standing in the dark at room temp for 18 hr. After extn with Et_2O the aq soln was adjusted to pH 7.5 by addn of 4 *N* HCl followed by NaCl to ppt crude Na salt of **96**. After several recrystn from H_2O the Na salt was dissolved in warm aq EtOH and crude **96** pptd by addn of 4 *N* HCl until pH 3.

Method 4G.—A mixt of the appropriate 4-R_1-3-R_2NH-5-sulfamylbenzoic acid (0.8 g of **97** and 0.5 g of **98**, resp), AcOH (20 20 ml for **99** and 5 ml for **100**, resp), and H_2O_2 (30% aq soln; 1.5 ml for **99** and 2.5 ml for **100**, resp) was stirred at room temp for several days, after which the crude reaction product was isolated by filtration.

Method 4H.—47 was reductive alkylated using the appropriate aldehyde as described[1] for the parent 4-Cl compd.

Method 4I.—A mixt of **47** (3 g), EtI (20 ml), and abs EtOH (20 ml) was refluxed for 5 days. After evapn *in vacuo* the residue was triturated with EtOH, and the solid contg the Et ester of **106** was collected by filtration. This crude ester was sapond and worked up following method 4C to yield crude **106**.

Method 4J.—Method 4C was followed except that the sapon was performed for 18 hr at room temp in the dark. For **110**, **70** was used as dehydrobromination occurred simultaneously.

Method 4K.—A mixt of the Na salt of **47** (4.95 g dried *in vacuo* at 115°; prepd by heating **47** in the calcd amt of 2.5 *N* NaOH followed by cooling and isolating of the pptd salt), abs MeOH (75 ml), and furfural (2.16 g) was refluxed for 5 hr. After cooling, $NaBH_4$ (2.5 g) was added in portions at 0–5° with stirring. After standing for 18 hr the mixt was evapd *in vacuo*, and the residue was redissolved in H_2O (45 ml). Adjusting the pH to 7.5 pptd crude Na salt of **114** (1.4 g), which was recrystd from H_2O. From a 0.5% soln of this Na salt in warm H_2O crude **114** pptd after addn of AcOH.

Method 4L.—Method 3B was followed, and the crude ester was sapond according to method 4C.

Method 4M.—An aq soln of **117** (0.5 g) was hydrogenated at pH 11 using PtO_2 catalyst (0.025 g). After the H_2 uptake had ceased, the catalyst was removed, and crude **118** was pptd by addn of 4 *N* HCl until pH 1.5.

Method 4N.—111 (1 g) was added to HSO_3Cl (5 ml) with stirring. The mixt was allowed to warm to 50°. After stirring for 10 min it was poured on ice to ppt crude **111** 4′-sulfochloride. The crude sulfochloride was added to concd aq NH_3 with stirring. Then the reaction mixt was heated on a steam bath to remove most of the excess of NH_3. After cooling the pH was adjusted to 2.5 to ppt crude **119**.

5-*n*-Butylsulfamyl-4-chloro-3-nitrobenzoic Acid (**120**).—To a cooled mixt of *n*-$BuNH_2$ (2.2 g) and 1 *N* NaOH (60 ml) 4-chloro-5-chlorosulfonyl-3-nitrobenzoic acid[1] was added in portions at −4 to −2° with stirring. The stirring was continued while the mixt was allowed to reach room temp. Filtration and addn of 1 *N* HCl (50 ml) to the filtrate provided sep of oily **120** crystg on standing for 16 hr. It was twice recrystd from aq EtOH to yield **120** (4.1 g), mp 196–198°. *Anal.* ($C_{11}H_{13}ClN_2O_8S$) C, H, N.

5-*n*-Butylsulfamyl-3-nitro-4-phenoxybenzoic Acid (**121**).—Method 1N was followed using **120** as starting material except that the Na salt failed to sep. Therefore crude **121** was pptd by acidification of the cooled reaction mixt. It was recrystd from aq EtOH and EtOH to yield **121** (49%), mp 191–192°. *Anal.* ($C_{17}H_{18}N_2O_7S$) C, H, N.

3-Amino-5-*n*-butylsulfamyl-4-phenoxybenzoic Acid (**122**).—Method 2G was followed using **121** as starting material. It was twice recrystd from aq EtOH to yield **122** (61%): mp 188–189°; nmr [$(CD_3)_2SO$, TMS], δ 0.8 (t, J = 6.3 H, 3 H, CH_3), 0.9–1.6 (m, 4 H, CH_2), 2.85 (m, 2 H, CH_2N), 5.20 (bs, 2 H, NH_2C), 6.8–7.5 (m, 5 H, C_6H_5O), 7.67 (s, 2 H, arom H), ~7.0 (b line, 2 H, COOH, $HNSO_2$). *Anal.* ($C_{17}H_{20}N_2O_5S\cdot0.5H_2O$)C*, H, N, H_2O.

