158. *Some Potentially Cytotoxic Methylnitrosamines.*

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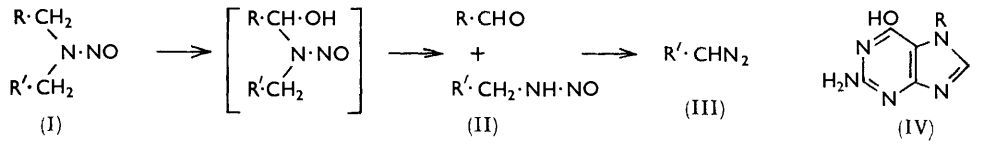
The synthesis of some compounds related to the hepatotoxic dimethylnitrosamine, bearing one or two methylnitrosamino-substituents attached to simple aliphatic, polyalcohol, amino-acid, or heterocyclic parents is described.

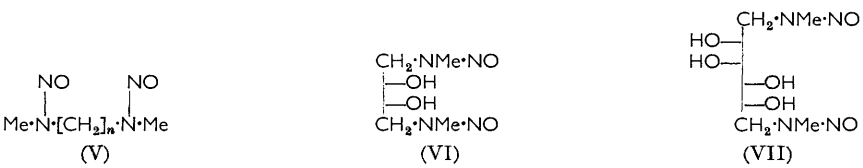
It has been postulated that the hepatocarcinogenicity of dialkylnitrosamines (I; R = R′ = Alkyl) is due to the formation of an alkylating metabolite,[1,2] a hypothesis supported by the isolation [3] of 7-methylguanine (IV; R = Me) from the hydrolysate of liver ribonucleic acid from rats treated with dimethylnitrosamine (I; R = R′ = H). Hepatotoxicity seems to be limited to those nitrosamines bearing a hydrogen substituent on an α-carbon atom,[4] and their activity has been ascribed to the formation of a diazoalkane (III) through an intermediate such as a monoalkylnitrosamine (II) after initial enzymic de-alkylation.[1,2,4,5] Although the effect of diazomethane on nucleic acids has not been fully characterised,[6] guanine derivatives (IV), alkylated in the 7-position, are commonly obtained by hydrolysis of guanylic acid [7] and ribo- or deoxyribo-nucleic acids [8] previously treated with alkylating agents.



(I) (II) (III) (IV)

In view of this hypothesis it was of interest to prepare various compounds bearing a methylnitrosamino-group in the expectation that these might give rise, *in vivo*, to diazoalkanes broadly related to the cytotoxic alkylating agents.

The bisnitrosamines (V) seemed likely precursors of diazoalkanes related to the difunctional sulphonyloxyalkanes of the " Myleran " type. Few such nitrosamines have previously been prepared [9,10] owing to the relative inaccessibility of the parent amines.[11–13]



(V) (VI) (VII)

Such amines may, in fact, readily be obtained by reduction of the corresponding αω-bis-*N*-methylcarboxamides with lithium aluminium hydride, and crystalline nitroso-derivatives (V; *n* = 3—6 and 10, Table 1) were prepared in the usual way.

Water-soluble bisnitrosamines (VI) and (VII) analogous to the hydroxylated " Myleran "

[1] Hultin, Arrhenius, Löw, and Magee, *Biochem. J.*, 1960, **76**, 109.
[2] Brouwers and Emmelot, *Expt. Cell Res.*, 1960, **19**, 467; Emmelot, Mizrahi, and Kriek, *Nature*, 1962, **193**, 1158.
[3] Farber and Magee, *Biochem. J.*, 1960, **76**, 58 P.
[4] Heath, *Nature*, 1961, **192**, 170.
[5] Müller, *Chem. Ber.*, 1960, **93**, 1541.
[6] Friedman, *Biochim. Biophys. Acta*, 1957, **23**, 215.
[7] Brookes and Lawley, *J.*, 1961, 3923.
[8] Brookes and Lawley, *Biochem. J.*, 1961, **80**, 496.
[9] Schneider, *Chem. Ber.*, 1895, **28**, 3076.
[10] Picon, *Compt. rend.*, 1922, **175**, 695.
[11] Boon, *J.*, 1947, 307.
[12] Martello and Giolitti, *Gazzetta*, 1955, **85**, 1224.
[13] Smith, *Org. Synth.*, 1956, **36**, 69.

derivatives [14] were prepared from 1,4-dibromo-1,4-dideoxyerythritol and 1,6-di-*O*-methanesulphonyl-D-mannitol, respectively, by treatment with a large excess of methylamine in dioxan, followed by *N*-nitrosation of the polyhydroxy-diamines.

