REACTIONS OF N-SUBSTITUTED 2-AMINOPYRIDINES WITH CHLOROACETYL CHLORIDE; FORMATION OF A NEW SERIES OF HETEROCYLIC BETAINES: 1-SUBSTITUTED 4-CHLOROMETHYL-2-OXOPYRIDO[1,2-*a*]PYRIMIDIN-5-IUM-3-OLATES

Hana HULINSKÁ[a], Miloš BUDĚŠÍNSKÝ[b], Jiří HOLUBEK[a], Oluše MATOUŠOVÁ[a], Hana FRYCOVÁ[a] and Miroslav PROTIVA[a]

[a] *Research Institute for Pharmacy and Biochemistry, 130 60 Prague 3 and*
[b] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6*

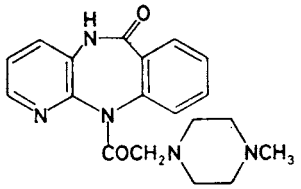
Received July 7, 1988
Accepted August 27, 1988

N-(2-Pyridyl)-2-chloroacetamide reacted with 1-methylpiperazine and gave the expected compound *III*. Attempts at preparing the N-substituted N-(2-pyridyl)-2-chloroacetamides by reactions of N-substituted 2-aminopyridines with chloroacetyl chloride in benzene in the presence of N,N-dimethylacetamide were negative and took an unexpected course. 2-Anilinopyridine and 2-(cyclohexylamino)pyridine afforded compounds which were identified by ^{1}H and ^{13}C NMR spectra as the heterocyclic betaines *IVa* and *IVb*. 2-(1-Butylamino)pyridine, 2-(benzylamino)-pyridine and 2-(2-phenylethylamino)pyridine gave similarly compounds *IVc*—*IVe*. The chloromethyl compounds *IVa*—*IVe* underwent normal substitution reactions with 1-methylpiperazine and gave the methylpiperazino compounds *Va*— *Ve*. Attempts to reduce the betaines with sodium borohydride in aqueous ethanol proceeded in one case as the hydrogenolytic displacement of the chlorine atom with hydrogen (product *VIa*), in another case as ethanolysis (product *VIIb*). Formation of *VIb* by treatment of *IVb* with hydrogen bromide in boiling acetic acid is probably the result of a disproportionation reaction. Compound *III* (dimaleate VÚFB-17 103) was practically equipotent with pirenzepine (*I*) as an anti-ulcer agent in the test of indomethacine-induced gastric lesions in rats but was much weaker in tests for anticholinergic and antisecretory activity.

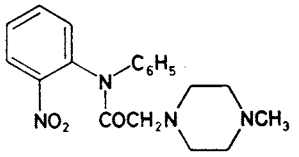
In a recent communication[1] it has been shown that even very simple open models (cf. *II*) of the antiulcer, antisecretory, and antimuscarinic agent pirenzepine (*I*) (ref.[2]) retain at least partly its pharmacological profile. The compounds described[1] were only aniline and diphenylamine derivatives and their molecules lacked the presence of the 2-aminopyridine fragment, typical for pirenzepine (*I*). This fact was the reason of attempts to prepare some N-substituted N-(2-pyridyl)-2-(4-methyl-1-piperazinyl)acetamides; these attempts are being described in the present communication.

N-(2-Pyridyl)-2-chloroacetamide[3] (obtained in moderate yield by reaction of 2-aminopyridine with chloroacetyl chloride in benzene) was reacted with 1-methylpiperazine in boiling chloroform; the substitution reaction afforded *III* which was

isolated and characterized as the bis(hydrogen maleate) (composition confirmed by the mass spectrum). As the N-substituted 2-aminopyridines, the following com-

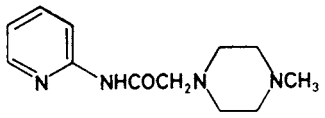


I



II

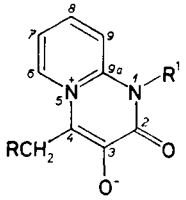
pounds were used and were prepared by literature methods: 2-anilinopyridine[4], 2-(cyclohexylamino)pyridine[5], 2-(1-butylamino)pyridine[6], 2-(benzylamino)pyridine[7], 2-(2-phenylethylamino)pyridine[8]. The attempts to prepare 2-(1-butylamino)pyridine by reaction of 2-bromopyridine with butylamine in ethanol at 160°C in the autoclave according to ref.[9] was unsuccessful (no reaction). The attempt to prepare 2-(benzylamino)pyridine by reaction of 2-bromopyridine with benzylamine at 115°C gave dibenzylamine as the only product to be isolated; it was isolated and characterized as the hydrobromide (ref.[10]) and the identity was confirmed by the mass spectrum; the rather high difference between our melting point (215–218°C) and the literature value (266°C) has to be explained by polymorphism rather than by inhomogeneity of our product.