Acknowledgment.—The author is greatly indebted to the staff of the Department of Pharmacology for the diuretic screening of the compounds described in this paper, and to N. Rastrup Andersen for nmr investigations.

Effect of 2-Anilinopyridines on Protein Synthesis

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A series of 2-(R-substituted anilino)pyridines has been synthesized by the interaction of 2-halopyridines with R-substituted anilines at elevated temperatures. These compounds have been assayed for their effect on protein synthesis. The 2-(4-bromo, 4-chloro, and 3,4-dichloroanilino)-6-methylpyridines were found to be as effective as chloramphenicol and *p*-fluorophenylalanine in their ability to block utilization of phenylalanine in a cell-free system. A structure–activity relationship was studied but no correlation between protein synthesis inhibition and *in vitro* microbial activity could be established.

During the course of studies with a cell-free system prepared from *Escherichia coli*, 2-(*m*-chloroanilino)-6-methylpyridine was found to inhibit the incorporation of phenylalanine into protein. Since protein synthesis inhibition is recognized as one of the major biochemical modes of action of a number of prominent antibiotics[1] (the tetracyclines, the erythromycins, chloramphenicol, lincomycin, novobiocin, etc.), a number of 2-anilinopyridines and related substances were prepared and assayed for their inhibitory effects on bacterial protein synthesis under cell-free conditions. The compounds were further evaluated for their ability to inhibit the growth of cultures of *E. coli* and *Staphylococcus aureus*. The results of these screens are recorded in Table I.

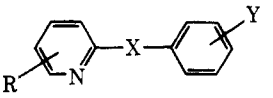
Selected members of the series (**2**, **3**, **5**, **8**, **10**, **11**, **18**, **26**, **27**) were screened *in vivo* for activity against *S. aureus*. These were found to be uniformly inactive.

Chemistry.—The 2-anilinopyridines were synthesized by cautiously heating to 150–170° a mixture of R-substituted-2-chloro- or -2-bromopyridine and 2 molar equiv of Y-substituted aniline. Alternatively, the second equivalent of Y-substituted aniline could be replaced with dimethylaniline. The reaction failed with *o*- and *p*-nitroaniline, and the derivatives **15** and **17**

(1) The following references are among many dealing with this topic: (a) E. F. Gale, *Pharmacol. Rev.*, **15**, 481 (1963); (b) D. S. Feingold, *N. Engl. J. Med.*, **206**, 900, 957 (1963); (c) I. H. Goldberg, *Amer. J. Med.*, **39**, 722 (1965); (d) T. J. Franklin, in "Biochemical Studies of Antimicrobial Drugs," Cambridge University Press, New York, N. Y., 1966, p 192 f; (e) F. E. Hahn, *Int. Congr. Microbiol., 9th, 1966*, 41 (1966); (f) R. E. Monro and D. Vazquez, *J. Mol. Biol.*, **28**, 161 (1967); (g) B. Weisblum and J. Davies, *Bacteriol. Rev.*, **32**, 493 (1968).

TABLE I

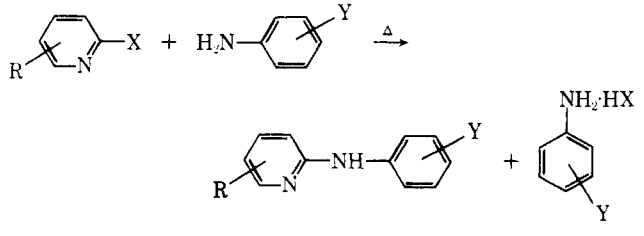
A COMPARISON OF THE EFFECTS OF 2-ANILINOPYRIDINES ON *in Vitro* PROTEIN SYNTHESIS AND MICROBIAL GROWTH