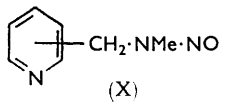
A series of α-methylnitrosamino-acids (VIII; R′ = H) and esters (VIII; R′ = Et) related to the common amino-acids has been prepared. Nitrososarcosine had previously been described as an oil [15] characterised by the formation of some crystalline salts. Brookes and Walker [16] prepared the *N*-nitroso-derivatives of sarcosine, *N*-methyl-leucine, *N*-methyl-valine, *N*-methylphenylalanine, and *N*-methylaspartic acid, but only the last two were obtained crystalline. We have prepared the *N*-methyl-*N*-nitroso-derivatives of the amino-acids listed in Table 2, all having the general structure (VIII; R′ = H), some of which were obtained crystalline. Esterification of the methylamino-acids gave the corresponding ester hydrochlorides (Table 3) which were nitrosated in the usual way, to give the methyl-nitrosamino-esters (VIII; R′ = Et) described in Table 4.

$Me{\cdot}N(NO){\cdot}CHR{\cdot}CO_2R'$ (VIII)

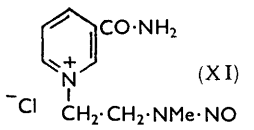
$Me{\cdot}N(NO){\cdot}CH_2{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H$ (IX)

As an analogue of " Azaserine," [17] *O*-(*N*-nitrososarcosyl)-DL-serine (IX) was prepared by coupling the mixed anhydride of benzyloxycarbonylsarcosine and isovaleric acid with *N*-benzyloxycarbonyl-DL-serine, followed by hydrogenolysis, and selective nitrosation of the secondary amino-group of the resulting *O*-sarcosyl-DL-serine.

In an effort to combine both potential alkylating and antimetabolite functions in the same molecule, several examples of heterocyclic compounds bearing a methylnitrosamino-substituent have been prepared. Nitrosation of the readily available picolylmethylamines gave the three isomeric (methylnitrosaminomethyl)pyridines (X). 2-Methyl-6-methylnitrosaminomethylpyridine was similarly obtained from 6-methyl-2-picolylmethylamine. Treatment of nicotinamide with 2-chloroethylmethylnitrosamine [18] (XII; R = Cl) gave the quaternary salt (XI). Attempts to alkylate other bases



(X)



(XI)

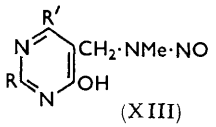
$ON{\cdot}MeN{\cdot}CH_2{\cdot}CH_2R$ (XII)

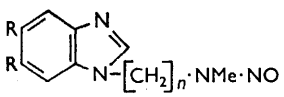
such as benzimidazole and 2-mercaptobenzothiazole with the chloro-compound (XII; R = Cl) met with little success. In the case of 2-mercaptobenzothiazole it is of interest that the nitroso-compound oxidised the thiol, giving a high yield of the corresponding disulphide, identical with that obtained by oxidation of 2-mercaptobenzothiazole with iodine.[19] The hitherto unknown 2-methylnitrosaminoethanol (XII; R = OH) and several of its derivatives were prepared.

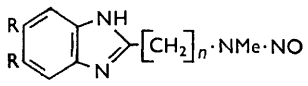
Treatment of 5-chloromethyluracil [20] with an excess of methylamine, followed by nitrosation of the crude product, gave 5-(methylnitrosaminomethyl)uracil (XIII; R = OH, R′ = H). An alternative route to such a 5-substituted pyrimidine is exemplified by the condensation of 4-hydroxy-6-methyl-2-piperidinopyrimidine-5-aldehyde [21] with methylamine followed by catalytic hydrogenation (platinum in ethanol) of the Schiff's base, and nitrosation to give the pyrimidine (XIII; R = $C_5H_{10}N$, R′ = Me). Unexpectedly, reduction of the Schiff's base with Raney nickel in ethanol gave 4-hydroxy-6-methyl-2-piperidinopyrimidine, presumably by hydrogenolysis of the 5-hydroxymethyl

[14] Brown and Timmis, *J.*, 1961, 3656.
[15] Schultzen, *Z. Chemie*, 1867, 616.
[16] Brookes and Walker, *J.*, 1957, 4409.
[17] De Wald, Behn, Morgan, Renfrew, and Moore, *J. Amer. Chem. Soc.*, 1959, **81**, 4367.
[18] Jones and Wilson, *J.*, 1949, 547.
[19] Sebrell and Boord, *J. Amer. Chem. Soc.*, 1923, **45**, 2396.
[20] Carbon, *J. Org. Chem.*, 1960, **25**, 1731.
[21] Hull, *J.*, 1957, 4845.

derivative. The substituted benzimidazoles (XIV and XV; $n = 1$; R = H or Cl) were prepared by reaction of the readily available chloromethyl compounds with methylamine, followed by nitrosation of the crude amines. The derivatives (XV; $n = 3$; R = H or


(XIII)


(XIV)


(XV)

Cl) were obtained by reduction of the corresponding benzimidazolylpropionic acid methylamides, followed by nitrosation.

The 1-substituted compound (XIV; $n = 4$; R = H) was prepared from *N*-*o*-aminophenyl-*N'*-methylsuccindiamide by reduction with lithium aluminium hydride followed by cyclisation of the triamine with formamidine acetate.[22] Attempts to prepare the 5,6-dichloro-analogue of this substance (XIV; $n = 4$; R = Cl) were frustrated by the very poor yield of the 6-nitro-derivative obtained on nitration of 3,4-dichlorophenylsuccinanilic acid.[23]

Experimental

Analyses were by Mr. P. R. W. Baker, Wellcome Research Laboratories, Beckenham, and by the Microanalytical Laboratory, Imperial College of Science and Technology.

