(**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.

Acknowledgment.—We thank Dr. W. A. Goss and Mr. E. B. Cimijotti for providing the antimicrobial data, Miss C. M. Martini and Dr. S. D. Clemans for assistance in spectral determinations, Dr. J. C. Collins and Dr. J. V. Boyle for helpful discussion, and Miss A. Smith for technical assistance.

Notes

Synthesis and Local Anesthetic Activity of Certain Piperazine and Ephedrine Derivatives

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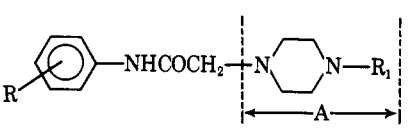
Since the introduction of lidocaine[1] the literature has recorded many derivatives containing the aminoacylamide grouping $PhNHCO(CH_2)_nN<$,[2] including the biological effect of compounds containing the piperazine moiety. In the field of 1,4-disubstituted piperazines, local anesthetic effect has been 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

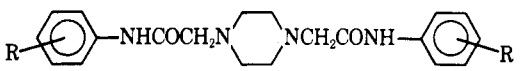
R_1 = Et, COOEt, CH_2CONH–(R)

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

(1) N. Löfgren, *Ark. Kemi Mineral. Geol.*, **22A**, 1 (1946).

(2) (a) V. Hach, *Cesk. Farm.*, **2**, 159 (1953); *Chem. Abstr.*, **49**, 2030*f* (1955). (b) N. Löfgren, C. Tegnér, and B. Takman, *Acta Chem. Scand.*, **11**, 1724 (1957). (c) N. Löfgren and C. Tegnér, *ibid.*, **14**, 486 (1960).

(3) (a) T. Klosa, German Patent 1,093,800 (1960); *J. Prakt. Chem.*, **17**, 340 (1962). (b) R. Dahlbom and A. Misiorny, *Acta Chem. Scand.*, **15**, 1367 (1961).

(4) (a) A. Larizza and G. Brancaccio, *Farm. Ed. Sci.*, **16**, 701 (1961). (b) W. O. Foye, H. B., Levine, and W. L. Mckenzie, *J. Med. Chem.*, **9**, 61 (1966).

(5) F. M. Schultz, Jr., and P. H. Barbour, Jr., *J. Pharmacol. Exp. Ther.*, **76**, 295 (1942).

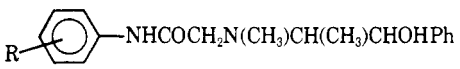
(6) O. Aschan, *Ber.*, **20**, 1523 (1887).

TABLE II
1,4-DISUBSTITUTED PIPERAZINES

No.	R	R_1	Prep	Yield, %	Mp, °C	Formula[f]	Ir absorption, cm^{-1}
11	H	COOEt	B	72	99–100	$C_{15}H_{21}N_3O_3$	1682–1684, 1710–1714
12	2-OH	COOEt	B	75	110–111	$C_{15}H_{21}N_3O_4$	1652–1656, 1686–1692
13	2-OEt	COOEt	B	85	109–110	$C_{17}H_{25}N_3O_4$	1686
14	4-OEt	COOEt	B	75	84–85	$C_{17}H_{25}N_3O_4$	1672, 1707
15	2-CH_3	COOEt	B	80	113–114	$C_{16}H_{23}N_3O_3$	1692
16	4-CH_3	COOEt	B	75	87–88	$C_{16}H_{23}N_3O_3$	1684
17	2-COOEt	COOEt	B	75	120–121	$C_{18}H_{25}N_3O_5$	1686
18	4-COOEt	COOEt	B	70	105–106	$C_{18}H_{25}N_3O_5$	1693, 1709
19	H	Et	C	69	223–224[a]	$C_{14}H_{21}N_3O$[b]	
20	2-OEt	Et	C	75	75–76	$C_{16}H_{25}N_3O_2$	1674
21	4-OEt	Et	C	65	215–216[a]	$C_{16}H_{25}N_3O$[b,c]	
22	4-CH_3	Et	C	70	222–223[a]	$C_{15}H_{23}N_3O$[b]	
23	4-CH_3	Et	C	70	219–220[a]	$C_{15}H_{23}N_3O$[b]	1690
24	2-COOEt	Et	C	70	188–189[a]	$C_{17}H_{25}N_3O_3$[b,d]	1680–1682
25	4-COOEt	Et	C	65	194–195[a]	$C_{17}H_{25}N_3O_3$[b,e]	

[a] Melting point of the dipicrate. [b] The analysis was carried out on the dipicrate. [c] C: calcd, 44.86; found, 44.36; N: calcd, 16.80; found, 16.20. [d] N: calcd, 16.21; found, 15.66. [e] C: calcd, 44.78; found, 45.79. [f] See Table I, footnote *a*.

