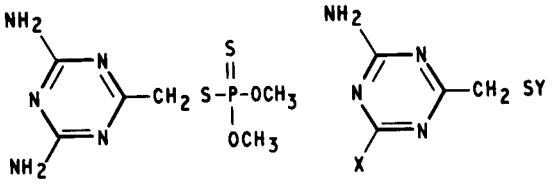
Imperial Chemical Industries Limited,
Industrial Hygiene Research Laboratories

Synthesis of Possible Metabolites of Menazon

M. A. Stevens and G. H. Walker

A product of the peracetic acid oxidation of 2,4-diamino-6-methylthiomethyl-1,3,5-triazine is identical with a major urinary metabolite of the aphicide, menazon, in the rat. This product has been shown to be the *S*-oxide 2,4-diamino-6-methylsulphinylmethyl-1,3,5-triazine and not the *N*-oxide.

Menazon or *O*,*O*'-dimethyl *S*-(4,6-diamino-1,3,5-triazin-2-yl)methyl phosphorodithioate (I), a compound with important aphicidal activity (1), is metabolised in the rat to a number of compounds then excreted in the urine. By dosing rats in separate



I

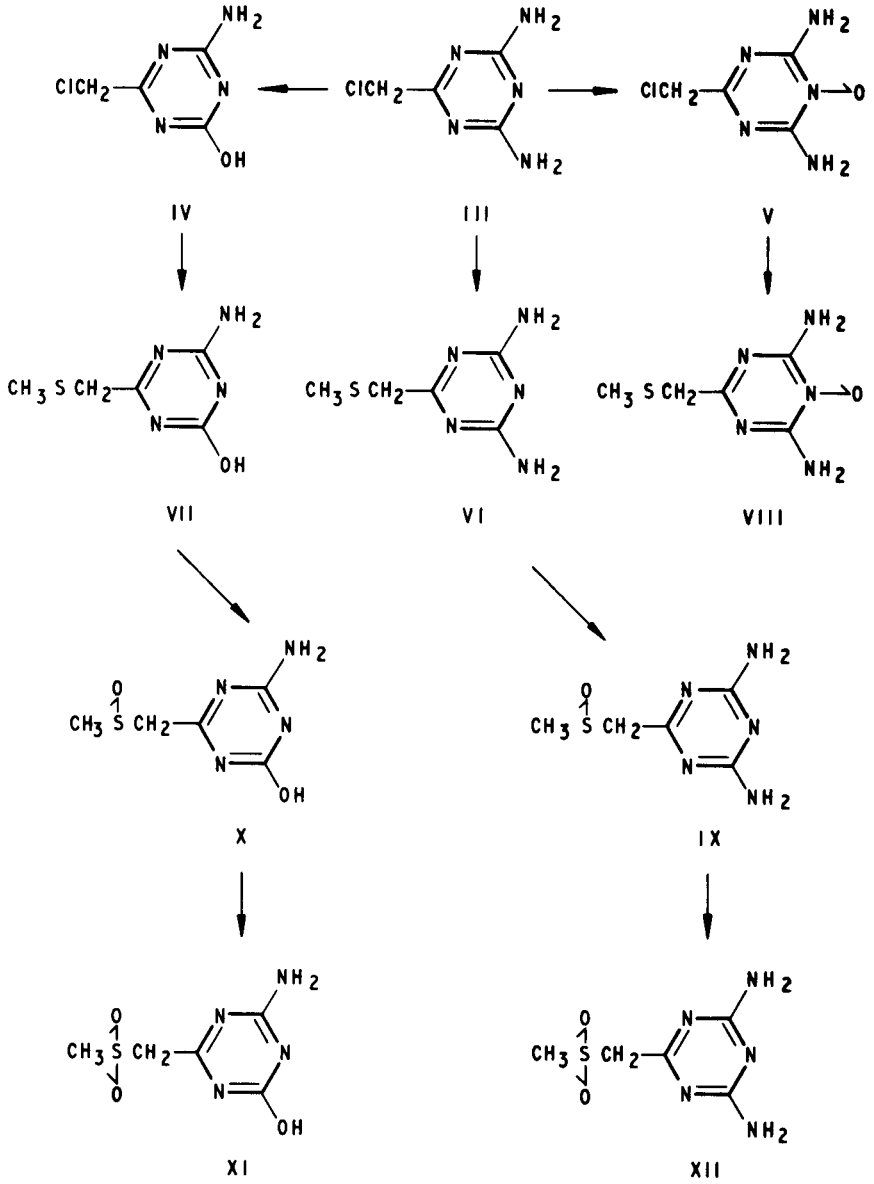
II

experiments with menazon labelled with carbon-14 on the 2-position of the triazine ring, with menazon labelled with phosphorus-32 and with menazon labelled with sulphur-35, it has been possible for Gage (2) to show that one major urinary metabolite possessed the triazine nucleus and the side chain sulphur atom but had lost the phosphate moiety. Since this metabolite did not correspond with any of a series of triazines prepared in a program (3) of synthesis of compounds related to menazon, further compounds containing the structural unit II (where X = OH or NH_2 and Y = CH_3) have now been made for comparison with rat urinary metabolites. The properties of the compounds prepared are listed in Tables I and II.

2,4-Diamino-6-methylthiomethyl-1,3,5-triazine (VI) prepared by the reaction of 2-chloromethyl-4,6-diamino-1,3,5-triazine (4) (III) with the sodium derivative of methanethiol was identical with the material obtained by Calderbank (3) by the hydrolysis of menazon with methanolic potash. 2-Amino-4-hydroxy-6-methylthiomethyl-1,3,5-triazine (VII) was similarly prepared from methanethiol and 2-amino-4-chloromethyl-6-hydroxy-1,3,5-triazine (3) (IV). Since neither of these compounds corresponded to the major rat metabolite of menazon and since it is known (5) that certain thiols are not only *S*-methylated but are also *S*-oxidised before excretion, the oxidised derivatives, the sulphoxides and sulphones, of 2,4-diamino-6-methylthiomethyl-1,3,5-triazine and 2-amino-4-hydroxy-6-methylthiomethyl-1,3,5-triazine were next prepared for comparison with the metabolites of menazon. Stepwise oxidations of these triazines with hydrogen peroxide in acetic acid produced 2,4-diamino-6-methylsulphinylmethyl-1,3,5-triazine (IX) and 2,4-diamino-6-methylsulphonylmethyl-1,3,5-triazine (XII) and 2-amino-4-hydroxy-6-methylsulphinylmethyl-1,3,5-triazine (X) and 2-amino-4-hydroxy-6-methylsulphonylmethyl-1,3,5-triazine (XI) respectively. Evidence for the structures assigned to these compounds consisted of elemental, ultra-violet and infra-red analyses. One of these compounds, 2,4-diamino-6-methylsulphinyl-1,3,5-triazine (IX), proved (2) to be identical with the metabolite.

To confirm that the first product of the oxidation of 2,4-diamino-6-methylthiomethyl-1,3,5-triazine is the sulphoxide rather than the isomeric *N*-oxide, the *N*-oxide of 2,6-diamino-6-methylthiomethyl-1,3,5-triazine was prepared by an unambiguous route for comparison. 2-Chloromethyl-4,6-diamino-1,3,5-triazine was converted by *m*-chloroperbenzoic acid oxidation (6) to 2-chloromethyl-4,6-diamino-1,3,5-triazine *N*-oxide (V). Reaction with the sodium derivative of methanethiol then yielded 2,4-diamino-6-methylthiomethyl-1,3,5-triazine *N*-oxide. By analogy with the oxidation product (7) of 2-methyl-4,6-diaminotriazine, these *N*-oxides are considered to bear the oxygen on the nuclear nitrogen between the two amino groups. 2,4-Diamino-6-methylthiomethyl-1,3,5-triazine 3-*N*-oxide (VIII) was found to be different in R_f on chromatography and in u.v. and i.r. absorption properties from the first product of the oxidation of 2,4-diamino-6-methylthiomethyl-1,3,5-triazine, thus giving further support for the sulphoxide structure for the latter compound. The *N*-oxides V and VIII have u.v. spectra differing from the triazines not bearing an *N*-oxide group by showing an additional and high absorption peak at 225 mμ at *p*H 7.0 similar to that observed (8) with purine *N*-oxides.