αω-Bismethylnitrosaminoalkanes (V; $n = 3$—6 and 10).—*NN'*-Dimethyltrimethylenediamine was commercially available; the homologous amines were prepared in 50—60% yield by stirring the corresponding αω-di-(*N*-methylcarboxamides) (0·1 mole) under reflux with lithium aluminium hydride (0·3 mole) in ether (600 ml.) for 24 hr. The complexes were decomposed with aqueous sodium hydroxide, and the amines isolated from the dried ethereal extracts. Addition of a slight excess of sodium nitrite solution to an ice-cold aqueous solution of the amine hydrochloride precipitated the corresponding *bisnitrosamine*, which, after desiccation, was crystallised from ethyl acetate–hexane. Pertinent data are recorded in Table 1.

Table 1.

αω-Bismethylnitrosaminoalkanes (V).

				Found (%)			Required (%)		
No.	*n*	M. p./Form	Formula	C	H	N	C	H	N
1	3	66—67°/Prisms	$C_5H_{12}N_4O_2$	37·6	7·5	35·1	37·5	7·6	35·0
2	4	71—72/Prisms	$C_6H_{14}N_4O_2$	41·0	7·4	32·2	41·4	8·1	32·2
3	5	45—46/Needles	$C_7H_{16}N_4O_2$	44·4	8·3	29·8	44·7	8·6	29·8
4	6	59—60/Rhombs	$C_8H_{18}N_4O_2$	47·3	9·2	27·8	47·5	9·0	27·7
5	10	63—65/Blades	$C_{12}H_{26}N_4O_2$	55·6	9·8	21·4	55·8	10·1	21·7

1,4-*Dideoxy*-1,4-*bismethylnitrosaminoerythritol* (VI). 1,4-Dibromo-1,4-dideoxyerythritol (24 g.) was added portionwise to an ice-cooled solution of 10N-methylamine in dioxan (200 ml.). The mixture was stirred magnetically in a pressure bottle for 15 hr. at room temperature, and the oily precipitate collected and crystallised from methanol–ether, to give 1,4-*dideoxy*-1,4-*bismethylaminoerythritol* (2·7 g.), needles, m. p. 169—171° (Found: C, 48·5; H, 10·7; N, 18·9. $C_6H_{16}N_2O_2$ requires C, 48·6; H, 10·9; N, 18·9%). When the reaction mixture was evaporated to dryness, and the solid product recrystallised from methanol–ether, the *diamine dihydrobromide* was obtained, as prisms, m. p. 188—189° (Found: C, 23·3; H, 5·9; Br, 52·7; N, 8·9. $C_6H_{18}Br_2N_2O_2$ requires C, 23·2; H, 5·9; Br, 51·6; N, 9·0%). Similar treatment of 1,4-di-*O*-methanesulphonylerythritol gave the *diamine dimethanesulphonate*, needles, m. p. 168—170° (Found: C, 28·5; H, 7·0; N, 8·4; S, 18·1. $C_8H_{24}N_2O_8S_2$ requires C, 28·2; H, 7·1; N, 8·2; S, 18·8%).

The diamine (2·7 g.) in ice-cold 2N-hydrochloric acid (20 ml.) was treated with sodium

[22] Taylor and Ehrhart, *J. Amer. Chem. Soc.*, 1960, **82**, 3138.
[23] Woolley and Pringle, *J. Biol. Chem.*, 1952, **194**, 729.

nitrite (2·75 g.) in water (5 ml.). After 30 min., 10N-hydrochloric acid (1 ml.) was added dropwise to pH 4, the solution evaporated to dryness, and the residue desiccated. Extraction with hot ethanol gave the *bis*-N-*nitroso-derivative* (1·5 g.), plates, m. p. 150—151° (Found: C, 35·0; H, 6·6; N, 27·3. $C_6H_{14}N_4O_4$ requires C, 35·0; H, 6·8; N, 27·2%).

1,6-*Dideoxy*-1,6-*bismethylnitrosamino*-D-*mannitol* (VII).—The reaction of 1,6-di-*O*-benzenesulphonyl- or 1,6-di-*O*-methanesulphonyl-D-mannitol with methylamine in dioxan gave (20—30% yield) 1,6-*dideoxy*-1,6-*dimethylamino*-D-*mannitol*, needles, m. p. 187—188°, $[\alpha]_D^{22}$ +11° (*c* 2 in H_2O) (Found: C, 46·2; H, 9·9; N, 13·5. $C_8H_{20}N_2O_4$ requires C, 46·1; H, 9·7; N, 13·5%), nitrosation of which, as before, gave the *bis*-N-*nitroso-derivative*, plates, m. p. 162—163°, $[\alpha]_D^{21}$ +26° (*c* 2 in H_2O) (Found: C, 36·4; H, 6·8; N, 20·7. $C_8H_{18}N_4O_6$ requires C, 36·1; H, 6·8; N, 21·0%).