No.	R	X	Y	Mp, °C or bp, °C (Torr)	Analysis[c]	—Microbial activity[a]— *E. coli*	*S. aureus*	Protein synthesis inhibition[b]
Chloramphenicol						1–2	6.25	100
Puromycin						7.8	7.8	114
p-Fluorophenylalanine						>250		105
1	6-CH_3	NH	*m*-Cl	137–139 (0.03)	C, H, N	250	125	87 ± 1
2	6-CH_3	NH	*m*-F	60–61	C, H, N	125	62.5	70
3	6-CH_3	NH	*m*-Br	39–42	C, H, N	31.3	31.3	73
4	6-CH_3	NH	*m*-I	63–65	C, H, N	125	7.8	52 ± 3
5	6-CH_3	NH	*m*-CF_3	56–58	N, F	50	50	68 ± 3
5a[d]				101–102	C, H, N	>100	>100	8
6	6-CH_3	NH	*m*-NO_2	134–136	C, H, N	125	15.6	± 3[e]
7	6-CH_3	NH	*m*-COOH	227–229	C, H, N	>125	>125	4 ± 2
8[f]	6-CH_3	NH	H	111–144 (1)		250	250	49 ± 3
9	6-CH_3	NH	3,5-Cl_2	124–126	C, H, N	>250	62.5	27 ± 6
10	6-CH_3	NH	*p*-Cl	85–86	C, H, N	31.3	62.5	104 ± 0.6
11	6-CH_3	NH	*p*-Br	80–82	C, H, N	25	25	104 ± 0.5
12	6-CH_3	NH	*p*-F	79–81	N, F	100	200	82 ± 2
13	6-CH_3	NH	*p*-CH_3	113–115 (0.02)	C, H, N	50	100	97 ± 1
14	6-CH_3	NH	*p*-OH	197–199	C, H, N	500	250	2
15	6-CH_3	NH	*p*-NO_2	177–179	C, H, N	>125	62.5	±4[e]
16	6-CH_3	NH	*o*-Cl	113–115 (0.03)	C, H, N	100	100	10
17	6-CH_3	NH	*o*-NO_2	91–93	C, H, N	>125	125	0
18	6-CH_3	NH	3,4-Cl_2	90–91	N, Cl	62.5	3.9	108 ± 0.7
19	6-CH_3	NH	3-CF_3-4-Cl	101–102	C, H, N	31.3	0.5	48 ± 3
20	5-CH_3	NH	*p*-Cl	129–131	C, H, N	>125	125	0
21	4-CH_3	NH	*p*-Cl	109–111	C, H, N	31.3	31.3	31 ± 2
22	3-CH_3	NH	*p*-Cl	80–81	C, H, N	31.3	31.3	50 ± 2
23	4,6-di-CH_3	NH	*p*-Cl	81–83	C, H, N	31.3	31.3	53
24	6-C_2H_5	NH	*p*-Cl	129–131 (0.02)	C, H, N	31.3	15.6	73
25[g]	H	NH	*p*-Cl	116–117		100	>100	54 ± 6
26	H	NH	*m*-CF_3	88–89	N, F[h]	100	50	91 ± 1
26a[d]				135–137	N, F	500	125	2
27	H	NH	*m*-Cl	92–93	N, Cl	100	50	87 ± 2
28	H	NH	*m*-F	60–63	N, F	125	62.5	74
29	H	NH	2,3-di-CH_3	113–114	C, H, N	125	62.5	5
29a[d]				108–109	C, H, N	>500	500	1
30[i]	H	NH	H	107–110		>100	>100	42 ± 7
31	5-OH, 6-CH_3	NH	*m*-CF_3	185–186	N, F	250	31.3	5 ± 2
32	5-OH, 6-CH_3	NH	*p*-Cl	224–225	N, Cl	125	31.3	0
33	6-CH_2OH	NH	H	126–127[j]	C, H, N	500	250	6
34	6-CH_2OH	NH	*p*-Cl	109–111	C, H, N	>125	125	83 ± 3
35[k]	6-CH_3	$NHCH_2$	*p*-Cl	84–86		62.5	62.5	27 ± 3
36[i]	H	C=O	*p*-Cl	60–63		250	125	10

[a] Minimum inhibitory concentration (MIC) in μg/ml. Compounds were considered active if their MIC was 31.3 μg/ml or less. [b] Relative % inhibition ±S.E. with chloramphenicol = 100%. [c] Except where noted, analytical values were within ±0.4% of theory. [d] Compounds labeled a are the corresponding pyridine *N*-oxides. [e] + values represent stimulation of protein synthesis. [f] U. S. Patent 2,784,195. [g] U. S. Patent 2,802,008. [h] Calcd F, 23.93; found F, 24.44. [i] Aldrich Chemical Co. [j] HCl salt. [k] 6-(*p*-Chlorobenzylamino)-2-picoline, G. Greczy, *Magy. Kem. Foly.*, **62**, 162 (1956).

were produced as a mixture by direct nitration with $Cu(NO_2)_2$ in HOAc.[2] The *m*-nitro isomer **6** could be prepared in the conventional manner but only in low yield. The halo pyridines were generally known materials and were synthesized by standard procedures.[3] The pyridine *N*-oxides could be synthesized by combining the appropriate halopyridine *N*-oxide and aniline, but were more efficiently produced by oxidizing the parent 2-anilinopyridine with 30% H_2O_2 in HOAc.[4]

