α-(N-Piperazino)dimethylacetanilides and Their Local Anesthetic Activity

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The preparation of several α-N-piperazino-2,5- and -2,6-dimethylacetanilides is reported. Determination of local anesthetic potencies showed them to possess somewhat less activity than procaine; their ionization constants are somewhat higher than those of more potent local anesthetics. A positive correlation between local anesthetic potency and carbonyl stretching frequency of the amide group was observed, and a direct relationship with the ionization constant is also apparent.

Since the introduction of lidocaine[1] as a local anesthetic, numerous analogs and derivatives have been prepared, but until quite recently no analogs containing a piperazine nucleus were reported. Several piperazine-containing analogs have appeared since this study was started,[2] but generally the piperazine ring has borne substituents on both nitrogen atoms. It appeared to us that a piperazine analog with one nitrogen unsubstituted might provide a potent local anesthetic. Albert[3] has pointed out that a large number of alkaloids, local anesthetics, and other nitrogen-containing drugs possess a pK_a value of about 8, thus making them 16% nonionized at pH 7.3, and allowing both the ionized and nonionized forms to be present under physiological conditions. The pK_a of piperazine is 9.82,[4] which should place the pK_a of appropriately substituted piperazines close to this value. It has also been claimed by Trevan and Boock[5] and later by Krahl, *et al.*,[6] that local anesthetics act in the undissociated form, although it is not known to what extent the ionized form may be involved in transport.

Accordingly, a series of piperazine analogs of lidocaine has been synthesized in which both mono-N- and di-N-substituted piperazine rings are included. Several substituted benzoylpiperazines were also prepared for comparison. In general, the procedures of Erdtman and Lofgren[7] and Dahlbom, *et al.*,[8] were employed in which N-chloroacetylanilines were allowed to condense with the appropriate piperazines. Mono-N-substituted piperazines were obtained by hydrogenolysis of N-carbobenzyloxypiperazines. Physical constants of the compounds prepared are recorded in Table I.

Determination of pK_a values showed that both the mono-N- and di-N-substituted piperazines had constants somewhat too high for good local anesthetic activity, assuming that a pK_a value of 8 represents the optimum (see Table II). Evaluation of local anesthetic effects was made using the rabbit cornea method of Rose,[9] and potencies were obtained in regard to both strength of threshold stimulus and duration of action. Results are expressed (Table II) in terms of potency comparable to that given by 0.5% procaine solution. The compounds tested possessed some activity, but were generally somewhat less potent and also of shorter duration of action than procaine. α-(N-Methyl-N′-piperazino)-2,6-dimethylacetanilide showed comparable activity to that of procaine by this assay, and this similarity of activity is reflected in the similar pK_a values.

A previous attempt[10] to correlate local anesthetic potency with ionization constants failed to uncover a relationship probably because of the wide range of structures examined. It appears from the data in Table II that a relation to ionic dissociation obtains when the comparison is limited to a narrow range of structures.

An attempt was also made to correlate local anesthetic potency with the bond order of the carbonyl linkage as measured by C=O stretching frequency. A previous correlation of this type has been reported by Galinsky, *et al.*,[11] with a series of diethylaminoethyl benzoate and cinnamate esters. Examination of Table II shows that a positive correlation does exist between the carbonyl absorption frequency and the local anesthetic potency, potency increasing with the frequency of the carbonyl stretching band. This order of activity represents the opposite of that found by Galinsky, *et al.*, who observed a decrease in local anesthetic potency (increase in ED_{50} values) with increase in carbonyl absorption frequency. Direct comparison of esters and amides perhaps should not be made, particularly with the hindered amides reported here, since amides in general are representative of a lower absorption frequency.[12] But, since the local anesthetic potencies observed here increased as absorption frequency approached the frequencies (1697–1710 $cm.^{-1}$) reported for the more active of the esters,[11] it is possible that an optimum absorption-frequency range exists for high anesthetic potency. It would follow then, that some double-bond character must be retained for optimum local anesthetic activity, and that binding with the receptor site should not become so strong as to be irreversible.

(1) N. Lofgren, *Arkiv Kemi Mineral. Geol.*, **22A**, 1 (1946).

(2) (a) J. 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); (c) A. Larizza and G. Brancaccio, *Farmaco* (Pavia), *Ed. Sci.*, **16**, 701 (1961); (d) E. Puscaru *et al.*, *Farmacia* (Bucharest), **10**, 35 (1962).

(3) A. Albert, "Selective Toxicity," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1960, p. 127.

(4) G. Schwarzenbach, B. Maissen, and H. Ackermann, *Helv. Chim. Acta*, **35**, 2333 (1952).