III

The reaction of 2-anilinopyridine[4] with chloroacetyl chloride was first attempted in boiling chloroform in the presence of triethylamine but it proceeded too slowly; chloroform was then substituted by boiling dioxane. After 6 h the mixture was processed and the obtained dark oil was chromatographed on silica gel. This procedure led to a small amount (18%) of a crystalline substance melting at 193·5–195·5°C. The same reaction was then carried out in benzene at 50°C in the presence of N,N-dimethylacetamide (for the method, cf. ref.[11]) and afforded the mentioned crystalline substance in a reasonable yield. A similar reaction of 2-(cyclohexylamino)pyridine[5] with chloroacetyl chloride (method *A*) afforded a similar crystalline substance melting at 184–187·5°C. Mass spectra (in agreement with analyses) determined for these two compounds the composition $C_{15}H_{11}ClN_2O_2$ and $C_{15}H_{17}ClN_2O_2$, respectively. A detailed analysis of their ^1H NMR spectra (200 MHz) and ^{13}C NMR spectra led to assigning structures *IVa* and *IVb* which represent a new type of heterocyclic betaines (cf. refs[12–14]). The ^1H NMR spectra proved the four spin system of mutually

interacting hydrogens of the pyridinium ring, out of which the CH proton adjacent to the nitrogen appears at extremely low field ($\vartheta \approx 10{\cdot}1$). In *IVb* the N-cyclohexyl was clearly demonstrated; *IVa* contains N-phenyl and both, *IVa* and *IVb* the group CH_2Cl. All the fragments mentioned were confirmed also by the ^{13}C NMR spectra, showing in addition the signals of three sp^2-carbons (at $\delta \approx 180$, 158, and 102, respectively) which do not carry any hydrogen atom. The numbers of C, H, and N atoms, derived from the NMR spectra, are in agreement with the mass spectra and the elemental analyses. For structural assignment of the sp^2-carbons and confirmation of the betaine-type structure, with *IVb* the undecoupled ^{13}C NMR spectrum and spectra with selective decoupling of hydrogens H-1′ (CH of cyclohexyl), H-1″ (CH_2Cl) and H-6 were measured. In this way, most of the coupling constants J(C, H) for *IVb* were obtained. The high value of ^1J(C-6, H-6) = 193·5 Hz, together with the low-field position of the hydrogen H-6 (δ 10·06) confirm the positive charge on nitrogen of the pyridinium ring (for comparison, the analogous ^1J(C, H) in pyridinium chloride is 191 Hz (ref.[15])). The carbonyl carbon C-2 with δ 158·35 was assigned by means of ^3J(C-2, H-1′) = 3·8 Hz, and carbons C-4 (δ 102·29) and C-3 (δ 178·17) using the geminal or vicinal interactions with hydrogens of the CH_2Cl group (^2J = 0·8 Hz, and ^3J = 3·7 Hz, respectively). It is possible to find for their chemical shifts some analogy e.g. with N-methylsydnone (values δ 96·8 and 169·2, respectively) (ref.[16]). 2-(1-Butylamino)pyridine[6], 2-(benzylamino)pyridine[7], and 2-(2-phenylethylamino)pyridine[8] were processed by similar reactions with chloroacetyl chloride (method *A*) and gave the betaines *IVc*–*IVe*. Their structures were confirmed by the mass, IR and ^1H NMR (80 MHz) spectra. Compounds *IVa*–*IVe* are assembled in Table I with the usual experimental data. Their spectra are summarized in Table II. Table III brings the ^{13}C–^1H coupling constants for compound *IVb*.



IV, R = Cl *VI*, R = H

V, R = N⟨⟩NCH$_3$ *VII*, R = OCH$_2$CH$_3$

In formulae *IV*–*VII*: *a*, R^1 =  *b*, R^1 = 

c, R^1=(CH$_2$)$_3$CH$_3$ *d*, R^1= CH$_2$C$_6$H$_5$ *e*, R^1= CH$_2$CH$_2$C$_6$H$_5$

In formulae of serie *a* R^1 should be an aromatic nucleus.

Compounds *IVa–IVe* reacted with 1-methylpiperazine in boiling chloroform (method *B*) and gave the products of substitution reactions *Va–Ve*; the dipole feature of the molecules was retained. The bases were mostly crystalline and partly were transformed to salts. The identity of the products was confirmed by the ^{1}H NMR (80 or 100 MHz) spectra, partly also by mass, UV, IR, and ^{13}C NMR spectra. These compounds are also assembled in Table I and their spectra are included in Table II. It was attempted to reduce *IVa* with sodium borohydride in aqueous ethanol; the obtained mixture was separated by chromatography on silica gel. The first to be eluted was the minor product which was identified as 2-anilinopyridine[4]. The main product was identified by analysis and spectra as *VIa* resulting from a hydrogenolytic displacement of the chlorine atom by hydrogen. A similar reaction of *IVb* gave a complex mixture from which chromatography separated a small amount of crystalline compound $C_{17}H_{22}N_2O_3$ (analysis) which was identified by ^{1}H (200 MHz) and ^{13}C NMR spectra as *VIIb* – product of ethanolysis. In the effort to destroy the dipole character and to impute an external anion (Br^-), *IVb* was treated with hydrogen bromide in boiling acetic acid. The only crystalline product, isolated from the mixture in minute yield, was identified as *VIb* (analysis, mass spectrum and ^{1}H NMR spectrum). It is suggested that we are dealing here with a product of disproportionation similar to that observed in reaction of α-bromodeoxybenzoin with water and resulting in a mixture of deoxybenzoin and benzil[17] (cf. also ref.[18]).