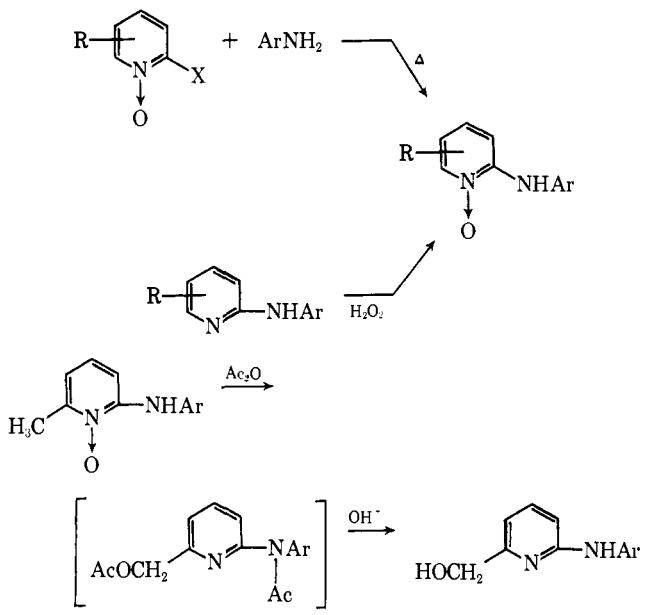


The 6-hydroxymethyl compounds **33** and **34** were produced by rearrangement of the pyridine *N*-oxides with acetic anhydride followed by base hydrolysis of

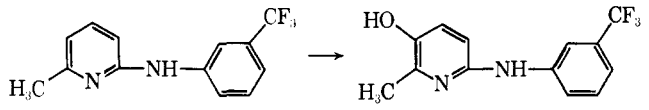
(2) We are grateful to Professor C. F. Koelsch for suggesting this procedure.

(3) C. F. H. Allen and J. R. Thirtle, "Organic Synthesis," Collect. Vol. 3, Wiley, New York, N. Y., 1955, p 136.

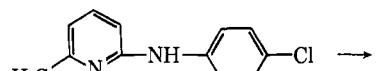
(4) D. M. Bailey, U. S. Patent 3,450,707 (1969). See also L. Pentimalli, *Gazz. Chim. Ital.*, **94**, 158 (1964).



the intermediate acyl derivatives.[5] In an attempt to produce hydroxymethyl and/or ring hydroxylated derivatives, **5** and **10** were incubated with *Aspergillus alliaceus* (ATCC 10060).[6] From these fermentations were isolated **31** and **32**, respectively, whose structures were deduced from elemental analyses and spectral measurements. In the case of **31** for example, C, H, and N analysis confirmed the introduction of 1 oxygen atom. Treatment with Ac_2O produced a mono-*O*-acetate. The 100-Hz nmr spectrum of **31** showed aromatic proton signals at 8.11 ppm (broad singlet; isolated proton), 6.98–7.76 ppm (ABC system), and an ortho coupled pair of protons at 7.1 and 6.6 ppm, $J \sim 8.5$ Hz. No meta coupling could be detected for the ortho coupled pair of protons, thus making it unlikely



5 **31**



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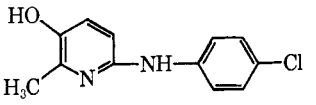

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that the hydroxyl group had entered the benzene ring. Confirmation that the ABC system and the isolated proton were in the same ring was obtained by double resonance experiments in which irradiation of the 8.11-ppm signal produced a sharpening of the ABC system without affecting the ortho coupled pair. Assignment of the hydroxyl function to position 5 rather than 3 was made on the basis of the ir spectrum at 0.001 *M* in CCl_4. A sharp band at 3620 cm^{-1} for the free ArOH group and absorptions at 2450 and 3426 cm^{-1} (NH) indicated that there was no intramolecular hydrogen bonding as would be expected for the 3-OH derivative.

Discussion

It is apparent from the data in Table I that the 2-anilino-6-methylpyridines **10**, **11**, **13**, and **18** are equivalent to chloramphenicol and *p*-fluorophenylalanine (*p*-FPA) in their ability to inhibit the utilization of phenylalanine. The positions for substitution in the benzene ring giving maximum protein synthesis inhibition appear to be para or meta,para. In the case of Cl the rank is meta,para (**18**) ~ para (**10**) > meta (**1**) ≫ ortho (**16**). In the *p*-chloroanilino series, removal of the CH_3 group from the pyridine ring (**25**) or shifting it to other positions on the ring (**20**, **21**, **22**) resulted in reduced activity. The introduction of a second CH_3 group to give **23** or **24** or alteration of the nitrogen bridge (**35**, **36**) was also deleterious. The *N*-oxides **5a**, **26a**, and **29a** were inactive.