(5) J. W. Trevan and E. Boock, *Brit. J. Exptl. Pathol.*, **8**, 307 (1927).

(6) M. E. Krahl, A. K. Keltch, and G. H. A. Clowes, *J. Pharmacol. Exptl. Therap.*, **68**, 330 (1940).

(7) H. Erdtman and N. Lofgren, *Svensk Kem. Tidskr.*, **49**, 163 (1937).

(8) R. Dahlbom, C. Tegner, and N. Willman, *Acta Chem. Scand.*, **13**, 1145 (1959).

(9) C. L. Rose, *Anesthesia Analgesia*, **10**, 159 (1931).

(10) J. Regnier, S. Bazin, and R. Berger, *Compt. rend. soc. biol.*, **135**, 1508 (1941).

(11) A. M. Galinsky, J. E. Gearien, A. J. Perkins, and S. V. Susina, *J. Med. Chem.*, **6**, 320 (1963).

(12) Lidocaine, for instance, has a carbonyl stretching frequency at 1660 $cm.^{-1}$ but has a more favorable pK_a value of 7.86, which presumably is of overriding importance by controlling the amount of drug that reaches the sites of action.

TABLE I

α-(N-PIPERAZINO)ACETANILIDES AND -ANILIDES

Compd.	Reaction solvent	Yield, %	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
α-(N-Methyl-N′-piperazino)-2,6-dimethylacetanilide·2HCl[a]	95% EtOH	38	265–268	$C_{15}H_{25}Cl_2N_3O$	53.89	54.04	7.54	7.70	12.57	12.71
α-(N-Carbobenzyloxypiperazino)-2,6-dimethylacetanilide	95% EtOH	18	130–132	$C_{22}H_{27}N_3O_3$	69.25	69.11	7.14	7.04	11.01	11.04
α-(N-Carbobenzyloxypiperazino)-2,5-dimethylacetanilide	95% EtOH	21	109–110	$C_{22}H_{27}N_3O_3$	69.25	69.12	7.14	7.12	11.01	11.25
α-(N-Piperazino)-2,6-dimethylacetanilide·2HCl	Abs. EtOH	29	225–230	$C_{14}H_{23}Cl_2N_3O$	52.50	52.30	7.24	7.16	13.12	12.51
α-(N-Piperazino)-2,5-dimethylacetanilide·2HCl	Abs. EtOH	30	233–238	$C_{14}H_{23}Cl_2N_3O$	52.50	51.50	7.24	7.60	13.12	12.95
N-Methyl-N′-*p*-nitrobenzoylpiperazine·HCl[b]	Benzene	57	319 dec.	$C_{12}H_{16}ClN_3O_3$	50.42	50.19	5.64	5.89		
N-Methyl-N′-*p*-aminobenzoylpiperazine·HCl	95% EtOH	51	155–157	$C_{12}H_{18}ClN_3O \cdot H_2O$	52.62	52.75	7.36	7.37	15.34	15.02

[a] Lit.[2b] m.p. 267–268°. [b] *Anal.* Calcd.: Cl, 12.69. Found: Cl, 12.21.

TABLE II

IONIZATION CONSTANTS (WATER, 25°), CARBONYL ABSORPTION FREQUENCIES, AND LOCAL ANESTHETIC POTENCIES

Compd.	pK_1	pK_2	Absorption frequency,[a] cm.$^{-1}$	Local anesthetic potency—Peak activity, cm. (min.)	Local anesthetic potency—Duration, min.
Piperazine[4] (20°)	9.82	5.68			
N-Methyl-N′-*p*-aminobenzoylpiperazine	8.9		1610	Slight	
α-(N-Carbobenzyloxypiperazino)-2,6-dimethylacetanilide	4.5		1650	8 (5)	20
α-(N-Piperazino)-2,6-dimethylacetanilide	9.4	4.2	1670	9 (10)	20
α-(N-Carbobenzyloxypiperazino)-2,5-dimethylacetanilide			1685	9 (5)	23
α-(N-Piperazino)-2,5-dimethylacetanilide	9.3	3.8	1690	9 (15)	28
α-(N-Methyl-N′-piperazino)-2,6-dimethylacetanilide	9.0	3.8	1710	7 (10)	25
Procaine	9.0[b]		1711	7 (12)	35

[a] Obtained with a Perkin-Elmer Model 137B recording spectrophotometer in Nujol mull. [b] J. Eisenbrand and H. Pieher, *Arch. Pharm.*, **276,** 1 (1938).

