296. *Nitro-compounds as Amœbicides. Part I. Compounds related to 2-Di-(2-hydroxyethyl)amino-5-nitropyridine.*

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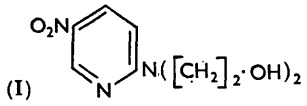
The discovery [1] that 2-di-(2-hydroxyethyl)amino-5-nitropyridine [2] is amœbicidal prompted the preparation of a number of related compounds by known methods. With certain exceptions, Mangini's method was suitable for reaction of 2-chloro-5-nitropyridine and the appropriate bases, including esters of amino-acids.

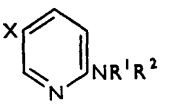
During routine screening of different compounds for chemotherapeutic activity it was observed [1] that 2-di-(2-hydroxyethyl)amino-5-nitropyridine [2,3] (I) had amœbicidal properties (*Entamoeba histolytica*) when tested in rats. Soon after this the antihistomonas (*Histomonas meleagridis*) properties of 2-amino-5-nitro-thiazole and -pyrimidine were

[1] Neal and Vincent, *Brit. J. Pharmacol.*, 1955, **10**, 434.
[2] Copp and Timmis, *J.*, 1955, 2021.
[3] B.P. 700,757.

announced [4] and further tests in our own laboratories [5] showed that both these two compounds and 2-amino-5-nitropyridine were also amœbicides. Many compounds, known and new, have since been prepared and tested and the compounds more closely related to the active compound (I) are here reported.



(I)



(II)

Most of the compounds containing the 5-nitro-group were obtained by condensing the appropriate amine and 2-chloro-5-nitropyridine in alcoholic sodium acetate [6] though the ease of reaction was much affected by steric factors. Thus, di-(2-hydroxyethyl)amine and 2-chloro-5-nitropyridine were shown [2] to react quite quickly under these conditions but 2-hydroxyethyl-2-hydroxypropylamine reacted with 2-bromo-5-nitropyridine only at 130° and in the absence of a diluent, whilst di-(2-hydroxypropyl)amine did not react with 2-chloro- or 2-bromo-5-nitropyridine without gross decomposition. 2-Bromo-5-chloro- and 2 : 5-dibromo- but not 2-bromo-5-iodo-pyridine gave the desired products (II; $R^1 = R^2 = [CH_2]_2{\cdot}OH$, X = Cl and Br respectively) when treated with neat diethanolamine at 180°. The iodo-compound (II; $R^1 = R^2 = [CH_2]_2{\cdot}OH$, X = I) was therefore prepared by iodination of the amine (II; $R^1 = R^2 = [CH_2]_2{\cdot}OH$, X = H) in the presence of mercuric acetate. It has been shown that under these conditions 2-aminopyridine gives 2-amino-5-iodopyridine.[7] Reduction of the compound (I) with cold stannous chloride gave the corresponding amine (II; $R^1 = R^2 = [CH_2]_2{\cdot}OH$, $X = NH_2$).

Although it has since been shown that the di-(2-hydroxyethyl)amine (I) has a direct inhibitory action upon amœba,[1] it originally seemed possible that the effective chemotherapeutic agent was not the di-(2-hydroxyethyl)amine *per se*, but a metabolite such as the acid (II; $R^1 = R^2 = CH_2{\cdot}CO_2H$, $X = NO_2$) which might conceivably act as an amino-acid antagonist. Unfortunately di(methoxycarbonylmethyl)amine could not be caused to react with 2-chloro- or 2-bromo-5-nitropyridine. In model experiments the ethyl esters of glycine, alanine, and sarcosine reacted readily with 2-chloro-5-nitropyridine under our usual conditions to give (II; $R^1 = H$, $R^2 = CH_2{\cdot}CO_2Et$; $R^1 = H$, $R^2 = CHMe{\cdot}CO_2Et$; and $R^1 = Me$, $R^2 = CH_2{\cdot}CO_2Et$, $X = NO_2$). The first two of these esters gave the corresponding acids on acid, but not alkaline, hydrolysis though the latter method easily gave the third parent acid. The corresponding amides and hydrazides were also prepared (except II; $R^1 = H$, $R^2 = CHMe{\cdot}CO{\cdot}NH_2$, $X = NO_2$, which was not investigated). Gruber [8] has since stated that glycine ethyl ester reacts with 2-fluoro- but not with 2-chloro-5-nitropyridine. This was probably due to the use of mildly alkaline conditions whereas we used more acid conditions (cf. Banks [9]).

