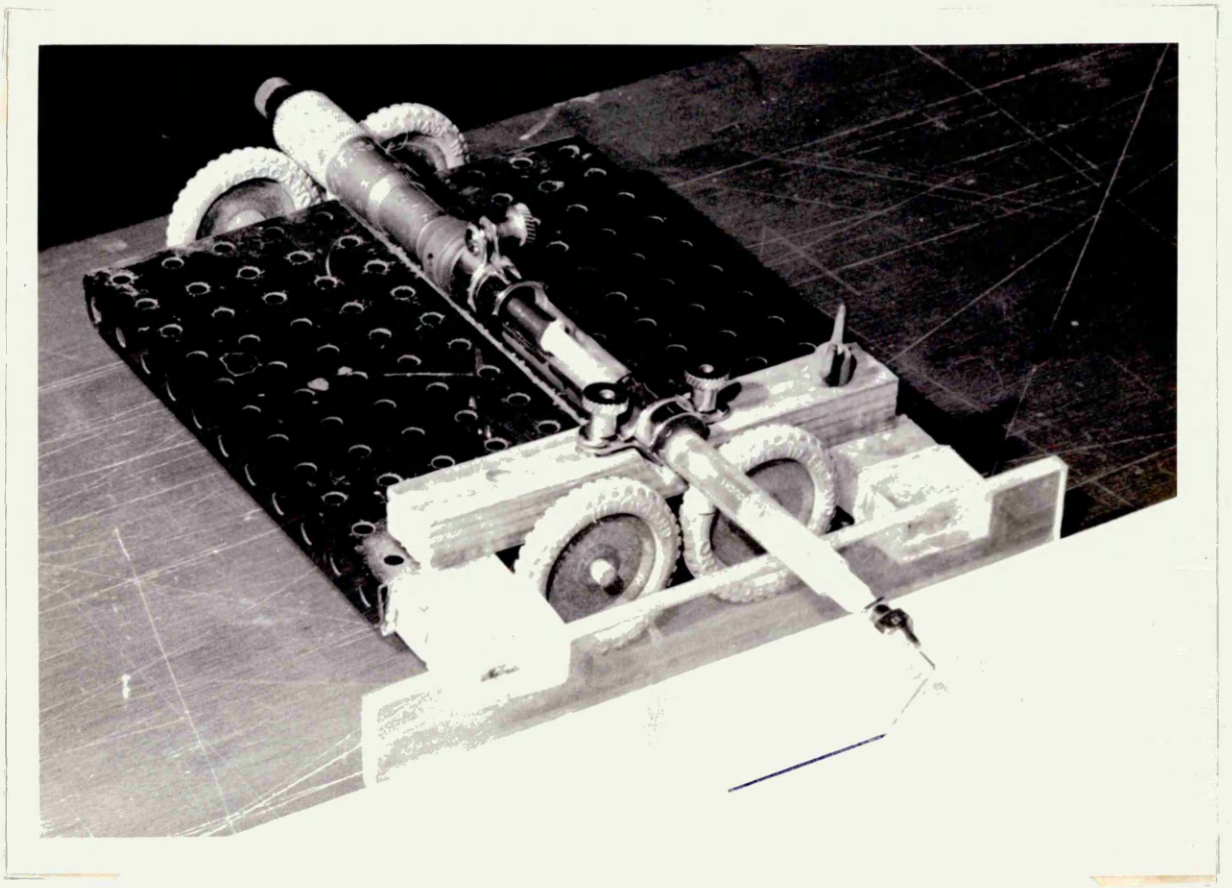


Fig. 5. Photograph of an Agla syringe mounted on a trolley used for applying solutions of menazon as bands on tlc plates.

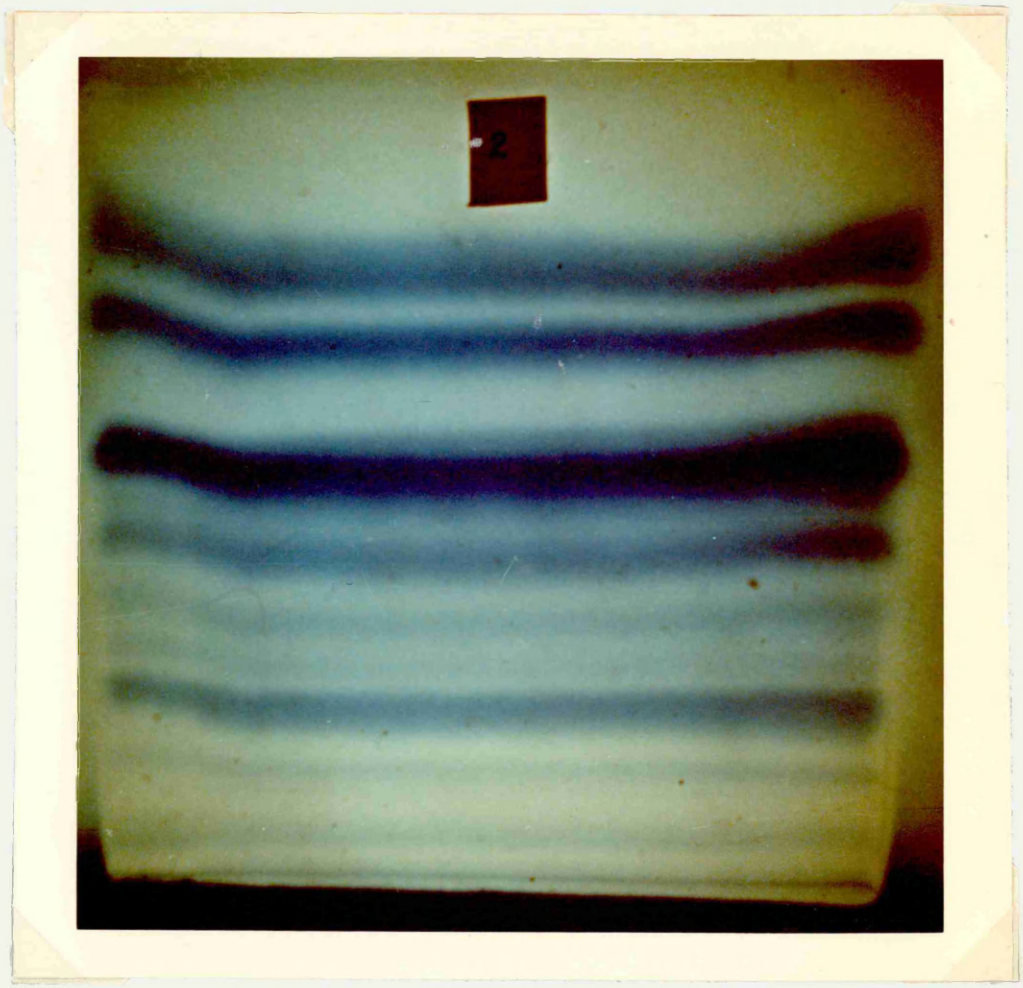


Fig. 9. Photograph taken under uv light of a tlc plate (silica gel HF_{254}; 0.25mm thick) loaded with 6mg of heated menazon applied as a band at the origin. Plate developed with BAW solvent for three hours.

uv quenching areas and the DBQ colour of the separated thermal breakdown products are given in Table 12 (page 52).

There were eight clearly separated areas that quenched uv light, each of these gave a colour reaction with DBQ. It would appear that some of the thermal breakdown products which had very close R_F values when chromatographed as spots (Table 11) are now appearing as a single band. However the separation of the eight uv quenching areas was good enough to warrant putting the procedure on a preparative scale.

The uv quenching bands have been labelled No.1 to No. 8 in order of decreasing R_F value (Table 12). This is the reference table for numbering the bands that will be applied throughout the remainder of this account.

It is also appropriate to note here that bands No. 1, No.2 and No.3 are very strong uv quenching areas, indicating that they probably contain the major breakdown products of menazon. Bands No.4 and No. 6 are medium strong uv quenching areas. Band No. 5 is a weak uv quenching area, and bands No. 7 and No. 8 are extremely weak uv quenching areas.

1.5 Preparative thin layer chromatography of heated menazon

With a view to reducing the total number of plates that would have to be handled, the thin layer chromatography described above (Section 1.4) was repeated with larger plates (40 x 20 cm) and a thicker layer of adsorbent (silica gel HF_{254}; 0.5 mm).

Table 12

Thin layer chromatography of menazon heated for 10 days at 80°, applied as a band

Band No.	R_F	uv quenching	DBQ colour
1	0.76	very strong	strong reddish brown and yellow
2	0.65	very strong	strong yellow
3	0.45	very strong	strong yellow
4	0.35	strong	strong yellow and pale brown
5	0.27	weak	pale brown
6	0.20	strong	pale brown
7	0.12	very weak	pale yellow
8	0.05	very weak	pale yellow

Solution of heated menazon obtained in dioxan-water (1:1 v/v). 6 mg applied as a band to tlc plate (20 x 20 cm; silica gel HF_{254}; 0.25 mm thick) and developed with BAW solvent for 3 hours. Plate examined under uv light and also sprayed with DBQ.

The dioxan-water solutions of heated menazon were again applied as a band, and the total loading per plate varied from 8 mg, 12 mg, 16 mg, 20 mg, 24 mg. It was found that up to 20 mg of heated menazon could be applied to a plate and still result in complete separation of the eight uv quenching areas.

The isolation and identification of the constituents of the samples of heated menazon, described in subsequent pages, was carried out on various batches of plates (Table 13, below).

Table 13

Various batches of preparative tlc plates run and their subsequent treatment

No. of plates	Adsorbent	Treatment after removing plate from developing tank and standing for 40 minutes
50	Silica gel HF_{254}	Bands removed and stored in bottles at room temperature for 14 days
12	Silica gel HF_{254}	Band No.2 removed, and solvent immediately removed under vacuum, and extracted with methanol at room temperature
50	Silica gel HF_{254}	Band No.2 removed, and solvent immediately removed under vacuum. Stored in deep freeze for 14 days Other bands also removed and stored in deep freeze for 14 days
20	Silica gel G/UV_{254}	The bands containing the two components which did not resolve in band No. 1 on preparative tlc plates coated with silica gel HF_{254}, removed and stored in deep freeze for 4 days

After development the plates were allowed to dry at room temperature (20^{o}) for about 40 minutes. After this time there was still a slight smell of the solvent.

The uv quenching areas were scraped off, and the silica gel from corresponding bands combined. The combined batches of silica gel received, in the progress of the present investigation, various treatments prior to solvent extraction of the constituents. These treatments were:

(a) storage in stoppered flasks in a cupboard at room temperature (approximately 20^{o})

(b) immediate transfer to a deep freeze unit ($-\ 20^{o}$) for storage

(c) immediate transfer to a distillation assembly for removal of solvent, and subsequent storage in a deep freeze unit.

2 Extraction of bands removed from preparative tlc plates

After having separated the breakdown products of menazon into eight uv quenching areas, by preparative tlc, it was necessary to extract the silica gel and isolate the actual components.

It was known that menazon had low solubility in water and common organic solvents (for e.g., methanol: 0.6% room temperature, 8% boiling; chloroform: 0.16% room temperature, 1.3% boiling[64]). Moreover it was thought desirable to avoid Soxhlet extraction which might cause further changes in the isolated components. Diamino-1,3,5-triazines are also known to have low solubility in common organic solvents. Methanol was chosen for the first extraction of silica gel. Literature comments[65] suggested that methanol might also remove "silicic acid" from the silica gel. In order to check on this possibility silica gel (50 g) was stirred for 12 hours with methanol (at room temperature) filtered by suction through a hardened filter paper (Whatman 541), and the solution re-filtered, without suction, through a hardened filter paper (Whatman 541). The methanol solutions were evaporated to dryness under vacuum, giving 350 mg of white residue. Portions of this residue were shaken separately with N,N-dimethyl-formamide, carbon tetrachloride, acetone, ethanol, dioxan, ethyl acetate and water. The suspensions were filtered and the filtrates evaporated to dryness. Under these conditions it was thus established that the material extracted by methanol from silica gel was insoluble in

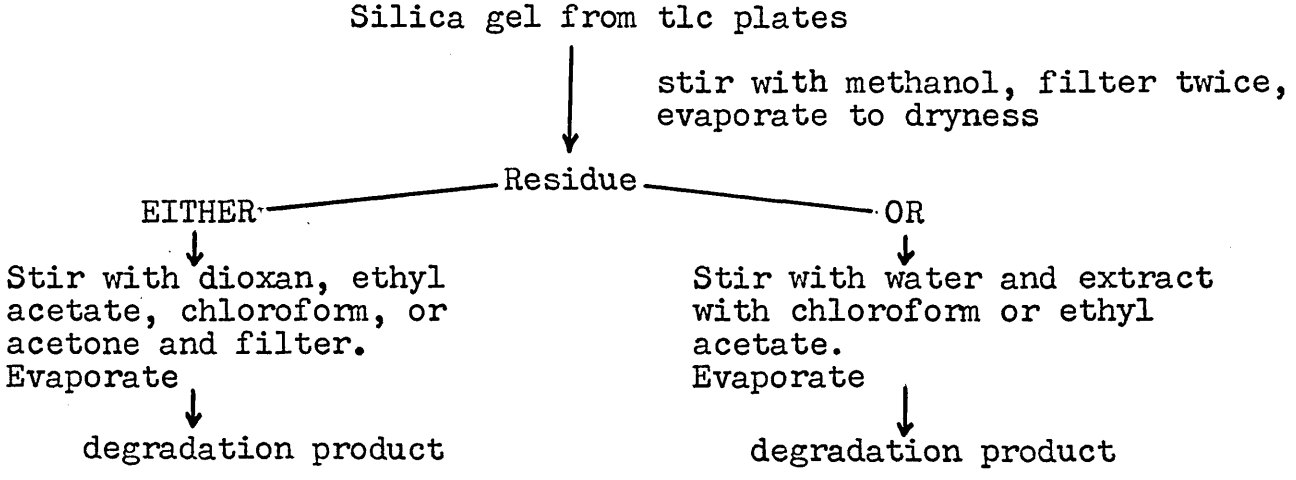
chloroform, acetone, dioxan and ethyl acetate.

On the basis of these observations two general extraction procedures were developed. The silica gel removed from the preparative tlc plates was stirred with methanol (at room temperature), filtered twice (with suction first) through a hardened filter paper, and the methanol removed by vacuum distillation. The residue was stirred with either chloroform, acetone, dioxan, or ethyl acetate, filtered, and the solvent removed in vacuo.

In certain cases it became necessary to remove the solvent under conditions whereby the temperature in the distillation flask was kept as low as possible. These instances are indicated in the text. Conventional vacuum distillation assemblies were used (B14 joint size), the receiver was cooled with an acetone/solid carbon dioxide mixture and the distillation flask immersed, as necessary, in warm water.

An alternative general extraction procedure was used. The residue that was obtained by removal of methanol from the initial extraction of the silica gel, was stirred with water. The whole was extracted with either chloroform or ethyl acetate.

These procedures are summarised as follows :



3 Identification of the compounds extracted from the thin layer chromatography bands

The system that is used in this section for numbering the tlc bands is given on page 51 . The relevant data for these bands is given in Table 12 (page 52).

3.1 Extraction, isolation and identification of the constituents of band No.3

3.1.1 Extraction and isolation

Heated menazon (10 days at 80°) was applied, as a solution in dioxan-water (1:1 v/v), to preparative tlc plates (silica gel HF_{254}; 0.5 mm). The plates were developed six at a time, in BAW solvent and allowed to dry by standing at room temperature for thirty minutes. The silica gel containing band No.3 (R_F = 0.45: strong uv quenching; brownish yellow colour with DBQ) was scraped off each batch of plates and stored in the deep freeze until the required 50 plates had been developed and treated in the same way. The maximum time a sample spent in the deep freeze was two weeks. In this way 1.0 g of heated menazon was chromatographed. The total bulk (50 g) of the silica gel containing band No.3 was placed in the round bottomed flask of a conventional vacuum distillation assembly. The receiver was immersed in a cold trap of solid carbon dioxide-acetone. A vacuum of 20 mm was maintained for three hours. After this treatment the silica gel did not smell of solvent. The silica gel was then stirred with methanol (300 ml) at room temperature for twelve hours. The

bulk of the silica gel was filtered off by suction, the filtrate was refiltered through a hardened filter paper (Whatman No. 541). The clear methanol filtrate was evaporated to dryness in a conventional vacuum distillation assembly in which the receiver was cooled in an acetone-solid carbon dioxide trap, and the distillation flask was occasionally immersed in a water bath (35°). These conditions for removal of solvent were used frequently and will henceforth in this account be referred to as removal of solvent under "cold" conditions. A white residue (350 mg) was left in the flask. Two dimensional paper chromatography of this residue showed a single uv quenching spot which did not give a colour with DBQ, and two non-quenching spots which both gave yellow colours with DBQ. The R_F values of these spots were:

R_F values		uv quenching	DBQ colour
BAW	HCMW		
0.45	0.47	Strong	No colour
0.48	0.78	Nil	Yellow
0.36	0.40	Nil	Yellow

In order to isolate the compound which quenched uv, the residue was shaken with dioxan (2 x 50 ml) at room temperature, filtered, and the solvent removed under "cold" conditions (see above). White crystals (30 mg) were obtained.

This material was rechromatographed on a silica gel plate (HF_{254}; 0.50 mm), being applied as a single spot (200 µg), using BAW. Only one quenching spot (R_F 0.45) could be detected

on the developed plate. The DBQ reagent did not produce any coloured areas. The isolated material was also subjected to two dimensional paper chromatography; 150 μg was applied as a single spot and the developed paper examined under uv light and sprayed with DBQ. There was a single uv quenching spot, the R_F values being 0.45 (solvent BAW) and 0.47 (solvent: HCMW). No areas of the paper gave a colour when sprayed with DBQ. It was concluded that, under these experimental conditions, the compound was pure.

3.1.2 Identification of the compound isolated from band No.3

Various spectra of the compound isolated from band No.3 were obtained, which enabled a structure to be proposed for the compound.

Ultraviolet spectroscopy

Spectra were obtained over the range 230 - 272 nm on aqueous solutions at pH1, pH7 and pH13. These spectra are recorded in Fig. 10 (App. page 133). The shape of the curves and the position of the maximum (λ_{max} 254 nm) and minimum (λ_{min} 238 nm) for the spectra obtained at pH7 and pH13 were indicative of a diamino-1,3,5-triazine ring system. Also, the change in shape of the spectra obtained at pH1 was also characteristic of a diamino-1,3,5-triazine system. It was also concluded from these spectra that there were no hydroxyl groups attached directly to the ring.

Infrared spectroscopy

The infrared spectrum (0.25% KBr disc) is given in Fig. 11 (App. page 142). Presence of a 1,3,5-triazine ring was deduced from the appearance of very strong bands at 1560 cm^{-1}, 1010 cm^{-1}, 815 cm^{-1}. In particular, the band at 815 cm^{-1} is strongly characteristic of the 1,3,5-triazine ring and has been attributed to a bending mode. The absorption bands at 3110 cm^{-1}, 3375 cm^{-1} and 3490 cm^{-1} were attributed to $-NH_2$ stretching modes and those at 1620 cm^{-1} and 1650 cm^{-1} to $-NH_2$ bending modes.

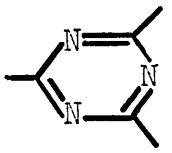
Thus the presence of a 1,3,5-triazine ring and at least one amino group was deduced from the infrared spectrum. The amino group appeared to be primary, although it was not possible to exclude the presence of an, additional, secondary amino group.

Mass spectrometry

The mass spectral data are recorded in Fig 12 and Table 14 (App. pages 156,157). The ion appearing at m/e 125 was assumed to be the molecular ion ($M^{\ddagger}$); this was also the base peak. A methyl group was thought to be present as evidenced by m/e 110 ($M^{\ddagger} - CH_3$). However, m/e 110 was of very low intensity so this deduction was viewed with caution.

The uv and infrared spectra suggested that amino groups were likely substituents on the triazine ring. Aromatic amines of the type $Ar.NH_2$ are known to show peaks due to $M^{\ddagger}$ - HCN ($M^{\ddagger}$ - 27). The peak at m/e 98 ($M^{\ddagger}$ - 27) was 6.1% of the base peak. This was at least consistent

with the presence of an $-NH_2$ group although loss of HCN did not appear to be a major fragmentation route. The units

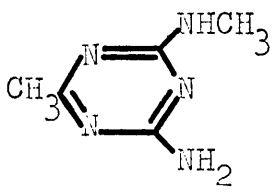


; $-CH_3$ and $-NH_2$

were indicated. This left $-NH_2$ unaccounted for in the molecular formula.

Nuclear magnetic resonance spectroscopy

The nmr spectra were obtained on solutions in trifluoroacetic acid using tetramethylsilane as internal standard. A typical spectrum is given in Fig. 13 (App. page 151). Two signals were evident, at 2.2τ (very broad signal, integrated for 4 protons) and 7.31τ (singlet, integrated for 3 protons). The high field signal suggested $-CH_3$ in an electron withdrawing environment. The very broad, low field, signal was indicative of amino groups and suggested two $-NH_2$ groups. Although the shape of this band made positive assignment difficult, it was felt reasonable to exclude from consideration the group -NHMe. The nmr spectrum of



[*]

is given in Fig. 14 (App. page151), for comparison.

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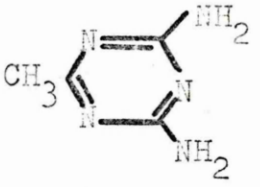
[*] Sample synthesised by Dr. J.B. Turner.

The spectroscopic data suggested that the triazinyl compound isolated from band No.3 was

2,4-diamino-6-methyl-1,3,5-triazine (I)



I

The spectroscopic data outlined above were compared with those obtained from an authentic sample of 2,4-diamino-6-methyl-1,3,5-triazine The comparative data and the location of the detailed results to facilitate reference are given below in Table 15.

Table 15

Location of the detailed results for compound isolated from band No.3 and 2,4-diamino-6-methyl-1,3,5-triazine

	Compound isolated from band No.3	2,4-diamino-6-methyl-1,3,5-triazine
Ultraviolet spectra pH 7, 13	Fig.10, App. p.133 λ_{max} 254nm ε_{max} 3200* λ_{min} 238nm ε_{min} 2400*	Fig. 15, App. p.134 λ_{max} 254nm ε_{max} 3250 λ_{min} 238nm ε_{min} 2400
Infrared spectra	Fig.11, App. p.142	Fig.16, App. p. 143
Nmr spectra	Fig.13, App. p.151	Fig.18, App. p. 152
Mass spectra	Fig.12 and Table 14, App. p.156,157	Fig.17 and Table 16, App. p. 158,159

* Calculated assuming molecular weight of 125.

Co-chromatography of the 1,3,5-triazinyl compound isolated from band No.3. and authentic 2,4-diamino-6-methyl-1,3,5-triazine

100 µg of each compound were superimposed as a single spot on chromatography paper. The paper was developed in one direction with BAW solvent and at right angles with HCMW solvent. A single, compact, uv quenching area was located at R_F = 0.53 (BAW) and 0.46 (HCMW). No areas gave a colour with DBQ.

This was regarded as additional evidence that structure I, postulated for the triazinyl compound isolated from band No.3, was correct. It was decided not to pursue, at this stage, the isolation of the two non-triazinyl constituents of band No.3.

3.2 Extraction, isolation and identification of the constituents of band No.2

3.2.1 Extraction and isolation

Heated menazon (10 days at 80°) was applied as a solution in dioxan-water (1:1 v/v), to fifty preparative tlc plates (silica gel HF_{254}; 0.5 mm). 20 mg was applied to each plate. The plates were developed, in batches of six, in BAW solvent and allowed to dry by standing at room temperature for thirty minutes. The silica gel containing band No.2 (R_F = 0.65; strong uv quenching; yellow colour with DBQ) was scraped off each batch of plates and stored in flasks at room temperature for two weeks until all fifty plates had been so treated.

The total bulk of silica gel (50g) containing band No.2 was digested with hot methanol for 15 minutes, filtered with suction and filtered again through a hardened filter paper (Whatman No. 541). The clear methanol filtrate was evaporated to dryness under vacuum, the receiver was at room temperature and the distillation flask was immersed in a water bath kept at 45°. A pale brown residue (400 mg) was left in the flask. This residue was extracted with cold dioxan (2 x 50 ml), filtered, and the dioxan removed under vacuum (distillation flask kept at 50°). White crystals (40 mg) remained in the flask. Chromatography of this material (150 μg applied as a single spot) on tlc plates (silica gel HF_{254}; 0.25 mm; solvent-BAW) gave a single uv quenching spot of R_F = 0.35, which slowly turned brown when sprayed with DBQ. This R_F is considerably lower than that of band No.2 before extraction (R_F 0.65). The chromatography was repeated two

dimensionally on paper (150 µg applied as a spot; solvents BAW and then HCMW). A single uv quenching spot was detected which slowly turned brown when sprayed with DBQ, of R_F values 0.31 (in BAW) and 0.31 (HCMW). These results indicated that the triazinyl compound initially present in band No.2 had been modified whilst the silica gel was stored during the extraction and work-up procedures. This product, of R_F value 0.31 - 0.35 (BAW) and 0.31 (HCMW), will henceforth be referred to as "No.2 modified".

Accordingly the preparative tlc was repeated and the isolation procedure altered. A further fifty preparative tlc plates were prepared and developed as before. The plates were dried by standing at room temperature for thirty minutes and the silica gel containing band No.2 (R_F = 0.65; strong uv quenching; yellow colour with DBQ) was removed from each batch of plates. Residual solvent was immediately removed using a conventional vacuum distillation assembly in which the receiver was immersed in a cold trap of solid carbon dioxide-acetone. A vacuum of 1.0 mm was maintained for three hours. After this treatment the silica gel did not smell of solvent, and was then stored in a deep freeze (-20^{o}) for two weeks until all the fifty plates had been treated likewise.

This total bulk (50 g) of silica gel, containing band No.2, was stirred with methanol (300 ml) at room temperature for twelve hours. Most of the silica gel was filtered off by suction, the filtrate was refiltered through a hardened filter paper (Whatman No. 541). The clear methanol filtrate was evaporated to dryness under "cold" conditions (page 58).

A white residue (360 mg) remained in the flask. The residue was digested with cold water (3 ml) and the whole extracted with chloroform (3 x 40 ml). The chloroform layer was separated, filtered, and evaporated to dryness under "cold" conditions. Very pale yellow crystals (40 mg) remained in the flask. Chromatography of this material (200 μg applied as a single spot) on tlc plates (silica gel HF_{254}; 0.25 mm; solvent-BAW) gave a single uv quenching spot of R_F = 0.65, which turned yellow when sprayed with DBQ. The chromatography was repeated two dimensionally on paper (150 μg applied as a spot; solvents BAW and then HCMW). A single uv quenching spot was detected, which turned yellow when sprayed with DBQ, of R_F values 0.67 (BAW) and 0.54 (HCMW). The triazinyl compound was regarded as sufficiently pure to warrant spectroscopic examination.

3.2.2 Identification of the compound isolated from band No.2

The results of spectroscopic examination enabled a structure to be proposed for the extracted compound.

Ultraviolet spectroscopy

Spectra were obtained over the range 230 - 272 nm on aqueous solutions at pH1, pH7 and pH13. These spectra are recorded in Fig. 19 (App. page 135). The position of the maximum (λ_{max} 258 nm) and minimum (λ_{min} 239 nm) and the loss of these in acid solution were characteristic of the diamino-1,3,5-triazine structure. It was also apparent from the spectra at pH13 that there were no hydroxyl groups attached

directly to the triazine ring.

Infrared spectroscopy

The infrared spectrum (0.25% KBr disc) is given in Fig. 20 (App. page 144). Presence of a 1,3,5-triazine ring system was deduced from the appearance of bands at 1550 cm^{-1}, 1005 cm^{-1}, 825 cm^{-1}. The band at 825 cm^{-1} was regarded as particularly characteristic of the 1,3,5-triazine system. Presence of a primary amino group was indicated by the strong bands at 3125 cm^{-1}, 3350 cm^{-1}, 3450 cm^{-1} (N-H stretching modes) and also at 1635 cm^{-1} and 1660 cm^{-1} (N-H bending modes).

Nuclear magnetic resonance spectroscopy

These spectra were obtained on a solution in trifluoroacetic acid using tetramethylsilane as internal standard, Fig. 21 (App. page 152). Three signals were evident at 2.30τ (very broad signal; relative intensity of the integrated signal was 4) 6.20τ (singlet, relative intensity of integrated signal was 2) and at 7.73τ (singlet; relative intensity of integrated signal was 3).

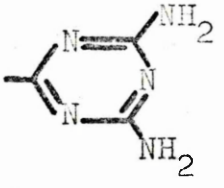
The singlet at 7.73τ was assigned to an isolated methyl group attached to an electron withdrawing group. Similarly the singlet at 6.20τ was assigned to an isolated methylene group situated in an electron withdrawing environment. The broad signal (2.30τ) was assigned to two primary amino groups.

Mass spectrometry

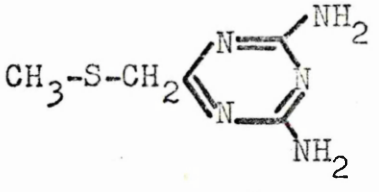
The mass spectral data are recorded in Fig. 22 and Table 17 (App. pages 160,161). Peaks were absent above m/e 173,

and m/e 156 was consistent with the sensible loss of Me from m/e 171. Accordingly m/e 171 was assigned to the molecular ion. The intensity of $M^{+\cdot}$ was too low to permit measurements at high resolution. m/e 173 was 5.2% of the intensity of m/e 171, this being consistent with the presence of one sulphur atom. The base peak was m/e 125. The occurrence of this peak together with a very intense fragment ion at m/e 43 (55% of base peak) and a significant peak at m/e 55 (10.5% of base peak) suggested that a diamino-1,3,5-triazine unit was part of the structure.

The spectroscopic data suggested the presence of

CH_3- ; -CH_2- ; -S- and  units.

Accordingly structure II was assigned to the triazinyl compound isolated from tlc band No.2



II

In order to obtain further support for this structure an authentic sample of 2,4-diamino-6-methylthiomethyl-1,3,5-triazine was obtained, by the alkaline hydrolysis of menazon[62]. Spectroscopic data were obtained to compare with that for the isolated compound. The comparative data and the location of the detailed results to facilitate reference are given in Table 18. All the data confirmed that the two compounds were identical. As a further check the two samples were co-chromatographed.