Little correlation could be made between the ability of compounds to block protein synthesis and their inhibitory effect on the *in vitro* growth of the two test organisms. Thus, although **10**, **11**, **13**, and **18** showed approximately the same potency in inhibiting protein synthesis, their effect on bacterial growth varied widely. On the other hand, **19** which only poorly blocked protein synthesis was quite effective in inhibiting the *in vitro* growth of *S. aureus*. The ineffectiveness of certain compounds against the intact bacterium may be due to an inability of the drug to enter the cell or to reach the site(s) of action within the cell. That metabolism to less effective products may play a role is suggested by the lack of protein synthesis inhibition activity in the fermentation products **31** and **32**. Although *p*-FPA effectively blocked the use of phenylalanine for protein synthesis in the cell-free system, it was inactive in the microbial assays. An effect of *p*-FPA on microbial growth might be blocked by tyrosine and phenylalanine in the broths as these amino acids can competitively antagonize the growth inhibitory properties of *p*-FPA.[7] Unlike *p*-FPA, however, some of the more active protein synthesis inhibiting anilinopyridines were able to elicit antibacterial activity when assayed under the same conditions.

That a direct complexing of phenylalanine and the anilinopyridines was probably not responsible for the latter's activity was demonstrated by an investigation[8] of the uv and tlc behavior of equimolar mixtures of phenylalanine and **10**. The uv spectrum of this mixture in the pH range of 7.5–10.0 was an exact summation of each compound alone and the tlc behavior in five systems excluded the formation of a third component.

It is apparent that interference in the utilization of phenylalanine may not be the only mechanism of action. More extensive mechanistic studies under way suggest that the use of several other amino acids is affected. The degree of inhibition noted for these amino acids, however, was considerably less than that

(5) S. Furukawa, *Yakugaku Zasshi*, **59**, 429 (1959).

(6) For examples of microbiological hydroxylation effected with other *Aspergillus* species see D. Rosi, G. Peruzzotti, E. W. Dennis, D. A. Berberian, H. Freele, B. F. Tullar, and S. Archer, *J. Med. Chem.*, **10**, 867 (1967), and D. Rosi, T. Lewis, R. Lorenz, H. Freele, D. A. Berberian, and S. Archer, *ibid.*, **10**, 877 (1967).

(7) E. D. Bergmann, S. Sicher, and B. E. Volcani, *Biochem. J.*, **54**, 1 (1953).

(8) These determinations were carried out by Mr. C. E. Joseph and Mr. A. VR. Crain of our Analytical Chemistry Department.

observed for phenylalanine. Finally, the fact that protein synthesis inhibition and antimicrobial activity did not necessarily parallel each other, would indicate that other modes of action may be involved.

Experimental Section

A. Biology.[9] **Preparation of Cell-Free System.**—The methods used to prep the *E. coli* cell-free system and its assay with modifications followed those described by Nirenberg and Matthaei.[10] Cultures of *E. coli* (ATCC 11229) were grown in tryptose phosphate broth (Difco) and harvested at the mid-log phase of growth. The cells were collected by continuous flow centrifugation and washed once with a fortified Tris buffer, pH 7.8, contg 0.014 *M* $MgCl_2$, 0.06 *M* NH_4Cl, and 0.001 *M* dithiothreitol. They were then centrifuged at 10,000*g* for 10 min. The packed cells were weighed and broken in a prechilled mortar with alumina equal to twice the wet wt of the cells. The broken cells were centrifuged at 20,000*g* for 20 min. The supernatant was centrifuged at 30,000*g* for 30 min. This last centrifugation was repeated on the supernatant, and the final supernatant (S-30) was dialyzed overnight in a cold room against 60 vol of the fortified Tris buffer. The S-30 was assayed for protein by the method of Lowry, *et al.*,[11] and then made to a protein concn of 10 mg/ml with the fortified Tris buffer.