Support for the sulphoxide structures for the first oxidation products of 2,4-diamino-6-methylthiomethyl-1,3,5-triazine and 2-amino-4-hydroxy-6-



methylthiomethyl-1,3,5-triazine is provided by the i.r. spectra which show strong bands in the area 1010-1025 cm^{-1} corresponding to S ⟶ O (9). The products of the further oxidation of these sulphoxides are considered to be the sulphones, 2,4-diamino-6-methylsulphonylmethyl-1,3,5-triazine (XII) and 2-amino-4-hydroxy-6-methylsulphonylmethyl-1,3,5-triazine (XI) rather than the isomeric *N*-oxide sulphoxides because of the presence in the i.r. spectrum of a strong band at 1290-1300 cm^{-1} corresponding to SO_2 (9), and the absence of the characteristic high absorption in the ultra-violet at 225 mμ displayed by closely related triazine *N*-oxides.

The technical assistance of Mr. P. J. Phillips and Mr. P. A. Lefèvre in this work is gratefully acknowledged.

EXPERIMENTAL

Preparation of 2,4-Diamino-6-methylthiomethyl-1,3,5-triazine (VI).

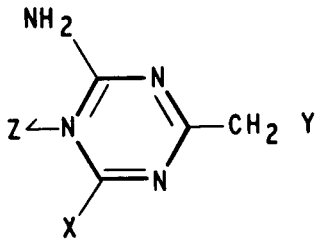
Methanethiol (14.5 ml.) was added with stirring to a solution of sodium (4.6 g.) in methanol (200 ml.) cooled to -70°, the mixture was allowed to warm to 0° to complete the reaction, then recooled to -70° and 2,4-diamino-6-chloromethyl-1,3,5-triazine (32 g.) was stirred in. The mixture was allowed to warm to room temperature then was refluxed for 15 minutes. The solution was cooled, water (100 ml.) was added, the solution was acidified to *p*H 4 with hydrochloric acid and then stirred overnight. The suspension was filtered and the residue (17.24 g., 50%) was washed with water, then recrystallized from hot water to yield 2,4-diamino-6-methylthiomethyl-1,3,5-triazine (13.12 g., 38%) as white needles.

Preparation of 2,4-Diamino-6-methylsulphinylmethyl-1,3,5-triazine (IX).

2,4-Diamino-6-methylthiomethyl-1,3,5-triazine (1.7 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