Table 18

Location of the detailed results for compound isolated from band No.2 and 2,4-diamino-6-methylthiomethyl-1,3,5-triazine

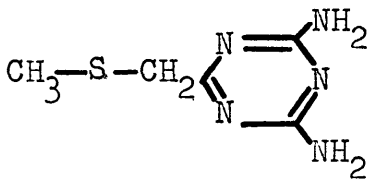
	Compound isolated from band No.2	2,4-diamino-6-methylthiomethyl-1,3,5-triazine
Ultraviolet spectra pH7, 13	Fig.19, App. p.135 λ_{max} 258nm ε_{max} 3975[M] λ_{min} 239nm ε_{min} 2200[M]	Fig.23, App. p. 136 λ_{max} 258nm ε_{max} 4000 λ_{min} 239nm ε_{min} 2175
Infrared spectra	Fig.20, App. p.144	Fig. 24, App. p.145
Nmr spectra	Fig.21, App. p.152	Fig.26, App. p. 153
Mass spectra	Fig.22 and Table 17, App. p.160, 161	Fig. 25 and Table 19, App. p.162 , 163

[M] Calculated assuming molecular weight of 171.

Co-chromatography of the triazinyl compound isolated from band No.2 and authentic 2,4-diamino-6-methylthiomethyl-1,3,5-triazine

100 µg of each compound were superimposed as a single spot on paper. The paper was developed in one direction with BAW and at right angles with HCMW. A single, compact uv quenching area was located at R_F = 0.67 (BAW) and R_F = 0.54 (HCMW). This was the only area which gave a colour reaction with DBQ (yellow).

On the basis of this evidence, structure II is postulated for the triazinyl compound isolated from band 2.



II

3.3 Extraction, isolation and identification of the compound previously referred to as "No. 2 modified"

3.3.1 Extraction, isolation

Initial attempts to isolate 2,4-diamino-6-methylthiomethyl-1,3,5-triazine from tlc band No.2 showed that the extracted compound had a R_F value in BAW, very different to that of the initial tlc band. Accordingly the modified compound was extracted and isolated as previously described (page 64). A total of 40 mg was isolated from fifty preparative tlc plates. The silica gel containing band No.2 was stored at room temperature for two weeks after development, and then extracted with hot methanol. After removal of solvent, the solid was extracted with cold dioxan which was subsequently removed under vacuum.

The material thus isolated (40 mg) gave a single uv quenching area on two dimensional paper chromatography with R_F = 0.31 (BAW) and R_F = 0.31 (HCMW). A brown colour slowly developed over this area (only) on spraying with DBQ. The usual spectroscopic data were obtained for this compound.

3.3.2 Identification of the compound previously referred to as "No.2 modified"

Spectra were obtained over the range 230-272 nm on aqueous solutions at pH1, pH7 and pH13. These spectra are recorded in Fig. 27 (App. page 137). The shape of the curves and the position of the maximum (λ_{max} 261nm) and minimum (λ_{min} 245 nm) for the spectra obtained at pH7 and pH13 indicated a 2,6-diamino-1,3,5-triazine ring system.

It was clear that there was an absence of hydroxyl groups attached directly to the ring.

Infrared spectroscopy

The infrared spectrum (0.25% KBr disc) is given in Fig. 28 (App. page 146). The absorption bands at 1560 cm^{-1}, 1020 cm^{-1}, 820 cm^{-1} strongly suggested the presence of a 1,3,5-triazine ring. Two intense bands at 3110 cm^{-1} and 3375 cm^{-1} were assigned to NH_2 stretching modes, enabling the band at 1645 cm^{-1} with a slight shoulder at 1665 cm^{-1} to be assigned to $-NH_2$ bending modes. As in previous cases it was not possible to deduce whether more than one amino group was present or whether secondary amino groups were also present.

Mass spectrometry

The mass spectral data are recorded in Fig. 29 and Table 20 (App. pages 164,165). Intense peaks appeared at m/e 125 (100%) and m/e 43 (67%) which, from earlier results suggested the presence of the 2,6-diamino-1,3,5-triazine unit. It was thought very likely that in fact, the diamino-triazine system would still be present, suggesting that m/e 172 was not the molecular ion, but a fragment ion. The ratio of m/e 172 to m/e 174 (4.7 %) supported the presence of one sulphur atom. However, no trace of a molecular ion could be found.

Nuclear magnetic resonance spectroscopy

The nmr spectra were obtained on solutions in trifluoro-acetic acid using tetramethylsilane as internal standard. A

typical spectrum is recorded in Fig. 30 (App. page 153). Several signals were obtained as follows :

τ	Relative Intensity	
7.56	1	singlet
6.82	3.58	singlet
5.52	2.36	doublet
2.35	3.85	broad

The doublet at τ = 5.52 did not appear to be due to normal proton coupling.

At this stage it seemed that it would be necessary to isolate more of the compound for elemental analysis and molecular weight determination. However, the compound in question had been isolated from silica gel stored at room temperature and quite likely containing traces of solvent. It was not unreasonable that oxidation might have caused the modification to the compound initially present in band 2.

Accordingly a sample of 2,4-diamino-6-methylsulphinyl-methyl-1,3,5-triazine was synthesised[66] and the spectroscopic data compared with that obtained for compound "No.2 modified". The comparative data and the location of the detailed results to facilitate reference are given below in Table 21.
The excellent agreement of the data suggested that "No.2 modified" was in fact 2,4-diamino-6-methylsulphinylmethyl-1,3,5-triazine. A molecular ion at m/e 187 should have been apparent in the mass spectrum of both compounds. However, the highest ion was m/e 172, presumably due to $M^+ - CH_3$, a

surprising finding.

Table 21

Location of the detailed results for compound referred to as "No. 2 modified" and 2,4-diamino-6-methylsulphinylmethyl-1,3,5-triazine

	"No.2 modified"	2,4-diamino-6-methylsulphinylmethyl-1,3,5-triazine
Ultraviolet spectra	Fig.27, App. p.137	Fig.31, App. p.138
pH 7,13	λ_{max} 261nm ε_{max} 3200*	λ_{max} 261nm ε_{max} 3300
	λ_{min} 245nm ε_{min} 2500*	λ_{min} 245nm ε_{min} 2550
Infrared spectra	Fig. 28, App. p. 146	Fig. 32, App. p.147
Nmr spectra	Fig. 30, App. p. 153	Fig. 34, App. p. 154
Mass spectra	Fig. 29 and Table 20, App. p.164, 165	Fig. 33 and Table 22, app. p.166, 167

* Calculated assuming molecular weight of 187.

The nmr spectrum could not be fully interpreted. On the assumption that the very broad signal at 2.35 τ was due to two $-NH_2$ groups the signals at 7.56, 6.82 and 5.52 τ corresponded to 1,3 and 2 protons respectively.

Accordingly the following assignments were made :

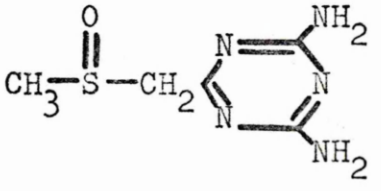
τ	Assignment	
6.82	$\underline{CH_3}$	
5.52	$\underline{CH_2}$	poorly resolved doublet

The splitting of the methylene group into a doublet may be due to the asymmetry of the sulphur atom.

Co-chromatography of compound "No.2 modified" and 2,4-diamino-6-methylsulphinylmethyl-1,3,5-triazine

100 µg of each compound were superimposed as a single spot on paper. The paper was developed in one direction with BAW solvent and at right angles with HCMW solvent. A single, compact uv quenching area was located at R_F = 0.32 (BAW) and R_F = 0.32 (HCMW). This was the only area which gave a colour reaction with DBQ (brown).

The following structure is proposed for compound "No.2 modified" :



The oxidation of 2,4-diamino-6-methylthiomethyl-1,3,5-triazine to 2,4-diamino-6-methylsulphinylmethyl-1,3,5-triazine

The extraction procedure that was developed to obtain 2,4-diamino-6-methylthiomethyl-1,3,5-triazine from band No.2 in a pure state showed that the oxidation did not take place during the development of the plate or during subsequent extraction. Accordingly it was thought desirable to confirm that leaving a developed plate at room temperature for several days caused the oxidation.

20 mg of 2,4-diamino-6-methylthiomethyl-1,3,5-triazine

was loaded as a band on a tlc plate (20 x 20 cm; silica gel HF_{254}; 0.5 mm) and developed in BAW solvent. The plate was allowed to dry by standing at room temperature for four hours, and was stored at room temperature for 14 days. The uv quenching band at R_F = 0.65 was then scraped off, and the silica gel stirred with cold methanol (150 ml) for 12 hours. The silica gel was filtered off and the clear methanol solution concentrated to about 4 ml under vacuum in a flask surrounded with a bath of cold water and connected to a receiver cooled in dry ice. Two dimensional paper chromatography of the concentrated solution showed two strong, and one weak, uv quenching spots:

R_F Value		uv quenching	DBQ colour
BAW	HCMW		
0.66	0.55	strong	Yellow
0.32	0.32	strong	slowly developing pale brown
0.52	0.42	weak	no colour

Thin layer chromatography (silica gel HF_{254}; 0.25 mm) of the same solution showed three uv quenching spots:

R_F Value	uv quenching	DBQ colour
BAW		
0.63	strong	yellow
0.33	strong	slowly developing pale brown
0.45	weak	no colour

It was known from the isolation of No.2 that actually developing in BAW solvent does not produce significant oxidation. These results showed that the oxidation of 2,4-diamino-6-methylthiomethyl-1,3,5-triazine occurred on the tlc plates during storage.

3.4 Extraction and isolation of the components of band No.1

3.4.1 Extraction and isolation

When the preparative plates used for the initial investigation of band No. 2 (page 64) were sprayed with DBQ it was noticed that the strong uv quenching band with R_F = 0.76 gave a strong reddish brown colour mixed with a strong yellow component. This suggested that band No.1 was a mixture of triazinyl components which did not separate on the preparative plates.

Heated menazon (10 days at 80°) was applied as a solution in dioxan-water (1:1 v/v) to twenty five preparative tlc plates (silica gel HF_{254}; 0.5 mm). 20 mg was applied to each plate. The plates were developed, in batches of six, in BAW and allowed to dry by standing at room temperature for thirty minutes. The silica gel containing band No.1 (R_F = 0.76) was scraped off each plate, pooled, and stored in the deep freeze for approximately seven days. The pooled silica gel (25g) was stirred with methanol (200 ml) at room temperature for 12 hours, and then filtered twice through hardened filter paper (Whatman 541). After concentration to about 3 ml by vacuum distillation, the methanol solution was chromatographed, two dimensionally, on paper. Two uv quenching spots were located as follows:

R_F Value		uv quenching	DBQ colour
BAW	HCMW		
0.84	0.70	medium strong	strong reddish brown
0.79	0.61	strong quench	strong yellow

It was now necessary to find a tlc adsorbent which would resolve these two compounds. Use was made of silica gel G/UV_{254} (with binder and fluorescent additive) which, although failing to separate adequately the other breakdown products did resolve band No.1 into two close but distinctly separate uv quenching bands. One of these two bands (R_F 0.89 in BAW) gave a strong reddish brown colour with DBQ, and the other (R_F 0.83 in BAW) gave a strong yellow colour with DBQ.[*] It was found that up to 50 mg of heated menazon could be applied as a band on a single tlc plate (40 cm x 20 cm; silica gel GUV_{254}; 0.5 mm) with subsequent resolution of band No.1 into two compounds by BAW solvent. Twenty such preparative plates were developed in BAW solvent, allowed to dry by standing at room temperature for approximately forty minutes, and the two bands scraped off and stored in the deep freeze. The two batches of silica gel (both approximately 30 g) were completely freed of any residual solvent using the vacuum distillation assembly described before (page 57), and stirred with cold methanol (300 ml) for twelve hours. The silica gel was filtered off twice through hardened filter paper (Whatman 541) and the methanol evaporated to dryness under vacuum (water bath 40°). A white residue (approximately 500 mg) was obtained with each of the two batches of silica gel.

The residue (300 mg) obtained from the band at R_F = 0.89 was shaken with cold dioxan (2 x 50 ml), filtered and evaporated to dryness under vacuum (water bath at 45°). White crystals

[*] The initial experimentation which revealed this separation was carried out by Mr. D. Pashley during project work for the Licentiateship of The Royal Institute of Chemistry. This project was successfully submitted in June, 1969.

(25 mg) were obtained. This material was rechromatographed on a tlc plate (silica gel HF_{254}) and gave a single uv quenching spot at R_F = 0.76 which gave a reddish brown colour with DBQ. This R_F value is the same as that recorded for band No.1 on the preparative plates. The isolated material was also subjected to two dimensional paper chromatography and gave a single uv quenching spot of R_F = 0.80 (BAW) and R_F = 0.70 (HCMW). This triazinyl compound was thus regarded as being sufficiently pure for spectroscopic examination.

The residue (300 mg) obtained from the band at R_F = 0.83 (silica gel GUV_{254}) was stirred with a little cold water (ca. 3 ml) and shaken with chloroform (3 x 40 ml). The chloroform layer was separated, filtered, and evaporated to dryness under vacuum at room temperature. Very pale yellow microcrystals (20 mg) were obtained. This material was re-chromatographed on a tlc plate (silica gel HF_{254}) and gave a single uv quenching spot at R_F 0.76 which gave a strong yellow colour with DBQ. This R_F value is the same as that recorded for band No.1 on preparative plates. It appeared to be pure by two dimensional paper chromatography, as only one uv quenching spot was located with R_F = 0.79 (BAW) and R_F = 0.61 (HCMW). This area also turned yellow when sprayed with DBQ. Accordingly both materials were judged to be suitable for spectroscopic examination.

3.4.2 Identification of the component of higher R_F value, separated on silica gel G/UV_{254}

The material extracted from the silica gel corresponding

to R_F = 0.89 (BAW solvent; silica gel G/UV_{254}) and which gave a reddish brown colour with DBQ, was thought to be menazon. Samples of the compound were subjected to the usual spectroscopic examination.

Infrared spectroscopy

The infrared spectra of the extracted component and of an authentic sample of menazon superimposed on the same chart is shown in Fig. 35 (App. page 148). They are identical. The presence of a 1,3,5-triazine ring was indicated by very strong bands at 810 cm^{-1}, and 1015 cm^{-1}. The presence of primary amino groups was indicated by two strong bands at 3375 cm^{-1} due to the stretching modes of vibration, and a strong band at 1630 cm^{-1} (with a slight shoulder at 1650 cm^{-1}) due to the bending modes. Thomas and Crittenden[16] state that the infrared spectrum of the P = S bond is characterised by two distinct bands, and that in tri-alkyldithiophosphates they lie within the regions 645 - 663 cm^{-1} and 790 - 833 cm^{-1}. In the spectra of menazon it is possible that the strong band on the extreme right of the chart at 660 cm^{-1} may be due to the lower frequency band of the P = S bond. The strong peak at 810 cm^{-1} has a shoulder at 820 cm^{-1} which may be due to the higher frequency band of the P = S bond.

Ultraviolet spectroscopy

Spectra of the isolated compound were obtained over the range 230 - 272 nm on aqueous methanol solutions at pH1, pH 7 and pH 12. These spectra and comparative ones for menazon

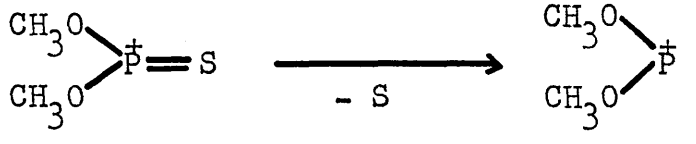
are recorded in Figs. 36, 37 (App. pages 139,140). The shape and position of the maxima and minima were consistent with a diamino-1,3,5-triazine, not containing hydroxyl groups attached directly to the ring. On the assumption that the molecular weight of the isolated compound was 281 (menazon) the molar extinction coefficients were in good agreement (Table 23, page 84).

Nuclear Magnetic Resonance Spectroscopy

^{1}H nmr spectra of the isolated compound and menazon were obtained on solutions in trifluoroacetic acid using tetramethylsilane as internal standard. These spectra are recorded in Figs. 38, 39 (App. pages 154,155). They were extremely similar and the following assignments made. The two very strong and sharp peaks at 5.94τ (integrated for 4 protons) and 6.20τ (integrated for 3 protons) formed a doublet with centre 6.07τ and were assigned to the alkyl protons of the methoxy groups, split by phosphorus. The peak at 5.65 τ (integrated for 1 proton) was one component of a doublet, the other component of which, was just resolved at 5.98τ in the ^{1}H nmr spectrum of the isolated compound, but masked by the strong peak at 6.02τ in the spectrum of authentic. This doublet was assigned to the methylene protons which were also split by phosphorus. The primary amino groups gave rise to a broad peak at 2.32τ (integrated to 3 protons if all the other signals were due to 8 protons).

Mass spectrometry

The mass spectral data of menazon and the isolated compound were again extremely similar and are recorded in Figs. 40, 41 and Tables 24,25 (App. pages 168-171). A fragment ion at m/e 250 was presumably due to the loss of a methoxy group from the molecular ion. The base peak was at m/e 156, and was thought to arise from the loss of the thiophosphate moiety, $(CH_3O)_2\dot{P}=S$, from the molecular ion. The strong fragment ion at m/e 125 was assigned to a similar cleavage of the molecular ion but with the positive charge residing on the thiophosphate moiety, and subsequent loss of sulphur to give the strong ion at m/e 93 as follows :



m/e 125 m/e 93

As in all other 1,3,5-triazine mass spectra studied, there was a strong fragment ion at m/e 43.

The above evidence shows conclusively that the isolated compound was, in fact, menazon. This also served as a check on the validity of the experimental procedure adopted for the extraction, isolation and characterisation of the breakdown products.

The comparative data and the location of the detailed results to facilitate reference are given below in Table 23.

Table 23

Location of the detailed results for isolated and authentic menazon

	Isolated menazon	Authentic menazon
Ultraviolet spectra	Fig.36, App. p.139	Fig.37, App. p.140
pH 7	λ_{max} 261nm ε_{max} 3400*	λ_{max} 261nm ε_{max} 3450
	λ_{min} 244nm ε_{min} 2450*	λ_{min} 244nm ε_{min} 2400
pH 12	λ_{max} 260nm ε_{max} 4570*	λ_{max} 260nm ε_{max} 4550
	λ_{min} 247nm ε_{min} 4100*	λ_{min} 247nm ε_{min} 4150
Infrared spectra	Fig.35, App. p. 148	Fig.35, App. p.148
Nmr spectra	Fig.38, App. p.154	Fig.39, App. p.155
Mass spectra	Fig.40 and Table 24, App. p.168, 169	Fig. 41 and Table 25, App. p.170, 171

* Calculated assuming molecular weight of 281.

3.4.3 Identification of the component, of lower R_F value, separated on silica gel G/UV_{254}

The material isolated from the silica gel corresponding to R_F = 0.83 (BAW solvent; silica gel G/UV_{254}) and which gave a yellow colour with DBQ was subjected to spectroscopic examination.

to R_F = 0.83 (BAW solvent; silica gel G/UV_{254}) and which gave

Ultraviolet spectroscopy

Spectra were obtained over the range 230 - 272 nm on aqueous solutions at pH1, pH 7 and pH10. These spectra are recorded in Fig. 42 (App. page 141). The shape of the curves, and the position of the maximum and minimum for the spectra obtained at pH7 and pH10 indicated a 2,4-diamino-1,3,5-triazine system. The mass spectrum suggested that the molecular weight was 203; on this basis the following molar extinction coefficients were calculated :

pH7	λ_{max} 260 nm	ε_{max} 4400
	λ_{min} 243 nm	ε_{min} 3600
pH10	λ_{max} 263 nm	ε_{max} 10550
	λ_{min} 239 nm	ε_{min} 7240

It was clear that there was an absence of hydroxyl groups attached directly to the ring.

Infrared spectroscopy

The infrared spectrum (0.25% KBr disc) is given in Fig. 43 (App. page 149). The fairly strong band at 825 cm^{-1} indicated the presence of a 1,3,5-triazine ring, this indication being supported by the strong bands at 1015 and 1550 cm^{-1}. Strong bands at 3450, 3315 and 3115 cm^{-1} were assigned to a primary amino group (stretching modes) and the strong band at 1635 cm^{-1} (slight shoulder at 1665 cm^{-1}) to

the corresponding bending mode. As in previous cases the presence of a secondary amino group as well as a primary amino group could not be ruled out. However, it seemed more likely that the primary amino group of menazon remained unmodified in the degradation product.

Nuclear magnetic resonance spectroscopy

These spectra were obtained on trifluoroacetic acid solutions using tetramethylsilane as internal standard, and are recorded in Fig. 44 (App. page 155). The relative intensity values are as follows:

τ	Relative intensity	
7.46	3	singlet
6.1	2	singlet
2.25	3	broad

However, amino protons at ca 2.25τ have not generally given accurate intensity values, due probably to the very broad nature of the signal. The spectrum was very similar to that given by 6-methylthiomethyl-1,3,5-triazine and it was thought at least possible that the broad signal at 2.25τ could be due to two primary amino groups. Secondary amino groups were considered to be absent. The signals at 7.46τ and 6.1τ were thought to be due to isolated methylene and methyl groups, both being adjacent to a sulphur atom.

Mass spectrometry

The mass spectral data are recorded in Fig. 45 and Table

26 (App. pages172,173)The peak of highest mass occurred at m/e 203. Although an intense ion occurred at m/e 157 (also odd mass number which was compatible with an intact 2,6-diamino-1,3,5-triazine ring) the ion at m/e 203 was regarded as the molecular ion for the following reasons :

(a) compatible with a species containing five nitrogen atoms (see later)

(b) the relative intensity of m/e 205 and m/e 203 was 9.2%, allowing the presence of two sulphur atoms (see later)

(c) the fragment ions nearest to m/e 203 occurred at m/e 188 and m/e 171. These correspond to sensible losses from M^{+}, probably a methyl group and sulphur atom respectively

(d) there was a small, but definite fragment ion at m/e 101.5.

Ions have been repeatedly detected at m/e values corresponding to half that for the molecular ion. This was particularly compelling evidence.

The base peak was m/e 157 corresponding to loss of 76 mass units. A strong fragment ion occurred at m/e 43. This fragment has been regarded as very good evidence for the presence in the parent molecule of the diamino-1,3,5-triazine structure.

Elemental analysis

Elemental analysis gave values of :

C 30.14%; H 4.38%; N 32.57%; S 31.00%

Assuming a molecular weight of 203 these values required the molecular formula to be $C_5H_9N_5S_2$ (4 double bond equivalents). This molecular formula required the following :

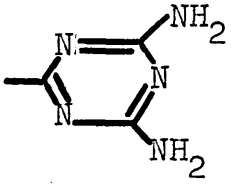
$$\frac{M^+}{M^+ + 1} \times 100 = 8.890\% \; ; \qquad \frac{M^+}{M^+ + 2} \times 100 = 8.430\%.$$

The measured ratios were :

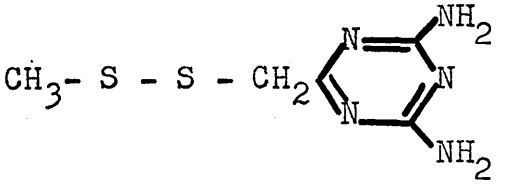
$$\frac{M^+}{M^+ + 1} \times 100 = 9.2\% \qquad \frac{M^+}{M^+ + 2} \times 100 = 8.43\%$$

The ultraviolet, infrared and mass spectra (intense fragment at m/e 43) suggested the presence of the diamino-1,3,5-triazine unit. This left two sulphur atoms and C_2H_5 to be incorporated into a final structure. It was tempting to postulate the following fragments :

$-CH_3$, $-CH_2$, -S-, -S-,



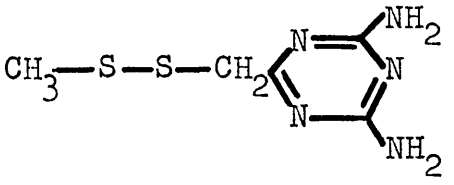
combined thus



Structure of the degradation product in band No.1.

As a confirmation of the presence of the disulphide linkage, a small amount (ca 2 mg) of the compound was reduced with zinc and dilute sulphuric acid. A strong smell of methyl mercaptan was detected.

All the evidence mentioned above shows that the degradation product in band No.1 has the structure



3.5 Investigation of Band No.4

The degradation product isolated from band No.2. was identified as 2,4-diamino-6-methylthiomethyl-1,3,5-triazine, (page 70). Since it was found that it oxidised to 2,4-diamino-6-methylsulphinylmethyl-1,3,5--triazine when allowed to stand on tlc plates at room temperature (page77), it was felt at least reasonable that the latter might in fact be one of the degradation products of menazon. Its R_F value (0.35) on tlc (silica gel HF_{254}; 0.25 mm; BAW solvent) indicated that it might be present in band No.4.

Six preparative tlc plates, each loaded with 20 mg of heated menazon, were developed in BAW and allowed to dry by standing at room temperature for forty-five minutes. The silica gel containing band No.4 (R_F = 0.35) was scraped off each plate, pooled, and stirred with methanol (50 ml) at room temperature for three hours. The solution was filtered by suction, and the filtrate concentrated to about 3 ml under "cold" conditions (page 58). The concentrated solution was chromatographed two dimensionally on paper. One uv quenching area, and a non-quenching area which gave a DBQ colour, were located as follows :

R_F Value		uv quenching	DBQ colour
BAW	HCMW		
0.32	0.32	medium strong	pale brown slowly
0.33	0.69	nil	strong yellow

The solution was also co-chromatographed two dimensionally on

2,4-diamino-

paper with 50 µg of 6-methylsulphinylmethyl-1,3,5-triazine. A single uv quenching area with R_F values 0.32 (BAW) and 0.32 (HCMW) was located which gave a pale brown colour with DBQ.

This chromatographic evidence suggested that 2,4-diamino-6-methylsulphinylmethyl-1,3,5-triazine might be present in band No.4.

Attempts to actually isolate the 1,3,5-triazinyl component in band No.4. were, however, unsuccessful. The silica gel containing band No.4 was also scraped off the preparative plates used for the isolation of the compound in band No.2 (page 65), and kept in a deep freeze (- 20°) for three weeks. The total bulk of silica gel was completely freed of any residual solvent using the vacuum distillation assembly described before (page 65). The silica gel (40 g) was stirred with cold methanol for twelve hours, and filtered off twice through hardened filter paper (Whatman 541). The clear methanol filtrate was evaporated to dryness under vacuum (water bath 40°). A white residue (200 mg) was obtained.

Attempts were made to isolate the 1,3,5-triazinyl component present in the residue. It was stirred with cold ethyl acetate, dioxan, chloroform, acetone for 6 hours, filtered, and the solvent evaporated. No compound was isolated. It was also digested with 3 ml of cold water, and shaken with chloroform and ethyl acetate (3 x 40 ml) which were then evaporated. Again no compound was isolated.

3.6 Investigation of Bands No.5, No.6, No.7 and No.8

The medium strong uv quenching band No.6, the weak quenching band No.5 and the very weak quenching bands No 7 and No.8 were scraped off 50 preparative tlc plates (silica gel UV_{254}; 0.25 mm) and stored in a deep freeze for ten days. The silica gel containing each of these bands had the solvent removed in a vacuum distillation assembly as mentioned previously (page 65).

The four batches of silica gel (30 gm each) were stirred with cold methanol (300 ml) for 12 hours, filtered twice, first by suction, and then through a hardened filter paper (Whatman No. 541), and the clear methanol filtrate evaporated to dryness under vacuum (water bath at 45° C), leaving a pale brown residue (ca 300 mg) in all four cases.

Two dimensional paper chromatography of the residue from band No.6 showed two uv quenching spots with R_F values and DBQ colour as follows :

R_F Value		uv quenching	DBQ colour
BAW	HCMW		
0.39	0.15	Medium strong	Pale brown
0.25	0.21	Medium strong	Pale yellow

In view of the fact that the two uv quenching spots were close together, it was not possible to conveniently isolate the two compounds by preparative paper chromatography. The residue was shaken with cold dioxan (2 x 50 ml) but no material

was left on evaporation of the dioxan solution to dryness. An unsuccessful attempt was also made to isolate at least one of the components by digesting the residue with water (3 ml) followed by solvent extraction with chloroform (3 x 40 ml) and evaporating the chloroform).

The residue from band No.5 on two dimensional paper chromatography showed no uv quenching spots but on spraying with DBQ showed a yellow spot with R_F = 0.20 (BAW) and R_F = 0.60 (HCMW). No spots were observed on the two dimensional paper chromatograms of the residue from band No.7 and from band No.8 either under uv light or on spraying with DBQ.

3.7 Investigation of the insoluble material left after digesting heated menazon in dioxan-water

The pale yellow insoluble material that was filtered off after heated menazon had been digested in dioxan-water (1:1 v/v) was insoluble in most solvents. The material (200 mg) was stirred for 10 minutes with cold ammonium hydroxide (6 ml; 2N). The ammonium hydroxide slowly turned pale yellow, and a white precipitate was left (120 mg) which was filtered.

The ammonium hydroxide filtrate was chromatographed on tlc plates (silica gel UV_{254}; 0.25 mm) and showed several uv quenching spots which seemed to correspond to those of bands No.1, No.2, No.3, No.4 and No.6.

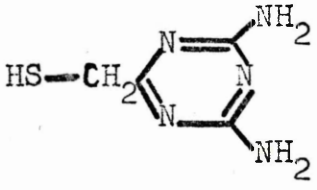
The white precipitate was also chromatographed on tlc plates (silica gel UV_{254}) and two dimensionally, on paper. The plates gave two uv quenching spots (R_F = 0.62 and 0.40) which developed a brown colour when sprayed with DBQ. Two uv quenching spots were also observed on the paper chromatograms, thus :

R_F Value		uv quenching	DBQ colour
BAW	HCMW		
0.45	0.14	strong	brown
0.31	0.08	strong	brown

Recrystallisation of the white precipitate in boiling water (30 mg in 40 ml) or in N,N-dimethylformamide (30 mg in 7 ml) did not separate the two triazinyl compounds.