Protein Synthesis Assay.—Protein synthesis was assayed in a medium, buffered to pH 7.8, contg 1 m*M* adenosine triphosphate, 0.06 m*M* guanosine triphosphate, 5 m*M* phosphoenolpyruvate, 15 μg of pyruvate kinase, 10.8 m*M* $MgCl_2$, 62 m*M* NH_4Cl, 0.01 m*M* of 20 nonradioactive amino acids, 0.2 m*M* dithiothreitol, 100 m*M* Tris buffer, and approx 100,000 dpm of uniformly labeled [^{14}C]phenylalanine. The amount of medium used per assay was 0.7 ml. Compds being assayed were added to the medium in a vol of 0.1 ml. The mediums contg either test compd or appropriate control were preincubated for 10 min at 37° in a Dubnoff metabolic shaker. The reaction was initiated by adding 0.2 ml of S-30 (2 mg of protein) and was stopped after 16 min by adding 1 ml of 10% Cl_3CCOOH. The samples were then heated at 90° for 20 min in a water bath and the ppt was collected on a filter (Millipore, HA 0.45 μ) and washed 5 times with 5-ml portions of 5% Cl_3CCOOH. The filter, with ppt, was transferred to a counting vial contg 1 ml of 0.25% NaOH, and the ppt was solubilized by gently shaking. The liq scintillator[12] (15 ml) was added, and the samples were assayed for radioactivity with a liq scintillation spectrometer, Packard Tri-Carb Model 3375.

Microbial Assay.—The compds were assayed for antimicrobial activity by the method of Goss and Cimijotti.[13] The test organisms were *E. coli* (ATCC 11229) and *S. aureus* (ATCC 6538). Compds were considered active if their minimal inhibitory concn (MIC) was 31.3 μg or less per ml of assay medium.

B. Chemistry.[14]—The following is an example of a std procedure which, when applied to compds in Table I, generally gave yields in the range 50–90%.

6-(*p*-Chloroanilino)-2-picoline (10).—A mixt of 26 g (0.15 mole) of 6-bromo-2-picoline and 48 g (0.38 mole) of *p*-chloroaniline was cautiously heated in an oil bath to 150–160° where an exothermic reaction set in. The bath was removed and the reaction mixt was cooled in ice to keep the temp near 160°. When the reaction subsided, the mixt was heated again at *ca.* 160° for 1.5 hr. After cooling, the mixt was dild with 60 ml of 10% NaOH and was steam distd until 2 l. of distillate had collected. The cooled residue was extd with Et_2O. The dried ($MgSO_4$) exts were charcoaled and stripped and the residue was crystd from hexane to give 28.7 g (84% yield) of white needles, mp 85–86°. *Anal.* ($C_{12}H_{11}ClN_2$) C, H, N.

***m*-[(6-Methyl-2-pyridyl)amino]benzoic Acid (7).**—A mixt of 14.0 g (0.082 mole) of 6-bromo-2-picoline, 12.3 g (0.082 mole) of methyl *m*-aminobenzoate, and 10.0 g (0.082 mole) of *N*,*N*-dimethylaniline was heated at 160° for 4 hr. After the mixt had cooled, 40 ml of 3 *N* NaOH was added, and the mixt was steam distd until 2 l. of distillate had collected. The residual slurry (200 ml) was cooled and 100 ml of Et_2O was added with shaking. The solid was collected and recrystd from DMF–H_2O to give 3.2 g (17%) of the desired product as a white powder, mp 227–229°. *Anal.* ($C_{13}H_{12}N_2O_2$) C, H, N.

1-[(6-Methyl-2-pyridyl)amino]phenol (14).—A mixt of 17.0 g (0.059 mole) of 6-(*p*-benzyloxyanilino)-2-picoline (mp 124–126°, prepd in 72% yield from 6-bromo-2-picoline and *p*-benzyloxyaniline), 40 ml of AcOH, 15 drops of $HClO_4$, and 1.0 g of 10% Pd/C was hydrogenated on a Parr app under 3.5 kg/cm^2 of H_2 (gauge) for 1 hr. Uptake of H_2 ceased after 50 min. The catalyst was removed and the soln was concd. The residue was mixed with enough satd $NaHCO_3$ to make the mixt slightly basic, and the ppt was collected and air-dried. Recrystn from EtOAc gave 9.3 g (78%) of product, mp 197–199°. *Anal.* ($C_{12}H_{12}N_2O$) C, H, N.

Nitration of 6-Anilino-2-picoline, 6-(*p*-Nitroanilino)-2-picoline (15), and 6-(*o*-Nitroanilino)-2-picoline (17).—A soln of 10 g (0.055 mole) of 6-anilino-2-picoline, 6.6 g (0.0275 mole) of $Cu(NO_3)_2 \cdot 3H_2O$, and 50 ml of AcOH was heated on a steam bath for 2 hr. After 1 hr of heating, an exothermic reaction took place, and the temp of the reaction rose to 118°. At the end of the heating period, the mixt was poured into 200 ml of H_2O and extd with $CHCl_3$. The $CHCl_3$ ext was dried (Na_2SO_4), charcoaled, and concd, and the resulting residue was triturated with 50 ml of PhH. The solid residue was recrystd from aq EtOH to give 4.0 g (32%) of 6-(*p*-nitroanilino)-2-picoline (**15**), mp 178–180°. *Anal.* ($C_{12}H_{11}N_3O_2$) C, H, N. The PhH filtrate from the trituration was concd and placed on a 20 × 2 in. dry alumina column and eluted with EtOAc–hexane, 1:4. The portion of the column R_f 7–9.5 was cut out and extd with MeOH. The MeOH ext was concd and the residue was recrystd from EtOH–H_2O to give 2.2 g (16%) of 6-(*o*-nitroanilino)-2-picoline (**17**), mp 91–93°. *Anal.* ($C_{12}H_{11}N_3O_2$) C, H, N.