Biological tests [5] have shown that of these compounds (and the related nitropyridines reported previously [2]), only (I), (II; $R^1 = [CH_2]_2{\cdot}OH$, $R^2 = CH_2{\cdot}CHMe{\cdot}OH$, $X = NO_2$), and the sarcosine derivatives (II; $R^1 = Me$, $R^2 = CH_2{\cdot}CO{\cdot}NH_2$ and $CH_2{\cdot}CO{\cdot}NH{\cdot}NH_2$, $X = NO_2$) have amœbicidal activity. The chloro-derivative (II; $R^1 = R^2 = [CH_2]_2{\cdot}OH$, X = Cl) appeared to be active in the original tests but these could not be confirmed (cf. ref. 3).

Experimental

2-Hydroxyethyl-3-hydroxypropylamine.—3-Hydroxypropylamine (39 g.) and ethylene oxide (20 ml.) were heated in ethanol (80 ml.) in an autoclave at 100° for 1 hr. The ethanol was then

[4] Waletzky, Clark, and Marson, *Science*, 1950, **111**, 720.
[5] Personal communication from Dr. Neal and Miss Vincent.
[6] Mangini and Frenguelli, *Gazzetta*, 1939, **69**, 86.
[7] Shepherd and Fellows, *J. Amer. Chem. Soc.*, 1948, **70**, 157.
[8] Gruber, *Canad. J. Chem.*, 1953, **31**, 1020.
[9] Banks, *J. Amer. Chem. Soc.*, 1944, **66**, 1127.

evaporated and the residue distilled *in vacuo*. The *product* (35 g.) was a viscous liquid, b. p. 160—162°/11 mm. (Found: C, 51·0; H, 10·8. $C_5H_{13}O_2N$ requires C, 50·4; H, 11·0%).

2-(2-Hydroxyethyl-2-hydroxypropylamino)-5-nitropyridine and Related Nitropyridines.—2-Hydroxyethyl-2-hydroxypropylamine [10] (10 g.) and 2-bromo-5-nitropyridine (8 g.) were heated at 130° for 3 hr. The cooled mixture was poured into excess of 2N-hydrochloric acid, the insoluble portion filtered off, and the parent base precipitated with potassium carbonate and then extracted with ethyl acetate. This base was a gum but its *hydrochloride* crystallised without difficulty (see Table).

Derivatives of 5-nitropyridine.

Substituent(s)	No.	Deriv.	M. p.	Solvent for crystn.
2-N<[CH$_2$]$_2$·OH / CH$_2$·CHMe·OH	1	HCl	131°	EtOH–EtOAc
2-NEt·[CH$_2$]$_2$·OH	2	HCl	147—148	EtOH
2-NPr·[CH$_2$]$_2$·OH	3	HCl	153—154	EtOH
2-N<[CH$_2$]$_2$·OH ..... / [CH$_2$]$_3$·OH	4	Base	68—69	—
	5	HCl	123—124	EtOH–EtOAc
2-N([CH$_2$]$_3$·OH)$_2$	6	Base	43—45	—
2-N([CH$_2$]$_2$·OH)$_2$; 3-Me	7	HCl	157	EtOH
2-N([CH$_2$]$_2$·OH)$_2$; 4-Me	8	HCl	138—139	EtOH–EtOAc
2-N([CH$_2$]$_2$·OH)$_2$; 6-Me	9	Base	108—109	EtOH