TABLE I

Elemental Analyses

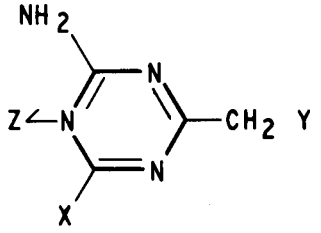


Subst. X	Subst. Y	Subst. Z	M.p. °C	Formula	Carbon % Calcd.	Carbon % Found	Hydrogen % Calcd.	Hydrogen % Found	Nitrogen % Calcd.	Nitrogen % Found
NH_2	SCH_3	-	206-207	$C_5H_9N_5S$	35.1	35.0	5.3	5.2	41.0	41.0
NH_2	$SOCH_3$	-	222-225 (a)	$C_5H_9ON_5S$	32.1	32.0	4.8	4.8	37.4	37.1
NH_2	SO_2CH_3	-	308-310 (a)	$C_5H_9O_2N_5S$	29.6	29.8	4.4	4.7	34.4	34.4
OH	SCH_3	-	270-305 (b)	$C_5H_8ON_4S$	34.8	34.8	4.7	5.0	32.6	32.7
OH	$SOCH_3$	-	over 270 (b)	$C_5H_8O_2N_4S$	32.0	32.0	4.3	4.4	30.0	30.1
OH	SO_2CH_3	-	over 300 (b)	$C_5H_8O_3N_4S$	29.4	29.7	3.9	4.1	27.5	27.9
NH_2	Cl	O	170	$C_4H_6ON_5Cl$	27.4	27.2	3.4	3.5	39.9	39.4 (c)
NH_2	SCH_3	O	210 (a)	$C_5H_9ON_5S$	32.1	31.9	4.8	4.6	37.4	37.4

(a) With decomposition. (b) Decomposes over range. (c) Chlorine: Calcd. 20.2%. Found: 20.2%.

TABLE II

Chromatographic and Spectral Properties



Subst. X	Subst. Y	Subst. Z	Rf in Solvents (a) A	B	C	Absorption maxima u.v. (mμ) (d)	i.r. (cm^{-1}) (e)
NH_2	SCH_3	-	0.67	0.50	0.54	258	832, 1005, 1150
NH_2	$SOCH_3$	-	0.32	0.49	0.36	262	824, 1012, 1140
							831, 895, 975, 1016
NH_2	SO_2CH_3	-	0.41	0.48	0.18	263	1116, 1158, 1290
OH	SCH_3	-	0.60	0.76	0.52	259	810, 952, 1012, 1060, 1158
OH	$SOCH_3$	-	0.22	0.78	0.30	256	810, 895, 970, 1024, 1142
OH	SO_2CH_3	-	0.36	0.76	0.27	262	818, 964, 1140, 1300
NH_2	Cl	O	0.66 (b)	0.71	0.39	225 (c) and 264	743, 915, 995, 1085, 1190, 1250
NH_2	SCH_3	O	0.71 (b)	0.76	0.44	225 and 261	785, 995, 1085, 1190

(a) A = nBuOH : AcOH : H_2O 12 : 3 : 5, B = 10 m*M* *p*H 7.0 phosphate buffer and C = Hexane : $CHCl_3$: MeOH : H_2O 5 : 5 : 10 : 2 (organic phase). All descending on Whatman No. 1 paper. (b) Chromatogram spots give red colour with $FeCl_3$ spray. (c) Shoulder. (d) In water. (e) In Nujol mull.

diluted with water (200 ml.) and evaporated to dryness under reduced pressure. The resulting yellow solid was recrystallised from hot water to yield white crystals of 2,4-diamino-6-methylsulphinylmethyl-1,3,5-triazine, yield 0.4 g., 21%.

Preparation of 2,4-Diamino-6-methylsulphonylmethyl-1,3,5-triazine (XII).

2,4-Diamino-6-methylthiomethyl-1,3,5-triazine (1.7 g.) was added with stirring to a solution of 100 volume hydrogen peroxide (2.5 ml.) and glacial acetic acid (25 ml.), allowed to stand at room temperature for 48 hours, then diluted with water (50 ml.) and the white solid precipitated was filtered off, washed with water and dried. The 2,4-diamino-6-methylsulphonylmethyl-1,3,5-triazine (0.4 g., 20%) so obtained decomposed on heating to 308-310°.

Preparation of 2-Amino-4-hydroxy-6-methylthiomethyl-1,3,5-triazine (VII).

Methanethiol (12.5 ml.) was added with stirring to a solution of sodium (4.6 g.) in methanol (200 ml.) cooled to -70°. The mixture was allowed to warm to 0° to complete the reaction, then recooled to -70° and 2-amino-4-chloromethyl-6-hydroxy-1,3,5-triazine (32 g.) was added with stirring. The mixture was allowed to warm to room temperature then was refluxed for 15 minutes. The solution was cooled, water (100 ml.) was added, the solution acidified to *p*H 4 with hydrochloric acid and then stirred overnight. The suspension was filtered and the residue was washed with water then recrystallized from hot water to yield 2-amino-4-methylthiomethyl-6-hydroxy-1,3,5-triazine as an off-white powder, yield 19 g., 55%.