Compound *III* in the form of dimaleate (VÚFB-17 103) was pharmacologically tested as a potential anti-ulcer agent and was compared with pirenzepine (*I*) as the standard. It was administered orally and the doses given were calculated per base. Acute toxicity in mice, LD_{50} = 706 mg/kg (pirenzepine, between 2 500 and 5 000 mg/kg). The anti-ulcer effect in the test of indomethacine-induced gastric lesions in rats; ED_{50} = 28·4 mg/kg (pirenzepine, 32·7 mg/kg). Mydriatic effect in mice was evaluated after the administration of doses of 100 mg/kg (fully active in all animals in the group of 10) and 10 mg/kg (inactive, pirenzepine still fully active). The affinity of the compound to muscarinic receptors in the rat brain using 0·5 nmol l^{-1} [^{3}H]-quinuclidinyl benzilate as the ligand; IC_{50} = 3 759 nmol l^{-1} (for pirenzepine 275·3). The effect on gastric secretion with pyloric ligated rats (for methods, cf. ref.[1]) was also evaluated: a dose of 50 mg/kg showed indication of activity (slight suppression of free hydrochloric acid and of the total acidity) (pirenezpine at 50 mg/kg was significantly active). In conclusion, compound *III* had comparable anti-ulcer activity with that of pirenzepine (*I*) but was much weaker in the other tests indicating therapeutic usefulness (anticholinergic and antisecretory activity).

Compounds *Vb* and *Ve* (both as dimaleates) were screened in several tests indicating neurotropic and psychotropic activity (oral administration, doses calculated per bases) but were found inactive. Acute toxicity in mice; LD_{50} : *Vb* > 700 mg/kg (caused lethality of 37·5% of the animals); *Ve*, >500 mg/kg (no lethality). Oral doses of

TABLE I

4-(Chloromethyl)- and 4-(4-methyl-1-piperazinyl)methyl-2-oxopyrido[1,2-*a*]pyrimidin-5-ium-3-olates

Compound	Method (yield %)	M.p. °C (solvent)	Formula (M.w.)	Calculated/Found			
				% C	% H	% Cl	% N
IVa	*A*[a]	193·5—195·5	$C_{15}H_{11}ClN_2O_2$	62·84	3·87	12·37	9·77
	(62)	(ethanol)	(286·7)	62·82	4·08	12·06	9·74
IVb	*A*	191·5—195	$C_{15}H_{17}ClN_2O_2$	61·54	5·85	12·11	9·57
	(41)	(ethanol–light petroleum)	(292·8)	61·45	5·77	12·47	9·55
IVc	*A*	97—98·5	$C_{13}H_{15}ClN_2O_2$	58·54	5·67	13·29	10·50
	(31)	(toluene–ether)	(266·7)	58·66	5·68	13·37	10·52
IVd	*A*	147·5—151	$C_{16}H_{13}ClN_2O_2$	63·90	4·36	11·79	9·31
	(76)	(ethanol)	(300·7)	63·67	4·33	12·10	9·13
IVe	*A*	184—187	$C_{17}H_{15}ClN_2O_2$	64·87	4·80	11·26	8·90
	(54)	(benzene)	(314·8)	64·92	4·82	11·39	8·92
Va	*B*[a]	155—158	$C_{20}H_{22}N_4O_2$	68·55	6·33	—	15·99
	(57)	(ethanol–light petroleum)	(350·4)	68·60	6·45	—	16·16

Vb	*B*	165—168·5	$C_{20}H_{28}N_4O_2$	67·39	7·92	—	15·72
	(66)	(hexane–ether)	(356·5)	67·42	8·23	—	15·49
Vb-2 M[b]		194·5—197·5 (decomp.)	$C_{28}H_{36}N_4O_{10}$	57·14	6·16	—	9·52
		(ethanol)	(588·6)	57·30	6·13	—	9·48
Vc-2 M[b,c]	*B*	166—170	$C_{26}H_{34}N_4O_{10}$	54·64	6·17	—	9·80
	(81)	(aqueous ethanol–ether)	$+ 0·5\ H_2O$	54·79	6·23	—	9·75
			(571·6)				
Vd	*B*	155·5—158	$C_{21}H_{24}N_4O_2$	69·21	6·64	—	15·37
	(83)	(ethanol–hexane)	(364·5)	68·81	6·65	—	15·67
Vd-2 F[d]		100—103	$C_{29}H_{32}N_4O_{10}$	58·38	5·41	—	9·39
		(ethanol)	(596·6)	58·40	5·30	—	9·79
Vd-1·5 M[e]		146·5—150·5	$C_{27}H_{30}N_4O_8$	60·22	5·61	—	10·40
		(ethanol)	(538·6)	60·20	5·70	—	9·97
Ve	*B*	164—166·5	$C_{22}H_{26}N_4O_2$	69·82	6·92	—	14·80
	(83)	(ethanol)	(378·5)	69·43	6·93	—	14·81
Ve-2 M[b]		157—164	$C_{30}H_{34}N_4O_{10}$	59·01	5·61	—	9·18
		(ethanol)	(610·6)	58·92	5·71	—	8·99

[a] See Experimental; [b] dimaleate; [c] hemihydrate, [d] difumarate; [e] sesquimaleate.