N-*Methyl*-N-*nitrosamino-acids* (VIII; R′ = H). A solution of sodium nitrite (7·6 g.) in water (12·5 ml.) was added dropwise to a heated (steam-bath) solution of the methylamino-acid (0·1 mole) in concentrated hydrochloric acid (11 ml.). Heating was continued for a further hour, after which the solution was cooled and extracted several times with ether or ethyl acetate. The organic layer was dried (Na_2SO_4) and the solvent removed by evaporation under reduced pressure, leaving an oil. In three cases the oil crystallised and the *methylnitrosamino-acids* were further purified by recrystallisation. Relevant data are recorded in Table 2. Yields were generally 50—60%.

TABLE 2.

N-Methyl-*N*-nitrosamino-acids (VIII; R′ = H).

No.	Deriv. of	R	Crystallised from	M. p.	Formula	Found (%) C	H	N	Required (%) C	H	N
1	Glycine	H	EtOAc–ligroin	75—77°	$C_3H_6N_2O_3$	30·5	5·1	24·0	30·5	5·1	23·7
2	Alanine	Me	EtOAc–ligroin	95—96	$C_4H_8N_2O_3$	36·6	6·3	21·6	36·4	6·1	21·2
3	Valine	Pr^i	—	—	$C_6H_{12}N_2O_3$	44·2	7·3	17·3	45·0	7·6	17·5
4	Leucine	Bu^i	C_6H_6–ligroin	68—69	$C_7H_{14}N_2O_3$	48·1	8·2	15·7	48·3	8·1	16·1
5	Norleucine	Bu^n	—	—	$C_7H_{14}N_2O_3$	47·8	7·6	16·5	48·3	8·1	16·1
6	Iso(+ alloiso)-leucine	CHMeEt	—	—	$C_7H_{14}N_2O_3$	48·1	7·9	15·7	48·3	8·1	16·1

N-*Methyl*-N-*nitrosamino-esters* (VIII; R′ = Et).—Methylamino-acids were esterified by the usual Fischer method. The *ethyl ester hydrochlorides* of *N*-methyl-leucine, -norleucine, and -phenylalanine were obtained crystalline and are described in Table 3. Sarcosine ester hydrochloride has already been described but the corresponding derivatives of alanine, valine, and isoleucine were obtained as gums and were nitrosated without further purification.

TABLE 3.

Ethyl α-methylamino-ester hydrochlorides.

No.	Ester hydrochloride of	Recryst. from	M. p.	Yield (%)	Formula	Found (%) C	H	Cl	N	Required (%) C	H	Cl	N
1	*N*-Methyl-leucine	Me_2CO–Et_2O	127—128°	95	$C_9H_{20}ClNO_2$	51·4	9·6	16·9	6·6	51·5	9·6	16·9	6·7
2	*N*-Methylnor-leucine	Me_2CO–Et_2O	97—98	80	$C_9H_{20}ClNO_2$	51·5	9·3	17·1	6·7	51·5	9·6	16·9	6·7
3	*N*-Methylphenyl-alanine	EtOH–Et_2O	159—160	75	$C_{12}H_{18}ClNO_2$	59·5	7·6	14·7	5·5	59·1	7·4	14·6	5·7

The methylamino-ester hydrochlorides (0·1 mole) were dissolved in water (10 ml.) to which a few drops of concentrated hydrochloric acid had been added and nitrosated by the method outlined for the acids. The *products* were distilled and are described in Table 4.

O-(N-*Nitrososarcosyl*)-DL-*serine* (IX). A solution of isovaleryl chloride (21 g.) in methylene chloride (40 ml.) was added dropwise at 0° during 30 min. to a stirred solution of benzyloxycarbonylsarcosine (36 g.) and triethylamine (18 g.) in methylene chloride (240 ml.). After a further 1½ hr. at 0° the mixture was treated with an ice-cold solution of benzyloxycarbonyl-DL-serine (43 g.) and triethylamine (18 g.) in methylene chloride (100 ml.). After 18 hr. at 5° triethylamine (18 g.) was added, and the solution was extracted with water (2 × 150 ml.). The methylene chloride solution was evaporated to an oil, which was dissolved in ethyl acetate

(200 ml.) and extracted with water (3 × 100 ml.). The aqueous phase was stirred at 3° with ethyl acetate (150 ml.) and acidified with 10N-hydrochloric acid (12 ml.). The organic layer was separated, the aqueous solution extracted with more ethyl acetate, and the combined extracts filtered through charcoal and evaporated to give oily *O*-(*N*-benzyloxycarbonylsarcosyl)-*N*-benzyloxycarbonyl-DL-serine (51 g.).

TABLE 4.

Ethyl *N*-methyl-*N*-nitrosamino-esters (VIII; R′ = Et).