The infrared spectrum (0.25% KBr disc) of the white precipitate recrystallised from N,N-dimethylformamide is shown in Fig. 46 (App. page 150), together with the infrared spectrum 0.25% KBr disc) of 2,4-diamino-6-mercaptomethyl-1,3,5-triazine. The latter spectrum was selected for comparison because the mercaptan had been prepared, characterised, and was known to be a sparingly soluble compound which gave a brown colour with DBQ. Two dimensional paper chromatography of a sample of the authentic mercaptan gave a uv quenching spot with R_F = 0.32 (BAW) and 0.08 (HCMW).

This chromatographic and infrared evidence suggest that the mercaptan



may be present in significant amounts in the insoluble residue.

4 Phosphorus determination on the triazinyl degradation products of menazon

A sensitive method for the determination of phosphorus on paper chromatograms has been described by Calderbank and Turner[9]. A brief account of the method is given on page 8 . The authors recommend that Whatman No. 1 paper washed in a solution of disodium ethylenediaminetetra-acetate should be used for chromatography to reduce the phosphate content of the paper. But in the present work it was found that washed paper gave poor separations of the triazinyl degradation products of menazon. Also ordinary non-washed paper gave sufficiently low blank values to warrant its use in the present work.

Accordingly duplicate two dimensional paper chromatograms similar to that in Fig. 8 (App. page 176), loaded with 350 µg of menazon heated for 10 days at 80° were developed. The eight uv quenching areas were cut out, digested for six hours with 1.0 ml of hot sulphuric-perchloric acid, neutralised with sodium hydroxide before colour development with Reagent C, and the absorbance was measured at 820 nm. Blank values for the paper were obtained by cutting out known areas of paper from the same chromatogram, ashed, neutralised and treated with Reagent C in the same manner. The absorbance of the solutions obtained from the eight uv quenching areas and from the paper blanks are given in Table 27 (page 97).

The uv quenching area of R_F values 0.84 (BAW), 0.69 (HCMW) was the only area which gave positive results. Its R_F values were identical to those of pure menazon. From the absorbance

Table 27

Phosphorus determination on two dimensional paper chromatograms of the triazinyl degradation products of menazon heated for 10 days at 80°.

R_F Values		uv quenching	Absorbance at 820 nm.
BAW	HCMW		(Reference distilled water)
0.84	0.69	medium strong	0.315, 0.310
0.79	0.61	strong	0.0275, 0.0225
0.66	0.54	strong	0.0125, 0.0120
0.47	0.48	very strong	0.0375, 0.0350
0.35	0.12	weak	0.0375, 0.0350
0.31	0.32	medium strong	0.010, 0.010
0.26	0.24	weak	0.030, 0.030
0.07	0.11	very weak	0.025, 0.035

Paper blanks (non-washed paper)

Area of paper	0	1.0	4.0	6.0 sq cm
Absorbance at 820 nm	0.0250	0.0225	0.040	0.025
(Reference distilled water)	0.0200	0.0220	0.025	0.030

Duplicate two dimensional paper chromatograms (Fig. 8, App. page 176) developed. The uv quenching areas ashed, neutralised, Reagent C added, and the absorbance measured at 820 nm.

of its solution and the gradient of curve B (App. page 174), the amount of menazon present in 350 µg of a sample of menazon heated for 10 days at 80° was calculated from the formula :

$$\text{Concentration of menazon } (\mu g) = \frac{\text{Absorbance}}{\text{Gradient of Curve B}} \times \text{Mol. wt of menazon}$$

It was calculated to be 13 µg, i.e. approximately 4%.

It is clear from the results that none of the triazinyl degradation products of menazon contain phosphorus.

EXPERIMENTAL

1 Heating of menazon sample

A sample of menazon (11.9 g) was recrystallised from methanol-water (1:1 v/v; 150 ml) giving white lustrous crystals (9.0 g). These were recrystallised from methanol (60 ml) giving white, needle-like crystals (6.0 g). The product was dried and the purity checked by paper chromatography (two-dimensional), and thin layer chromatography (Section 2, below). One uv quenching area was located, which also gave a reddish-brown colour with the DBQ spray reagent. No other areas of the chromatogram gave a colour reaction with DBQ. The menazon sample was concluded to be pure within the limits of detection of these procedures.

The menazon was spread out on the bottom of a petrie dish to form a layer approximately 0.5 cm thick, and heated for 18 days in an oven set at 80°. No alterations to atmospheric conditions were made. Samples were taken after periods of 2,3,6,10,18 days, and examined by paper and thin layer chromatography.

2. Paper Chromatography and thin layer chromatography plates

2.1 Paper chromatography

Watman No.1 chromatography paper was used as strip (19 cm wide), or sheet (46 x 56 cm) as the occasion demanded.

When it was intended to estimate the phosphorus content of selected areas of the chromatogram, the paper was at first washed, prior to loading and development, with a solution of

disodium ethylenediaminetraacetate[63] to reduce the phosphate content of the paper. Later this was omitted.

Thin layer chromatography

Two adsorbents were used (supplied by Alderman and Company Limited, the U.K. suppliers of E. Mercks Laboratory Chemicals, Darmstadt, Germany).

Merck silica gel HF_{254} (without binder)
Merck silica gel G/UV_{254} (with binder)

Both adsorbents contain a fluorescent indicator which facilitates the location of uv quenching areas.

Silica gel HF_{254} (30 g) was added to distilled water (65 ml) and shaken for 2 minutes. The slurry was applied to glass plates (20 x 20 cm or 40 x 20 cm) by means of a "Desaga" spreader set to apply a layer 0.25 or 0.50 mm thick. The plates were allowed to set for 10 minutes, and then activated by heating in an oven set at 120°, overnight. Silica gel G/UV_{254} was used to separate two components of heated menazon which appeared as one band on silica gel HF_{254} plates. This adsorbent was applied to glass plates as described above, except that a slurry of slightly different composition (30 g in 55 ml of distilled water) was used.

3 Composition of solvent systems

Butanol-1 : glacial acetic acid:water (12:3:5) (abbreviated in the text to BAW). Reagent grade solvents were used without further purification. Freshly prepared solvent mixtures were used for each development.

n-Hexane : chloroform:methanol:water (5:5:10:2) (abbreviated in the text to HCMW). The bottom layer was used for development. Reagent grade solvents were used without further purification. Freshly prepared solvent mixtures were used for each development.

4 Loading and development of paper chromatograms and analytical thin layer chromatography plates

4.1 Menazon and other 1,3,5-triazine derivatives required as chromatography standards were dissolved in methanol, methanol-water (1:1, v/v) or dioxan-water (1:1, v/v). Solutions containing about 100 mg/2 ml were used, as 10 µl (which is a convenient quantity to apply as a single spot) then corresponds to about 50 µg.

4.2 Samples (100 mg) of menazon which had been heated for 2, 3, 6, 10 and 18 days at 80°C were added to dioxan-water (1:1 v/v; 2 ml), and warmed to 45° in order to obtain a concentrated solution. The residue was filtered off, dried and weighed. The actual concentration of the solutions of heated menazon could thus be calculated. The strength of the solutions obtained were typically of the order of 30 mg/ml (i.e., 30 µg/µl).

4.3 In order to know accurately the weight of sample applied to the paper and thin layer plates, an "Agla" micrometer syringe was used. This was a glass syringe with a micrometer head attached to it, so that rotation of the micrometer head moved the plunger of the syringe by a known amount, thus delivering a known volume of

solution through the needle. The micrometer head was divided into 50 divisions, and one complete revolution of the micrometer head advanced the plunger by 0.5 mm delivering 0.01 ml of solution. Rotation of the micrometer head through one division delivered 2 µl of solution.

4.4 The amount of 1,3,5-triazine derivative applied as a spot to paper and thin layer plates varied, according to the purpose, from 50 µg to 400 µg.

4.5 Papers were developed in BAW solvent for 12 hr (descending) or 18 hr (ascending), and in HCMW solvent for 6 hr (ascending).

4.6 Thin layer plates were developed in BAW solvent for 3 hr.

5 Preparative thin layer chromatography

5.1 Adsorbent (silica gel HF_{254} or silica gel G/UV_{254}) was applied to plates (40 x 20 cm) as a layer 0.25 mm or 0.50 mm thick, as described above in section 2.2. The relevant solution was applied as a narrow (about 5 mm) band using the apparatus shown in Fig. 5 (page 49). This consists essentially of an Agla syringe mounted on a trolley. The wheels of the trolley rotate the head of the syringe thus applying the solution to the adsorbent via narrow gauge (0.25 mm) polythene tubing. In one pass approximately 0.10 ml of solution is applied. A typical procedure used fifty plates (40 x 20 cm), the adsorbent being 0.50 mm thick, to which was applied

a total of 1.0 g of heated menazon. This involved 8 passes of the trolley per plate. The final width of the band was about 5 mm and the surface of the silica gel remained undisturbed. The fifty plates were developed in BAW solvent (in batches of six) and allowed to dry at room temperature.

6 Detection of the breakdown products of menazon on paper and thin layer chromatograms

6.1 By examination in ultraviolet light Paper and thin layer chromatograms were viewed in ultraviolet light of 254 nm. Areas containing compounds that possessed a 1,3,5-triazine ring appeared as either a dark spot against a weakly fluorescent background (paper), or as a dark spot (or band) against a brilliant yellow-green fluorescent background (plates). The fluorescence in the latter case was due to the fluorescent additive in both samples of the silica gel.

6.2 By spraying with 2,6-dibromoquinone-N-chloroimide (DBQ) Paper and thin layer chromatograms were sprayed with a chloroform solution of 2,6-dibromoquinone-N-chloroimide (0.5% w/v) and lightly oversprayed with glacial acetic acid. The colours developed very slowly at room temperature, thus the sprayed papers and plates were normally heated in an oven (100°) for about ten minutes. It was necessary to keep freshly prepared DBQ solution for about three days before using as a spray reagent.

7 Removal of bands from developed thin layer chromatography plates

The boundaries of the required bands were marked with a pointed glass rod, and the bands scraped off with a spatula, whilst the plate was held in a semi-vertical position over a sheet of paper. The various bands were kept in separate flasks. These flasks were either stored at room temperature, stored in a deep freeze (-20^{o}) or immediately connected to a vacuum distillation (20 mm) assembly for removal of solvent.

8 Spectra

The ultraviolet spectra of the 1,3,5-triazinyl breakdown products of menazon were taken on the Unicam SP 500 in aqueous solutions of concentration 5 mg/1.0 ml. Spectra at pH 13 and pH 1 were taken by addition of NaOH or HCl, and for pH 2 and 12 ammonia buffer was added to the aqueous solution.

The absorbance of the molybdophosphate complex formed by organophosphorus compounds in the phosphorus determination (Section 8) was measured at 820 nm on the Unicam SP 200.

All infrared spectra were taken on samples which had been dried in a vacuum pistol, and pressed in KBr discs at 0.25% concentration. On account of the low solubility of phosphorylated diamino-1,3,5-triazines in ordinary organic solvents, their nmr spectra were taken in trifluoroacetic acid at a concentration of 0.3% using the Varian A60.

The mass spectra were obtained on a AE1 MS 9 mass spectrometer by using a direct insertion probe. A typical set of operating conditions is as follows.

INLET	VAC. LOCK	RATE	4 x 200	TEMP(S)	220° C
I.V.	70	SPEED	1 x 8	PRESSURE(S)	
		EM	500		4×10^{-7}
I.A.	90	SLIT(S)	3		
RANGE	5	SLIT(C)	7		

9 Estimation of phosphorus in menazon and its breakdown products

The method of Chen, Toribara and Warner[11] as applied by Calderbank and Turner[9] was used without modification. It was necessary to use distilled water instead of de-ionised water. The observation made in other laboratories that the use of the Roche brand of ascorbic acid was obligatory for low reagent blanks was confirmed.

10 Determination of inorganic phosphate

As a preliminary check on the reproducibility of the method for the determination of inorganic phosphate described by Calderbank and Turner[9], curves A and B were drawn (Fig. 6, App. page 174).

Volumes of aliquots from 0 to 2.0 ml of a solution containing 0.061 mole of potassium dihydrogen orthophosphate per ml were adjusted to 2.0 ml with distilled water. The blue colour of the molybdophosphate complex developed directly with 2.0 ml of reagent C, and the absorbance measured at 820 nm to give curve A. To obtain curve B the 2.0 ml aliquots of test solution were evaporated to dryness under an infrared lamp, each residue digested for 6 hours with 0.5 ml of sulphuric-

perchloric acid, neutralised with sodium hydroxide, and the blue colour developed with 2.0 ml of reagent C.

11 Preparation of model compounds for comparison with the degradation products of menazon

(a) 2,4-diamino-6-mercaptomethyl-1,3,5-triazine[62]

Menazon (2.00 g) was heated on the steam-bath at 95 - 100° for 1 hour with N-hydrochloric acid (100 ml). Hydrogen sulphide was evolved. The clear solution was cooled, made just alkaline with ammonium hydroxide, and the solid (520 mg decomp. 200 - 202°) collected. Recrystallisation of this material from boiling water (500 ml) gave lustrous needles (340 mg) m.p. 230 - 232° (decomp.) of 2,4-diamino-6-mercaptomethyl-1,3,5-triazine monohydrate. Yield 30%.

(b) 2,4-diamino-6-methylthiomethyl-1,3,5-triazine[62]

Menazon (8.00 g) was heated under reflux for 1 hour with 10% potassium hydroxide in methanol (80 ml). Solid precipitated from the solution on cooling. Water (80 ml) was added, and the solid (3.30 g, m.p. 204 - 206°) collected, washed with water and recrystallised from methanol (80 ml). Pale yellow needles (2.10 g, m.p. 206 - 208°) of 2,4-diamino-6-methylthiomethyl-1,3,5-triazine separated on standing. Yield 43%.

(c) 2,4-diamino-6-methylsulphinylmethyl-1,3,5-triazine[66]

2,4-diamino-6-methylthiomethyl-1,3,5-triazine (1.70 g) was dissolved in glacial acetic acid (125 ml) at 70°, 100

volume hydrogen peroxide (2.5 ml) was added and 10 minutes later the solution was diluted with water (200 ml) and evaporated to dryness under reduced pressure. The resulting yellow solid was recrystallized from hot water (10 ml) to yield white crystals (0.50 g) of m.p. 220-223° (decomp.)

2,4-diamino-6-methylsulphinylmethyl-1,3,5-triazine. Yield 26%.

DISCUSSION

1 Chromatography of heated menazon

Samples of menazon which have been heated for 2,3,6,10 and 18 days at 80° have been examined by paper and tlc. When the chromatograms were observed under uv light of 254 nm, the same number of uv quenching areas were located with the corresponding areas having identical R_F vales. The same colour pattern was obtained in all cases when the chromatograms were sprayed with DBQ reagent. The uv quenching areas containing the products of thermal degradation were however more intense for the longer heated samples, and also developed more distinct colours with DBQ. It is thus apparent that, as expected, thermal degradation occurs to a greater extent in the longer heated samples, but surprisingly the nature of the products is the same regardless of the heating time. A rough assessment of the extent of thermal degradation can be quickly obtained by weighing the amount of insoluble residue left when a heated sample is digested with dioxan-water. For the isolation of the degradation products a 10 day sample was found to be quite convenient to use.

The one dimensional paper chromatogram (developed with BAW solvent) on Whatman No.1 paper of a 10 day heated sample (Fig. 7, App. page 175) at 150 µg loading showed eight uv quenching areas and three non-quenching areas which were located by their DBQ colour. Since some of the areas were very closely spaced, the same sample was subjected to two dimensional paper chromatography (developed with BAW solvent

and then with HCMW) at 350 µg loading (Fig. 8, App. page 176) for more efficient separation. Eight uv quenching areas were observed and six non-quenching areas which gave a colour with DBQ. It appears therefore that within the limits of detection there are at least eight 1,3,5-triazinyl components in the heated sample, and six components which presumably contain the phosphorus moiety but not the triazine ring.

The tlc on silica gel HF_{254} (developed with BAW solvent) of the 10 day heated sample loaded as a spot containing 400 µg (Table 11, page 47) showed nine uv quenching areas and a non quenching area located by its DBQ colour. Only eight uv quenching areas were located on the paper chromatograms probably because their detection is less sensitive on paper. On the other hand only one of the six non-quenching areas located on the two dimensional paper chromatogram was resolved on the tlc plates, and due to this some of the uv quenching areas on the tlc plates are not homogeneous.

2 Separation of the degradation products of menazon by preparative tlc

Preparative tlc was envisaged as a convenient method for the isolation of the 1,3,5-triazinyl degradation products. Plates (40 x 20 cm) coated with silica gel HF_{254} of thickness 0.50 mm were used. 20 mg of heated menazon was loaded on each plate. It was thus possible to load a total of 1.0 g of heated menazon on 50 plates. After development (BAW solvent), the silica gel from corresponding uv quenching bands was combined and kept in a deep freeze unit (- 20°) to

avoid further breakdown of the degradation products. Loading at this level gives slightly poorer resolution of the 1,3,5-triazinyl components, since only eight uv quenching bands were observed on the preparative tlc plates (compared with the nine uv quenching areas observed when loaded as a spot containing 400 μg), and none of the non-triazinyl components were resolved into distinct bands. It is considered that this loss of resolution is offset by the advantages of using considerably higher loading for preparative work.

Before isolation of the 1,3,5-triazinyl degradation products, the uv quenching bands were examined to find out if they were homogeneous. This was done by extraction with cold methanol, and concentration of the solution before examination by two dimensional paper chromatography.

3 Isolation and structure of the degradation products of menazon

The photograph of a typical tlc plate under uv light is shown in Fig. 9 (page 50). The system that is used in this discussion for numbering the uv quenching tlc bands is given on page 51 . The relevant data for these bands is given in Table 12 (page 52).

3.1 Isolation and structure of the components in band No.1

Band No.1 was in fact a mixture of two 1,3,5-triazinyl compounds when subjected to two dimensional paper chromatography. The two components were separated on a preparative scale by tlc on silica gel G/UV_{254} (with binder) of thickness

0.50 mm. One of the components was isolated (25 mg) by extraction with cold methanol followed by treatment with dioxan. Its uv, infrared, nmr and mass spectra and their comparison (see Table 23, page 84 for location of the detailed results) with those of an authentic sample showed it to be in fact menazon. This indicates

(a) that the menazon structure is unaffected by the solvent system (BAW) and the silica gel adsorbent used for chromatography,

and

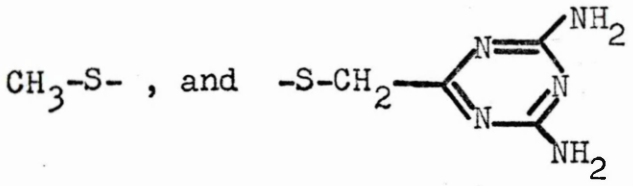
(b) that cold extraction of the tlc band with methanol and dioxan leaves the menazon structure intact.

It is thus plausible to assume that the other triazinyl components located on tlc are genuine degradation products and not artifacts produced by interaction of the heated menazon with the solvent system or the adsorbent. Also if the bands are extracted under "cold" conditions, there is little danger of alteration of the structure of the degradation products. Should this happen the R_F value on tlc of the isolated compound will be different from that of the band from which it has been extracted.

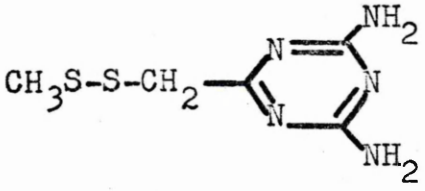
The other component present with menazon in band No.1 was extracted, after separation on silica gel GUV_{254}, with cold methanol followed by treatment with chloroform. The isolated material (20 mg) was subjected to spectroscopic examination.

The uv spectrum (Fig. 42, App. page 141) and the infrared spectrum (Fig. 43, App. page 149) showed the presence of a

diamino-1,3,5-triazine structure. The nmr spectrum (Fig. 44, App. page 155) was strongly similar to that of 2,4-diamino-6-methylthiomethyl-1,3,5-triazine (Fig. 26, App. page 153). This suggested the presence of the units



In the mass spectrum (Fig. 45 and Table 26, App. pages 172,173). the peak at highest mass occurred at m/e 203. It was very weak, but it was regarded as the molecular ion since a small but definite fragment was observed at m/e 101.5. Ions have been repeatedly detected at m/e values corresponding to half that of the moecular ion. The intensities of the $M^{\cdot+}$ + 1, $M^{\cdot+}$ + 2 isotope peaks were consistent with the presence of two sulphur atoms. Assuming a molecular weight of 203, the elemental analysis values required the molecular formula to be $C_5H_9N_5S_2$. All the evidence was compatible with the structure



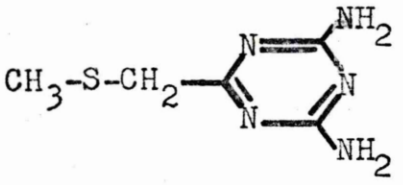
The presence of the disulphide -S-S- linkage was confirmed by reduction with zinc and dilute sulphuric acid. The smell of methyl mercaptan was detected.

This degradation product is a novel compound, but its synthesis has not been attempted in the present work.

3.2 Isolation and structure of the degradation product in band No.2

Band No.2 was very strong uv quenching, suggesting that it contains a major 1,3,5-triazinyl degradation product. The band was homogeneous when subjected to two dimensional paper chromatography, and the degradation product (40 mg) was isolated by extracting it with cold methanol followed by treatment with chloroform.

The uv, infrared, nmr, and mass spectra were compatible with the structure,



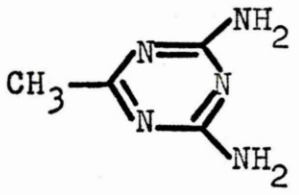
This was confirmed by comparison of the spectra (see Table 18, page 69 for location of the detailed results) with those of a sample of 2,4-diamino-6-methylthiomethyl-1,3,5-triazine previously characterised by A. Calderbank and prepared by the alkaline hydrolysis of menazon[62]. Menazon is a good methylating agent, and this degradation product may be formed by methylation of the initially formed mercaptan (see below).

3.3 Isolation and structure of the triazinyl component in band No.3.

Band No.3 was very strong uv quenching, suggesting that it contains a major 1,3,5-triazinyl degradation product. The band gave a yellow colour when sprayed with DBQ. When subjected

to two dimensional paper chromatography, it was found to contain two non-triazinyl components located by their yellow colour when sprayed with DBQ, and a triazinyl component which gave no colour with the reagent but which appeared as a quenching area under uv. The latter has been isolated (30 mg) in a pure state by extracting it with cold methanol followed by treatment with dioxan.

The uv, infrared, nmr, and mass spectra suggested the structure,



This was confirmed by comparison of the spectra (see Table 15, page 62 for location of the detailed results) with those of a sample of 2,4-diamino-6-methyl-1,3,5-triazine. This compound must be formed by splitting of the S-C bond in menazon on heating, and its thermodynamic stability probably accounts for its formation as a major degradation product.

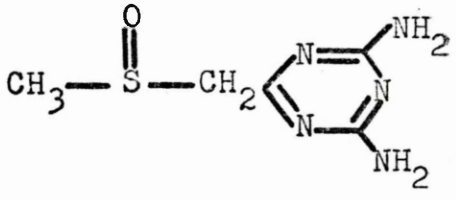
3.4 Structure of the triazinyl component in band No.4

The uv quenching band No.4 was not as intense as band Nos 1,2,3. When subjected to two dimensional paper chromatography, it was found to contain a 1,3,5-triazinyl component which appeared as a quenching area under uv and gave a pale brown colour when sprayed with DBQ, and a non-quenching area located by its yellow colour with DBQ. Attempts made to actually isolate the triazine compound from band No.4 were

unsuccessful probably because it was not present in adequate amounts.

However, in an initial attempt to isolate the degradation product in band No.2, band No.2 was stored for two weeks at room temperature before being extracted with methanol and treated with dioxan. A compound was isolated (40 mg) which had a lower R_F on tlc than band No.2, clearly indicating that the compound originally present in band No.2 had been modified.

The uv, infrared, nmr, and mass spectra (see Table 21, page 74 for location of the detailed results) were extremely similar to those of 2,4-diamino-6-methylsulphinylmethyl-1,3,5-triazine,



This compound must be formed by oxidation of the 2,4-diamino-6-methylthiomethyl-1,3,5-triazine originally present in band No.2. In the present work it has been shown that the oxidation occurs when a tlc plate (silica gel HF_{254}; BAW solvent) loaded with 2,4-diamino-6-methylthiomethyl-1,3,5-triazine is allowed to stand at room temperature for two weeks. It was thought at least possible that it might be one of the other degradation products of menazon. Its R_F value on tlc was identical with that of band No.4. Further both band No. 4 and 2,4-diamino-6-methylsulphinylmethyl-1,3,5-triazine co-chromatographed as a single uv quenching area which gave a pale brown colour with DBQ, when subjected to two dimensional

paper chromatography.

There is therefore chromatographic evidence that the 1,3,5-triazinyl degradation product in band No.4 may in fact be 2,4-diamino-6-methylsulphinylmethyl-1,3,5-triazine. This compound has been identified previously as a major rat urinary metabolite of menazon and characterised by M.A. Stevens and G.H. Walker[66] by oxidation of 2,4-diamino-6-methylthiomethyl-1,3,5-triazine with peracetic acid. Its formation as a thermal degradation product of menazon may be due to atmospheric oxidation of 2,4-diamino-6-methylthiomethyl-1,3,5-triazine, identified in band No.2.

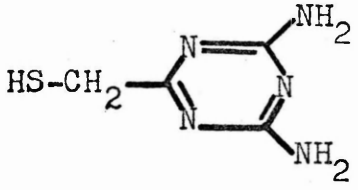
3.5 The degradation products in bands No.5, No.6, No.7 and No.8

The uv quenching band No.6 is of about the same intensity as band No.4, and contains two 1,3,5-triazinyl components of unknown identity when chromatographed two dimensionally on paper. Bands No.5, No.7 and No.8 are so weak that it is doubtful if the triazine compounds present in them could be isolated in sufficient amounts for structure determination. Their importance as degradation products may be minimal.

3.6 Examination of the residue left after heated menazon is digested with dioxan-water

When heated menazon is digested with dioxan-water, a pale yellow almost intractable residue is left. After stirring with dilute ammonium hydroxide, the residue was chromatographed

two dimensionally on paper. Two uv quenching areas were located, and one of them had the same R_F values and gave the same colour (brown) when sprayed with DBQ as 2,4-diamino-6-mercaptomethyl-1,3,5-triazine



The infrared spectra of the residue and of the mercaptan were also taken and found to be identical (Fig. 46, App. page 150). It thus appears likely that the mercaptan may be present in considerable amounts in the residue. This mercaptan has been characterised previously by A. Calderbank[62] as a sparingly soluble compound produced by hydrolysis of menazon with dilute hydrochloric acid. Its formation as a thermal degradation product is not unexpected since its S-methylated derivative has conclusively been identified as the degradation product in band No.2.

4 Phosphorus determination on menazon and its triazinyl degradation products

Phosphorus determination, using the method described by A. Calderbank and J.B. Turner[9], on a two dimensional paper chromatogram loaded with 350 µg of heated menazon gave negative results for all the uv quenching areas, except the one which contains menazon (Table 27, page 97). The authors do not discuss the limits of detection of the method, but it may be deduced as

follows.

The smallest loading of menazon detected on paper by uv quenching is 10 μg,[9] and this may be set as the limit of detection of triazine compounds on paper by uv quenching. The average uv adsorbance for paper blanks over 18 recorded determinations[9] is 0.081. The limit of detection of the phosphorus determination may be set at twice this value, 0.162, which corresponds to 7.2 μg of menazon. This is of the same order as the limit of detection on paper by uv quenching.

It is thus clear that if the triazinyl degradation products did contain phosphorus, positive results would be expected. The phosphorus containing moiety of the menazon molecule must break off easily on heating, and this is additional evidence for the structures previously proposed for the degradation products.

5 The mass spectra of diamino-1,3,5-triazines and of menazon

5.1 General

In this section some comments will be made on the common features present in the mass spectra of 2,4-diamino-1,3,5-triazines. There is a scarcity of literature[45,67] in this area, and the multiplicity of ions and fragmentation pathways possible make interpretation difficult. Even in cases where the elemental composition of the fragment ions have been obtained by high resolution, there is often no evidence for the structures postulated and they should be viewed with caution. Further complications arise particularly with diamino-1,3,5-triazines, since hydrogen rearrangement may occur in the mass spectrometer to give tautomeric imino structures. Evidence for similar rearrangements has been given elsewhere[69,70] in the study of the mass spectra of pyrimidines. The fragmentation mechanisms of 2-amino-pyrimidine have been shown by deuterium labelling to involve initially hydrogen rearrangement of the molecular ion to give a tautomeric imino ion,

In the present work the mass spectrum of 2,4-diamino-

6-methyl-1,3,5-triazine has been studied in some detail since it was thought in view of its relatively simple structure that it would reveal more clearly fragments arising from degradation of the diamino-1,3,5-triazine ring. The mass spectrum of menazon is then discussed since it is expected that in addition phosphorus containing fragments will also be observed.

5.2 Mass spectrum of 6-methyl-2,4-diamino-1,3,5-triazine

The mass spectrum of this compound is shown in Fig. 17 and the accompanying Table 16 (App. pages 158,159) which also gives the formulae of some of the ions as determined by high resolution measurements.

Transitions involving ions of the following m/e values have been found to occur by the observation of metastable transitions using Jennings' adaptation[70] :

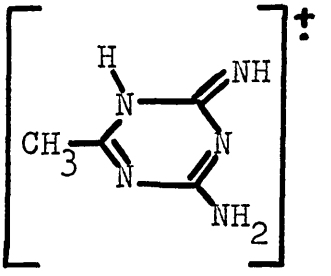
43 ⟵ 84
42 ⟵ 43, 83
41 ⟵ 43, 42, 68, 83
40 ⟵ 41, 42
84 ⟵ 125
68 ⟵ 110

Broad metastable peaks occurred in the mass spectrum at m/e values 67.2, 55.1 and these were due to the transitions :

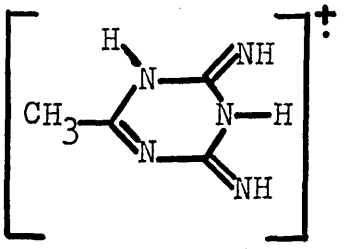
98 ⟵ 125
83 ⟵ 125

The base peak of the spectrum at m/e 125 is due to the molecular ion, which as in the case of pyrimidines could exist

as imino structures, e.g. 22 and 22a.