TABLE II

Mass, UV, IR, and NMR spectra of compounds *IVa*—*IVe* and *Va*—*Ve*

Compound	Spectrum	Data
IVa	MS	286 (M^+, $C_{15}H_{11}ClN_2O_2$, 18), 251 (2), 237 (100), 223 (7), 181 (45), 154 (9), 77 (73), 51 (58), 39 (64).
	UV	230 (4·23), 262 (4·28), infl. 318 (3·94), 341 (4·08).
	IR (KBr)	702, 753, 771 (5 and 4 adjacent Ar–H); 1 505, 3 080 (Ar); 1 630 (C═C—O^-); 1 675 (CON).
	^{1}H NMR (200 MHz)	4·86 s, 2 H (CH_2Cl); 7·16 ddd, 1 H (H-9, J(9, 6) = 0·9; J(9, 7) = 1·3; J(9, 8) = 8·7); 7·26 ddd, 1 H (H-7, J(7, 6) = 6·7; J(7, 8) = 7·4; J(7, 9) = 1·3); 7·41—7·65 m, 5 H (C_6H_5); 7·63 ddd, 1 H (H-8, J(8, 6) = 1·3; J(8, 7) = 7·4; J(8, 9) = 8·7); 10·13 ddd. 1 H (H-6, J(6, 7) = 6·7); J(6, 8) = 1·3; J(6, 9) = 0·9).
	^{13}C NMR (50·3 MHz)	46·44 t (CH_2Cl); 102·12 s (C-4); 106·88 d (C-9); 127·01 d (C-2′, C-6′); 129·50 d (C-4′); 130·02 d (C-6, C-3′, C-5′); 131·55 s (C-1′); 133·06 d (C-8); 137·24 s (C-9a); 158·30 s (C-2); 178·92 s (C-3).
IVb	UV	230 (4·17), 264 (4·32), 317 (4·02), 343 (4·12).
	IR (KBr)	759 (4 adjacent Ar–H); 1 511, 3 030, 3 050, 3 100 (Ar); 1 618 (C═C—O^-); 1 656 (CON).
	^{1}H NMR (200 MHz)	1·38—1·83 m, 10 H (5 CH_2 of cyclohexyl); 4·53 tt, 1 H (H-1′, J(1′, 2′ ax) = J(1′, 6′ ax) = 12·4; J(1′, 2′ eq) = J(1′, 6′ eq) = 3·8); 4·84 s, 2 H (CH_2Cl); 7·16 ddd, 1 H (H-7, J(7, 6) = 6·6; J(7, 8) = 7·4; J(7, 9) = 1·3); 7·42 ddd, 1 H (H-9, J(9, 6) = 0·9; J(9, 7) = 1·3; J(9, 8) = 8·8); 7·68 ddd, 1 H (H-8, J(8, 6) = 1·3); J(8, 7) = 7·4; J(8, 9) = 8·8); 10·06 ddd, 1 H (H-6, J(6, 7) = 6·6; J(6, 8) = 1·3; J(6, 9) = 0·9).
	^{13}C NMR (50·3 MHz)	24·92 t (C-4′); 25·67 t (C-3′, C-5′); 29·66 t (C-2′, C-6′); 46·25 t (CH_2Cl); 51·95 d (C-1′); 102·29 s (C-4); 107·11 d (C-9); 115·42 d (C-7); 129·78 d (C-6); 132·15 d (C-8); 136·32 s (C-9a); 158·35 s (C-2); 178·17 s (C-3).
IVc	MS	266 (M^+, $C_{13}H_{15}ClN_2O_2$, 40), 231 (10), 224 (20), 218 (50), 210 (10), 175 (10), 161 (100), 78 (40).
	IR	768 (4 adjacent Ar–H); 1 513, 1 593, 3 050, 3 110 (Ar); 1 630 (C═C—O^-); 1 662 (CON).
IVd	MS	300 (M^+, $C_{16}H_{13}ClN_2O_2$, 20), 299 (10), 251 (40), 118 (2), 91 (100).
	UV	228·5 (4·20), 263 (4·34), infl. 317 (3·99), 342 (4·13).
	IR	700, 760 (5 and 4 adjacent Ar–H); 1 512, 3 060, 3 080 (Ar); 1 610 (C═C—O^-); 1 679 (CON).
	^{1}H NMR (80 MHz)	4·80 s, 2 H (CH_2Cl); 5·18 s, 2 H ($ArCH_2$); 7·10 m, 2 H (H-7, 9); 7·30 s, 5 H (C_6H_5); 7·60 m, 1 H (H-8); 10·00 bd, 1 H (H-6, J = 5·0).