No.	Deriv. of	R	B. p./mm.	Yield (%)	n_D^{22}	Formula	Found (%) C	H	N	Required (%) C	H	N
1	Glycine	H	81—85°/0·5 *	65	1·4500 *	$C_5H_{10}N_2O_3$	41·0	7·3	19·3	41·1	6·9	19·2
2	Alanine	Me	57—59°/0·1	30 †	1·4485	$C_6H_{12}N_2O_3$	44·8	7·5	17·8	45·0	7·5	17·5
3	Valine	Pr^i	54—56°/0·05	25 †	1·4486	$C_8H_{16}N_2O_3$	51·0	9·0	14·8	51·1	8·6	14·9
4	Leucine	Bu^i	89—90°/0·2	62	1·4480	$C_9H_{18}N_2O_3$	53·3	9·1	14·0	53·4	9·0	13·8
5	Norleucine	Bu^n	100—101°/0·2	60	1·4449	$C_9H_{18}N_2O_3$	53·2	9·1	13·5	53·4	9·0	13·8
6	Iso(+ alloiso)-leucine	CHMeEt	86—87°/0·2	40 †	1·4509	$C_9H_{18}N_2O_3$	53·2	9·1	14·0	53·4	9·0	13·8
7	Phenylalanine	·CH_2Ph	126—128°/0·1	58	1·5170	$C_{12}H_{16}N_2O_3$	61·0	6·6	12·1	61·0	6·8	11·9

* Lit., b. p. 70—73°/0·3 mm., n_D^{25} 1·4471. † Based on amino-acid (2 stages).

A solution of this product in ethanol (240 ml.) containing N-hydrochloric acid (120 ml.) was stirred in a stream of hydrogen with 5% palladium–charcoal for 24 hr. The mixture was filtered, the residue extracted with water (100 ml.), and the extract evaporated, to give O-*sarcosyl*-DL-*serine hydrochloride* (7 g.), m. p. 164—166° (decomp.). Recrystallisation from aqueous ethanol gave plates, m. p. 173—175° (decomp.) (inserted at 160°) (Found: C, 33·8; H, 6·2; Cl, 16·3; N, 13·0. $C_6H_{13}ClN_2O_4$ requires C, 33·9; H, 6·2; Cl, 16·7; N, 13·2%).

The hydrochloride (1·1 g.) in ice–water (25 ml.) was treated with sodium nitrite (0·56 g.) in water (7 ml.) in one portion; the solution was left for 1 hr., then passed through a column (2 × 17 cm.) of an acid-washed 1 : 1 mixture of " Hyflo " and charcoal. The column was eluted with water, and three 100 ml. fractions were collected, and then evaporated to dryness. These yielded sodium nitrite and sodium chloride; elution was continued with 4% aqueous acetone, and the next three fractions gave white solids (strong Liebermann and ninhydrin reactions), each melting at *ca.* 130° (effervescence). Further elution gave small amounts of gums which were not investigated. These solids were combined, dissolved in water (5 ml.), and precipitated with ethanol (15 ml.), to give O-(N-*nitrososarcosyl*)-DL-*serine* (0·46 g.), plates, m. p. 138—139° (effervescence) (Found: C, 35·5; H, 5·4; N, 20·9. $C_6H_{11}N_3O_5$ requires C, 35·1; H, 5·4; N, 20·5%). The R_F values of this product, of serine, and of sarcosine in Bu^{n}OH–EtOH–$EtCO_2H$–water (10 : 5 : 2 : 5) were 0·37, 0·22, and 0·32, respectively.

2-*Methylnitrosaminoethanol* (XII; R = OH). 5N-Hydrochloric acid (60 ml.) was added dropwise to a stirred solution of 2-methylaminoethanol (25 g.) and sodium nitrite (25 g.) in water (100 ml.) during 0·5 hr. The temperature of the mixture rose spontaneously to 45° and the mixture was stirred for a further 0·5 hr. The crude product was isolated by continuous extraction with ether for 12 hr. The yellow oil (19·1 g.) obtained by evaporation of the dried ($MgSO_4$) ether extract was distilled to give 2-methylnitrosaminoethanol as a yellow oil (14·0 g.), b. p. 90—93°/0·2 mm., n_D^{25} 1·4578 (Found: C, 34·7; H, 7·7; N, 27·3. $C_3H_8N_2O_2$ requires C, 34·6· H, 7·7; N, 26·9%). A mixture of the nitrosamino-alcohol (3·1 g.(and phenyl isocyanate (4·0 g.), kept in a stoppered tube for 12 hr., gave the *phenylurethane* as white needles (3·0 g.), m. p. 77—78° (from benzene) (Found: C, 53·8; H, 5·7; N, 19·2. $C_{10}H_{13}N_3O_3$ requires C, 53·8; H, 5·9; N, 18·8%).