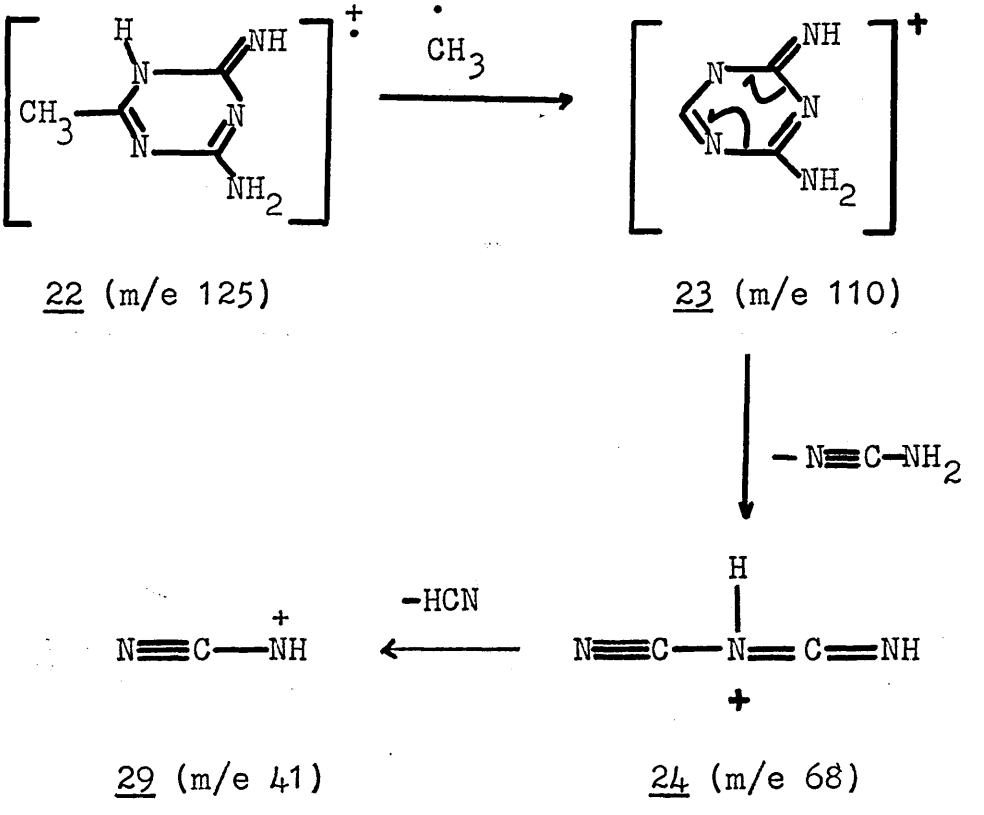


22



22a

Loss of $\dot{C}H_3$ from the molecular ion is expected, but only a weak ion 23 (m/e 110) was observed. The latter strongly eliminates $H_2N-C\equiv N$ to give 24 (m/e 68), which decomposes further with ejection of HCN to give 29 (m/e 41)

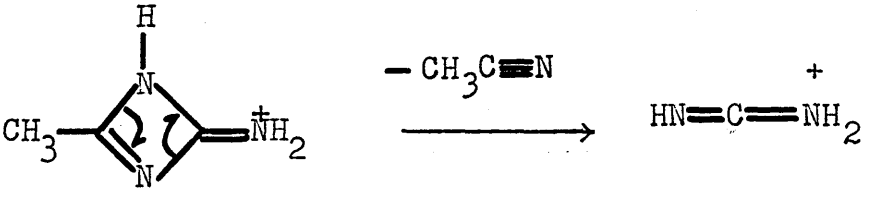


22 (m/e 125) 23 (m/e 110)

29 (m/e 41) 24 (m/e 68)

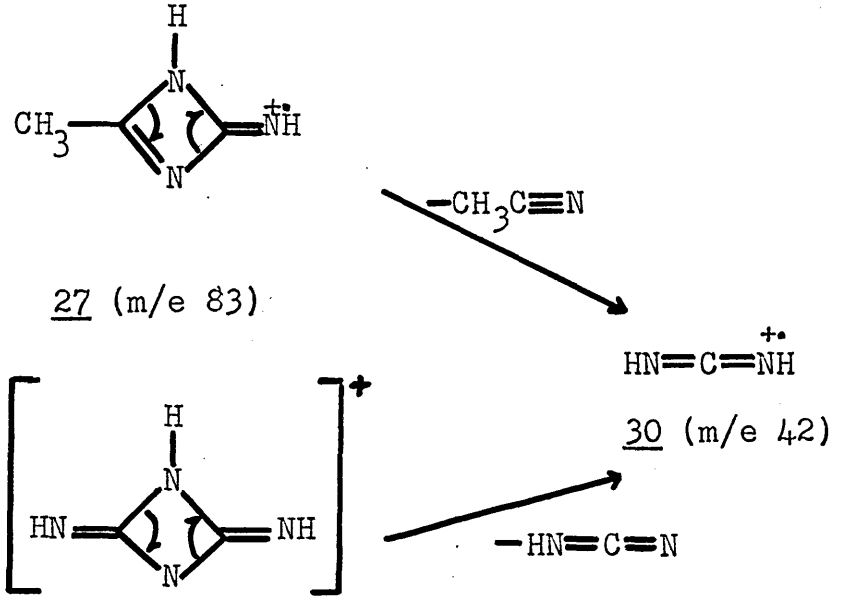
Loss of HCN is known to occur in aromatic amines and in

2-amino-pyrimidines[68], and the peak at m/e 98 is consistent with sensible loss of HCN from the molecular ion. The ion of m/e 84 may have the structure 25, and elimination of $CH_3C{\equiv}N$ then leads to the intense ion of structure 26 at m/e 43. The high intensity of this ion suggests that it may well be formed from the molecular ion itself.



25 (m/e 84) 26 (m/e 43)

The peak at m/e 83 is a doublet when observed at high resolution, and the components of the doublet may be attributed the structures 27, 28. Elimination of $CH_3C{\equiv}N$ from 27 or HN=C=N from 28 both lead to 30 (m/e 42)



27 (m/e 83)

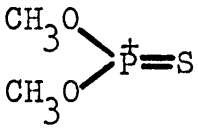
28 (m/e 83)

The ions of m/e 83, 68, 43, 42, 41 present in the mass spectrum of 2,4-diamino-6-methyl-1,3,5-triazine, are also often present in the mass spectra of diamino-1,3,5-triazines generally. There are two other ions of m/e 82, 55 which are also frequently encountered in the mass spectra of diamino-1,3,5-triazines, and they are especially prominent in the mass spectrum of 2,4-diamino-6-methylsulphinylmethyl-1,3,5-triazine (Fig. 33 and Table 22, App. pages 166,167). Unfortunately structures cannot be postulated for them since high resolution measurements are not available; but the latter spectrum shows a broad metastable peak at m/e 37 which indicates that m/e 55 may be derived from m/e 82 by elimination of HCN. Another feature of diamino-1,3,5-triazine spectra is the consistent presence of a small but definite fragment at an m/e value equal to half that of the molecular ion. This fact is very useful in cases where the molecular ion is in doubt, and hence in the identification of unknown derivatives.

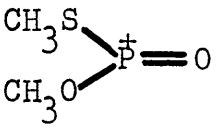
5.3 Mass spectrum of menazon

The mass spectrum of menazon (Fig. 41 and Table 25, App. pages 170,171) shows a distinct molecular ion at m/e 281. Fragments formed by degradation of the diamino-1,3,5-triazine ring are expected, and are in fact observed at m/e 82, 68 (24), 55,43 (26), 42, 41. Comparing its spectrum with that of methyl trithion (Fig. 3, page 33), typical phosphorus fragments are observed at m/e 125 (7, 7a), 93 (8), 79 (10), 63, 47, the last two fragments having structures PS^+ and PO^+

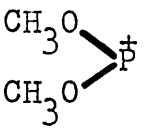
respectively (see Introduction section 9).



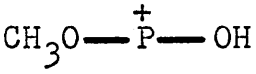
7 (m/e 125)



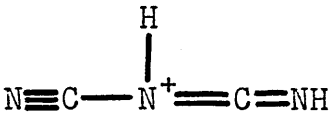
7a (m/e 125)



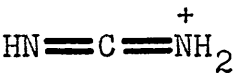
8 (m/e 93)



10 (m/e 79)

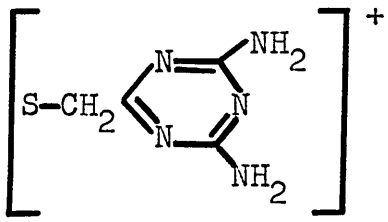


24 (m/e 68)



26 (m/e 43)

The base peak of the spectrum occurs at m/e 156, and is due to the stable fragment ion 31 formed by loss of $(CH_3O)_2\dot{P}=S$ from the molecular ion. This assignment is supported by the fact that the mass spectrum of N-methyl menazon* shows a base peak at m/e 170, which is clearly due to the N-methyl analogue of the species 31



31 (m/e 156)

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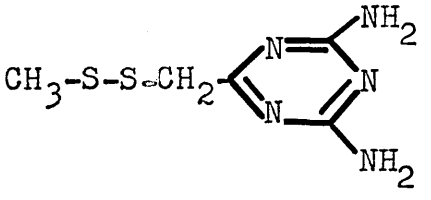
* Private collection belonging to Dr. J.B. Turner.

6 Suggestions for further work

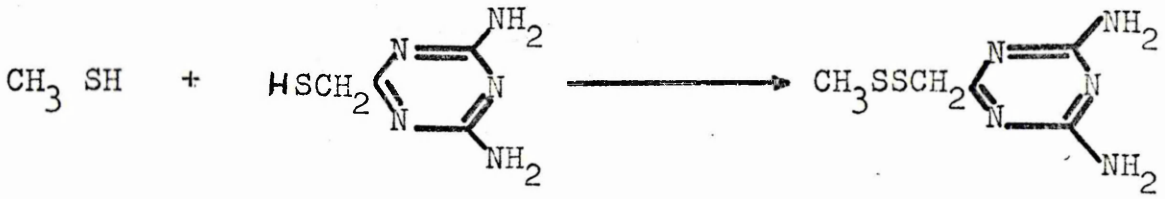
On the main problem of identification of the degradation products of menazon, there are two fairly strong uv quenching bands, No. 4, No.6 (see page 51 for the system of numbering of the tlc bands) which have to be extracted. Bands Nos. 5, 7, 8 are so weak that their extraction may not be worthwhile. There are also two components in the residue left after heated menazon is digested with dioxan-water and stirred with ammonium hydroxide. Previous experience[3,62] has shown that various 1,3,5-triazine derivatives can be isolated pure from mixtures by preparative paper chromatography on sheets of Whatman 3 MM paper. Bands No.4, No.6 may be extracted with cold methanol, and the solution concentrated before applying on Whatman 3 MM paper. The separated triazinyl components may then be located by observation under uv, the quenching areas eluted with methanol, followed by evaporation of the solution to isolate the pure triazine compounds. The same basic procedure may be used for isolating the components in the residue.

The degradation product present in band No.1 has been identified in the present work as



This is a novel compound, and its synthesis is required as a final confirmation of its identity. It may be possible to

prepare it in statistical proportion by reaction of methyl mercaptan with 2,4-diamino-6-mercaptomethyl-1,3,5-triazine in the presence of iodine, and isolated from the other disulphides formed by tlc.



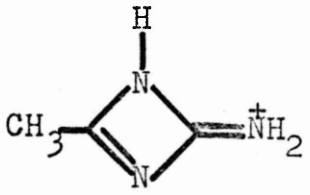
It may also be oxidised to its sulphoxides with peracetic acid and these may be the unidentified degradation products of menazon.

Six non triazinyl degradation products, which presumably contain phosphorus, have been located on two dimensional paper chromatograms of heated menazon. The phosphorus fragments are probably responsible for the unpleasant smell of heated menazon, and gas liquid chromatography is a possible way to investigate the fate of the phosphorus end of menazon.

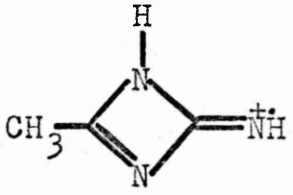
Regarding the heating of the menazon sample, it may be advantageous to carry out the heating in a solvent. This may give larger quantities of degradation products and permit one to heat at a lower temperature, in which case a build-up of the various products may occur after different periods of time, and it would be easier to follow the course of the reaction by tlc.

Finally, on the mass spectrometry side, it would be desirable to have high resolution measurements done on the peaks at m/e 82, 55 in the mass spectrum of 2,4-diamino-6-methyl-sulphinylmethyl-1,3,5-triazine (Fig. 33, App. page 166)

since they frequently occur in the mass spectra of diamino-1,3,5-triazines. Ions of m/e 84, 83 are also often present in the spectra of diamino-1,3,5-triazines and structures 25 and 27 have been postulated for them in the mass spectrum of 2,4-diamino-6-methyl-1,3,5-triazine (Fig. 17, App. page 158).



25 (m/e 84)



27 (m/e 83)

It would be useful to compare it with the mass spectrum of 2,4-diamino-1,3,5-triazine to verify if these structures are correct.

REFERENCES

1. A.A.B. Swan, Imperial Chemical Industries Ltd., Ind. Hygiene Toxicol. Report, 1961, IHL/142.
2. A. Calderbank and J. B. Turner, Plant Protection Experimental Report, 1962, PP/E/165.
3. A. Calderbank and J. B. Turner, Plant Protection Experimental Report, 1962, PP/E/161.
4. A. Calderbank and J.A. Silk, Plant Protection Experimental Report, 1960, PP/E/56.
5. E.M. Smolin and L. Rapoport, "s-Triazines and Derivatives" in "The Chemistry of Heterocyclic Compounds", ed. A. Weissberger. Interscience, New York, 1959, vol. 13, p. 234.
6. K.H. Slotta and R. Tschesche, Ber., 1929, 62, 1390.
7. F.H.S. Curd, J.A. Hendry, T.S. Kenny, A.G. Murray, and F.L. Rose, J. Chem. Soc., 1948, 1634.
8. C.G. Overberger, F.W. Michelotti, and P.M. Carabateas, J. Amer. Chem. Soc., 1957, 79, 941.
9. A. Calderbank and J.B. Turner, Analyst, 1962, 87, 273.
10. J.J. Menn, W.R. Erwin, and H.T. Gordon, J. Agric. Food Chem., 1957, 5, 601.
11. P.S. Chen, T.Y. Toribara, and H. Warner, Analyt. Chem., 1956, 28, 1756.
12. F.S. Mortimer, Spectrochim. Acta, 1957, 9, 270.
13. L.S. Maiants, E.M. Popov, and M.I. Kabachnik, Optics and Spectroscopy, 1959, 7, 108.
14. R.A. Nyquist and W.W. Muelder, Spectrochim. Acta, 1966, 32, 1563.

15. L.C. Thomas, R.A. Crittenden, Spectrochim. Acta, 1964, 20, 467.

16. R.A. Crittenden and L.C. Thomas, Spectrochim. Acta, 1964, 20, 1679.

17. L.J. Bellamy, "Advances in Infrared Group Frequencies", Methuen, London, 1968.

18. L.C. Thomas and R.A. Crittenden, Spectrochim. Acta, 1964, 20, 489.

19. L.J. Bellamy, "Infrared Spectra of Complex Molecules", Methuen, London, 1958.

20. A. R. Katritzky and A.P. Ambler, "Physical Methods in Heterocyclic Chemistry", Academic Press, New York, 1963, vol. 2.

21. J. Govbeau, E.L. Jahn, A. Kreutzberger, and C. Grundmann, J. Phys. Chem., 1954, 58, 1078.

22. S. Califano and B. Crawford, Spectrochim. Acta, 1960, 16, 900.

23. C. Grundmann and A. Kreutzberger, J. Amer. Chem. Soc., 1954, 76, 5646.

24. J.E. Lancaster and N.B. Colthup, J. Chem. Phys., 1954, 22, 1149.

25. J.E. Lancaster, R.F. Stamm, and N.B. Colthup, Spectrochim. Acta, 1961, 17, 155.

26. H.K. Reimschuessel and N.T. McDevitt, J. Amer. Chem. Soc., 1960, 82, 3756.

27. W.A. Heckle, H.A. Ory, and J.M. Talbert, Spectrochim. Acta, 1961, 17, 600.

28. W.M. Padgett and W.F. Hamner, J. Amer. Chem. Soc., 1958, 80, 803.

29. W.J. Jones and W.J. Orville Thomas, Trans. Faraday Soc., 1959, 55, 203.

30. J.W. Emsley, J. Feeney, and L.H. Sutcliffe, Progress in Nuclear Magnetic Resonance Spectroscopy, 1967, vol. 2.

31. G. Mavel and A. Besnard, Compt. rend., 1963, 257, 898.

32. G. Mavel and G. Martin, Compt. rend., 1961, 252, 110.

33. J.B. Hendrickson, M.L. Maddox, J.J. Sims, and H.D. Kaesz, Tetrahedron, 1964, 20, 449.

34. G. Mavel and G. Martin, J. Chim. phys., 1965, 62, 475.

35. G. Mavel and G. Martin, J. Chim. phys., 1962, 59, 762.

36. G. Mavel and G. Martin, Compt. rend., 1962, 254, 260.

37. L.H. Keith, A.W. Garrison, and A.L. Alford, J. Assoc. Offic. Anal. Chem., 1968, 51, 1063.

38. H. Babad, W. Herbert, and M. C. Goldberg, Analyt. Chim. Acta, 1968, 41, 259.

39. J.F. Nixon and R. Schmutzler, Spectrochim. Acta, 1962, 22, 565.

40. T.H. Siddall and C.A. Prohaska, J. Amer. Chem. Soc., 1962, 84, 2502.

41. T.H. Siddall and C.A. Prohaska, J. Amer. Chem. Soc., 1962, 84, 3467.

42. J.S. Waugh, and F.A. Cotton, J. Phys. Chem. 1961, 65, 562.

43. F. Kaplan, G. Singh, and H. Zimmer, J. Phys. Chem. 1963, 67, 2510.

44. F. Declerck, R. Degrotte, J. De Lannoy, R. Nasielski-Hinkens, and J. Nasielski, Bull. Soc. chim. belges, 1965, 74, 119.

45. J. Jorg, R. Houriet, and G. Spiteller, Monatsh, 1966, 97, 1064.

46. J.N. Damico, J. Assoc. Offic. Anal. Chem., 1966, 1027.

47. J.B. McPherson and G.A. Johnson, J. Agric. Food Chem., 1956, 4, 42.

48. T.A. Bryce and J.E. Maxwell, Chem. Comm., 1965, 206.

49. J.H. Beynon, R.A. Saunders and A.E. Williams, Ind. chim. belge., 1964, 29, 311.

50. J.H. Beynon, "Mass Spectrometry and its Applications to Organic Chemistry", Elsevier, New York, 1960, p. 269.

51. A. Quayle, Advances in Mass Spectrometry, 1959, p. 365.

52. F.W. McLafferty, Analyt. Chem., 1956, 28, 213.

53. D.A. Bafus, E.J. Gallegos, and R.W. Kiser, J. Phys. Chem., 1966, 70, 2614.

54. H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Mass Spectrometry of Organic Compounds", Holden-Day, San Francisco, 1967.

55. S.F. Mason, J. Chem. Soc., 1959, 1247.

56. S.F. Mason, "Physical Methods in Heterocyclic Chemistry", Academic Press, New York, 1963, vol. 2.

57. M. Kasha, Discussions Faraday Soc., 1950, 9, 14.

58. C.G. Overberger and S.L. Chapiro, J. Amer. Chem. Soc., 1954, 76, 1855.

59. C.G. Overberger and S.L. Chapiro, J. Amer. Chem. Soc., 1954, 76, 93.

60. C.G. Overberger and S.L. Chapiro, J. Amer. Chem. Soc., 1954, 76, 97.

61. C.G. Overberger and S.L. Chapiro, J. Amer. Chem. Soc., 1954, 76, 1061.

62. A. Calderbank, J. Chem. Soc., 1966, 56.

63. L.V. Eggleston and R. Hems, Biochem. J., 1952, 52, 157.

64. A. Calderbank, Plant Protection Experimental Report, 1960, PP/E/66.

65. K. Randerath, "Thin Layer Chromatography", Academic Press, New York, 1966.

66. M.A. Stevens and G.H. Walker, J. Heterocyclic Chem., 1967, 4, 268.

67. P.C. Kearney, D.D. Kaufman, and T.J. Sheets, J. Agric. Food Chem., 1965, 13, 369.

68. J. Rice, G. Dudek, and M. Barber, J. Amer. Chem. Soc., 1965, 84, 4569.

69. T. Nishiwaki, Tetrahedron, 1966, 22, 3117.

70. K.R. Jennings, J. Chem. Phys., 1963, 43, 4176.

APPENDIX

1 Ultraviolet spectra

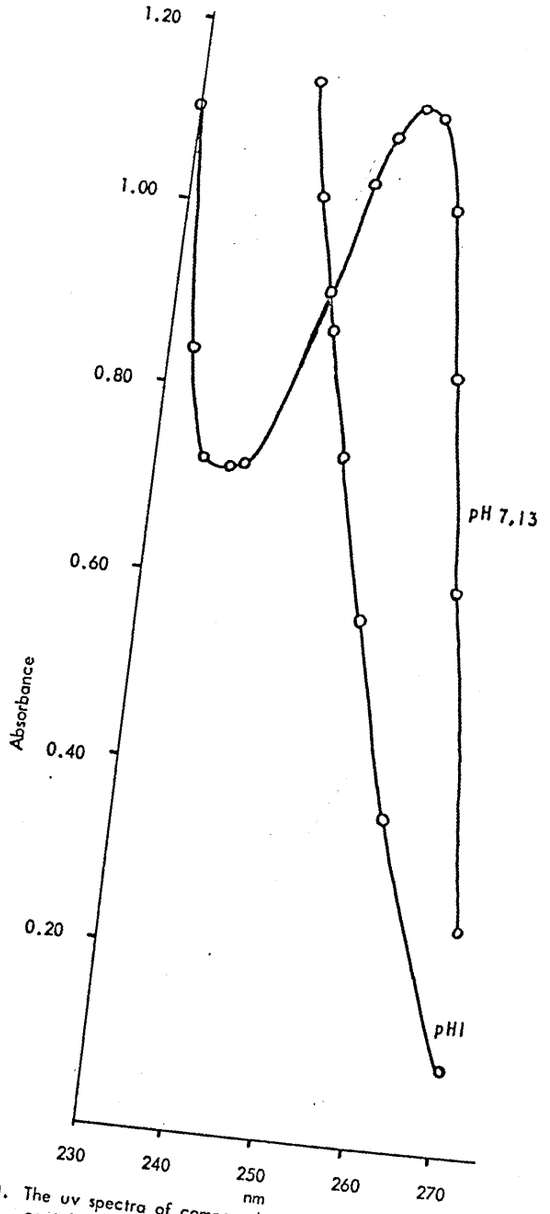


Fig. 10. The uv spectra of compound isolated from band No. 3 in aqueous solution containing 44.0 mg per litre at pH 1, 7 and 13.

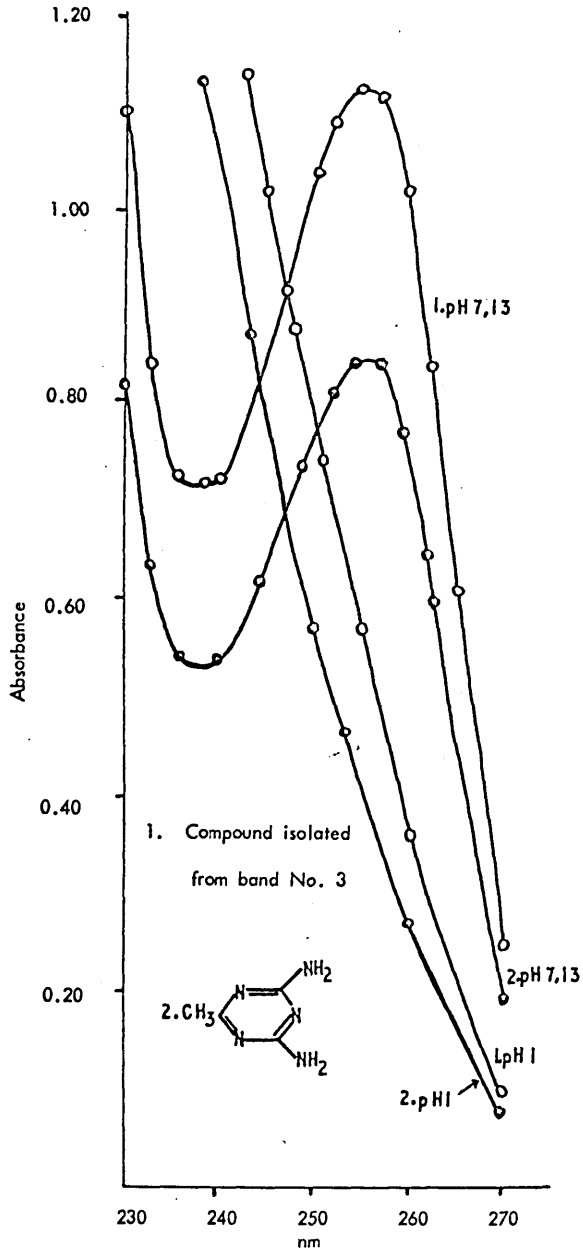


Fig. 15 The uv spectra of (1) compound isolated from band No. 3 in aqueous solution containing 44.0 mg per litre and (2) authentic 2,4-diamino-1,3,5-triazine in aqueous solution containing 33.0 mg per litre at pH 1, 7 and 13.

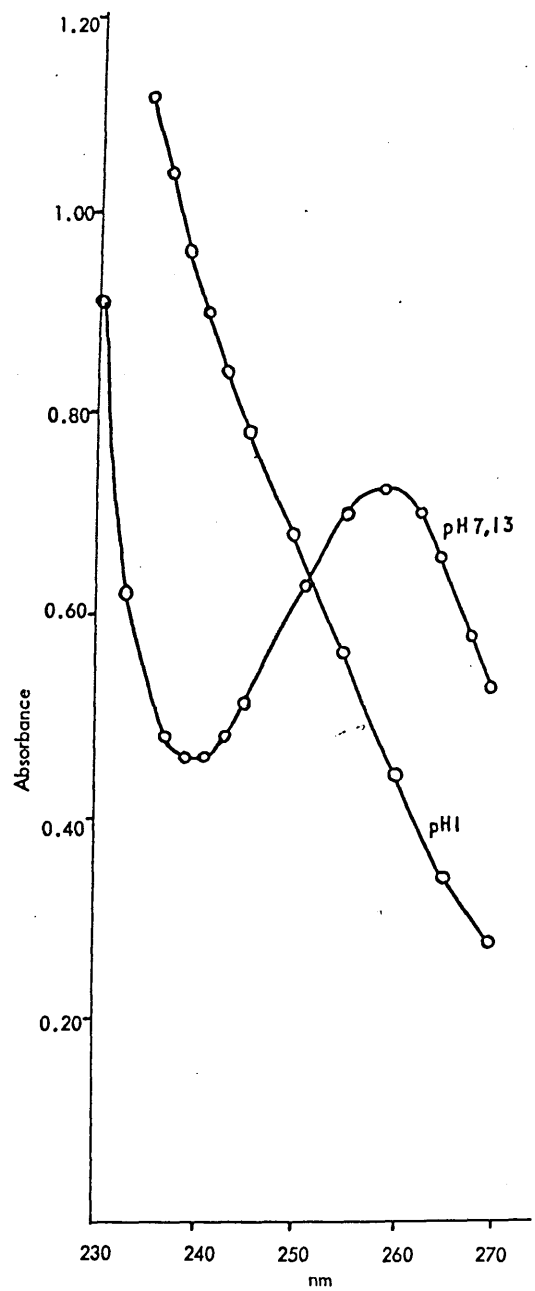


Fig. 19 The uv spectrum of compound isolated from band No. 2 in aqueous solution containing 30.8 mg per litre at pH 1, 7 and 13.

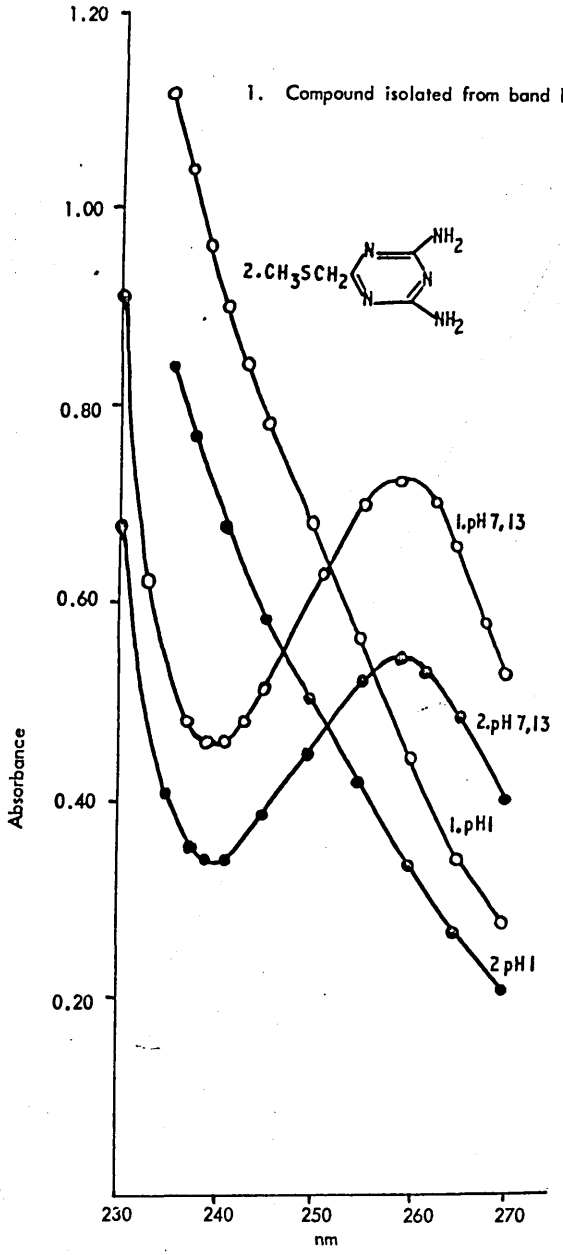


Fig. 23. The uv spectra of (1) compound isolated from band No. 2 in aqueous solution containing 30.8 mg per litre and (2) authentic 6-methylthiomethyl-2,4-diamino-1,3,5-triazine in aqueous solution containing 23.1 mg per litre at pH 1, 7 and 13.