6-(*p*-Chloroanilino)-2-picoline *N*-Oxide.—A soln of 28.5 g (0.13 mole) of 6-(*p*-chloroanilino)-2-picoline, 25 ml of 30% H_2O_2, and 60 ml of AcOH was heated at 75° for 5 hr. An addnl 10 ml of 30% H_2O_2 was then added, and heating at 75° was continued for an addnl 5 hr. The reaction mixt was poured into 400 ml of H_2O, extd with $CHCl_3$, and the exts were washed with satd $NaHCO_3$. Evapn of the dried (Na_2SO_4) and charcoaled exts left a residue which was triturated with hot hexane and filtered. The filter residue was dissolved in hot MeCN, charcoaled, and cooled. The resulting ppt was collected and recrystd from MeCN giving 10.7 g (35%) of the desired product, mp 126–128°. *Anal.* ($C_{12}H_{11}ClN_2O$) C, H, N.

6-(*p*-Chloroanilino)-2-pyridinemethanol (34).—A soln of 10.2 g (0.043 mole) of the above *N*-oxide and 60 ml of Ac_2O was heated under reflux for 2 hr, poured into 500 ml of H_2O, and stirred for 1 hr. The resulting soln was made basic with 40% NaOH and was extd with Et_2O. The exts were dried (Na_2SO_4), charcoaled, and concd. The residue was combined with 60 ml of 10% NaOH, heated under reflux for 2 hr, cooled, and extd with $CHCl_3$. The $CHCl_3$ ext was dried (Na_2SO_4) and concd, and the residue was crystd from EtOH–Et_2O giving 2.3 g (20%) of a white powder, mp 109–111°. *Anal.* ($C_{12}H_{11}ClN_2O$) C, H, N.

Microbiological Oxidation.[15] **Conversion of 5 into 31.**—Two 10-l. fermentations were carried out in a soy–dextrose medium at 28–29°. The fermentations were sparged with 4 l./min of sterile air and agitated at 450 rpm. After an initial 24-hr growth, 8 g of 2-methyl-6-[*m*-(trifluoromethyl)anilino]pyridine was added to each tank and the fermentation continued for 72 hr. The tanks were then extd with CH_2Cl_2 (2 × 20 l. each) which was evapd *in vacuo* to a thick brown residue. On addn of hexane a semisolid product was obtained. This material was crystd once from $CHCl_3$ and twice from EtOAc resulting in 2.2 g of white crystals

(9) Adenosine triphosphate, guanosine triphosphate, phosphoenolpyruvate, pyruvate kinase, *p*-fluorophenylalanine, alumina, and Tris buffer were purchased from Sigma Chemical Company, St. Louis, Mo.; dithiothreitol from Calbiochem, Los Angeles, Calif; and [^{14}C]phenylalanine from New England Nuclear Corp, Boston, Mass..

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(12) The liquid scintillator contained 8 g of Omnifluor (New England Nuclear Corp.), 100 g of naphthalene, 130 ml of bis(2-methoxyethyl) ether, and *p*-dioxane to 1 l.

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(14) Melting points were determined on a Mel-Temp apparatus and are uncorrected. Microanalytical determinations were carried out by Instranal Laboratories, Inc., Rensselaer, N. Y., and Galbraith Laboratories, Inc., Knoxville, Tenn.; nmr analyses were carried out with 60 and 100 MHz Varian instruments. Chemical shifts (δ) are reported relative to tetramethylsilane (δ 0.00, internal standard). Infrared spectra were determined using a Perkin-Elmer Model 1R21, and uv spectra were measured with a Cary Model 15 recording spectrophotometer.

(15) Under the direction of Mr. D. Rosi of our fermentation chemistry group.

(31), mp 185–186°, mono-*O*-acetate (C_5H_5N–Ac_2O) mp 119–120°.