2-(*Methylnitrosaminomethyl*)*pyridine* (X). 2-Picolylmethylamine (12·2 g.) and sodium nitrite (7·6 g.) were stirred with water (30 ml.) and treated with 5N-hydrochloric acid (22 ml.) during 0·5 hr. After cooling, the solution was saturated with salt and extracted with ethyl acetate (3 × 50 ml.). Evaporation of the combined dried extracts gave the crude *nitroso-compound.* Distillation of this product gave a clear yellow oil (10·0 g.), b. p. 100—101°/0·2 mm.,

n_D^{25} 1·5439 (Found: C, 55·6; H, 5·8; N, 27·3. $C_7H_9N_3O$ requires C, 55·6; H, 6·0; N, 27·8%).

The following methylnitrosaminomethylpyridines were prepared by the method described above:

3-(*Methylnitrosaminomethyl*)*pyridine* (X), from 3-picolylmethylamine (12·2 g.) as a yellow oil (10·8 g.), b. p. 115—116°/0·2 mm., n_D^{25} 1·5450 (Found: C, 55·8; H, 6·1; N, 28·1. $C_7H_9N_3O$ requires C, 55·6; H, 6·0; N, 27·8%) [methiodide (prepared in acetone), prisms, m. p. 129—130° (from ethanol) (Found: C, 32·8; H, 4·2; I, 44·3; N, 14·8. $C_8H_{12}IN_3O$ requires C, 32·8; H, 4·1; I, 43·3; N, 14·3%].

4-(*Methylnitrosaminomethyl*)*pyridine* (X), from 4-picolylmethylamine (12·2 g.) as a yellow oil (7·0 g.), b. p. 114—115°/0·2 mm., n_D^{25} 1·5440 (Found: C, 55·7; H, 6·1; N, 27·4%).

2-*Methyl*-6-(*methylnitrosaminomethyl*)*pyridine*, from 6-methyl-2-picolylmethylamine (16·5 g.) as a pale yellow solid (9·9 g.), m. p. 48—49°, b. p. 99—100°/0·1 mm. (Found: C, 58·0; H, 6·6; N, 25·2. $C_8H_{11}N_3O$ requires C, 58·2; H, 6·7; N, 25·4%).

3-*Carbamoyl*-1-(2-*methylnitrosaminoethyl*)*pyridinium chloride* (XI). Nicotinamide (2 g.) and 2-chloro-*N*-methyl-*N*-nitrosoethylamine [13] (2 g.) were heated in boiling ethanol (10 ml.) for 1 hr. The solution was cooled and the crude product recrystallised from methanol, to give the pure quaternary *salt* (3·3 g.), m. p. 170—171° (Found: C, 44·1; H, 5·5; Cl, 14·3; N, 22·6. $C_9H_{13}ClN_4O_2$ requires C, 44·2; H, 5·4; Cl, 14·5; N, 22·9%).

5-(*Methylnitrosaminomethyl*)*uracil* (XIII; R = OH, R′ = H).—5-Chloromethyluracil [20] (2·0 g.) was added portionwise to ice-cold 10N-methylamine in dioxan (50 ml.), and the mixture stirred in a pressure bottle for 18 hr. Ethanol (30 ml.) was added to dissolve the precipitated oil, a small amount of insoluble material was filtered off, and the filtrate was evaporated to a sticky solid. This was dissolved in 2N-hydrochloric acid and treated with sodium nitrite (0·9 g.) in water (2 ml.); a cream-coloured solid slowly crystallised. After 30 min. this was collected, washed, and dried, to give 5-(*methylnitrosaminomethyl*)*uracil* (0·4 g.), rhombs, m. p. 235—240° (effervescence). A portion recrystallised from aqueous ethanol had m. p. 237—238° (effervescence) (inserted at 220°) (Found: C, 38·8; H, 4·4; N, 29·4. $C_6H_8N_4O_3$ requires C, 39·1; H, 4·4; N, 30·4%).

4-*Hydroxy*-6-*methyl*-5-(*methylnitrosaminomethyl*)-2-*piperidinopyrimidine* (XIII; R = $C_5H_{10}N$, R′ = Me).—4-Hydroxy-6-methyl-2-piperidinopyrimidine-5-aldehyde [21] (16 g.) was stirred with 2N-methylamine in dioxan (150 ml.) at 100° until dissolution was complete (15 min.). The solution was then refrigerated, and the product collected and recrystallised from dioxan to give the Schiff's *base* (12 g.) (blades), m. p. 197—199° (decomp.) (Found: C, 61·6; H, 7·8; N, 23·9. $C_{12}H_{18}N_4O$ requires C, 61·5; H, 7·7; N, 23·9%). Hydrogenation of this product at 40 atm. in ethanol over Raney nickel and crystallisation from ethanol gave a 51% yield of 4-hydroxy-6-methyl-2-piperidinopyrimidine (needles) (Found: C, 62·4; H, 7·8; N, 22·0. Calc. for $C_{10}H_{15}N_3O$; C, 62·2; H, 7·8; N, 21·8%), identical with an authentic specimen; the samples gave the same 5-nitroso-derivative. When the Schiff's base (3·7 g.) was hydrogenated at room temperature and pressure in acetic acid (40 ml.) over Adams catalyst (0·2 g.) uptake of 1 mol. was complete in 40 min. The filtered solution was evaporated to an oil, which was dissolved in water (30 ml.) and treated with sodium nitrite (1·4 g.) in water (5 ml.). The precipitated solid was recrystallised from ethanol, to give the *nitrosamine* (2·5 g.), needles, m. p. 209—210° (decomp.) (Found: C, 54·6; H, 6·9; N, 26·6. $C_{12}H_{19}N_5O_2$ requires C, 54·3; H, 7·2; N, 26·4%).