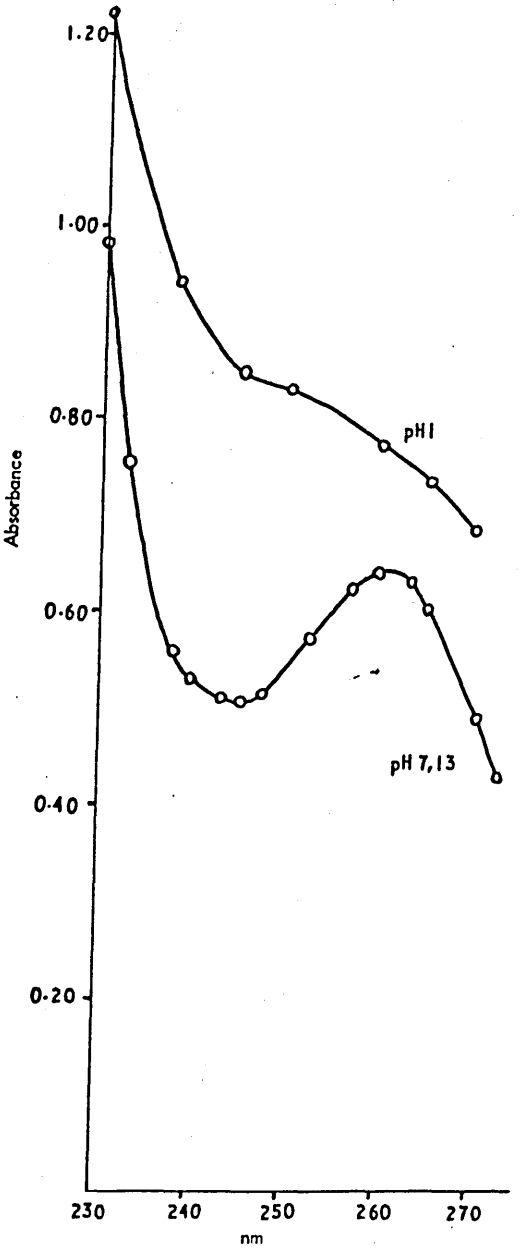


Fig. 27. The uv spectrum of compound referred to as "No. 2 modified" in aqueous solution containing 37.6 mg per litre at pH 1,7 and 13.

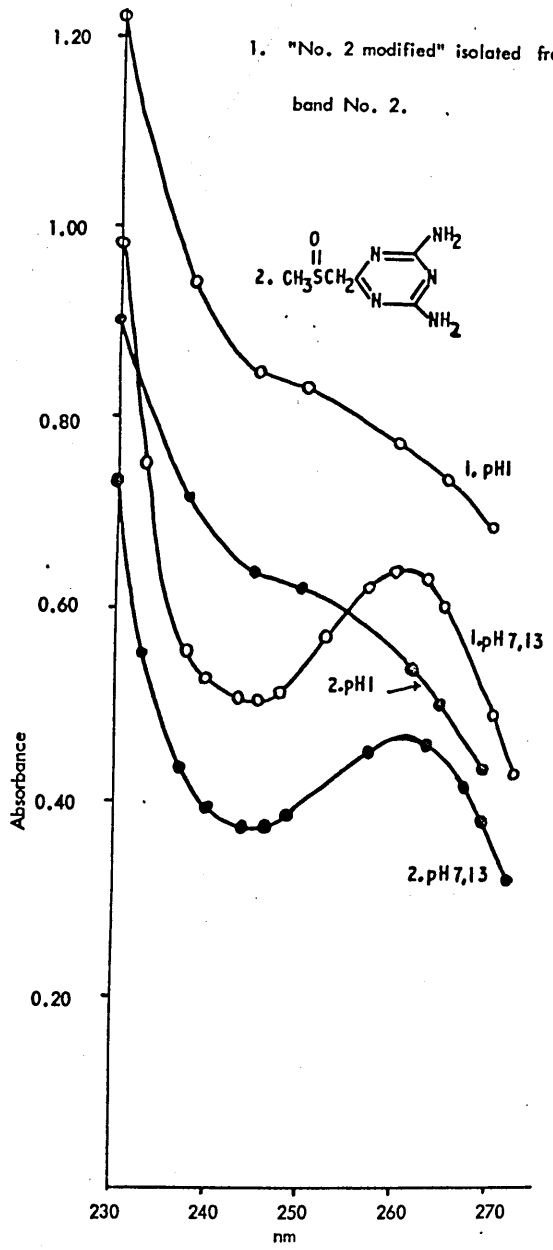


Fig. 31. The uv spectra of (1) compound referred to as "No. 2 modified" in aqueous solution containing 37.6 mg per litre and (2) authentic 6-methylsulphinylmethyl-2,4-diamino-1,3,5-triazine in aqueous solution containing 28.2 mg per litre at pH 1,7 and 13.

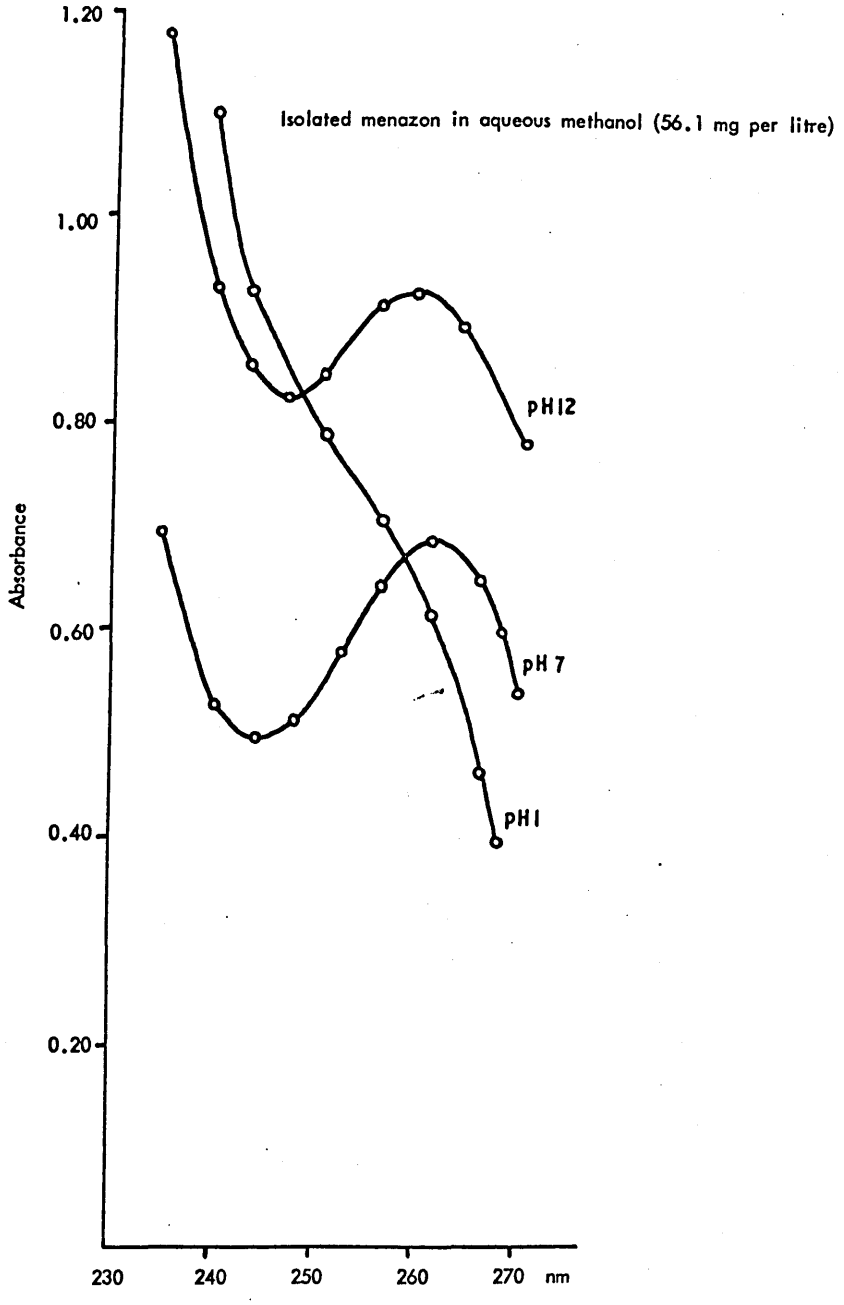


Fig. 36. The uv of isolated menazon.

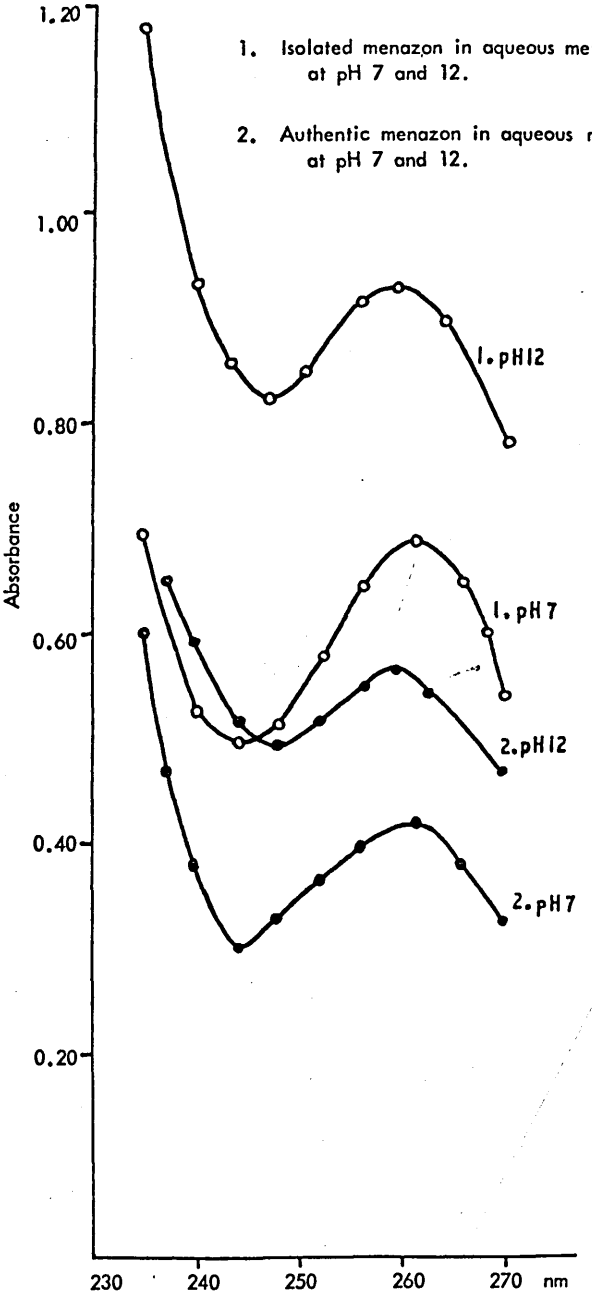


Fig. 37. The uv of isolated and authentic menazon.

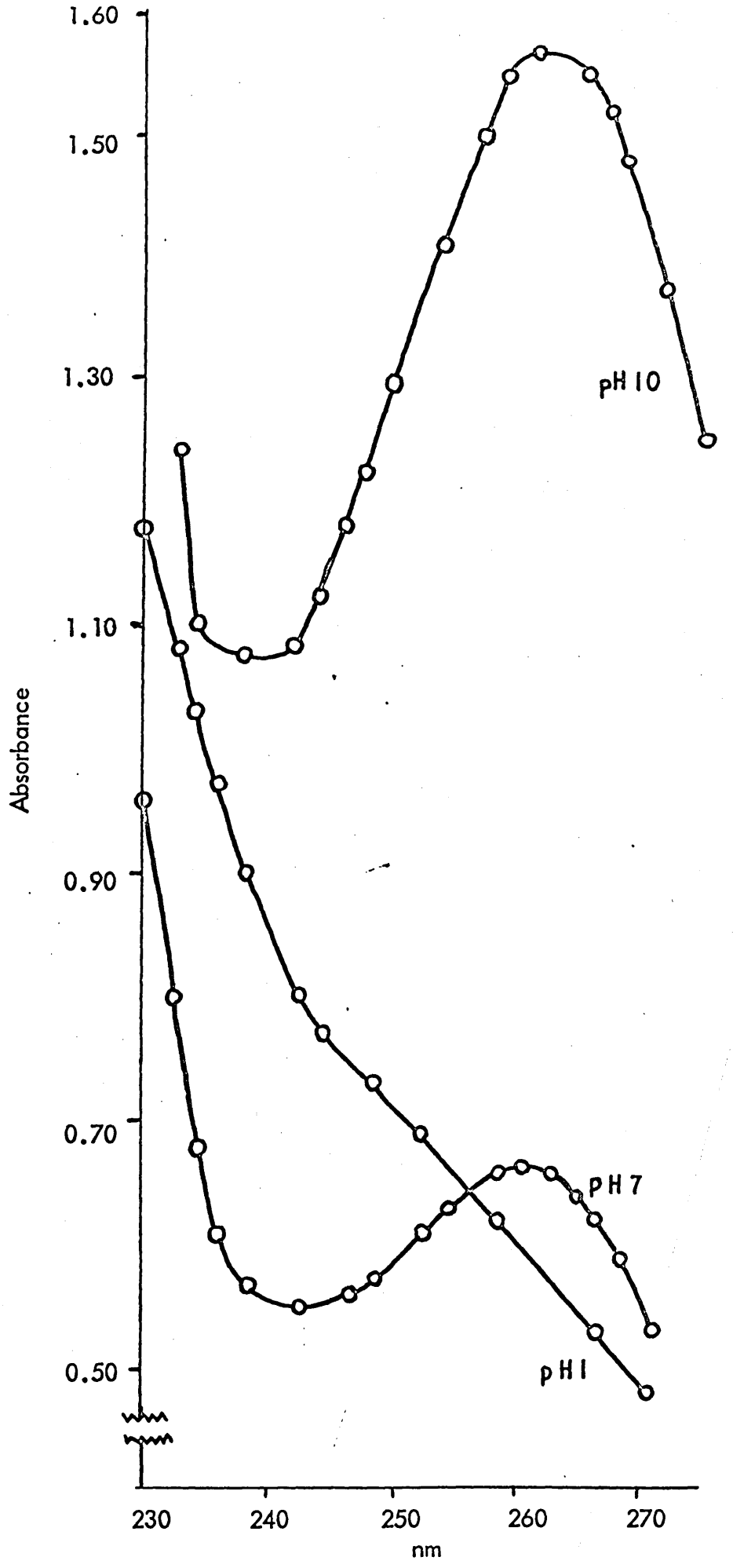


Fig. 42. The uv spectrum of the degradation product present in band No. 1 in aqueous solution containing 30.0 mg per litre at pH 1,7 and 10.

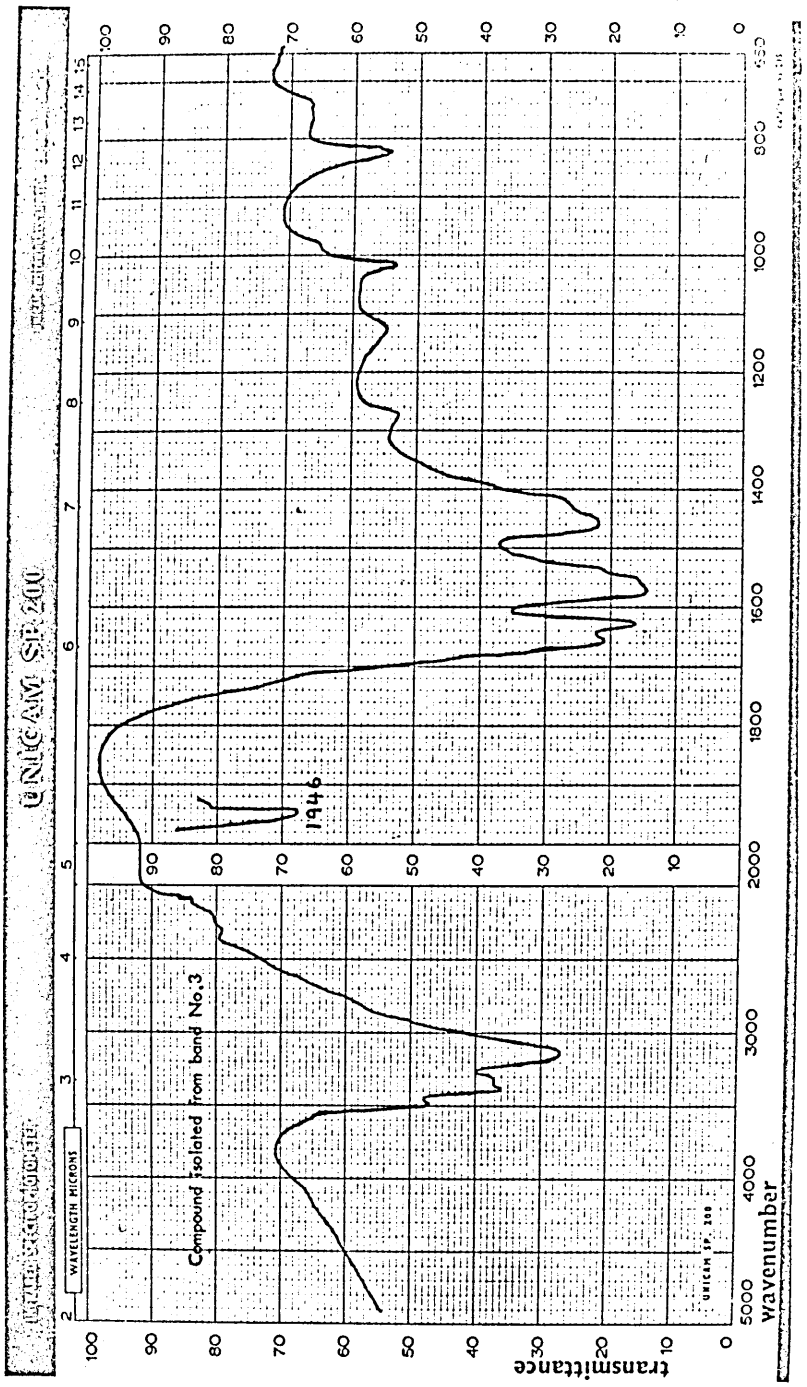


Fig. 11. The infrared spectrum of compound isolated from band No. 3. Spectrum taken in pressed KBr disc, 0.25% concentration.

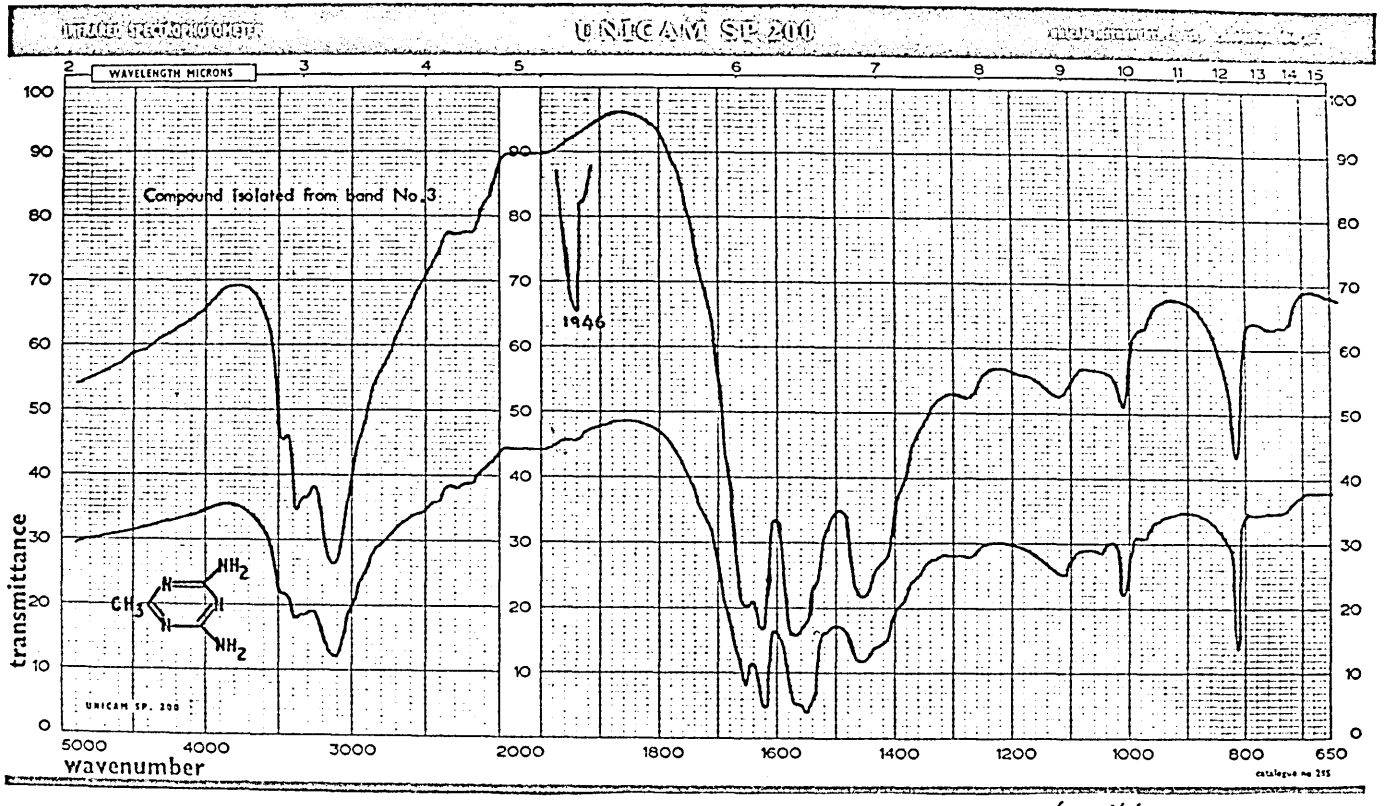


Fig. 16. The infrared spectra of compound isolated from band No. 3 and of 6-methyl 2,4-diamino-1,3,5-triazine. Spectra taken in pressed KBr discs, 0.25% concentration.

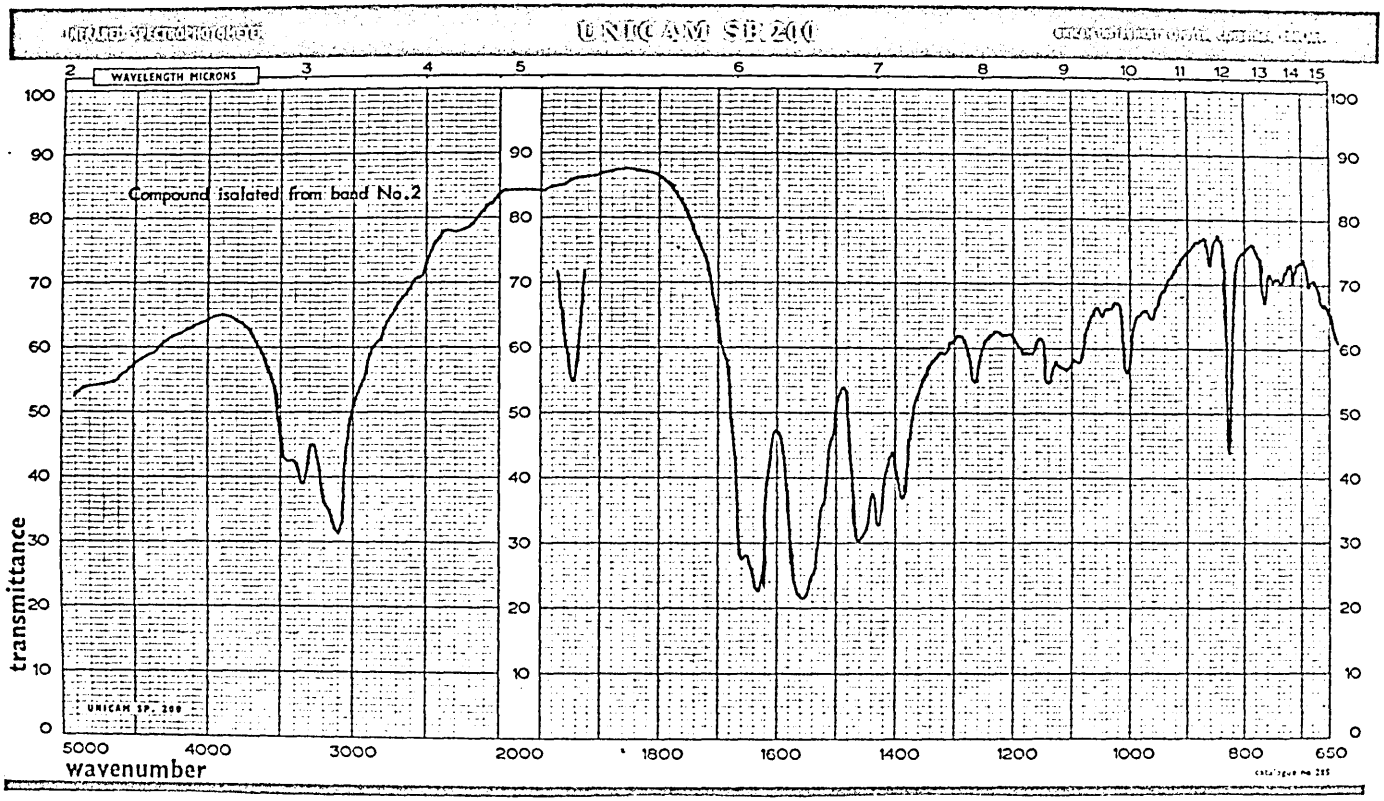


Fig: 20. The infrared spectrum of compound isolated from band No. 2. Spectrum taken in pressed KBr disc, 0.25% concentration.

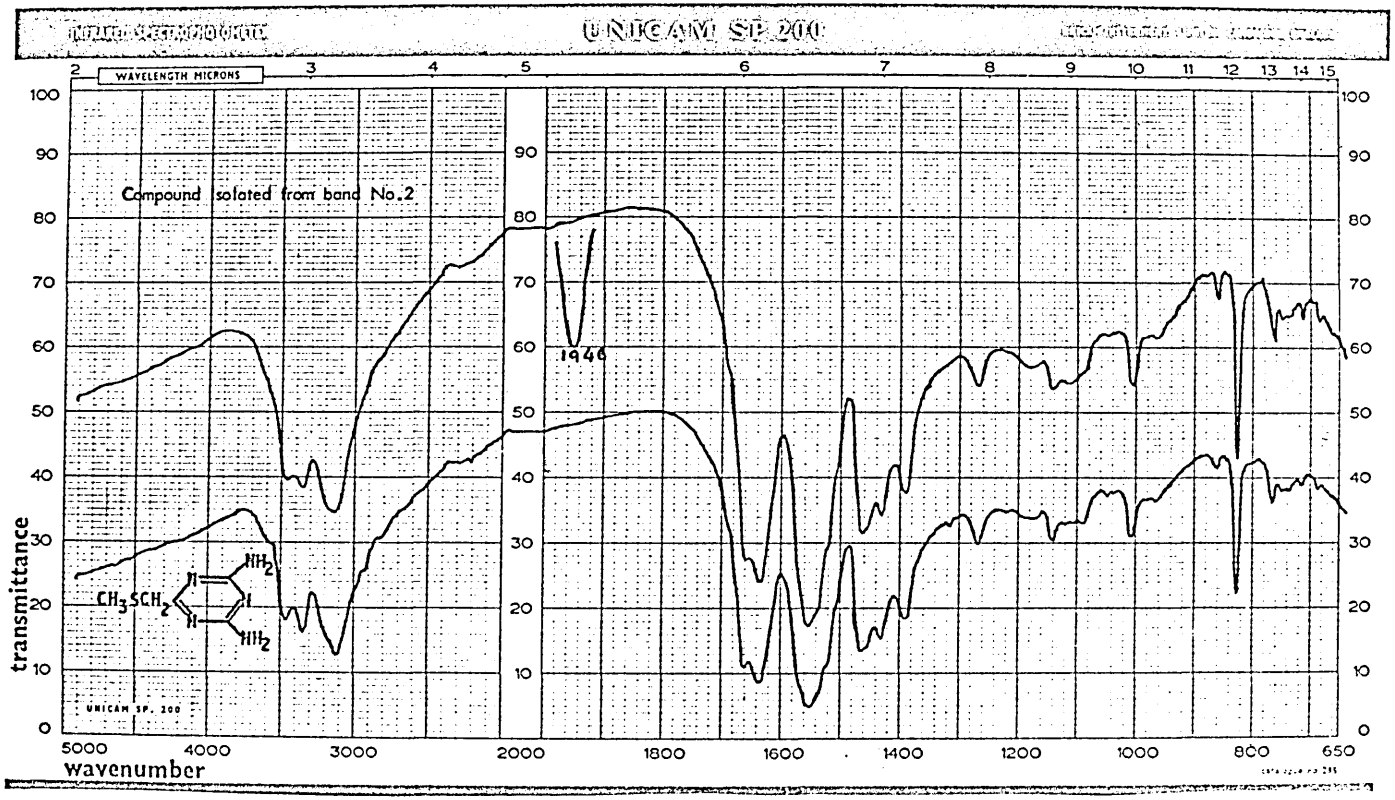


Fig. 24. The infrared spectra of compound isolated from band No. 2 and of 6-methylthiomethyl-2,4-diamino-1,3,5-triazine. Spectra taken in pressed KBr discs, 0.25% concentration.