Preparation of 2-Amino-4-hydroxy-6-methylsulphinylmethyl-1,3,5-triazine (X).

2-Amino-4-hydroxy-6-methylthiomethyl-1,3,5-triazine (1.7 g.) was dissolved in glacial acetic acid (125 ml.) at 70°, 100 volume hydrogen peroxide (2.5 ml.) was added and ten minutes later the solution was diluted with water (200 ml.) and evaporated to dryness under reduced pressure. The resulting yellow solid was extracted with hot water; the hot water extract was run through a bed of decolourising charcoal + Supercel then cooled and the pale yellow crystals (0.5 g., 32%) separating were filtered from the solution. The 2-amino-4-hydroxy-6-methylsulphinylmethyl-1,3,5-triazine so obtained decomposes slowly on heating over 270°.

Preparation of 2-Amino-4-hydroxy-6-methylsulphonylmethyl-1,3,5-triazine (XI).

2-Amino-4-hydroxy-6-methylthiomethyl-1,3,5-triazine (2 g.) was dissolved in a mixture of 100 volume hydrogen peroxide (6 ml.) and glacial acetic acid (80 ml.) then the solution was stirred at 70° for two hours. At the end of this period paper chromatography of an aliquot of the solution indicated that the sulphoxide first formed had been largely converted into the required sulphone. The reaction mixture was diluted with water (200 ml.), the solid was filtered from the solution; the filtrate was evaporated to a white solid, and the two batches of product were combined. The 2-amino-4-hydroxy-6-methylsulphonylmethyl-1,3,5-triazine (0.74 g., 31%) decomposed on heating over 300°.

Preparation of 2,4-Diamino-6-chloromethyl-1,3,5-triazine 3-*N*-oxide (V).

2,4-Diamino-6-chloromethyl-1,3,5-triazine (7.5 g.) was added to glacial acetic acid (95 ml.) the solution stirred at 50° and a suspension of *m*-chloroperbenzoic acid (17 g.) in glacial acetic acid (70 ml.) was added over half-an-hour. The solution was stirred a further three-quarters of an hour at 50-60° and was then poured into an excess (825 ml.) of cold distilled water. The suspension was extracted with chloroform (3 x 150 ml.) to remove *m*-chloroperbenzoic acid and the aqueous layer evaporated under vacuum at 40-45° until separation of a white solid occurred. The contents of the flask were warmed to 50-55° to redissolve the precipitate, then cooled to cause crystallization of 2,4-diamino-6-chloromethyl-1,3,5-triazine 3-*N*-oxide, yield 2.25 g., 27%.

Preparation of 2,4-Diamino-6-methylthiomethyl-1,3,5-triazine 3-*N*-oxide (VIII).

2,4-Diamino-6-chloromethyl-1,3,5-triazine (4 g.) was dissolved in 50% aqueous methanol (100 ml.), the temperature was reduced to -70° and the mixture was stirred whilst a solution of sodium hydroxide (0.91 g.) in water (2 ml.) was added. The mixture was kept a further 15 minutes at -70°, then a solution of methanethiol (1.45 ml.) in methanol (2 ml.) was added, and the mixture was allowed to warm up to 20°. The solution was evaporated to dryness *in vacuo* and the residue was redissolved in water (100 ml.) at 40-50° then treated with decolourising charcoal (0.5 g.), evaporated to small bulk and allowed to crystallise. 2,4-Diamino-6-methylthiomethyl-1,3,5-triazine 3-*N*-oxide (2.3 g., 54%) was obtained as a white solid.

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Received March 27, 1967

Alderley Park, Nr. Macclesfield, Cheshire, England