IVe	MS	314 (M^+, $C_{17}H_{15}ClN_2O_2$, 7), 265 (8), 210 (50), 161 (100), 147 (10), 119 (10), 105 (40), 91 (10).
	IR (KBr)	693, 703, 756 (5 and 4 adjacent Ar–H); 1 518, 3 035, 3 048, 3 120 (Ar); 1 588 (C=C—O^-); 1 670 (CON).
	^{1}H NMR (80 MHz)	3·05 t, 2 H ($ArCH_2$, J = 6·0); 4·20 t, 2 H (NCH_2, J = 6·0); 4·80 s, 2 H (CH_2Cl); 6·50—7·20 m, 7 H (C_6H_5 and H-7, 9); 7·40 m, 1 H (H-8); 9·90 bd, 1 H (H-6, J = 5·0).
Va	UV	230 (4·31), 261·3 (4·38), infl. 313 (4·00), 340 (4·13).
	IR	717, 773 (5 and 4 adjacent Ar–H); 1 511, 3 000, 3 040, 3 055 (Ar); 1 590 (C=C—O^-); 1 689 (CON); 2 768, 2 800 (N–CH_2).
	^{1}H NMR (80 MHz)	2·28 s, 3 H (NCH_3); 2·55 m, 4 H ($CH_2N^4CH_2$ of piperazine); 2·72 m, 4 H ($CH_2N^1CH_2$ of piperazine); 3·92 s, 2 H (N–CH_2–C); 7·00—7·80 m, 8 H (C_6H_5 and H-7, 8, 9); 10·20 bd, 1 H (H-6, J = 5·0).
Vb	MS	356 (M^+, $C_{20}H_{28}N_4O_2$, 4), 313 (3), 300 (3), 286 (10), 273 (8), 258 (30), 229 (15), 161 (30), 147 (35), 113 (40), 91 (100).
	UV	219·6 (4·56), 229·8 (4·57), 264·4 (4·53), 310·5 (4·19), 342 (4·16).
	^{1}H NMR (100 MHz)	1·00—2·80 m, 18 H (5 CH_2 of cyclohexyl and 4 CH_2N of piperazine); 2·10 s, 3 H (NCH_3); 3·70 s, 2 H (N–CH_2–C); 4·40 m, 1 H (H-1′); 7·30 m, 1 H (H-8); 7·75 m, 2 H (H-7, 9); 10·02 bd, 1 H (H-6, J = 7·5).
Vc	MS	330 (M^+, $C_{18}H_{26}N_4O_2$, 4); 287 (3), 274 (3), 232 (50); 203 (25), 161 (15), 113 (50), 70 (100).
	^{1}H NMR (100 MHz)	1·00 t, 3 H (terminal CH_3 in butyl); 1·20—2·00 m, 4 H (CH_2CH_2 in positions 2 and 3 of butyl); 2·36 s, 3 H (NCH_3); 2·62 bm, 4 H ($CH_2N^4CH_2$ of piperazine); 2·80 bm, 4 H ($CH_2N^1CH_2$ of piperazine); 3·96 s, 2 H (N–CH_2–C); 4·03 t, 2 H (NCH_2 of butylamino, J = 7·0); 7·00—7·80 (m, 3 H (H-7, 8, 9); 10·20 bd, 1 H (H-6, J = 6·0).
Vd	^{1}H NMR (100 MHz)	2·30 s, 3 H (NCH_3); 2·57 bm, 4 H ($CH_2N^4CH_2$ of piperazine); 2·76 bm, 4 H ($CH_2N^1CH_2$ of piperazine); 2·76 bm, 4 H ($CH_2N^1CH_2$ of piperazine); 3·96 s, 2 H (N–CH_2–C); 5·18 s, 2 H ($ArCH_2N$); 6·98—7·60 m and 7·32 s, 8 H (C_6H_5 and H-7, 8, 9); 10·17 bd, 1 H (H-6, J = 6·0).
	^{13}C NMR (25·142 MHz)	43·25 ($ArCH_2N$); 46·09 (NCH_3); 53·86 (C-2, 6 of piperazine); 55·13 (C-3, 5 of piperazine); 63·64 (N–CH_2–C); 103·45 (C-3); 106·30 (C-9); 116·08 (C-7); 127·58 (C-3, 5 of phenyl), 128·34 (C-4 of phenyl); 129·15 (C-2, 6 of phenyl); 129·90 (C-6); 131·69 (C-8); 134·98 (C-4); 136·32 (C-1 of phenyl); 158·50 (C-9a); 184·87 (C-2).
Ve	MS	378 (M^+, $C_{22}H_{26}N_4O_2$, 2·5); 361 (0·1), 347 (0·4), 335, 334 (2), 322 (2·4), 308 (9), 280 (35), 251 (20), 176 (70), 113 (59), 70 (100).
	UV	230 (4·39), 264 (4·42), 312 (4·10), 340 (4·12).
	IR	700, 752, 760 (5 and 4 adjacent Ar–H); 1 520, 3 120 (Ar); 1 590 (C=C—O^-); 1 680 (CON); 2 800 (N–CH_3).
	^{1}H NMR (100 MHz)	2·23 s, 3 H (NCH_3); 2·60 bm, 4 H ($CH_2N^4CH_2$ of piperazine); 2·76 bm, 4 H ($CH_2N^1CH_2$ of piperazine); 3·06 t, 2 H ($ArCH_2$, J = 7·0); 3·94 s, 2 H (N–CH_2–C); 4·20 t, 2 H ($CONCH_2$, J = 7·0); 6·68 bd, 1 H (H-9, J = 8·0); 7·00—7·50 m, 7 H (C_6H_5 and H-7, 8); 10·10 bd, 1 H (H-6, J = 6·0).

50 mg/kg of both compounds lack protective activity towards the toxicity of adrenaline and noradrenaline administered intravenously in mice, towards the ulcerogenic effects of reserpine in rats, do not influence apomorphine-induced stereotypies and agitation in rats; in oral doses of 100 mg/kg they do not influence the convulsant action of pentetrazole in mice, in oral doses of 10 mg/kg they have not protective activity in the test of histamine aerosol in guinea pigs.