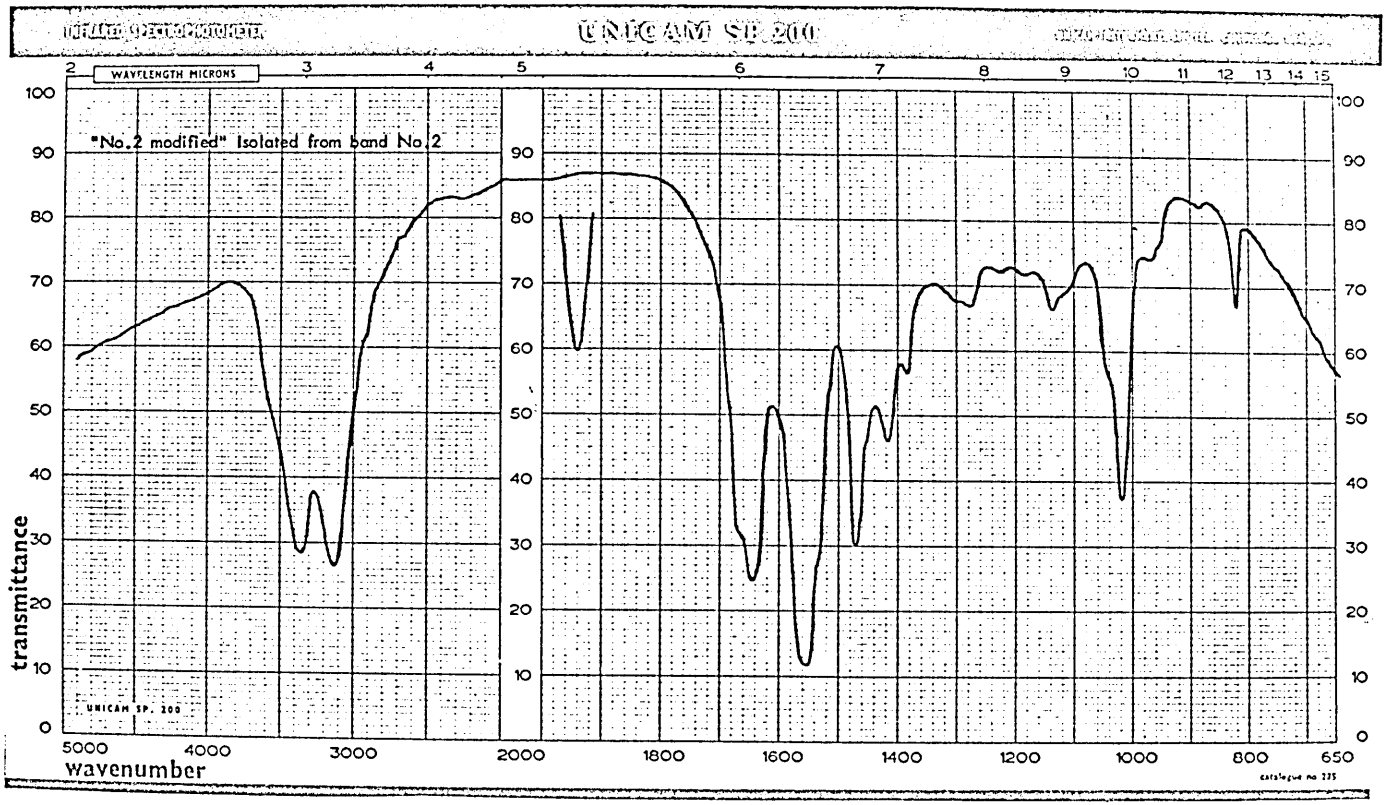


Fig. 28. The infrared spectrum of compound referred to as "No. 2 modified" isolated from band No. 2. Spectrum taken in pressed KBr disc, 0.25% concentration.

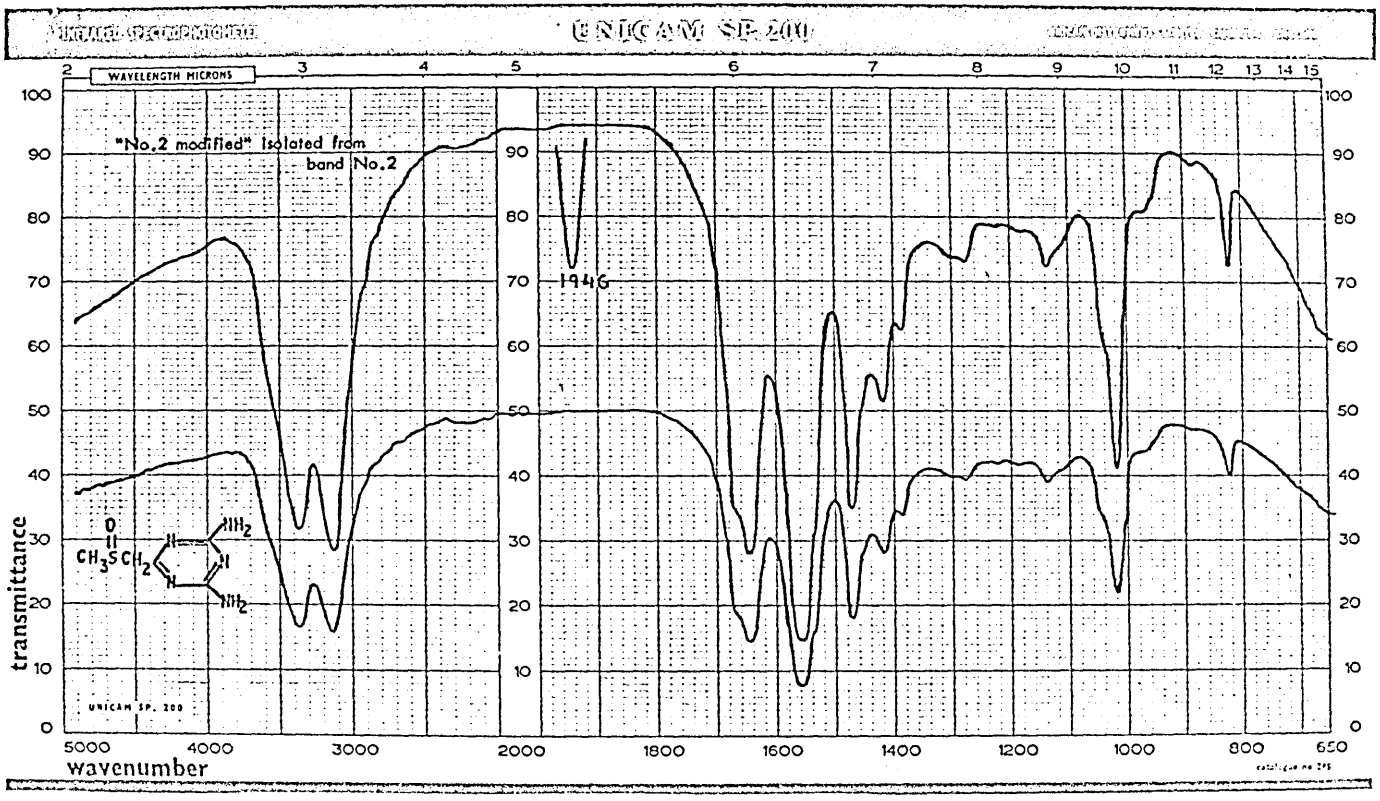


Fig. 32, The infrared spectra of compound referred to as "No. 2 modified" and of 6-methylsulphinylmethyl-2,4-diamino-1,3,5-triazine. Spectra taken in pressed KBr discs, 0.25% concentration.

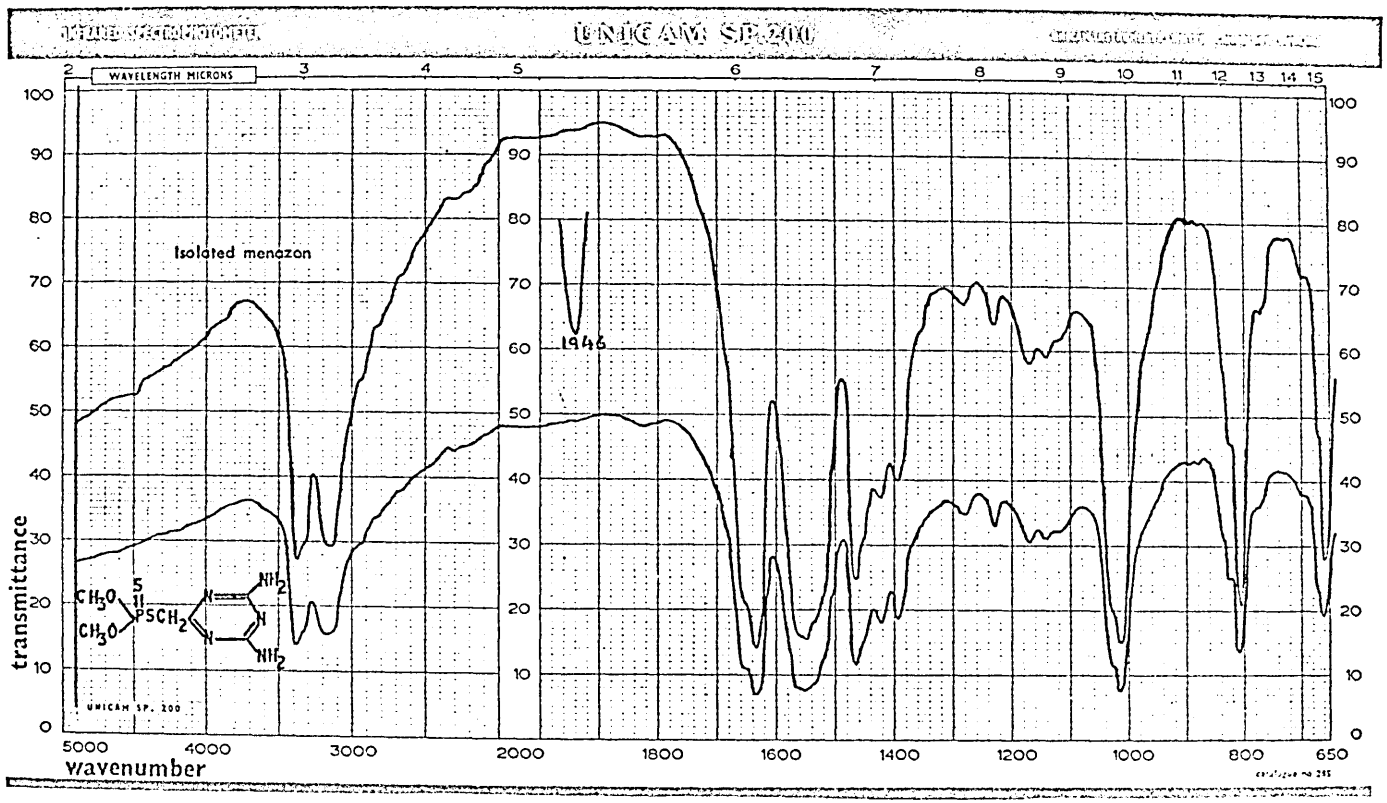


Fig. 35. The infrared spectra of isolated and authentic menazon. Spectra taken in pressed KBr discs, 0.25% concentration.

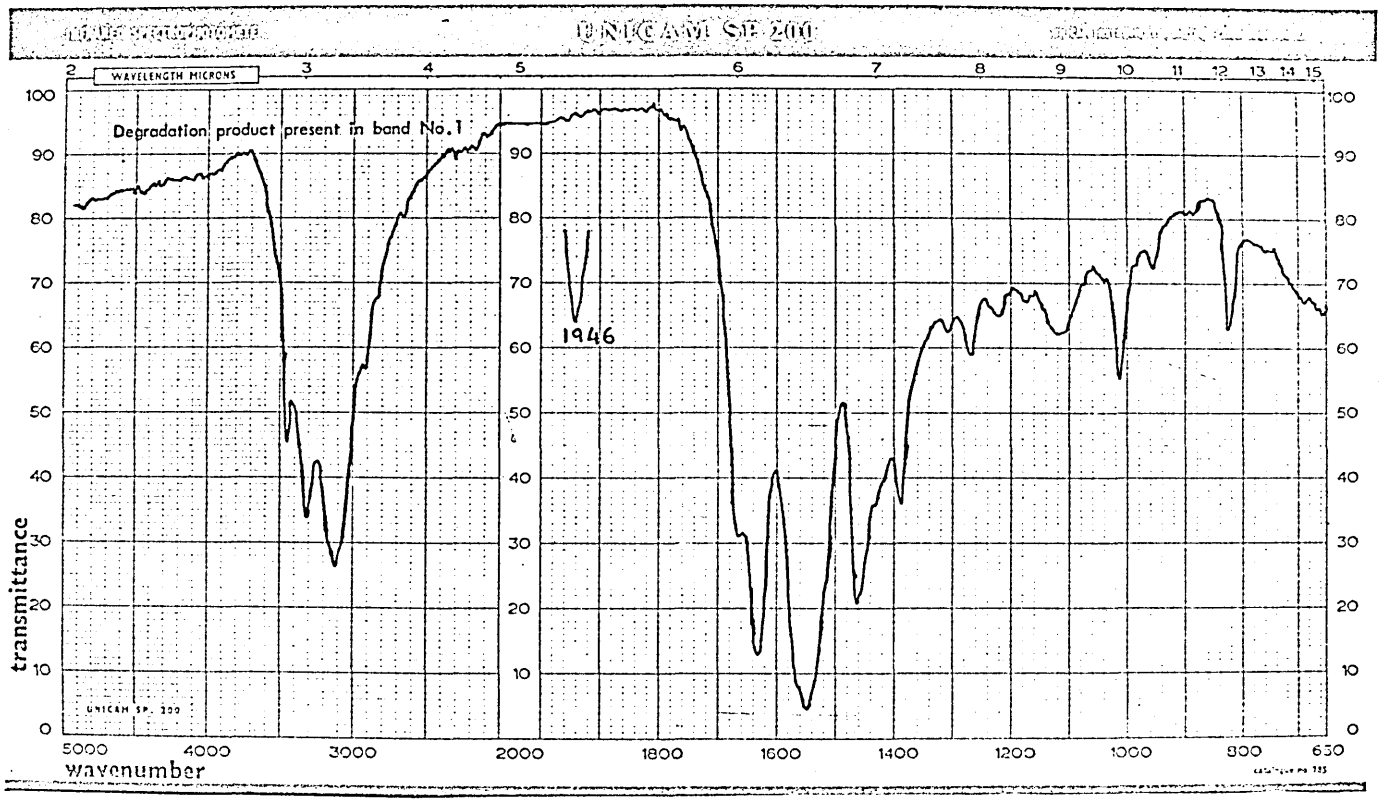


Fig. 43. The infrared spectrum of degradation product present in band No. 1. Spectrum taken in pressed KBr disc, 0.25% concentration.

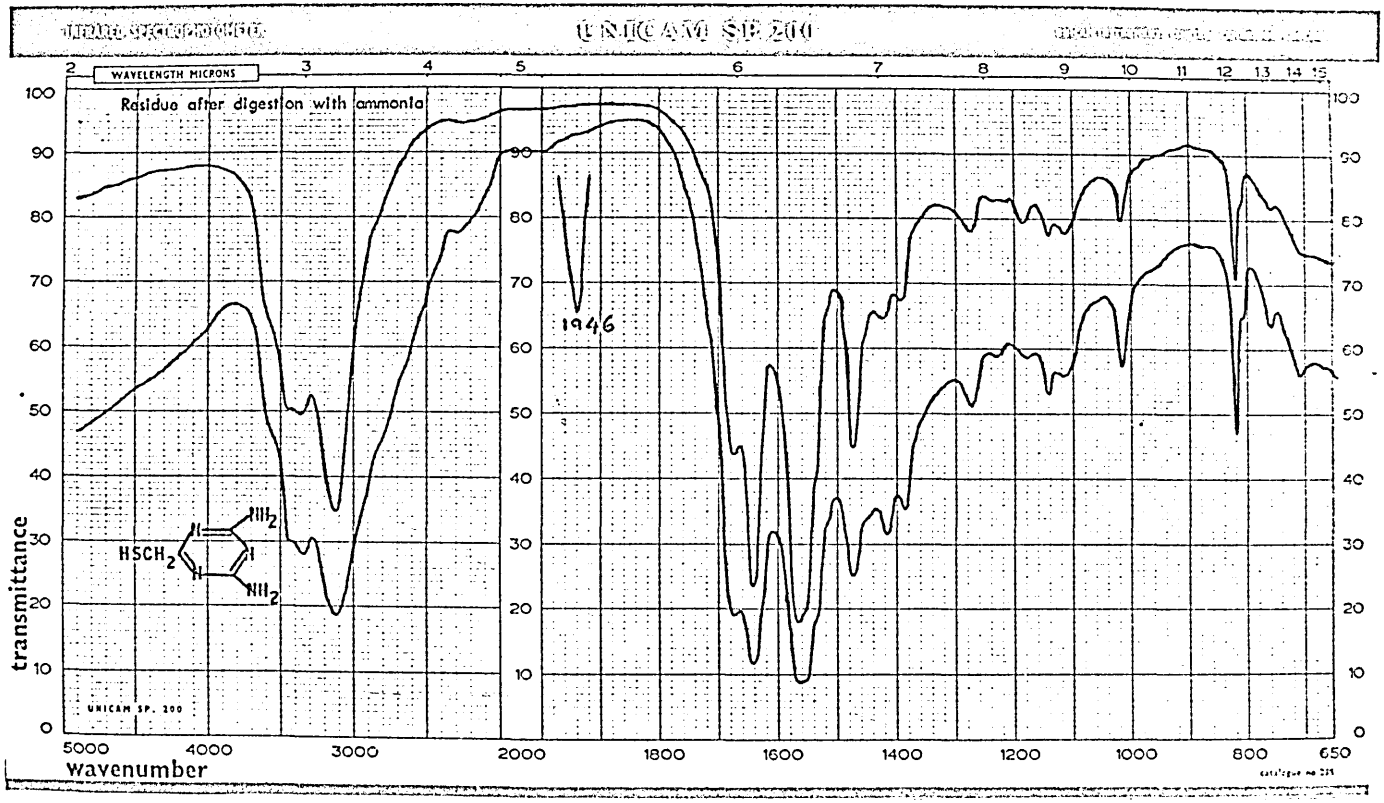
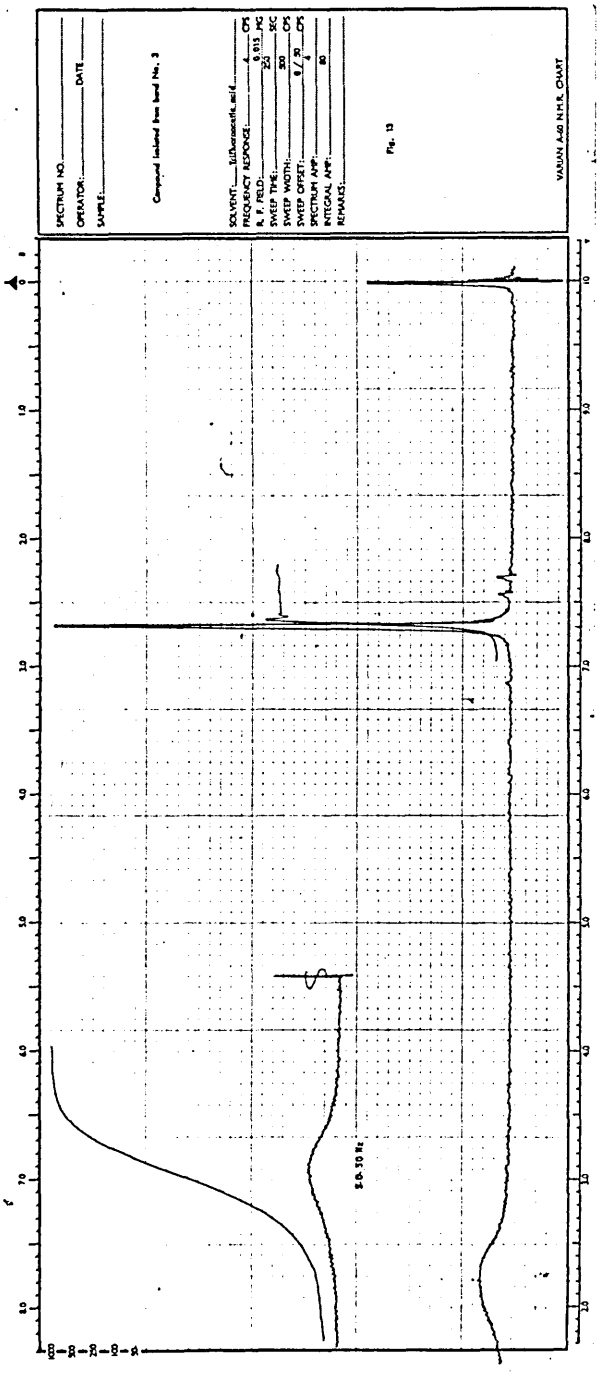
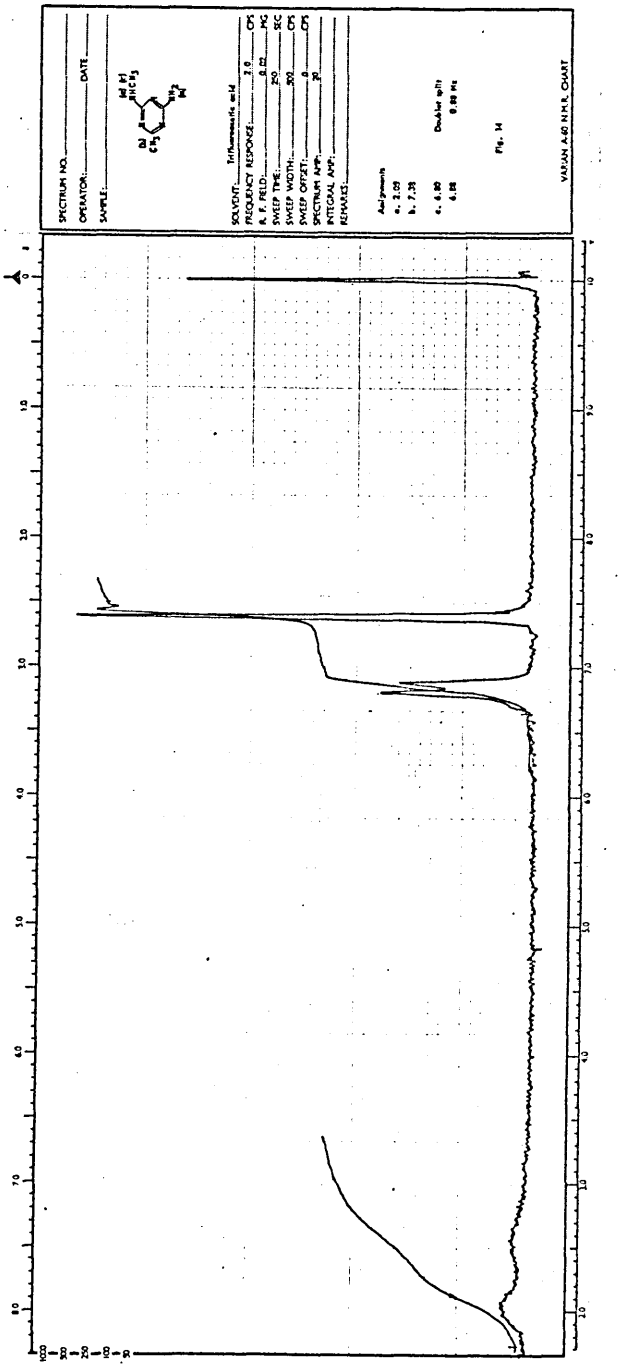
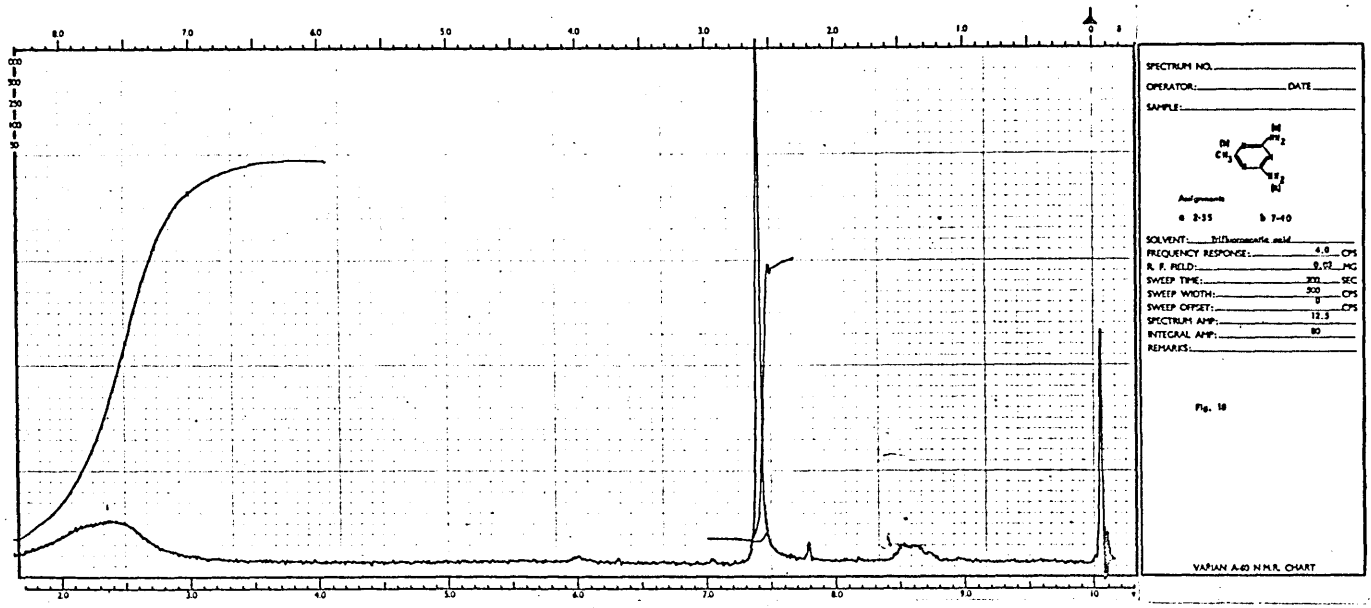


Fig. 46. The infrared spectra of residue after digesting with ammonia and of 6-mercaptomethyl-2,4-diamino-1,3,5-triazine. Spectra taken in pressed KBr discs, 0.25% concentration.