2-(*Methylnitrosaminomethyl*)- *and* 5,6-*Dichloro*-2-(*methylnitrosaminomethyl*)-*benzimidazole* (XV; *n* = 1; R = H or Cl).—2-Chloromethylbenzimidazole hydrochloride was prepared by boiling a solution of *o*-phenylenediamine (50 g.) and chloroacetic acid (65 g.) in 4N-hydrochloric acid for 1 hr. The dried product was crystallised from dioxan (250 ml.) (yield, 42 g.; m. p. 150—151°). The 5,6-*dichloro-derivative* (cf. ref. 24), prepared likewise (95% yield), formed needles (from ethanol), which darkened and sublimed at *ca.* 215° (Found: C, 35·1; H, 2·0; Cl, 51·7; N, 10·3. $C_8H_6Cl_4N_2$ requires C, 35·3; H, 2·2; Cl, 52·2; N, 10·3%).

The chloromethylbenzimidazoles were treated in the usual way with an excess of methylamine in dioxan; the crude reaction products were extracted with methanol–ether, methylamine hydrochloride was filtered off, and the filtrates were evaporated to oils which were nitrosated as before. This method gave 2-(*methylnitrosaminomethyl*)- (36%), rhombs (from acetone–hexane), m. p. 179—180° (Found: C, 57·4; H, 5·5; N, 29·5. $C_9H_{10}N_4O$ requires C, 56·8; H, 5·3; N, 29·5%), and 5,6-*dichloro*-2-(*methylnitrosaminomethyl*)-*benzimidazole* (56%), plates (from

[24] Lettré, Fritsch, and Porath, *Chem. Ber.*, 1951, **84**, 719.

ethyl acetate–hexane), m. p. 185—187° (Found: C, 41·3; H, 2·8; Cl, 26·9; N, 21·1. $C_9H_8Cl_2N_4O$ requires C, 41·7; H, 3·1; Cl, 27·4; N, 21·6%).

1-(*Methylnitrosaminomethyl*)- *and* 5,6-*Dichloro*-1-(*methylnitrosaminomethyl*)-*benzimidazole* (XIV; $n = 1$; R = H or Cl).—Benzimidazole and 5,6-dichlorobenzimidazole were hydroxymethylated by Bachman and Heisey's procedure;[25] 5,6-*dichloro*-1-*hydroxymethylbenzimidazole* (95% yield) was precipitated from the reaction mixture as blades, m. p. 205—207° (effervescence) (Found: C, 44·2; H, 2·7; Cl, 33·0; N, 13·1. $C_8H_6Cl_2N_2O$ requires C, 44·3; H, 2·8; Cl, 32·7; N, 12·9%). The compound lost formaldehyde on attempted recrystallisation from the common solvents. Reaction of the 1-hydroxymethylbenzimidazoles with an excess of thionyl chloride gave the hydrochlorides of the 1-chloromethyl derivatives; 5,6-*dichloro*-1-*chloromethylbenzimidazole hydrochloride* (79% yield) formed needles (from ethanol), m. p. 196—199° (decomp.) (Found: N, 10·3. $C_8H_6Cl_2N_2O$ requires N, 10·3%). Treatment of the chloromethyl compounds with methylamine and nitrosation of the crude reaction products, as before, gave 1-(*methylnitrosaminomethyl*)*benzimidazole* (38%), blades (from ethyl acetate), m. p. 94—95° (Found: C, 57·0; H, 5·2; N, 28·6. $C_9H_{10}N_4O$ requires C, 56·8; H, 5·3; N, 29·5%), and 5,6-*dichloro*-1-*methylnitrosaminomethylbenzimidazole* (23%), rhombs (from ethyl acetate), m. p. 164—165° (Found: C, 41·5; H, 2·9; Cl, 27·3; N, 21·6. $C_9H_8Cl_2N_4O$ requires C, 41·7; H, 3·1; Cl, 27·4; N, 21·6%).