Molecular Complex Analysis.[8]—Solns of phenylalanine and **10** were prepd in H_2O–MeOH (1:1) at a concn of 100 μg/ml. A 1:1 mixt of the two solns showed no cloudiness or pptn. The uv spectrum of the mixt was an exact summation of the spectrum of each compd alone, showing no alteration of either the phenylalanine or **10** spectrum. This procedure was repeated using pH 7.5 phosphate buffer–MeOH (1:1) and pH 10.0 borate buffer–MeOH (1:1). In each case no cloudiness or pptn occurred and no alteration of either uv spectrum was observed.

For tlc investigations 0.5% solns of each compd were prepd in H_2O–MeOH (1:1) and a (1:1) mixt of the two made as before. Equiv aliquots of the three solns were spotted for development in the following systems: on silica gel, EtOAc–PhH (95:5), *n*-BuOH–H_2O (80:20), *n*-BuOH–concd. NH_4OH–H_2O (80:10:10), *n*-BuOH–gl HOAc–H_2O (80:10:10); on cellulose, *n*-BuOH–PhH (1:1) satd with H_2O. Spots were detected under short wavelength uv light (**10**) and after spraying with 0.2% ninhydrin in EtOH and heating (phenylalanine). In each case the mixt showed only two spots with size and R_f corresponding to those of **10** and phenylalanine. No third spot, possibly representing a mol complex, was found.

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Notes

Synthesis and Local Anesthetic Activity of Certain Piperazine and Ephedrine Derivatives

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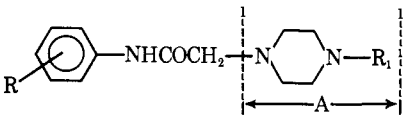
Since the introduction of lidocaine[1] the literature has recorded many derivatives containing the aminoacylamide grouping $PhNHCO(CH_2)_nN<$,[2] including the biological effect of compounds containing the piperazine moiety. In the field of 1,4-disubstituted piperazines, local anesthetic effect has been reported.[3,4] The prolongation of local anesthesia is obtained by low doses of vasoconstrictors, a rationale for the study of cinnamylephedrine by Schultz.[5]

This report describes the pharmacological testing and the chemistry involved in the preparation of certain structures including the acylamide moiety together with piperazine or ephedrine.



(1)

R = H, OH, OEt, CH_3, COOEt

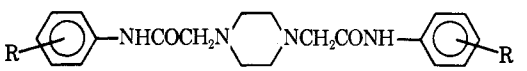
R_1 = Et, COOEt, CH_2CONH–C$_6$H$_4$–R

A = $N(CH_3)CH(CH_3)CH(OH)C_6H_5$

Chemistry.—The piperazine and ephedrine derivatives in Tables I–III, were obtained by the general procedure of Foye.[4] However, when piperazine, 1-ethylpiperazine, and ephedrine were allowed to react with 2-chloro-2′-hydroxyacetanilide, three identical products of the same melting point were isolated. The compound appeared devoid of the phenolic group. This is explained by an intramolecular cyclization of 2-chloro-2′-hydroxyacetanilide under the influence of the basic amine present leading to 1,4-benzoxazin-3-one, previously prepared by Aschan[6] by treating alcoholic KOH with 2-chloro-2′-hydroxyacetanilide. The identity of the product was substantiated by microanalytical data, ir, and molecular weight determination.

TABLE I

SYMMETRICAL PIPERAZINE DERIVATIVES



No.	R	Prep	Yield, %	Mp, °C	Formula[a]	Ir absorption, cm^{-1}
3	H	A	74	259–260	$C_{20}H_{24}N_4O_2$[b]	1652–1654
4	4-OH	A	70	283–284	$C_{20}H_{24}N_4O_4$	1662
5	2-OEt	A	80	209–210	$C_{24}H_{32}N_4O_4$	1685
6	4-OEt	A	75	206–207	$C_{24}H_{32}N_4O_4$	1670
7	2-CH_3	A	78	223–124	$C_{22}H_{28}N_4O_2$	1686
8	4-CH_3	A	70	240–241	$C_{22}H_{28}N_4O_2$	1674
9	2-COOEt	A	75	178–179	$C_{26}H_{32}N_4O_6$	1686
10	4-COOEt	A	70	203–204	$C_{26}H_{32}N_4O_6$	1697–1707

[a] All compds were analyzed for C, H, N. [b] C: calcd, 68.10; found, 67.09.

Since the formation of the phenoxide anion is favored by the strongly basic amine, our assumption finds a theoretical support in the fact that cyclization takes place when piperazine, 1-ethylpiperazine, or ephedrine (pK_b = 4.2, 3.4, and 4.6, resp) are the reacting amines, but not with 1-carbethoxypiperazine which is less basic (pK_b = 5.96).

Biological Testing.—Water-soluble compounds were screened for their local anesthetic action using the

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