3 'H nmr spectra







SPECTRUM NO. _____

OPERATOR: _____ DATE _____

SAMPLE: _____

Assignments

a 2-35 b 7-40

SOLVENT: Trifluoroacetic acid

FREQUENCY RESPONSE: 4.0 CPS

R. F. FIELD: 0.92 MG

SWEEP TIME: 200 SEC

SWEEP WIDTH: 500 CPS

SWEEP OFFSET: 0 CPS

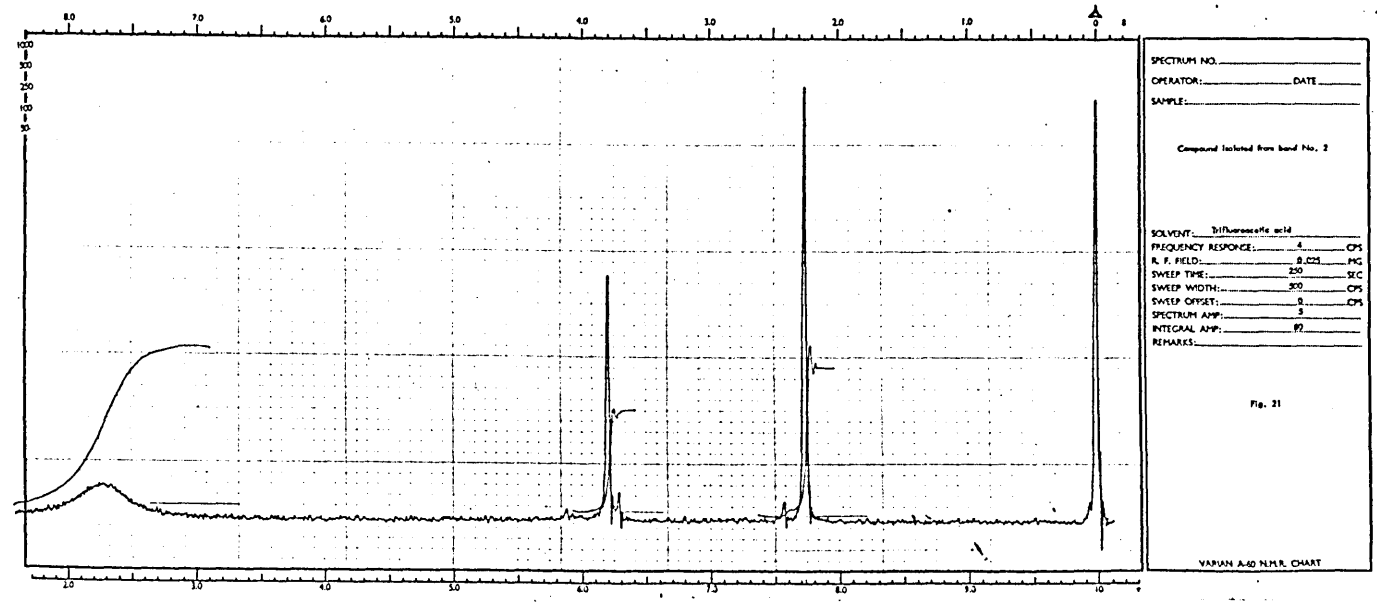
SPECTRUM AMP: 12.5

INTEGRAL AMP: 80

REMARKS: _____

Fig. 18

VARIAN A-60 N.M.R. CHART



SPECTRUM NO. _____

OPERATOR: _____ DATE _____

SAMPLE: _____

Compound isolated from band No. 2

SOLVENT: Trifluoroacetic acid

FREQUENCY RESPONSE: 4 CPS

R. F. FIELD: 0.025 MG

SWEEP TIME: 250 SEC

SWEEP WIDTH: 500 CPS

SWEEP OFFSET: 0 CPS

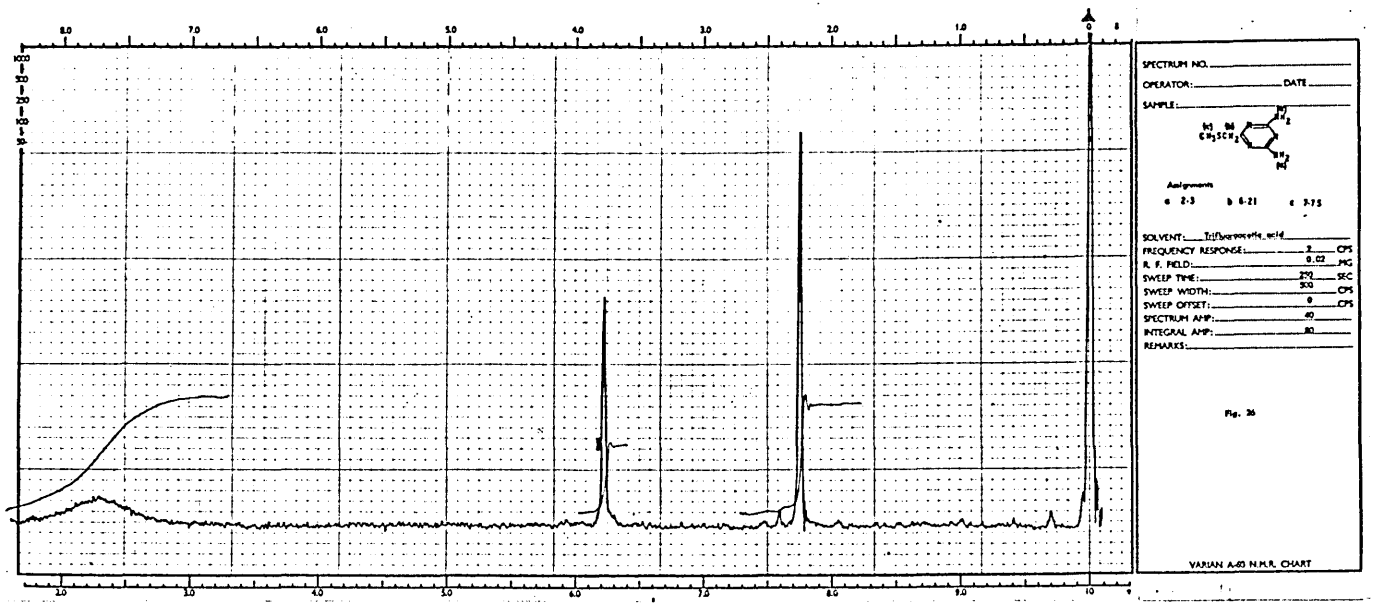
SPECTRUM AMP: 5

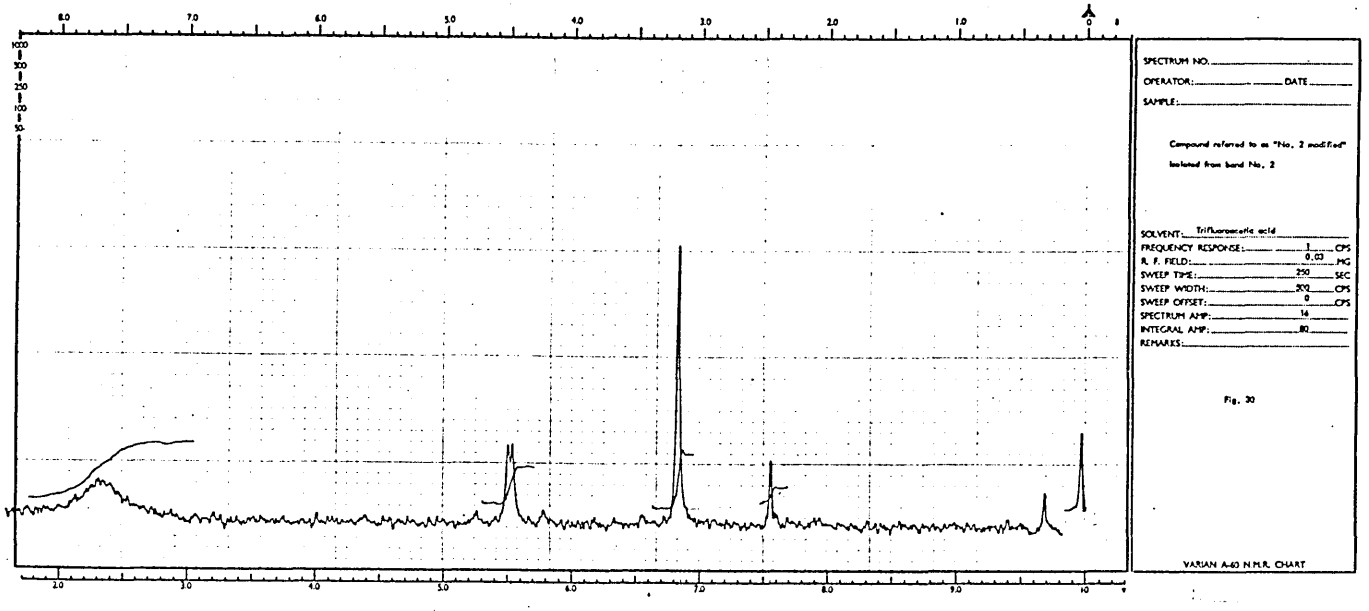
INTEGRAL AMP: 80

REMARKS: _____

Fig. 21

VARIAN A-60 N.M.R. CHART


SPECTRUM NO.
OPERATOR: DATE
SAMPLE:
Assignments
a 2-3 b 6-21 c 7-75
SOLVENT: Trifluoroacetic acid
FREQUENCY RESPONSE: 2 CPS
R. F. FIELD: 0.03 MG
SWEEP TIME: 250 SEC
SWEEP WIDTH: 500 CPS
SWEEP OFFSET: 0 CPS
SPECTRUM AMP: 40
INTEGRAL AMP: 80
REMARKS:
Fig. 26
VARIAN A-60 N.M.R. CHART


SPECTRUM NO.
OPERATOR: DATE
SAMPLE:
Compound referred to as "No. 2 modified"
Isolated from band No. 2
SOLVENT: Trifluoroacetic acid
FREQUENCY RESPONSE: 1 CPS
R. F. FIELD: 0.03 MG
SWEEP TIME: 250 SEC
SWEEP WIDTH: 500 CPS
SWEEP OFFSET: 0 CPS
SPECTRUM AMP: 16
INTEGRAL AMP: 80
REMARKS:
Fig. 30
VARIAN A-60 N.M.R. CHART

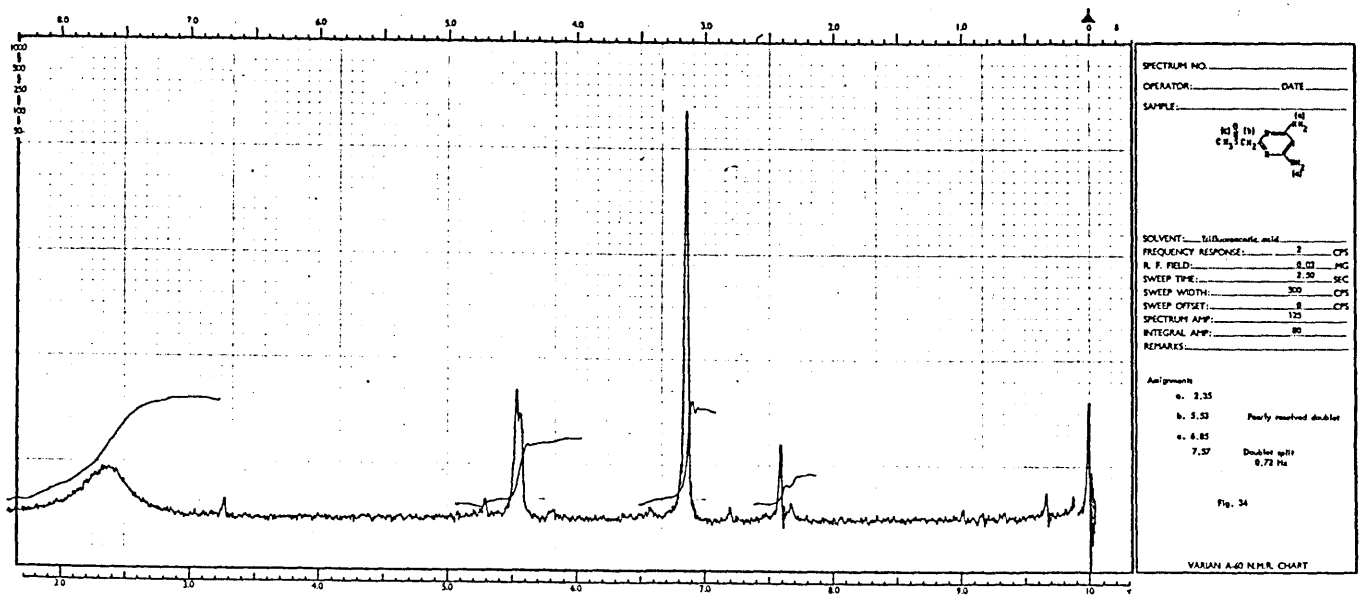

SPECTRUM NO.
OPERATOR: DATE
SAMPLE:
SOLVENT: Trifluoroacetic acid
FREQUENCY RESPONSE: 2 CPS
R. F. FIELD: 0.03 MG
SWEEP TIME: 2.50 SEC
SWEEP WIDTH: 500 CPS
SWEEP OFFSET: 0 CPS
SPECTRUM AMP: 125
INTEGRAL AMP: 80
REMARKS:
Assignments
a. 2.35
b. 5.53 Poorly resolved doublet
c. 6.85
7.57 Doublet split 0.72 Hz
VARIAN A-60 N.M.R. CHART

Fig. 34

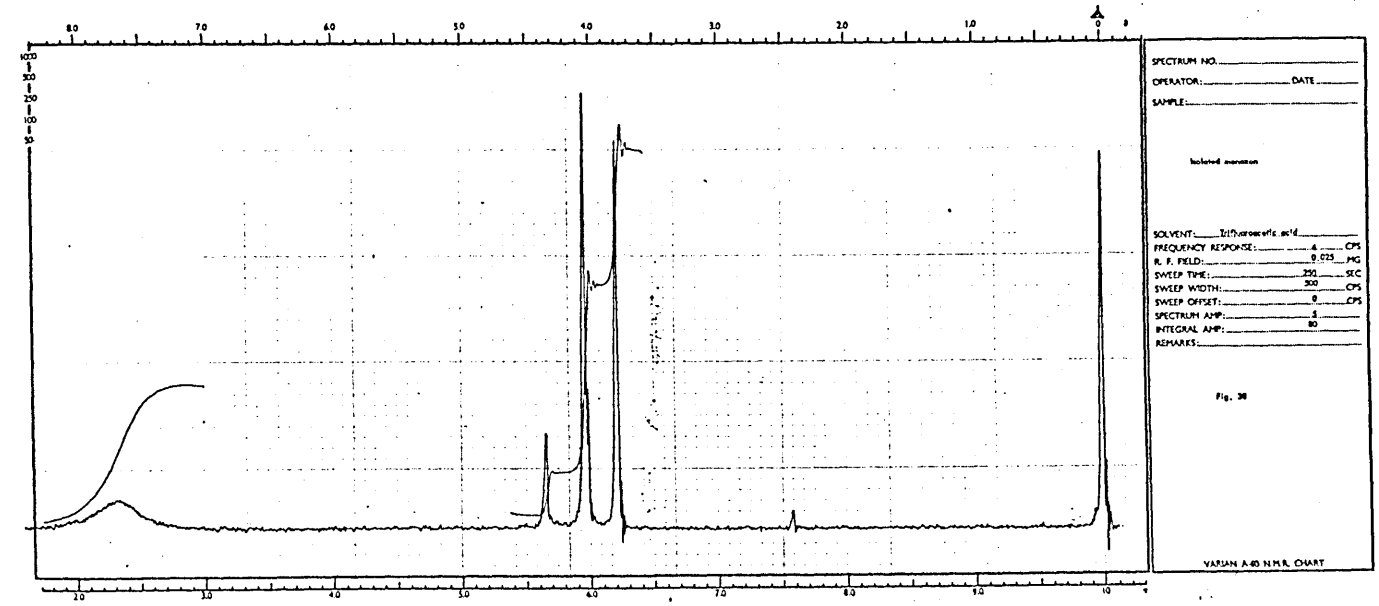
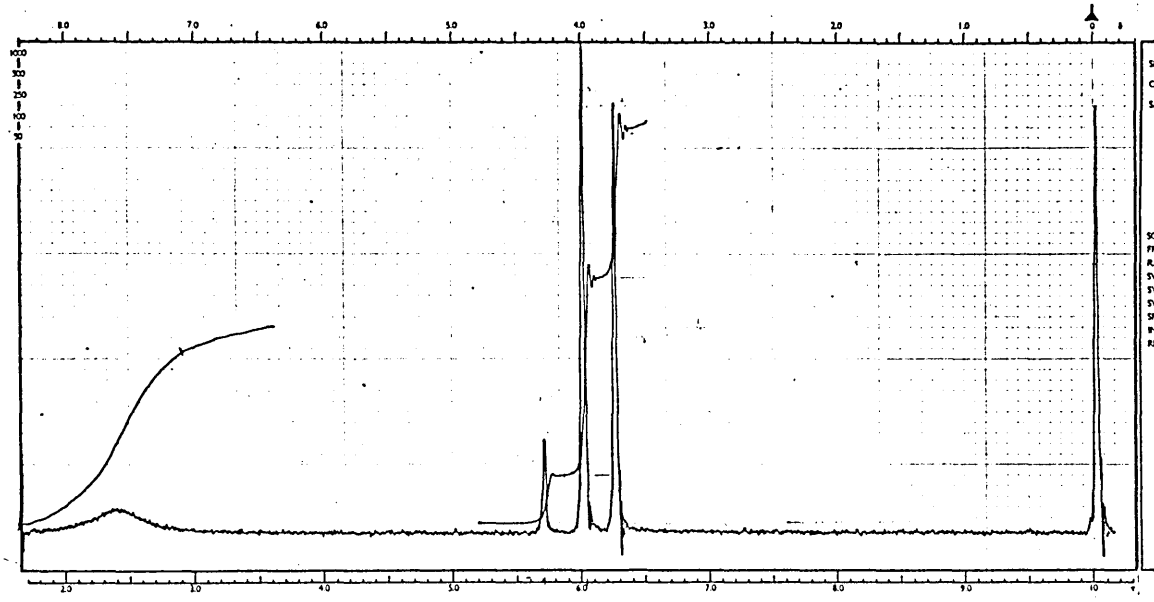
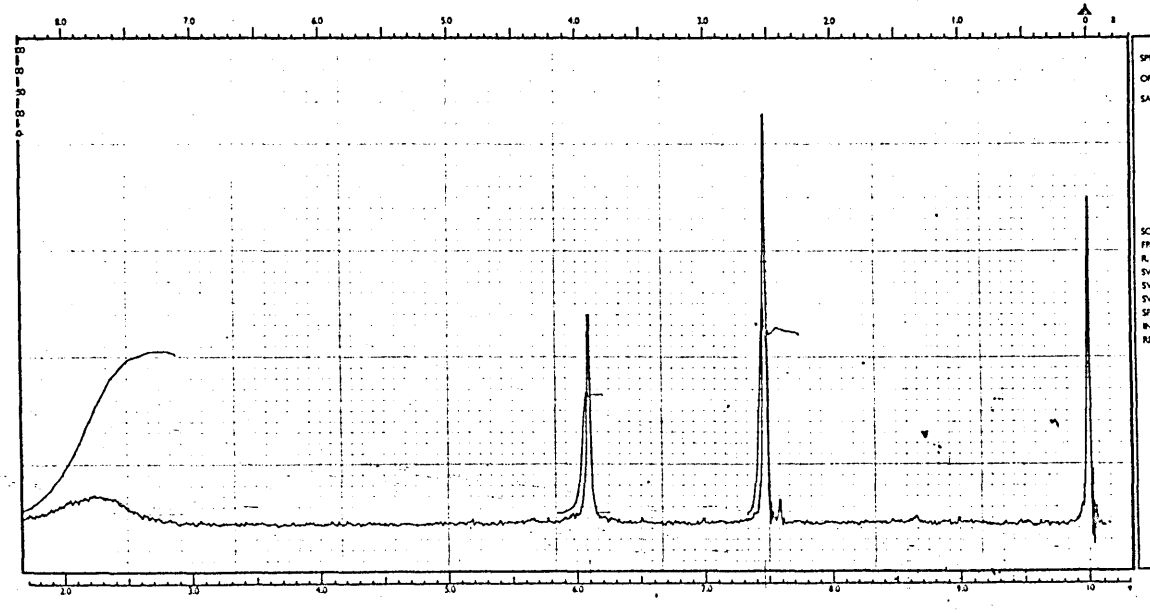

SPECTRUM NO.
OPERATOR: DATE
SAMPLE:
Isolated monomer
SOLVENT: Trifluoroacetic acid
FREQUENCY RESPONSE: 4 CPS
R. F. FIELD: 0.025 MG
SWEEP TIME: 250 SEC
SWEEP WIDTH: 500 CPS
SWEEP OFFSET: 0 CPS
SPECTRUM AMP: 5
INTEGRAL AMP: 80
REMARKS:
VARIAN A-60 N.M.R. CHART

Fig. 38


8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
δ
100
90
75
50
SPECTRUM NO.
OPERATOR:
DATE
SAMPLE:
SOLVENT: Trifluoroacetic acid
FREQUENCY RESPONSE: 4.0 CPS
R. F. FIELD: 0.02 MG
SWEEP TIME: 250 SEC
SWEEP WIDTH: 500 CPS
SWEEP OFFSET: 0 CPS
SPECTRUM AMP: 12.5
INTEGRAL AMP:
REMARKS:
Assignments
a. 2.4
b. 6.72
6.04
Doublet split
0.32 Hz
c. 6.02
6.36
Doublet split
0.34 Hz
Fig. 39
VARIAN A-60 N.M.R. CHART


8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
δ
100
90
80
70
60
SPECTRUM NO.
OPERATOR:
DATE
SAMPLE:
Degradation product present in band No. 1
SOLVENT: Trifluoroacetic acid
FREQUENCY RESPONSE: 2 CPS
R. F. FIELD: 0.025 MG
SWEEP TIME: 250 SEC
SWEEP WIDTH: 500 CPS
SWEEP OFFSET: 0 CPS
SPECTRUM AMP: 6.3
INTEGRAL AMP: 80
REMARKS:
Fig. 44
VARIAN A-60 N.M.R. CHART

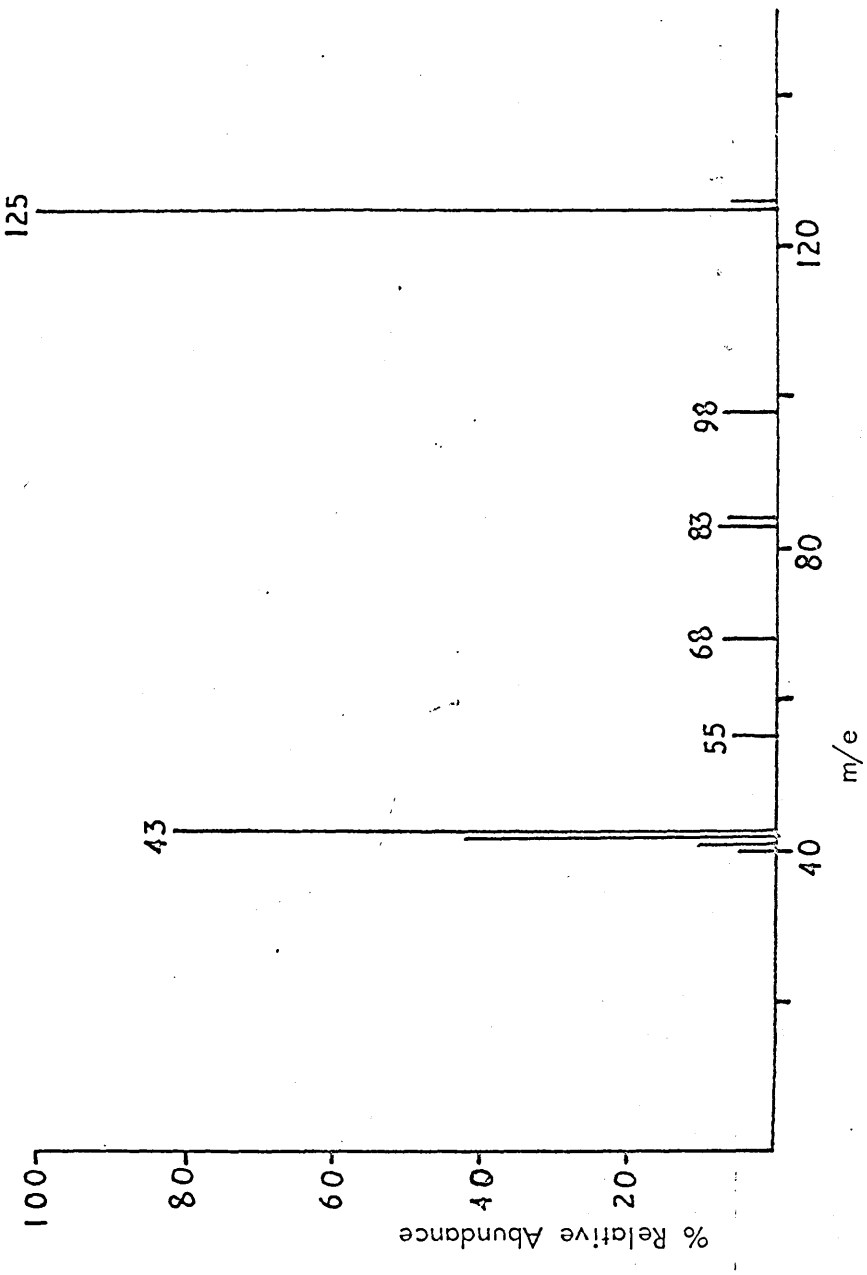
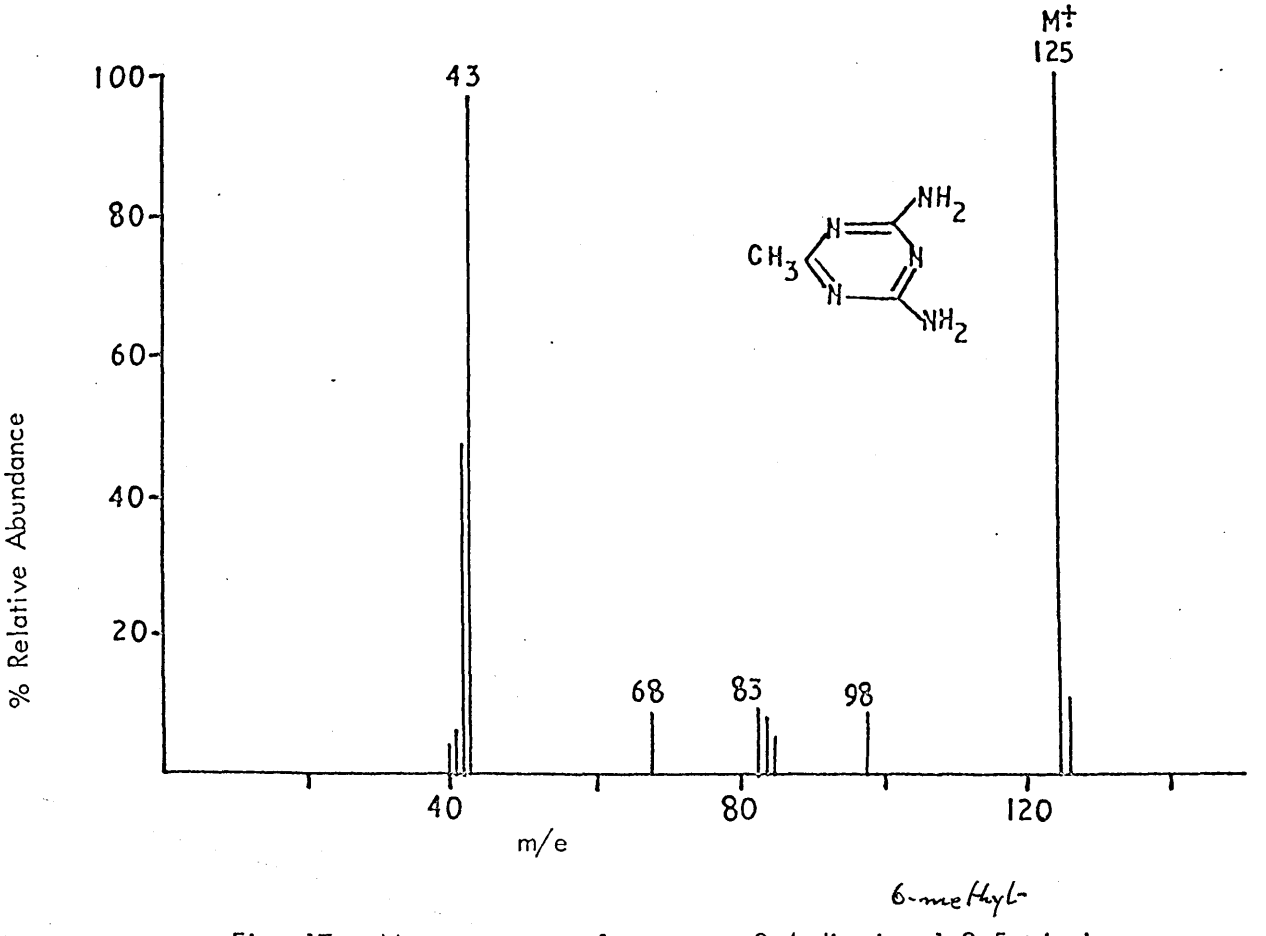


Fig. 12 Mass spectrum of compound isolated from band No. 3

Table 14. Relative Abundance of ions in the mass spectrum of compound isolated from band No.3

m/e	% Abundance	m/e	% Abundance
126	6.1	82	2.5
125	100	68	7.6
110	1.7	55	5.0
98	7.2	43	82
85	3.0	42	42
84	7.2	41	10.5
83	8.0	40	5.0



6-methyl-

Fig. 17 Mass spectrum of [illegible]-2,4-diamino-1,3,5-triazine

Table 16. Relative abundance of ions in the mass spectrum of 6-methyl-2,4-diamino-1,3,5-triazine.

m/e	% Abundance	High Resolution Formula
126	11.2	
125	100	
110	1.7	
98	8.9	
85	4.9	
84	8.5	$C_3H_6N_3$
83	9.7	$C_3H_5N_3$, $C_2H_3N_4$
82	2.2	
68	9.2	$C_2H_2N_3$
43	97	CH_3N_2
42	47	CH_2N_2, CH_4N
41	6.4	CHN_2, C_2H_3N

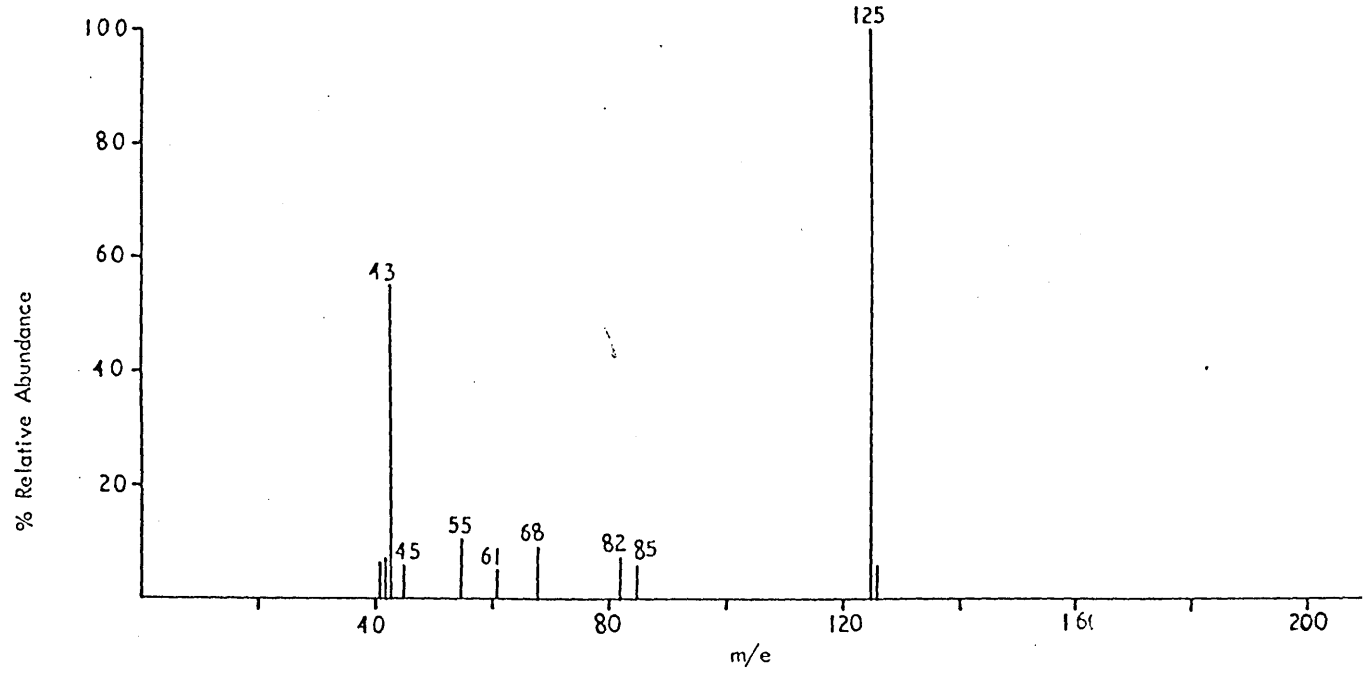


Fig. 22 Mass spectrum of compound isolated from band No. 2

Table 17 Relative Abundance of ions in the mass spectrum of compound isolated from band No. 2

m/e	% Abundance	m/e	% Abundance
171	2.7	61	4.7
126	6.3	55	10.5
125	100	45	5.9
85	5.7	43	55.0
82	6.7	42	7.0
68	9.3	41	6.4