	Found (%)					Required (%)			
No.	C	H	N	Cl	Formula	C	H	N	Cl
1	43·3	5·5	—	—	$C_{10}H_{15}O_4N_3$,HCl	43·3	5·8	—	—
2	—	—	—	14·2	$C_9H_{13}O_2N_3$,HCl	—	—	—	14·3
3	—	—	—	14·0	$C_{10}H_{15}O_3N_3$,HCl	—	—	—	13·6
4	50·1	6·1	17·2	—	$C_{10}H_{15}O_4N_3$	49·8	6·3	17·4	—
5	—	—	—	12·9	$C_{10}H_{15}O_4N_3$,HCl	—	—	—	12·7
6	51·9	6·25	16·8	—	$C_{11}H_{17}O_4N_3$	51·75	6·7	16·5	—
7	43·2	5·8	14·7	—	$C_{10}H_{15}O_4N_3$,HCl	43·3	5·8	15·1	—
8	43·3	5·8	—	—	$C_{10}H_{15}O_4N_3$,HCl	43·3	5·8	—	—
9	49·7	6·1	17·0	—	$C_{10}H_{15}O_4N_3$	49·8	6·3	17·4	—

The more closely related nitropyridines are described in the Table. They were prepared from 2-chloro-5-nitropyridine and the appropriate base according to the previously used method.[3, 6] 2-(Ethyl-2-hydroxyethylamino)-5-nitropyridine has already been prepared.[11] Apart from the hydrochlorides, the nitropyridines described in this paper were bright yellow.

2-Amino-5-chloro- and 2-Amino-5-bromo-pyridine.—The first of these compounds was prepared by chlorination of 2-aminopyridine in 20% sulphuric acid as previously described [12] but the crude product was more easily purified by steam-distillation, almost pure 2-amino-5-chloropyridine being thus obtained; it was converted directly into 2-bromo-5-chloropyridine by established methods.[13, 14]

2-Amino-5-bromopyridine was similarly prepared but the product from steam-distillation was a mixture. This was dissolved in a slight excess of hot 2N-acetic acid. On cooling, the feebly basic 2-amino-3:5-dibromopyridine crystallised. This was filtered off and the filtrate basified to give 2-amino-5-bromopyridine, m. p. 136—137° (49%), which was then converted into 2:5-dibromopyridine.[13–15]

5-Chloro- and 5-Bromo-2-di-(2-hydroxyethyl)aminopyridine.—2-Bromo-5-chloropyridine (20 g.) and diethanolamine (24 g.) were warmed in a Carius tube to 100°. The mixture was well shaken at this temperature, an essential precaution, and then heated to 170° for 8 hr. After cooling, the crude mixture was poured into excess of 4N-ammonia and repeatedly extracted with ethyl acetate. The combined extracts were dried (K_2CO_3) and evaporated. The residue was converted into its *hydrochloride*, which was crystallised once from ethanol and ether and then from propan-2-ol, m. p. 143° (12·1 g.) (Found: C, 42·8; H, 5·7; N, 10·7; Cl, 28·1. $C_9H_{13}O_2N_2Cl$,HCl requires C, 42·7; H, 5·6; N, 11·1; Cl, 28·1%). The parent *base* was extracted

[10] Cottle, Jeltsch, Stoudt, and Walter, *J. Org. Chem.*, 1946, **11**, 286.
[11] Bremer, *Annalen*, 1936, **521**, 286.
[12] English, Clark, Clapp, Seeger, and Ebel, *J. Amer. Chem. Soc.*, 1946, **68**, 458.
[13] Craig, *ibid.*, 1934, **56**, 231.
[14] Case, *ibid.*, 1946, **68**, 2574.
[15] Leese and Rydon, *J.*, 1954, 4039.

from a basified solution of the above hydrochloride with chloroform. It slowly solidified and had m. p. 23—25° (Found : C, 49·8; H, 5·9; N, 12·6. $C_9H_{13}O_2N_2Cl$ requires C, 49·9; H, 6·0; N, 12·9%).