The antimicrobial effects of the compounds in the tests in vitro are negligible (microorganisms and the minimum inhibitory concentrations in μg/ml – unless they exceed 128 and 50 μg/ml, respectively): *Streptococcus pyogenes*, *III* 128; *Streptococcus faecalis*, *III* 128; *Staphylococcus aureus*, *III* 128, *Ve* 128; *Pseudomonas aeruginosa*, *III* 128; *Escherichia coli*, *III* 128; *Proteus vulgaris*, *III* 128; *Trichophyton mentagrophytes*, *III* 50; *Vb* 50, *Ve* 50.

EXPERIMENTAL

The melting points of analytical samples were determined in the Kofler block and were not corrected. The samples were dried in vacuo of about 60 Pa at room temperature or at a suitably elevated temperature. The UV spectra (in methanol, λ_{max} in nm ($\log \varepsilon$)) were recorded on

TABLE III

^{13}C–^{1}H Coupling constants in the compound *IVb* (C-1″ and H-1″ relates to CH_2Cl)

Carbon	^1J	^2J	^3J
C-2	—	—	J(C-2, H-1′) = 3·8
C-3	—	—	J(C-3, H-1″) = 3·7 (2×)
C-4	—	J(C-4, H-1″) ≈ 0·8 (2×)	J(C-4, H-6) ≈ 0
C-6	J(C-6, H-6) = 193·5	J(C-6, H-7) ≈ 6·5	J(C-6, H-8) ≈ 7·0
C-7	J(C-7, H-7) = 170·0	J(C-7, H-6) ≈ 4·0 J(C-7, H-8) ≈ 2·0	J(C-7, H-9) = 7·5
C-8	J(C-8, H-8) = 168·0	J(C-8, H-7) < 1 J(C-8, H-9) < 1	J(C-8, H-6) = 6·5
C-9	J(C-9, H-9) = 171·0	J(C-9, H-8) < 1	J(C-9, H-7) = 8·0
C-9a	—	J(C-9a, H-9) < 1	J(C-9a, H-6)[a] J(C-9a, H-8) = 8·5 J(C-9a, H-1″)[a]
C-1′	J(C-1′, H-1′) ≈ 137	[a]	[a]
C-2′ (C-6′)	J(C-2′, H-2′) ≈ 128 (2×)	[a]	[a]
C-3′ (C-5′)	J(C-3′, H-3′) ≈ 128	[a]	[a]
C-4′	J(C-4′, H-4′) = 128	[a]	[a]
C-1″	J(C-1″, H-1″) = 151·8 (2×)	—	—

[a] The values could not be determined.

a Unicam SP 8 000 spectrophotometer, the IR spectra (mostly in Nujol, ν in cm^{-1}) with a Perkin–Elmer 298 spectrophotometer, the NMR spectra (in $CDCl_3$ unless stated otherwise, δ in ppm, J in Hz) (i) with a CW-NMR spectrometer Tesla BS 487 C (^1H at 80 MHz), (ii) with an FT-NMR spectrometer Varian XL-200 (^1H at 200 MHz; ^{13}C at 50·3 MHz), (iii) with an FT-NMR spectrometer Tesla BS 567A (^1H NMR at 100 MHz; ^{13}C NMR at 25·142 MHz), and the mass spectra (m/z, %) with Varian MAT 44S and MCH 1 320 spectrometers. The homogeneity of the substances and composition of the mixtures were checked by thin-layer chromatography (TLC) on silica gel (Silufol). The extracts were processed by drying with $MgSO_4$ or K_2CO_3 and evaporation under reduced pressure on a rotating evaporator.

N-(2-Pyridyl)-2-(4-methyl-1-piperazinyl)acetamide (*III*)

A mixture of 8·5 g N-(2-pyridyl)-2-chloroacetamide[3], 10 g 1-methylpiperazine and 50 ml chloroform was stirred for 4h at 50°C. After cooling 100 ml water were added, the organic layer was separated, dried and evaporated; 6·7 g (57%) of oily *III*.

Bis(*hydrogen maleate*), m.p. 150—153·5°C (ethanol). Mass spectrum: 234 (M^+, $C_{12}H_{18}N_4O$, 12), 191 (2·4), 164 (2·8), 121 (4·4), 113 (72·8), 98 (14·4), 70 (100), 42 (80). For $C_{20}H_{26}N_4O_9$ (466·4) calculated: 51·50% C, 5·62% H, 12·01% N; found: 51·37% C, 5·58% H, 11·66% N.

Dibenzylamine

A mixture of 17·1 g 2-bromopyridine and 29 g benzylamine was stirred for 7 h at 115°C. The solidified mixture was dissolved in boiling ethanol and the solution was allowed to crystallize; 9·0 g (45%) of dibenzylamine hydrobromide, m.p. 215—218°C (ethanol). Mass spectrum: 198 $(M + H)^+$, 197 (M^+, $C_{14}H_{15}N$, 76), 196 (76), 120 (6), 118 (5), 106 (59), 91 (100). For $C_{14}H_{16}$.BrN (278·2) calculted: 60·45% C, 5·80% H, 28·72% Br, 5·03% N; found: 60·01% C, 5·83% H, 28·97% Br, 5·01% N. Ref.[10], m.p. 266°C.