A word might be added concerning the absorption spectrum of those compounds having an additional carbonyl function in the carbobenzyloxy group. In the case of α-(N-carbobenzyloxypiperazino)-2,6-dimethylacetanilide, two amide I peaks are visible at 1650 and 1700 cm.$^{-1}$, the latter apparently due to the urethan group which absorbs at greater frequencies than amides.[13] With the carbobenzyloxy derivative of the 2,5-dimethyl isomer, however, only one amide I peak was found at approximately 1685 cm.$^{-1}$.

Experimental Section

Melting points were taken on either a Mel-Temp or Fisher-Johns block and are corrected. Analyses for carbon, hydrogen, and nitrogen were done by Weiler and Strauss, Oxford, England. The following procedures are representative.

N-Chloroacetylanilines.—To 24.2 g. (0.2 mole) of 90% 2,6-dimethylaniline in 200 ml. of ether was added dropwise 11.2 g. (0.1 mole) of chloroacetyl chloride in 200 ml. of ether. The reaction was carried out in an ice–water bath (stirring) for 1 hr. The precipitate of 2,6-dimethylaniline hydrochloride was extracted with three 200-ml. portions of water. The ether layer was filtered and evaporated to dryness. The solids were combined and recrystallized from 40% ethanol. A yield of 10.5 g. (54%) of **N-chloroacetyl-2,6-dimethylaniline** was obtained, m.p. 147–148°.

Anal. Calcd. for $C_{10}H_{12}ClNO$: C, 60.76; H, 6.13; N, 7.09. Found: C, 60.78; H, 6.38; N, 7.14.

N-Chloroacetyl-2,5-dimethylaniline (13.9 g., 70%) was obtained similarly; m.p. 151–153°.

Anal. Calcd. for $C_{10}H_{12}ClNO$: C, 60.76; H, 6.13. Found: C, 60.5; H, 5.9.

α-(N-Carbobenzyloxypiperazino)-2,6-dimethylacetanilide.—A mixture of 7.9 g. (0.04 mole) of N-chloroacetyl-2,6-dimethylaniline, 8.8 g. (0.04 mole) of 1-benzyloxycarbonylpiperazine,[14] and 3.36 g. (0.04 mole) of $NaHCO_3$ in 200 ml. of 95% ethanol was refluxed for 5 hr. The alcohol was removed under reduced pressure, and the residue was recrystallized from 95% ethanol giving 2.7 g. (18%) of product melting at 130–132°.

α-(N-Piperazino)-2,6-dimethylacetanilide Dihydrochloride.—In a Parr hydrogenation bottle was placed 1.9 g. (0.005 mole) of α-(N-carbobenzyloxypiperazino)-2,6-dimethylacetanilide in 100 ml. of absolute ethanol along with 0.5 g. of 10% palladium–charcoal. The bottle was evacuated, twice-filled with hydrogen, and shaken with hydrogen (2–3 atm.) for 1 hr. The mixture was filtered through sintered glass, and the filtrate was saturated with HCl. The solution was concentrated to 50 ml. and cooled in an ice bath. The crystalline product was separated, washed with ether, and recrystallized from 95% ethanol yielding 0.56 g. (29%), m.p. 225–230°.

Determination of pK_a Values.—Solutions of 0.005 mole of base in 100 ml. of distilled water were titrated with 0.1 *N* HCl at 25°, employing a Beckman Zeromatic pH meter. The hydrochlorides were titrated with 0.1 *N* NaOH, or the solutions were first brought to a pH of approximately 11 with 0.1 *N* sodium hydroxide and then titrated with the standard acid.

Determination of Local Anesthetic Potencies.—Solutions (2%) of the compounds were prepared in normal saline, and 2–3 drops were placed on the rabbits' cornea. A solution of procaine hydrochloride (0.5%) was used as standard. Each soluble compound was tested in the same eye (of each of the rabbits) as had been

(13) N. B. Colthup, L. H. Daly, and S. E. Wiberley, "Introduction to Infrared and Raman Spectroscopy," Academic Press Inc., New York, N. Y., 1964, p. 266.

(14) W. O. Foye and L. R. Fedor, *J. Am. Pharm. Assoc.*, **48,** 412 (1959).

used for the standard procaine solution, by noting the response to a measured electrical stimulus applied by electrodes. Threshold determinations were made at 5-min. intervals following 1-min. applications of the compounds to the corneal surface. The strength of threshold stimulus (setting in centimeters of the secondary coil) was plotted *vs.* time, and relative potencies were thus obtained. Results are expressed in Table II in terms of averaged activity (in three or more animals) relative to procaine, both in regard to threshold stimulus and duration of action at the same setting (10 cm.). Due to variations in response in different animals, use of figures to express these potencies indicates a greater degree of quantitative accuracy than the method is capable of giving.