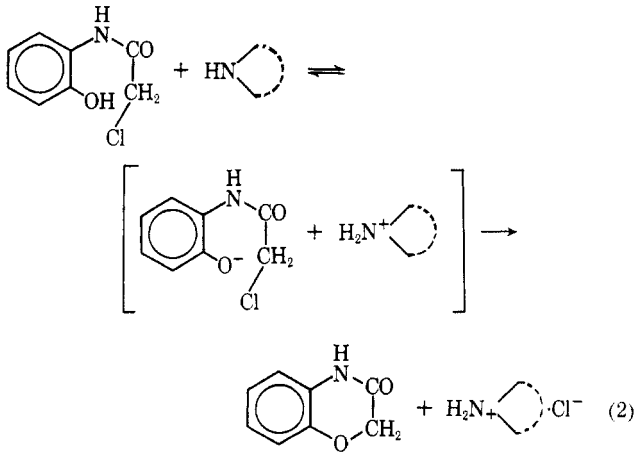
TABLE III
EPHEDRINE DERIVATIVES



No.	R	Prep	Yield, %	Mp, °C	Formula[a]	Ir absorption, cm^{-1}
26	H	D	40	134–135	$C_{18}H_{22}N_2O_2$	1678
27	2-OEt	D	68	99–100	$C_{20}H_{26}N_2O_3$	1661
28	4-OEt	D	55	190–191	$C_{20}H_{26}N_2O_3$	1658
29	2-CH_3	D	65	98–99	$C_{19}H_{24}N_2O_2$	1675
30	4-CH_3	D	65	137–138	$C_{19}H_{24}N_2O_2$	1674
31	2-COOEt	D	60	90–91	$C_{21}H_{26}N_2O_4$	1700
32	4-COOEt	D	50	138–139	$C_{21}H_{26}N_2O_4$	1670

[a] See Table I, footnote *a*.

rabbit's corneal reflex test. Table IV lists the duration of corneal anesthesia of these compounds. Compounds **11, 12, 16, 17,** and **19–23** were found to be nonactive in concentrations up to 4%. The HCl salts of **3–10, 15, 18, 28,** and **32** were difficultly soluble in saline and therefore were not tested.



(2)

Table IV shows also that all the active compounds contain nuclear substituents of either nucleophilic or electrophilic character. The introduction of Me in either ortho or para positions yielded compounds of very weak activity or no activity at all.

TABLE IV
DURATION OF CORNEAL ANESTHESIA

			Time, min — Concn of the base, %		
No.	R	R_1(A)	1	2	4
13	2-OEt	COOEt	3, 5	14, 15	29, 34
14	4-OEt	COOEt	No effect	No effect	12, 18
24	2-COOEt	Et	(5, 6)[a] 26, 29	32, 34	
25	4-COOEt	Et	2, 2	7, 9	14, 16
26	H	$N(CH_3)CH(CH_3)\underset{\underset{OH}{|}}{C}HC_6H_5$	(−)[a] 8	12, 12	
27	2-OEt	$N(CH_3)CH(CH_3)\underset{\underset{OH}{|}}{C}HC_6H_5$	(3, 5)[a] 17, 18	25, 25	
29	2-CH_3	$N(CH_3)CH(CH_3)\underset{\underset{OH}{|}}{C}HC_6H_5$		11, 14	15, 15
30	4-CH_3	$N(CH_3)CH(CH_3)\underset{\underset{OH}{|}}{C}HC_6H_5$	3, 3	6, 8	
31	2-COOEt	$N(CH_3)CH(CH_3)\underset{\underset{OH}{|}}{C}HC_6H_5$	(8, 11)[a] 13, 13	28, 28	
	Lidocaine	$N(CH_3)CH(CH_3)\underset{\underset{OH}{|}}{C}HC_6H_5$		6, 14	

[a] 0.5% concn.