2-(3-*Methylnitrosaminopropyl*)- *and* 5,6-*Dichloro*-2-(3-*methylnitrosaminopropyl*)-*benzimidazole* (XV; $n = 3$; R = H or Cl).—Methyl 2-benzimidazolylpropionate[26] and *methyl* 2-(5,6-*dichlorobenzimidazolyl*)*propionate*[24] (Found: Cl, 25·6; N, 10·4. $C_{11}H_{10}Cl_2N_2O_2$ requires Cl, 26·0; N, 10·3%) were treated with an excess of methylamine in dioxan at 100°; the *methylamide* of 2-benzimidazolylpropionic acid (65% yield) formed needles (from acetone–methanol), m. p. 239—241° (Found: C, 65·3; H, 6·6; N, 20·5. $C_{11}H_{13}N_3O$ requires C, 65·0; H, 6·5; N, 20·7%), and the 5,6-*dichloro-derivative* (71% yield) formed prisms (from ethyl acetate–ethanol), m. p. 243—245° (Found: C, 48·8; H, 4·0; Cl, 25·5; N, 15·5. $C_{11}H_{11}Cl_2N_3O$ requires C, 48·5; H, 4·1; Cl, 26·1; N, 15·4%). The methylamides were boiled under reflux for 40 hr. with lithium aluminium hydride (2 mol.) in ether; the complexes were decomposed with 2N-sodium hydroxide, the precipitated salts were collected, dissolved in 2N-hydrochloric acid, and treated with aqueous sodium nitrite. The mixtures were adjusted with ammonia to pH 7·5, then extracted with chloroform, to give 2-(3-*methylnitrosaminopropyl*)- (53%), plates (from ethyl acetate–hexane), m. p. 130—132° (Found: C, 61·2; H, 6·5; N, 25·3. $C_{11}H_{14}N_4O$ requires C, 60·5; H, 6·5; N, 25·7%), and 5,6-*dichloro*-2-(3-*methylnitrosaminopropyl*)-*benzimidazole*, (38%), blades (from ethyl acetate–hexane), m. p. 141—142° (Found: C, 46·0; H, 4·2; Cl, 24·4; N, 18·5. $C_{11}H_{12}Cl_2N_4O$ requires C, 46·0; H, 4·2; Cl, 24·5; N, 19·5%).

1-(4-*Methylnitrosaminobutyl*)*benzimidazole* (XIV; $n = 4$; R = H).—*o*-Nitrosuccinanilic acid (25 g.) was boiled under reflux for 6 hr. with thionyl chloride (30 g.) and pyridine (0·1 ml.) in ether (250 ml.). The acid chloride (m. p. 160—162°) was collected, washed with ether, and added to N-methylamine in dioxan (200 ml.). After 1 hr. the mixture was diluted with ether (200 ml.); the precipitated *methylamide* crystallised from ethanol as needles (20 g.), m. p. 149—150° (Found: C, 52·5; H, 5·2; N, 16·1. $C_{11}H_{13}N_3O_4$ requires C, 52·6; H, 5·2; N, 16·7%). Hydrogenation of this product (20 g.) in ethanol (400 ml.) over 5% palladium–charcoal (2 g.) gave N-*methyl*-N′-o-*aminophenylsuccindiamide* (16 g.), needles (from ethanol), m. p. 195—196° (Found: C, 59·4; H, 6·8; N, 18·9. $C_{11}H_{15}N_3O_2$ requires C, 59·7; H, 6·8; N, 19·0%). This amide (6·3 g.) was stirred under reflux for 12 hr. with lithium aluminium hydride (4·5 g.) in ether (150 ml.); the complex was decomposed with 2N-sodium hydroxide, and the product extracted with ether and distilled, to give (after elimination of a trace of *o*-phenylenediamine) N-*methyl*-N′-o-*aminophenyltetramethylenediamine*, b. p. 144—146°/0·03 mm. (4·0 g.), as a yellow oil, n_D^{25} 1·5735 (Found: N, 21·2. $C_{11}H_{19}N_3$ requires N, 21·7%).

This triamine (3·1 g.) was boiled under reflux with formamidine acetate (1·8 g.) in ethanol (30 ml.) for 2·5 hr. (*i.e.*, until test portions of the solution no longer gave an intense red colour on diazotization). The solution was evaporated to an oil, a small portion of which, treated with ethanolic picric acid, gave 1-4′-*aminobutylbenzimidazole dipicrate*, rhombs (from ethanol), m. p. 191—193° (Found: C, 43·7; H, 3·7; N, 18·8. $C_{24}H_{33}N_9O_{14}$ requires C, 43·6; H, 3·5; N, 19·1%).

[25] Bachman and Heisey, *J. Amer. Chem. Soc.*, 1946, **68**, 2496.
[26] Chatterjee, *J.*, 1929, 2965.

Nitrosation of the remainder of the amine in the usual way gave an oily product which was isolated by extraction with chloroform; the glass so obtained crystallised, to give **1-4'-*methyl-nitrosaminobutylbenzimidazole*** (1·8 g.), plates (from ethyl acetate–hexane), m. p. 91—93° (Found: C, 62·8; H, 6·7; N, 24·2. $C_{12}H_{16}N_4O$ requires C, 62·1; H, 6·9; N, 24·1%). The *picrate* of this product formed plates (from ethanol–2-methoxyethanol), m. p. 142—143° (Found: C, 46·8; H, 4·1; N, 21·3. $C_{18}H_{19}N_7O_8$ requires C, 46·9; H, 4·2; N, 21·3%).

This investigation has been supported by grants to this Institute from the Medical Research Council, the British Empire Cancer Campaign, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

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