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2-Hydroxyacetophenetidine, a New Metabolite of Acetophenetidine

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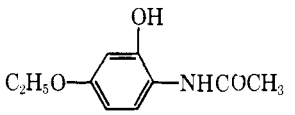
2-Hydroxyacetophenetidine (2-hydroxy-4-ethoxyacetanilide) conjugated with glucuronic acid has been shown to be a metabolite found in the urine of dogs, cats, and human subjects treated with acetophenetidine.

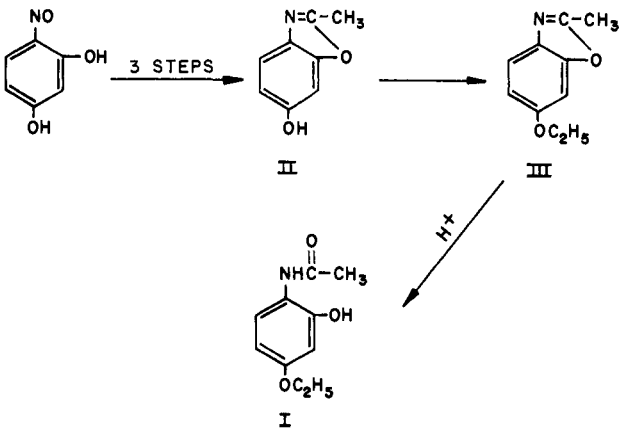
Brodie and Axelrod[1] have shown that N-acetyl-*p*-aminophenol is the major metabolite of acetophenetidine. In this communication we report the identification of a new metabolite of acetophenetidine, 2-hydroxyacetophenetidine (2-hydroxy-4-ethoxyacetanilide, I) in the urine of cats, dogs, and humans. Qualitative paper chromatography was used to detect the presence of this hydroxylated derivative which was then isolated and positively identified by comparison with a specimen of I obtained by unequivocal synthesis.



I

SCHEME I

SYNTHESIS OF 2-HYDROXYACETOPHENETIDINE (2-HYDROXY-4-ETHOXYACETANILIDE, I)



Results and Discussion

A. Preliminary Chromatographic Studies on Urinary Metabolites.—Three dogs received oral doses of acetophenetidine, and urine was collected and pooled. The $CHCl_3$ extract of urine, hydrolyzed with β-glucuronidase, was examined by paper chromatography. When the paper chromatogram was sprayed with diazotized sulfanilic acid (Pauly reagent), an orange spot was detected. Although this unknown substance could be detected in $CHCl_3$ extracts from unhydrolyzed urine, the concentration determined chromatographically was about 10 times as great in $CHCl_3$ extracts of urine treated with β-glucuronidase. Since the metabolite coupled with diazotized sulfanilic acid, it appeared that it was a ring-hydroxylated derivative of acetophenetidine, possibly I.

B. Synthesis of 2-Hydroxyacetophenetidine from Resorcinol.—2-Hydroxyacetophenetidine of unequivocal structure was synthesized as shown in Scheme I. The oxazole ring of II was used both to confirm the position of the potential 2-hydroxyl with respect to the future acetamido group, and to protect this hydroxyl during the ethylation of the 4-hydroxyl to the eventual 4-ethoxy group. The synthesis of I started with resorcinol, ensuring *meta* orientation of the final hydroxyl and ethoxyl groups. Nitrosation[2a] gave the 4-nitroso compound shown. Reduction of the nitroso group (Fe and HCl in aqueous ethanol) was followed by acetic anhydride treatment, thermal cyclization, and gentle saponification, to give 2-methyl-6-hydroxybenzoxazole (II).[2b] The phenolic impurity reported by Haginiwa was found to be II which was purified and combined with that produced by saponification of the acetoxy compound, to give an over-all yield of 20%.

Ethylation of the potassium salt of II with ethyl iodide to III, followed by cautious hydrolysis of III, led to 2-hydroxyacetophenetidine (I), m.p. 169–171° (varies with speed of heating). It formed a monoacetate (addition of acetic anhydride to the sodium salt in water) with a melting point more reproducible than that of I.

C. Isolation and Identification of the Metabolite as 2-Hydroxyacetophenetidine.—The β-glucuronidase-

(1) B. B. Brodie and J. Axelrod, *J. Pharmacol. Exptl. Therap.*, **97**, 58 (1949).

(2) (a) J. Haginiwa, *J. Pharm. Soc. Japan*, **73**, 1316 (1953). Two commercially available samples of 4-nitrosoresorcinol could not be reduced successfully. (b) The procedures described in the Experimental Section are modifications of Haginiwa's methods.