2 : 5-Dibromopyridine (6 g.) was similarly treated with diethanolamine (6 g.) for 6 hr. at 170°. The product was isolated in the same way and characterised as its *hydrochloride* (2·6 g.), m. p. 145—146° (Found : C, 36·5; H, 4·4; N, 9·7. $C_9H_{13}O_2N_2Br,HCl$ requires C, 36·3; H, 4·7; N, 9·4%). The base did not crystallise.

2-Di-(2-hydroxyethyl)amino-5-iodopyridine.—A solution of 2-di-(2-hydroxyethyl)aminopyridine [2, 16] (5·3 g.) in water (25 ml.) was treated with mercuric acetate (4·0 g.). The mixture was heated for 2 min. on a steam-bath, then cooled to 70°, and a solution of iodine (6·8 g.) in hot dioxan (30 ml.) was rapidly added. The iodine colour disappeared and precipitation occurred. The suspension was then stirred at 70° for 45 min. during which the original precipitate dissolved and a little red solid separated. After cooling to 0°, the insoluble portion was filtered off but it gave no identifiable products. The filtrate was evaporated *in vacuo* until an oil appeared. After addition of potassium iodide (10 g.), this mixture was extracted several times with ethyl acetate. The combined extracts were dried and evaporated. The residue was purified as its *hydrochloride* (5·1 g.), m. p. 116—117°, from ethanol–propan-2-ol (Found : N, 8·0; Cl^-, 10·2; I, 36·6; O, 9·7. $C_9H_{13}O_2N_2I,HCl$ requires N, 8·1; Cl, 10·3; I, 36·8; O, 9·3%). The regenerated *base*, crystallised from benzene, had m. p. 72—73° (Found : I, 41·0. $C_9H_{13}O_2N_2I$ requires I, 41·2%).

5-Amino-2-di-(2-hydroxyethyl)aminopyridine.—2-Di-(2-hydroxyethyl)amino-5-nitropyridine (9·2 g.) was reduced in 2N-hydrochloric acid (200 ml.) with stannous chloride (28·0 g.) at 15—20° in nitrogen. After removal of the tin with hydrogen sulphide, the filtrate was evaporated immediately *in vacuo*. The residual *dihydrochloride* solidified when ground with propan-2-ol and recrystallised from methanol on addition of propan-2-ol as colourless needles (6·8 g.), m. p. 178—179·5°, which darkened very rapidly in air (Found : C, 40·2; H, 6·3. $C_9H_{15}O_2N_3,2HCl$ requires C, 40·3; H, 6·3%).

N-(5-Nitro-2-pyridyl)amino-acid Esters.—Glycine ethyl ester hydrochloride (16·8 g.), 2-chloro-5-nitropyridine (19·2 g.), and anhydrous sodium acetate reacted in boiling ethanol (100 ml.) during 3 hr. Addition of water gave a solid precipitate which was heated at 100° *in vacuo* to remove unchanged 2-chloro-5-nitropyridine. The residual *N*-(5-nitro-2-pyridyl)glycine ethyl ester crystallised from ethanol as yellow needles, m. p. 138—139° (10·3 g.) (Found : C, 48·0; H, 4·7. Calc. for $C_9H_{11}O_4N_3$: C, 48·0; H, 4·9%). (Gruber [8] obtained this substance as " white needles," m. p. 142—143°.) Similar preparations gave *N-(5-nitro-2-pyridyl)alanine ethyl ester*, m. p. 115—116° (Found : C, 50·5; H, 5·4. $C_{10}H_{13}O_4N_3$ requires C, 50·2; H, 5·5%), and *N-(5-nitro-2-pyridyl)sarcosine ethyl ester*, m. p. 78—79° (from ethanol) (Found : C, 50·4; H, 5·2; N, 17·5. $C_{10}H_{13}O_4N_3$ requires C, 50·2; H, 5·5; N, 17·55%).