4-(Chloromethyl)-2-oxo-1-phenylpyrido[1,2-*a*]pyrimidin-5-ium-3-olate (*IVa*) (Method *A*)

A stirred solution of 4·0 g 2-anilinopyridine[4] in 20 ml benzene was treated with 4·5 g N,N-dimethylacetamide and then with 5·84 g chloroacetyl chloride, added dropwise over 10 min. The mixture was stirred for 1 h at 50°C, evaporated in vacuo, the residue was dissolved in 200 ml chloroform and the solution was washed with water. Processing gave 7·0 g of semisolid residue which was crystallized from ethanol; 4·15 g (62%), m.p. 193·5—195·5°C (ethanol). For analysis, cf. Table I; for spectra, cf. Table II.

4-(4-Methyl-1-piperazinyl)methyl-2-oxo-1-phenylpyrido-
[1,2-*a*]pyrimidin-5-ium-3-olate (*Va*) (Method *B*)

A mixture of 1·0 g *IVa*, 1·05 g 1-methylpiperazine, and 5 ml chloroform was stirred for 2·5 h at 50°C. It was then diluted with 75 ml chloroform and washed with water. Processing gave 0·7 g (57%) of *Va*, m.p. 155—158°C (ethanol–light petroleum). For analysis and spectra, cf. Tables I and II.

4-Methyl-2-oxo-1-phenylpyrido[1,2-*a*]pyrimidin-5-ium-3-olate (*VIa*)

A suspension of 1·5 g *IVa* in 75 ml ethanol was stirred and treated at 50°C with a solution of 0·1 g $NaBH_4$ in 10 ml ethanol containing 4 drops of 25% NaOH. The mixture was stirred for 9 h at 50°C and refluxed for 2 h. The addition of a solution of 0·1 g $NaBH_4$ in 5 ml ethanol was repeated three times and the mixture was stirred for 24 h at 50°C. TLC showed that the starting

IVa disappeared from the mixture. It was decomposed with 10% hydrochloric acid and the mixture was neutralized with 10% NaOH. It was extracted with chloroform, the extract was processed and the residue was chromatographed on 20 g silica gel. Elution with chloroform gave first a small amount (0·25 g) of 2-anilinopyridine, m.p. 106·5—108°C. The analysis is in agreement with the composition $C_{11}H_{10}N_2$ and the ^{1}H NMR spectrum (80 MHz) appears as a unresolved multiplet at δ 6·50—8·30. Ref.[4], m.p. 100—102°C.

The product eluted afterwards was characterized as *VIa* (0·35 g), needles melting at 224 to 226·5°C (chloroform). Mass spectrum: 252 (M^+, $C_{15}H_{12}N_2O_2$, 100), 237 (85), 181 (20), 78 (25), 77 (37), 51 (32). UV spectrum: 228 (4·36), 260 (4·47), 312 (4·00), 339 (4·05). IR spectrum (KBr): 690, 713, 750, 768 (5 and 4 adjacent Ar—H); 1 509, 1 606, 3 070, 3 100 (Ar); 1 628 (C=C—O$^-$); 1 680 (CON). For $C_{15}H_{12}N_2O_2$ (252·3) calculated: 71·41% C, 4·80% H, 11·11% N; found: 71·09% C, 4·76% H, 10·78% N.

1-Cyclohexyl-4-methyl-2-oxopyrido[1,2-*a*]pyrimidin-5-ium-3-olate (*VIb*)

A mixture of 2·0 g *IVb* and 21·8 g 36% HBr in acetic acid was heated for 8·5 h to 110°C. After cooling the yellow crystals (1·7 g) were filtered and chromatographed on silica gel. Elution with chloroform gave a small homogeneous fraction (0·50 g) which crystallized from a mixture of chloroform and light petroleum, m.p. 150—151·5°C and was identified as *VIb*. Mass spectrum: 258 (M^+, $C_{15}H_{18}N_2O_2$, 40), 241 (1), 203 (1), 176 (90), 161 (100), 134 (10), 133 (10), 106 (20), 105 (20), 91 (20), 78 (30). ^{1}H NMR spectrum (100 MHz): 1·20—2·40 m, 10 H (5 CH_2 of cyclohexyl); 2·65 s, 3 H (CH_3); 4·58 m, 1 H (H-1'); 7·00—7·70 m, 3 H (H-7, 8, 9); 10·22 bd, 1 H (H-6, J = 6·0). For $C_{15}H_{18}N_2O_2$ (258·3) calculated: 69·75% C, 7·02% H, 10·84% N; found: 69·75% C, 7·21% H, 10·75% N.