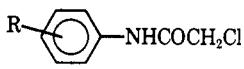
Experimental Section

Melting points were determined in capillary tubes in oil bath and are uncorr. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Ir spectra were determined in KBr by El-Nasr Company, U.A.R., using a Beckmann spectrometer IR-20. The spectra were carried out using high resoln in the range of 1800–1500 cm^{-1}. The mol wt was determined by the Rast method.

***N*-Chloroacetylanilines.**—To 18.6 g (0.2 mole) of redistd $PhNH_2$ in 15.6 (0.2 mole) of pyridine, and 200 ml of PhH, 22.6 g (0.2 mole) of $ClCOCH_2Cl$ in 100 ml of PhH was added dropwise. The reaction was carried out in an ice–water bath with stirring for 1 hr. The solvents were removed under reduced pressure and the oily residue was dissolved in 50 ml of ETOH and poured into 100 ml of cold H_2O previously acidified with 5 ml of 1 *N* HCl. The sepd *N*-chloroacetyl aniline was crystd from 40% ETOH; yield 26 g (76%), mp 137–138° as reported.[7]

The data for the other 2-chloro-2′-(or 4′-)substituted acetanilides are given in Table V.

TABLE V



R	Yield, %	Mp, °C	Ref
2-OH	30	136	*a*
4-OH	23	142	*b*
2-OEt	84	72	*c*
4-OEt	84	142–144	*d*
2-Me	80	112	*e*
4-Me	80	158–160	*f*
2-COOEt	75	74	*g*
4-COOEt	75	116	*h*

[a] W. A. Jacobs, M. Heidelberger, and I. P. Rolf, *J. Amer. Chem. Soc.*, **41**, 458 (1919). [b] W. A. Jacobs and M. Heidelberger, *ibid.*, **39**, 1442 (1917). [c] M. Heidelberger and W. A. Hacobs, *ibid.*, **41**, 1452 (1919). [d] A. Bistrzycki and F. Ulffers, *Ber.*, **31**, 2790 (1898). [e] W. Abenius, *J. Prakt. Chem.*, **38**, 299 (1888). [f] H. Eckenroth and A. Donner, *Ber.*, **23**, 3288 (1890). [g] W. A. Jacobs, M. Heidelberger and I. P. Rolf, *J. Amer. Chem. Soc.*, **41**, 469 (1919). [h] G. Sanna and M. Granata, *Chem. Zentraibl.*, **108**, 3313 (1937); *Chem. Abstr.*, **33**, 5827 (1939).

1-Carbethoxypiperazine was obtained in 70% yield, bp 237°;[8] pK_a = 8.04 ± 0.04, potentiometrically at 30° following a procedure modeled upon the method of Albert and Serjeant.[9] **1-Ethylpiperazine·2HCl** was obtained in 80% yield.[8]

Piperazine and Ephedrine Derivatives (Tables I–III). (A) 1,4-Bis[(2- or 4-substituted)phenylcarbamoylmethyl]piperazine (3–10).—To a soln of 0.01 mole of 2-chloro-2′-(or 4′-) substituted acetanilide in 30 ml of EtOH was added 1.0 g of anhyd Na_2CO_3 followed by a soln of 0.97 g (0.005 mole) of piperazine in EtOH. The mixt was stirred while refluxing for 12 hr. The alcohol was distd off and 50 ml of H_2O was added to the residue. A solidifying oily layer sepd which was filtered, washed (H_2O), and recrystd twice from propylene glycol.

(B) 1-Carbethoxy-4-[(2- or 4-substituted)phenylcarbamoylmethyl]piperazine (11–18).—These compds were obtained as in A, using 0.01 mole of 2-chloro-2′-(or 4′-) substituted acetanilide (1.58 g), 0.01 mole of 1-carbethoxypiperazine, and 0.5 g of anhyd Na_2CO_3. The products were recrystd from 50% EtOH.

(C) 1-Ethyl-4-[(2- or 4-substituted)phenylcarbamoylmethyl]-piperazine (19–25).—To a soln of 0.46 g (0.02 g-atom) of Na in 30 ml of EtOH, 1.87 g (0.01 mole) of ethylpiperazine·2HCl was added, and the mixt was refluxed for 1 hr. To the cooled mixt was added 0.5 g of anhyd Na_2CO_3 and 0.01 mole of 2-chloro-2′-(or -4′-)substituted acetanilide. The mixt was refluxed with stirring for 12 hr. After removal of EtOH, 50 ml of H_2O was added to dissolve the oily residue. The aq soln was extd 3 times with 20-ml portions of Et_2O. The Et_2O soln was dried (Na_2SO_4), filtered, and evapd. The HCl salts were hygroscopic. For analysis, the dipicrates were prepd and recrystd from EtOH.