N-(5-Nitro-2-pyridyl)amino-acids.—A solution of *N*-(5-nitro-2-pyridyl)glycine ethyl ester (2·25 g.) in 4N-hydrochloric acid (12·5 ml.) was heated under reflux for 1 hr. and its pH then adjusted to 4—5 by ammonia. The precipitate was filtered off and recrystallised from hot water, to give *N-(5-nitro-2-pyridyl)glycine hydrate* (1·6 g.), m. p. 205° (decomp.) (Found : C, 39·4; H, 4·1; loss at 100° *in vacuo*, 8·5. $C_7H_7O_4N_3,H_2O$ requires C, 39·1; H, 4·2; H_2O, 8·4%). Its *copper salt* was precipitated from aqueous solution as green needles, decomp. 193—196° (Found : Cu, 14·2. $C_{14}H_{12}O_8N_6Cu$ requires Cu, 13·9%). *N-(5-Nitro-2-pyridyl)-alanine*, similarly prepared, had m. p. 175° (from methanol) (Found : C, 46·0; H, 4·4. $C_8H_9O_4N_3$ requires C, 45·5; H, 4·3%).

N-(5-Nitro-2-pyridyl)sarcosine ethyl ester (2·0 g.) was hydrolysed with potassium hydroxide (0·5 g.) in boiling methanol for 1 hr. The potassium salt which separated was filtered off and the parent *N-(5-nitro-2-pyridyl)sarcosine* crystallised from 10% aqueous alcohol; it had m. p. 146° (decomp.) (Found : C, 45·7; H, 3·9; N, 20·1. $C_8H_9O_4N_3$ requires C, 45·5; H, 4·3; N, 19·9%). Its *copper salt* was obtained as green needles (from water), m. p. 194—195° (Found : Cu, 12·9. $C_{16}H_{16}O_8N_6Cu$ requires Cu, 13·2%).

Amides.—A suspension of *N*-(5-nitro-2-pyridyl)glycine ethyl ester (7·7 g.) in saturated methanolic ammonia (70 ml.) was stirred at 40° for 8 hr. in an autoclave. After evaporation the residual *N-(5-nitro-2-pyridyl)glycine amide* crystallised from hot water and had m. p. 213° (2·15 g.) (Found : C, 43·2; H, 4·2; N, 28·5; O, 24·8. $C_7H_8O_3N_4$ requires C, 42·9; H, 4·1; N, 28·55; O, 24·5%). *N-(5-Nitro-2-pyridyl)sarcosine amide*, similarly prepared, had m. p.

[16] Weiner and Kaye, *J. Org. Chem.*, 1949, **14**, 868.

158° (from methanol) (Found: C, 46·1; H, 4·7; N, 25·7. $C_8H_{10}O_3N_4$ requires C, 45·7; H, 4·8; N, 26·7%).

Hydrazides.—*N*-(5-Nitro-2-pyridyl)glycine ethyl ester (8 g.) was treated with a boiling solution of hydrazine hydrate (3·5 g.) in ethanol (30 ml.) for 4 hr. The N-(*5-nitro-2-pyridyl*)-*glycine hydrazide* which separated recrystallised from boiling water, having m. p. 212° (5·1 g.) (Found: C, 40·2; H, 4·2; N, 32·9. $C_7H_9O_3N_5$ requires C, 39·8; H, 4·3; N, 33·2%). Similar preparations gave N-(*5-nitro-2-pyridyl*)*alanine hydrazide*, m. p. 200° (from methanol) (Found: C, 42·6; H, 5·0; N, 30·4; O, 21·65. $C_8H_{11}O_3N_5$ requires C, 42·7; H, 4·9; N, 31·1; O, 21·3%), and N-(*5-nitro-2-pyridyl*)*sarcosine hydrazide*, m. p. 175° (from ethanol) (Found: C, 42·4; H, 4·8; N, 30·6; O, 21·15. $C_8H_{11}O_3N_5$ requires C, 42·7; H, 4·9; N, 31·1; O, 21·3%).

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