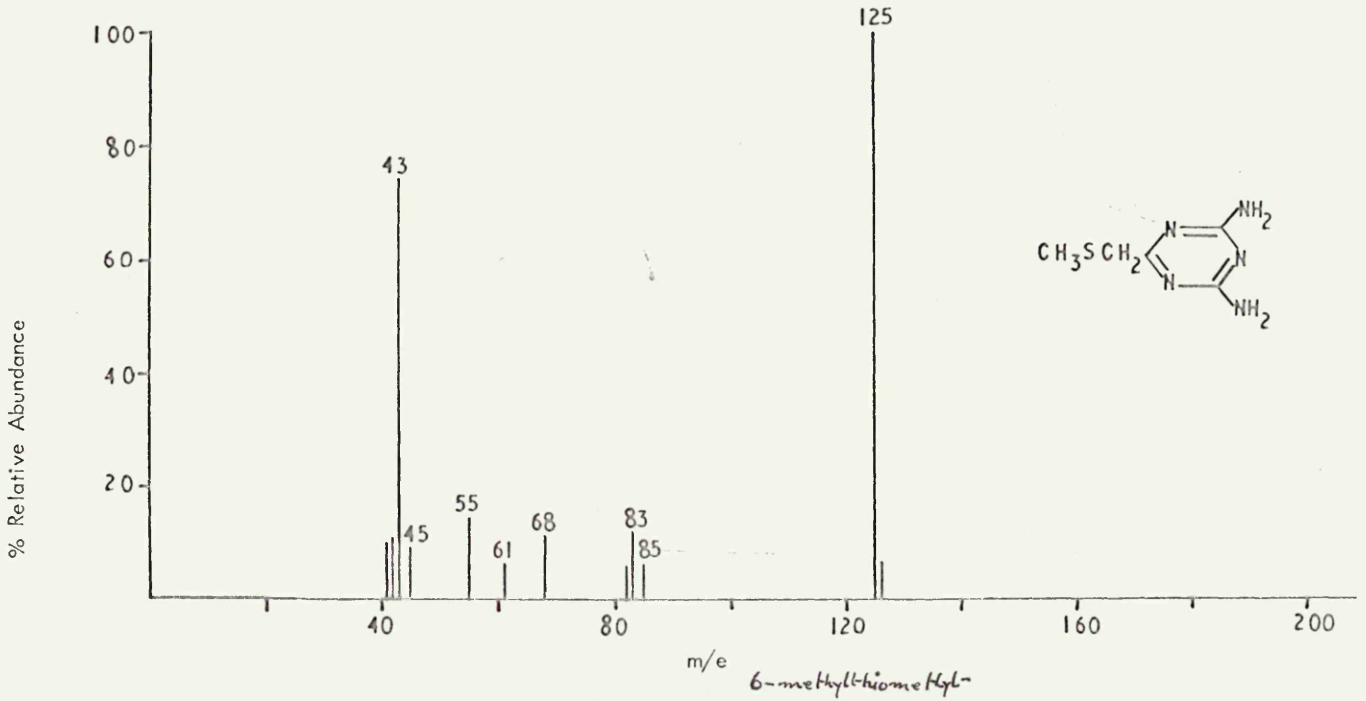


Fig. 25 Mass spectrum of [redacted] 6-methylthiomethyl-2,4-diamino-1,3,5-triazine

Table 19 Relative Abundance of ions in the mass spectrum of 6-methylthiomethyl-2,4-diamino-1,3,5-triazine.

m/e	% Abundance	m/e	% Abundance
171	2.9	61	6.3
126	6.6	55	14.5
125	100	45	9.3
85	6.3	43	74
83	11.8	42	11.0
82	6.3	41	10.0
68	11.4		

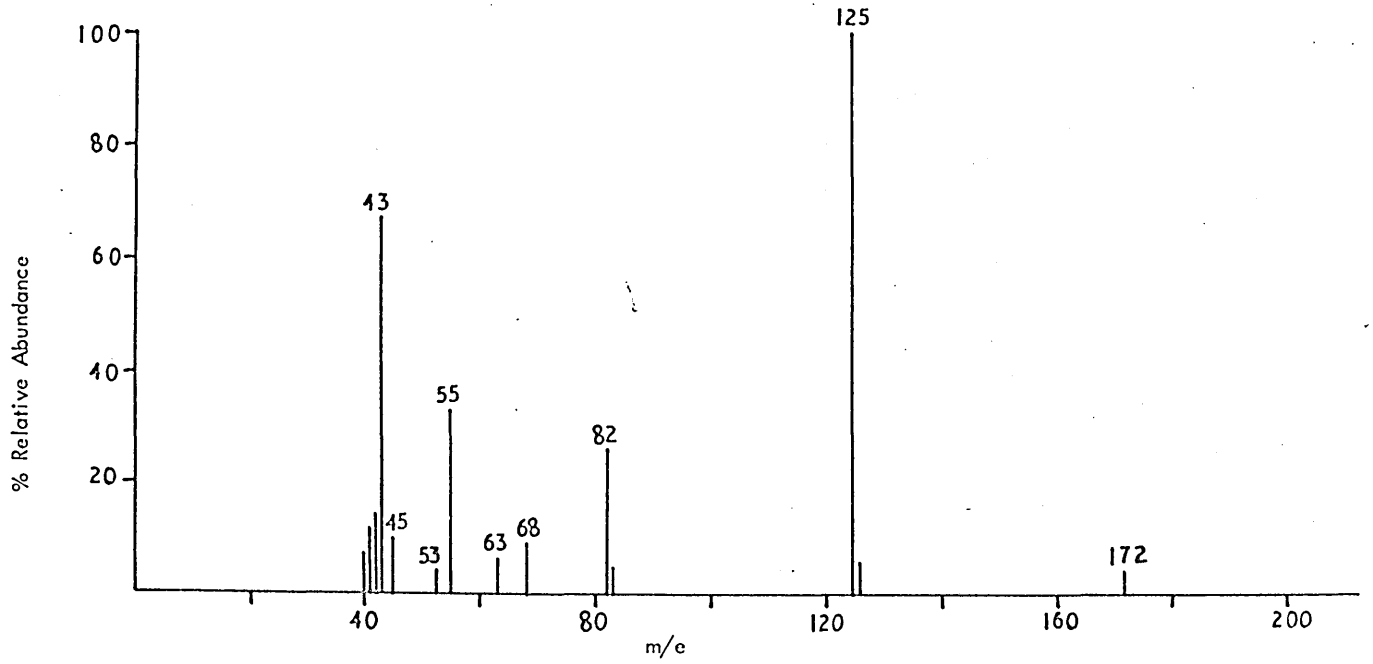


Fig. 29 Mass spectrum of compound referred to as "No. 2 modified"

Table 20 Relative Abundance of ions in the mass spectrum of compound referred to as "No. 2 modified" isolated from band No.2

m/e	% Abundance	m/e	% Abundance
172	3.9	55	32.5
126	6.2	53	3.8
125	100	45	10.0
183	4.8	43	67
82	26	42	13.5
68	9.4	41	11.5
63	6.5	40	7.0

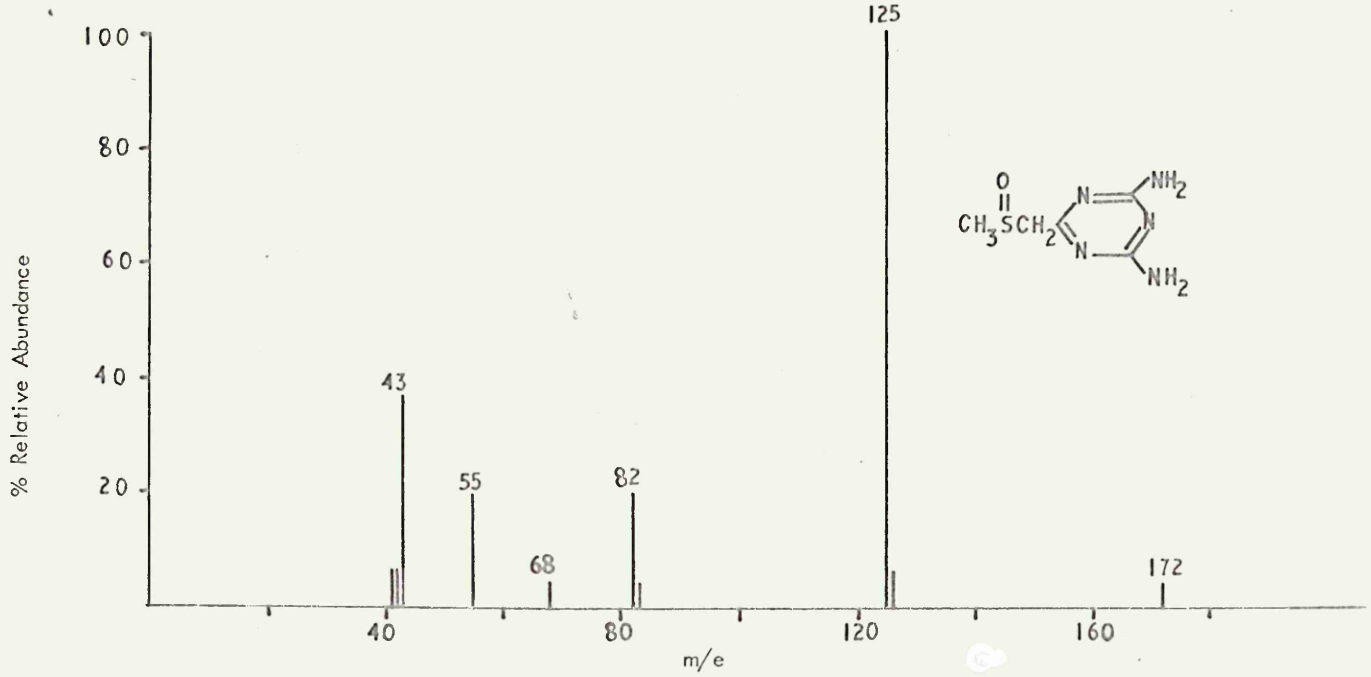


Fig. 33 Mass spectrum of 6-methylsulphinylmethyl-2,4-diamino-1,3,5-triazine

Table 22 Relative Abundance of ions in the mass spectrum of 6-methylsulphinylmethyl-2,4-diamino-Δ1,3,5-triazine

m/e	% Abundance	m/e	% Abundance
172	4.1	55	20
126	6.4	43	37.2
125	100	42	5.6
83	3.8	41	5.6
82	20	40	3.1
68	4.5		

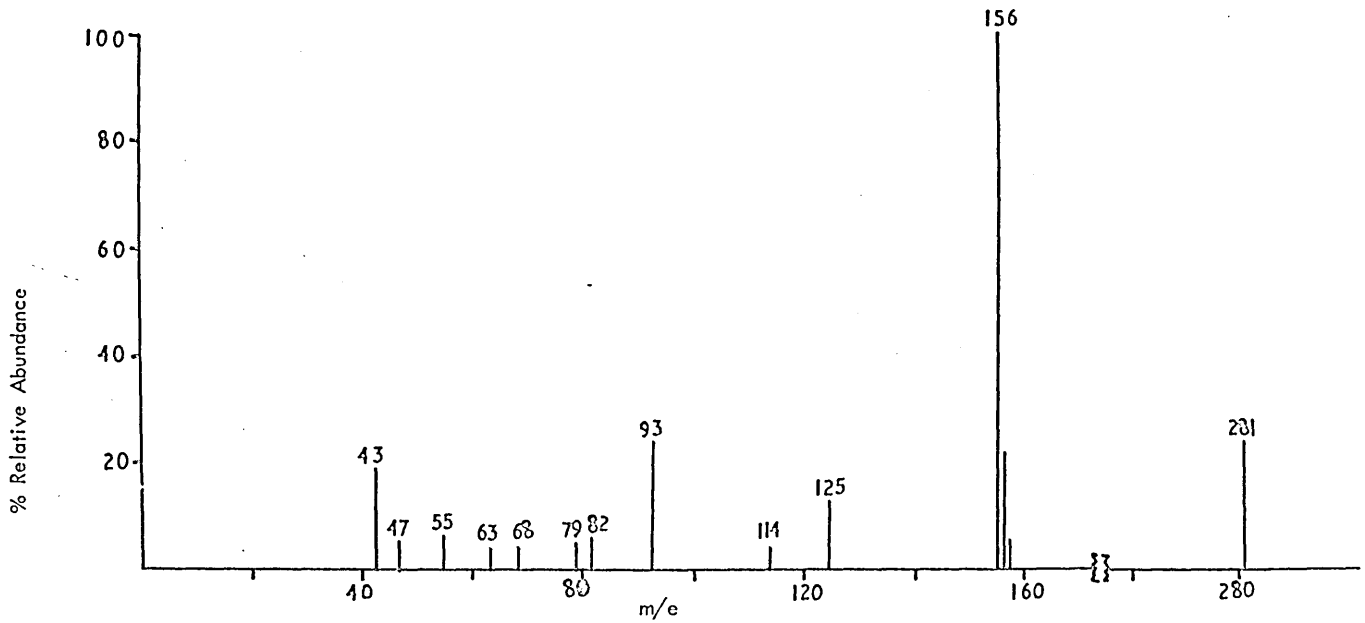


Fig. 40 Mass spectrum of isolated menazon

Table 24 Relative Abundance of ions in the mass spectrum of isolated menazon

m/e	% Abundance	m/e	% Abundance
281	23.6	79	5.3
250	1.4	68	4.1
158	6.3	63	3.9
157	22.0	55	6.5
156	100	47	4.5
125	13.0	43	19.1
114	4.2	42	3.0
93	24.5	41	3.4
82	5.6		

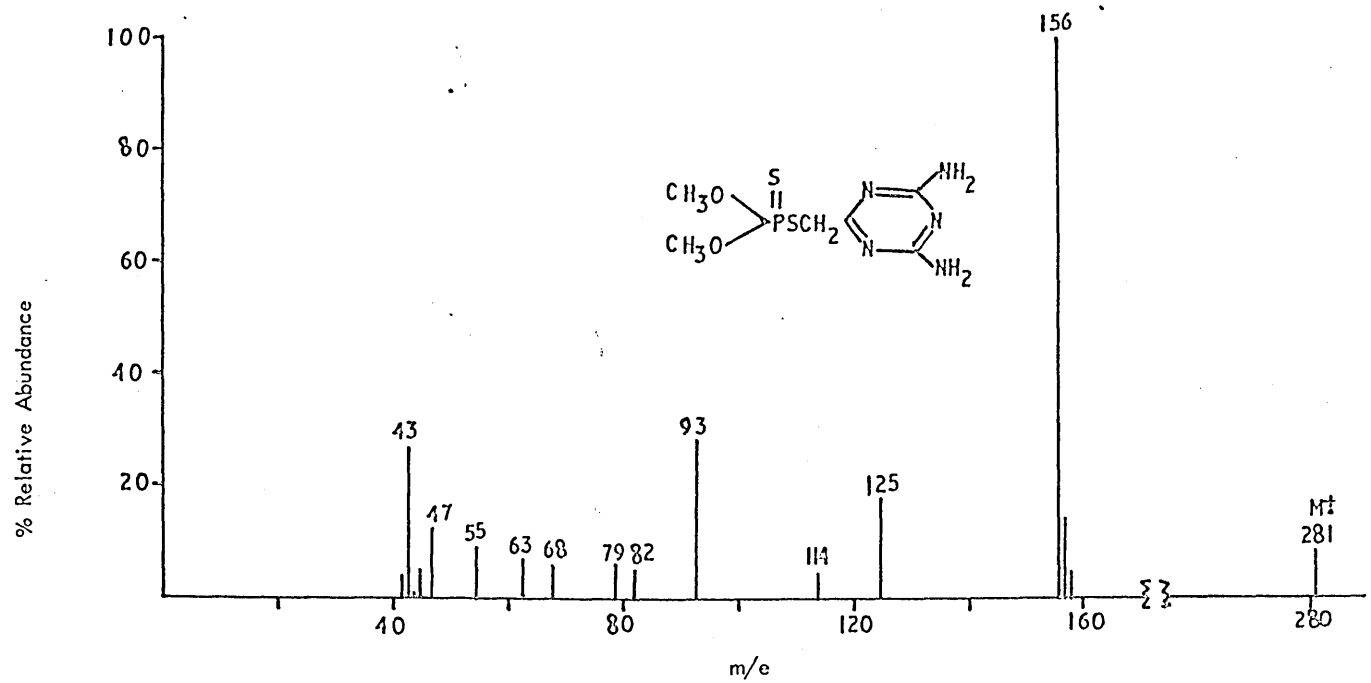


Fig. 41 Mass spectrum of authentic menazon

Table 25 Relative Abundance of ions in the mass spectrum of authentic menazon

m/e	% Abundance	m/e	% Abundance
281	9.3	79	6.6
250	0.85	68	5.6
158	5.2	63	6.7
157	15	55	8.7
156	100	47	12
125	18.5	45	4.8
114	4.5	43	27.0
93	28.5	42	3.7
82	4.9	41	3.4

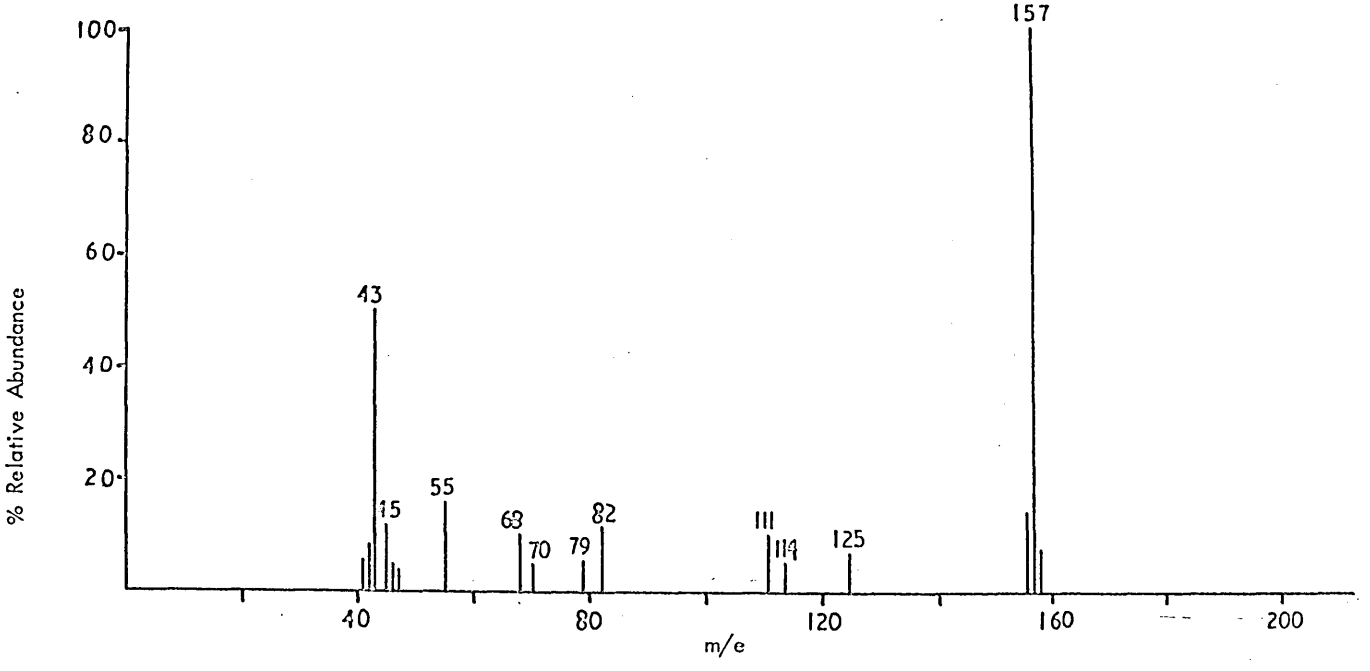


Fig. 45 Mass spectrum of degradation product present in band No. 1

Table 26 Relative Abundance of ions in the mass spectrum of the degradation product present in band No.1

m/e	% Abundance	m/e	% Abundance
203	0.9	70	4.9
188	1.0	68	10.0
158	7.5	55	16.0
157	100	47	4.3
156	14.0	46	5.1
125	7.1	45	12.0
114	6.2	43	49.3
111	10.0	42	8.1
82	12.0	41	5.7
79	5.5		

5 Miscellaneous

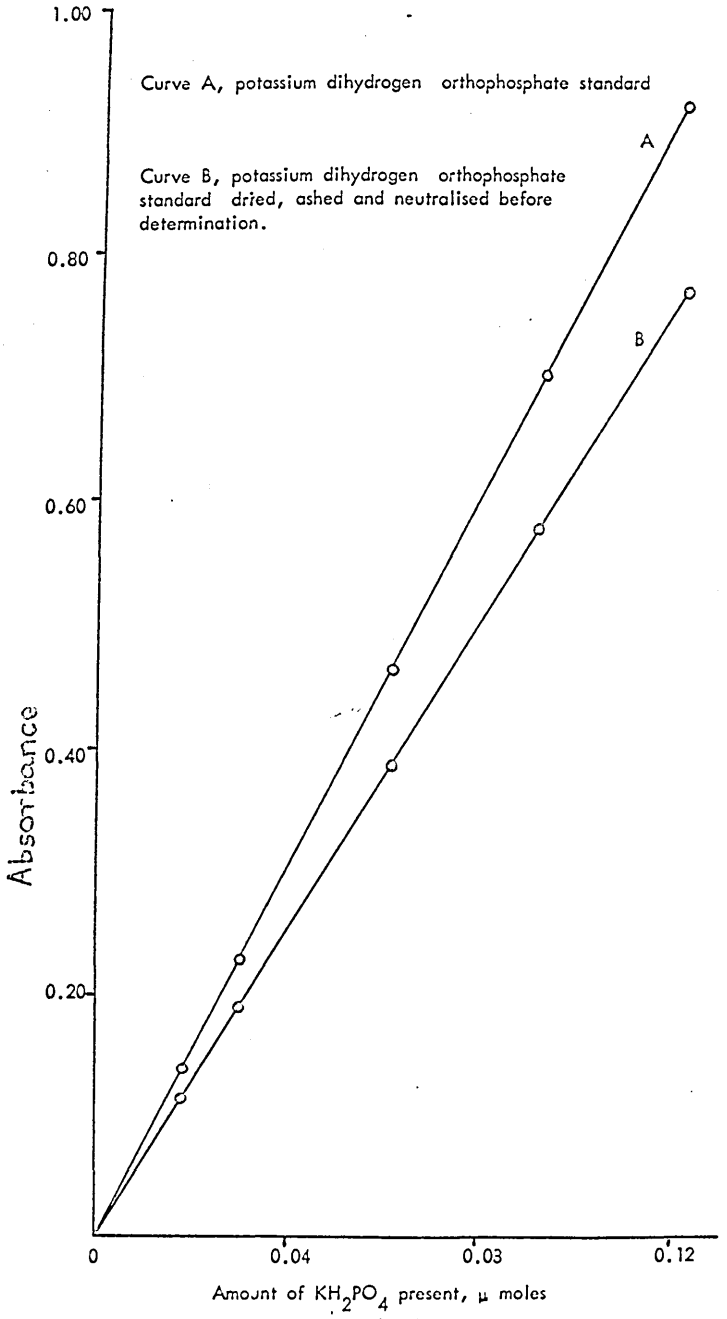
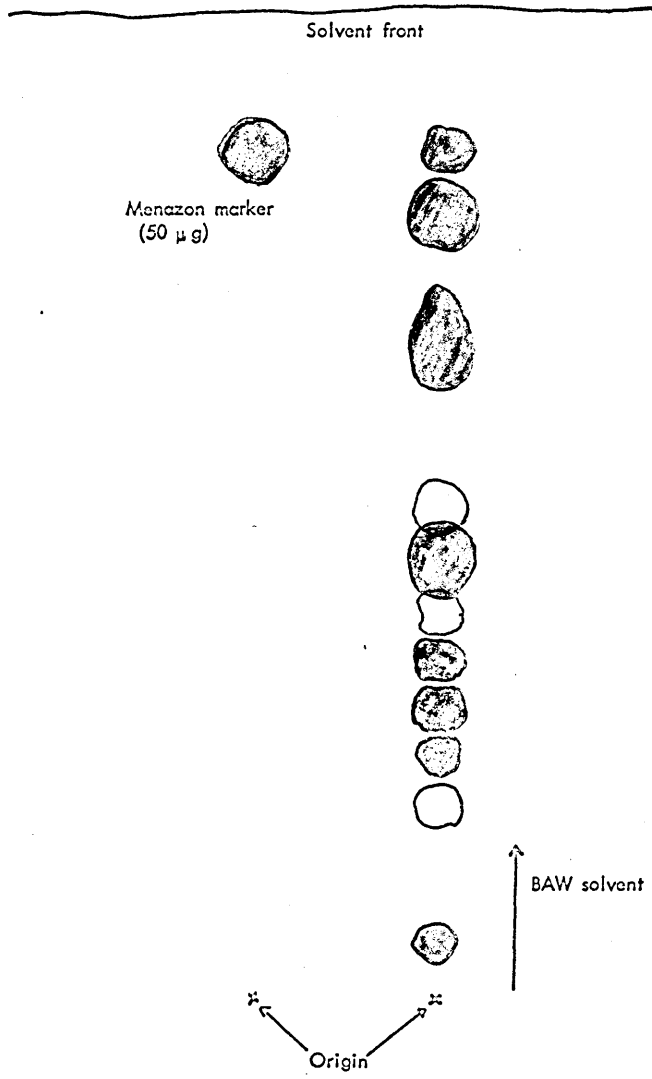


Fig. 6 Determination of inorganic phosphate

Fig. 7. One dimensional paper chromatogram of menazon heated for 10 days at 80°.



N.B.

Area located by quenching under uv.

Area located by spraying with DBQ

Chromatogram developed with BAW solvent (ascending)

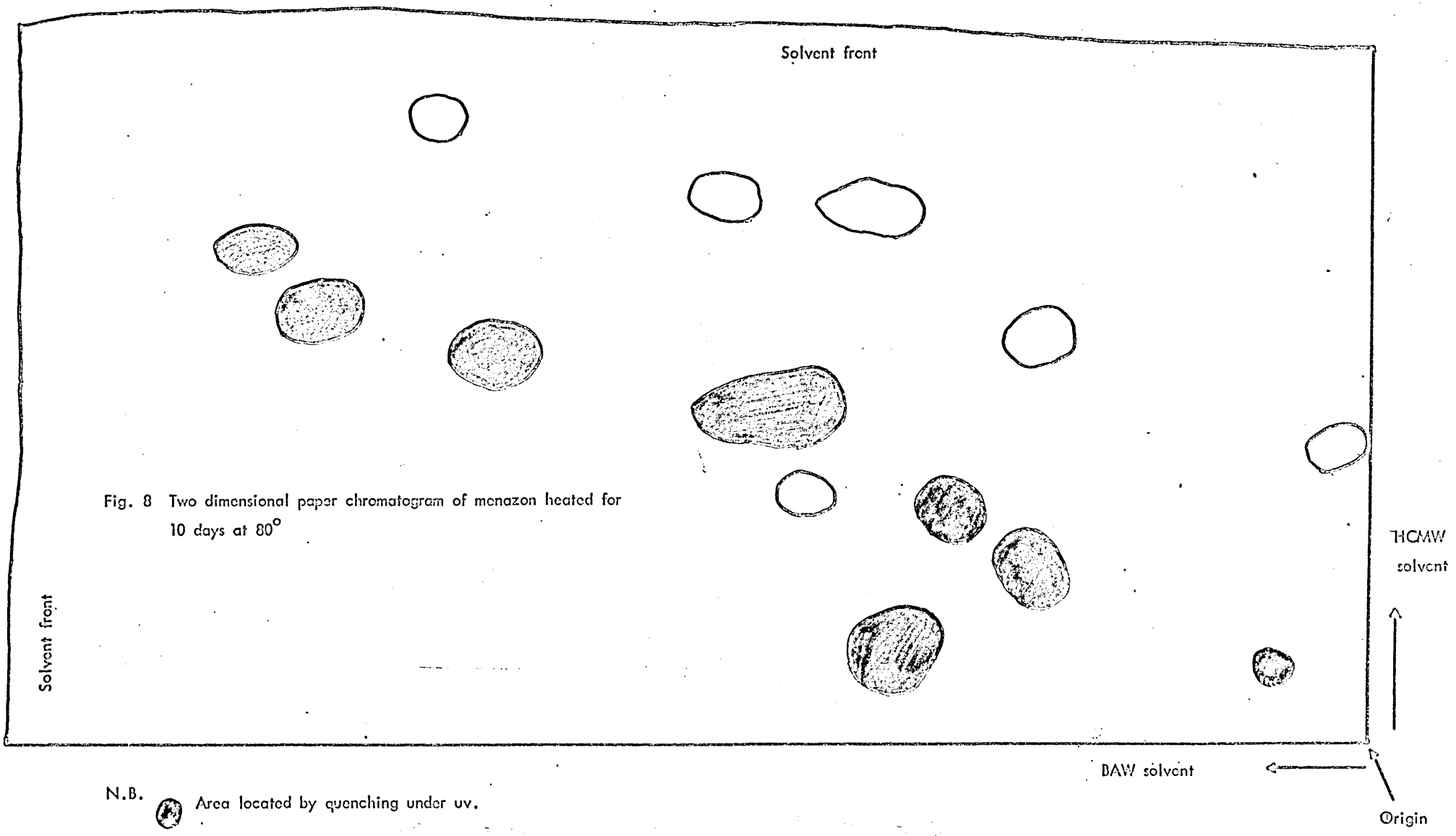


Fig. 8 Two dimensional paper chromatogram of menazon heated for 10 days at 80°

N.B. Area located by quenching under uv.

Area located by spraying with DBQ

Chromatogram developed with BAW solvent (descending) and at right angles in HCMW solvent (ascending).

Postgraduate course of studies.

The following series of lectures given to postgraduate students at the University of Sheffield were attended.

1. Physical chemistry of mass spectrometry, by Dr. K.R. Jennings (6 lectures).
2. Organic applications of mass spectrometry, by Dr. C.P. Falshaw (6 lectures).
3. Principles of nuclear magnetic resonance and chemical applications, by Dr. W.T. Raynes (6 lectures).