(7) Th. Zincke and O. Kegel, *Ber.*, **23**, 243 (1890).

(8) T. S. Moore, M. Boyle, and V. M. Thorn, *J. Chem. Soc.*, 39 (1929).

(9) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Methuen & Co., Ltd., London, 1962.

(D) 2-(2-Hydroxy-1,*N*-dimethyl-2-phenylethylamino)-2′- (or 4′-)substituted Acetanilide (26–32).—To a soln of 0.23 g (0.01 g-atom) of Na in 30 ml of EtOH, 2 g (0.01 mole) of ephedrine·HCl was added and the mixt was refluxed for 1 hr. To the cooled mixt were added 0.5 g of anhyd Na_2CO_3 and 0.01 mole of 2-chloro-2′-(or 4′-)subst acetanilide. The mixt was refluxed with stirring for 12 hr. After distn of EtOH, the residue was extd 3 times with 20-ml portions of Et_2O. The Et_2O soln was extd exhaustively with 1 *N* HCl. The combined acidic solns were neutralized with concd NH_4OH and the milky white colloidal soln was warmed and then left overnight. The residue was recrystd from 60% EtOH.

Test for Corneal Anesthesia.—The HCl salt solns of the compds were prepared in normal saline, and 0.25 ml was placed in the rabbit's cornea. Three concns for each compd in the range 0.5–4% of the base were tested on both eyes. The sol HCl salts were found to have a pH 3.8–4.0; an attempt to raise the pH to the neutral side resulted in the pptn of the bases. Corneal reflex detn was made at 2-min intervals following the start of anesthesia.

Acknowledgment.—The authors are indebted to Dr. A. M. Afifi for invaluable help in the pharmacological part of the present investigation.

A Stable, Biologically Active Indoxyl

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While many indole derivatives are known to be biologically active[1] little is known about the activity of indoxyls (indol-3-ol). The scarcity of information[2] on such compounds is most probably due to their instability as they tend, unless disubstituted at position 2, to undergo oxidation and or dimerization.[3]

The purpose of this work was to synthesize stable indoxyl derivatives and test them for biological activity. In a previous report,[4] it was shown that *o*-nitrobenzaldehyde reacted with keto steroids to yield steroidal indoxyls. The effect of the steroidal nucleus on the (potential) biological activity of the indoxyl derivatives rendered steroidal indoxyls unsuitable for our purpose.

The reaction of *o*-nitrobenzaldehyde with some cyclic ketones (cyclopentanone, cyclohexanone) proceeded vigorously and lead to intractable tars, presumably *via* unstable indoxyls. Mechanistic consideration indicated that a 1,4-cyclodione would lead to stable indoxyl derivatives, where C_2 of the indoxyl moiety will constitute the α position of an α,β-unsaturated ketone. It was envisaged that such a conjugation would stabilize the indoxyl structure. Indeed, the reaction of *o*-nitrobenzaldehyde with 1,4-cyclohexanedione in 5% methanolic KOH gave Ia and Ib in 32 and 20% yield, resp. The products were separated by chromatography.

(1) W. M. McIsaacs and R. T. Harris, *Advan. Pharmacol.*, **6**, 247 (1968); A. Hoffman, *Med. Res. Ser.*, **2**, 221 (1968).

(2) R. G. Taborsky, *Int. J. Neuropharmacol.*, **7**, 483 (1968); A. Pelczarska, *Arch. Immunol. Ther. Exp.*, **17**, 118 (1969).

(3) (a) A. Hassner and M. J. Haddadin, *J. Org. Chem.*, **28**, 224 (1963). (b) A. Albert, "Heterocyclic Chemistry," Athlone Press, London, 2nd ed, 1968, pp 209 and 241. (c) R. M. Acheson, "An Introduction to the Chemistry of Heterocyclic Compounds," Interscience, New York, N. Y., 2nd ed, 1967, p 163. (d) Reference 3c, p 155.

(4) A. Hassner, M. J. Haddadin, and P. Catsoulacos, *J. Org. Chem.*, **31**, 1363 (1966).