1-Cyclohexyl-4-ethoxymethyl-2-oxopyrido[1,2-*a*]pyrimidin-5-ium-3-olate (*VIIb*)

A solution of 1·0 g *IVb* in 50 ml ethanol was treated with a solution of 106 mg $NaBH_4$ and 200 mg NaOH in a mixture of 5 ml ethanol and 5 ml water. The mixture was stirred for 6 h at 65°C and refluxed for 20 min, decomposed with 10% hydrochloric acid, neutralized with 10% NaOH, ethanol was evaporated in vacuo and the aqueous residue was extracted with toluene. Processing of the extract and chromatography of the residue on 15 g silica gel gave 100 mg of *VIIb*, m.p. 127·5—130·5°C. ^{1}H NMR spectrum (200 MHz): 1·32 t, 3 H (CH_3, $J(CH_3, CH_2)$ = = 7·0); 1·35—2·25 m, 10 H (5 CH_2 of cyclohexyl); 3·70 q, 2 H (OCH_2 of ethoxyl, $J(CH_2, CH_3)$ = = 7·0); 4·52 tt, 1 H (H-1', J(1',2' ax) = J(1', 6' ax) = 12·4; J(1', 2' eq) = J(1', 6' eq) = 3·8); 4·79 s, 2 H (C–CH_2O); 7·13 ddd, 1 H (H-7, J(7, 6) = 6·7; J(7, 8) = 7·4; J(7, 9) = 1·3); 7·34 ddd, 1 H (H-9, J(9, 8) = 8·8; J(9, 7) = 1·3; J(9, 6) = 0·9); 7·58 ddd, 1 H (H-8, J(8, 6) = 1·3; J(8, 7) = = 7·4; J(8, 9) = 8·8); 10·16 ddd, 1 H (H-6, J(6, 7) = 6·7; J(6, 8) = 1·3; J(6, 9) = 0·9). ^{13}C NMR spectrum (50·3 MHz): 15·08 q (CH_3); 25·08 t (C-4'); 25·82 t (C-3', C-5'); 29·86 t (C-2', C-6'); 51·92 d (C-1'); 66·86 t (OCH_2 of ethoxyl); 73·20 t (C–CH_2O); 102·20 s (C-4); 106·90 d (C-9); 115·24 d (C-7); 129·61 d (C-6); 131·04 d (C-8); 135·84 d (C-9a); 158·25 s (C-2); 184·32 s (C-3). For $C_{17}H_{22}N_2O_3$ (302·4) calculated: 67·52% C, 7·33% H, 9·27% N; found: 67·07% C, 7·51% H, 8·92% N.

The authors wish to thank their colleagues at the Research Institute for Pharmacy and Biochemistry for their contributions to the present study: Drs I. Koruna and M. Ryska (mass spectra); Dr E. Svátek, Mrs A. Hrádková, and Mrs Z. Janová (UV *and* IR *spectra*); *Mrs J. Komancová, Mrs V. Šmídová and Mrs A. Svatošová (elemental analyses); Drs J. Metyš and M. Valchář, Mrs S. Schubertová, Mrs Z. Paduanová, Mrs L. Horáková, and Mrs E. Šulcová (pharmacology and biochemical pharmacology); Dr V. Holá (microbiological screening).*

REFERENCES

1. Hulinská H., Polívka Z., Jílek J., Šindelář K., Holubek J., Svátek E., Matoušová O., Budějšínský M., Frycová H., Protiva M.: Collect. Czech. Chem. Commun. *53*, 1820 (1988).
2. Kitagawa H., Kurahashi K., Fuhiwara M., Kohei H.: Arzneim.-Forsch. *28*, 2122 (1978).
3. Hach V., Protiva M.: Chem. Listy *47*, 729 (1953); Collect. Czech. Chem. Commun. *18*, 684 (1953).
4. Ito Y., Hamada Y., Hirota M.: Chem. Pharm. Bull. *20*, 2678 (1972).
5. Bergstrom F. W., Sturz H. G., Tracy H. W.: J. Org. Chem. *11*, 239 (1946); Chem. Abstr. *40*, 4720 (1946).
6. Vajda T., Kovács K.: Rec. Trav. Chim. Pays-Bas *80*, 47 (1961); Chem. Abstr. *55*, 16547 (1961).
7. Kemal O., Reese C. B.: J. Chem. Soc., Perkin Trans. 1 *1981*, 1569.
8. Biniecki S., Modrzejewska W.: Acta Polon. Pharm. *19*, 103 (1962); Chem. Abstr. *59*, 1613 (1963).
9. Bernstein J., Stearns B., Dexter M., Lott W. A.: J. Am. Chem. Soc. *69*, 1147 (1947).
10. Limpricht H.: Justus Liebigs Ann. Chem. *144*, 305 (1867).
11. Weisz I., Ötvös L.: Arch. Pharm. *318*, 766 (1985).
12. Mosby W. L. in: *The Chemistry of Heterocyclic Compounds* (A. Weissberger, Ed.), Vol. 15, pt. 2, 1141. Interscience Publishers, New York 1961.
13. Ramsden C. A.: Adv. Heterocycl. Chem. *26*, 1 (1980).
14. Ollis W. D., Stanforth S. P., Ramsden C. A.: Tetrahedron *41*, 2239 (1985).
15. Seel H., Günther H.: J. Am. Chem. Soc. *102*, 7051 (1980).
16. Kalinowski H.-O., Berger S., Braun S. in: ^{13}C *NMR-Spektroskopie*, p. 361. Thieme, Stuttgart 1984.
17. Limpricht H.: Justus Liebigs Ann. Chem. *155*, 68 (1870).
18. Jílek J. O., Svátek E., Metyšová J., Pomykáček J., Protiva M.: Collect. Czech. Chem. Commun. *32*, 3186 (1967).

Translated by the author (M.P.).