ACTA CHEMICA SCANDINAVICA 8 (1954) 1806—1812

Studies on Local Anesthetics X[1–9]

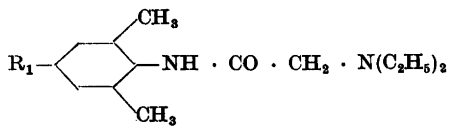
NILS LÖFGREN and CLAËS TEGNÉR

Institute of Organic Chemistry and Biochemistry, University of Stockholm, Stockholm, Sweden, and School of Pharmacy, University of Lausanne, Lausanne, Switzerland

Xylocaine, *i.e.* ω-diethylamino-2,6-dimethylacetanilide, and a fairly large number of compounds related to it have been synthesized by Löfgren *et al.*[1–9] The compounds have been tested pharmacologically, especially for their local anesthetic action. On certain of the compounds, some physico-chemical measurements have been carried out, and relations of physico-chemical properties to structure and anesthetic activity studied [5,10,11].

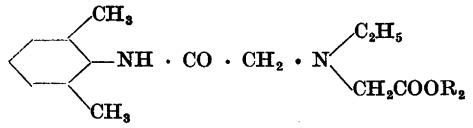
In the present investigation six new compounds have been synthesized and their local anesthetic action studied.

The structure of three of these compounds (I, II, III) is represented by the formula



where R_1 = HO (I), CH_3O (II), or C_3H_7COO (III).

The three other compounds (IV, V, VI) belong to another series



where R_2 = C_2H_5 (IV), *n*-C_3H_7 (V), or *n*-C_4H_9 (VI).

Compounds I, II, and III were prepared by chloroacetylation of the actual aromatic amine, using Löfgren's acetate buffer method [1,5], and subsequent treatment of the chloroacetyl derivative with diethylamine.

To obtain compound II, ω-diethylamino-4-methoxy-2,6-dimethylacetanilide, we first tried to methylate I, ω-diethylamino-4-hydroxy-2,6-dimethylacetanilide, with diazomethane; but this reaction failed. Nor did we succeed to methylate ω-chloro-4-hydroxy-2,6-dimethylacetanilide with the aid of the same reagent. Owing to the risk of side reactions methylation with dimethyl sulphate was not tried. (The tertiary base may give a quaternary compound; the chloro derivative may be sensitive to alkali.)

For the chloroacetylation of 4-hydroxy-2,6-dimethylaniline the above mentioned acetate method was modified because of the slight solubility of the

aminophenol in glacial acetic acid. The chloroacetylation was made by adding first chloroacetyl chloride to a water solution of the hydrochloride and then, without any delay, an aqueous solution of sodium acetate, this mixture being at once shaken vigorously . — The aminophenol, 4-hydroxy-2,6-dimethylaniline, was obtained by nitrosating 3,5-dimethylphenol followed by reduction with ammonium sulphide. This aminophenol has been previously prepared by treating 2,6-dimethylphenylhydroxylamine with diluted sulphuric acid [12], and by reduction of 4'-nitro-4-hydroxy-3,5-dimethylazobenzene [13]. — An efficient synthesis for 4-methoxy-2,6-dimethylaniline — the starting amine for preparing compound III — has been devised by Saunders and Watson [14]. They coupled 3,5-dimethylphenol with benzenediazonium chloride and methylated the resulting azo compound with dimethyl sulphate. The methoxyazo compound was then reduced under high pressure with hydrogen and Raney nickel. We followed their method, but for the reductive cleavage we used sodium hydrosulphite instead of hydrogen and Raney nickel.

Compounds IV, V, and VI were obtained by reaction between ω-ethylamino-2,6-dimethylacetanilide and the actual alkyl bromoacetate. (*Cf.* Löfgren and Widmark [4] who previously used the same method for preparation of ω-(N-methyl-carbethoxymethylamino)-2-methylacetanilide).

The compounds were tested for their local anesthetic potency on the rabbit cornea and compared with xylocaine, the method of Wiedling [15] being followed. As a measure of the potency, the *relative activity* according to Wiedling is used, *i.e.* an activity which will be found by dividing the concentration of a xylocaine standard solution by the concentration of the actual compound at which the duration of anesthesia is the same as that of the standard solution.

By this method 0.25 ml of the test solution is instilled into the rabbit's conjunctival sac, so that the cornea is covered, and allowed to remain for 30 seconds. Simultaneously the same volume of 2 % xylocaine hydrochloride solution of the same pH value is applied to the animal's other conjunctival sac. The time elapsing from the application of the local anesthetic solution to the onset of anesthesia is termed the *latent period*. The latent period and *duration* of complete anesthesia are established by the use of a rigid mechanical irritant, *i. e.* a graphite point. The pressure is adjusted so that a depression of the cornea is just elicted.

The 2 % xylocaine · HCl (0.0738 *M*) is said to have unit activity. A concentration of the actual compound at which the duration is the same as that of the xylocaine standard is selected. By dividing the molarity of the standard (= 0.0738) by the selected molarity of the test solution we thus obtain a value which we term the *relative activity* of the compound *.

As a rule, the test solutions are applied to the cornea at a pH of about 6. Sometimes, however, it may happen that one or other of the compounds within a series must be measured at a lower pH value (sometimes down to pH 4) since otherwise the compound can not be brought into solution. The duration at this lower pH value may differ slightly from that which would have been obtained at the higher pH value **; but since the stand-

* This way of calculating the relative activity differs from that of Wiedling who takes the quotient between the percentages instead of dividing the molarities.

** Thus for instance the 2 % hydrochloride solution of xylocaine gives durations from about 13 minutes down to about 10 minutes if the pH value is varied from 7 to 4. (The figures for the duration are given as average values from many experiments.) When, however, *minimal effective concentrations* are measured to evaluate local anesthetics, the influence of pH is very great (*cf.* Trevan and Boock [16], Löfgren [5]).

ard solution of xylocaine is measured at the same lower pH value, the calculated relative activity will be found to be consistent with the values of the other compounds.

In the first series of compounds (*cf.* the general formula given on p. 1806), I and III gave no anesthesia when tested as 2 % solutions on the rabbit cornea (solutions of the hydrochlorides, containing 2 % of the bases; pH = 6.0). They may therefore be described as inactive. Compound II gave a slight relative activity, the value being estimated to 0—0.5.

In a recent paper of 1951, Büchi *et al.*[17] report on a compound, ω-diethylamino-4-*n*-butoxy-2,6-dimethylacetanilide, which differs from our compound II in having a *n*-butoxy group instead of a methoxy group. They found the compound to be much more active than xylocaine on the rabbit cornea. We synthesized this butoxy compound following the directions given by Büchi *et al.* who describe the compound as being an oil at room temperature. On the contrary we found the compound to be a solid (m. p. 37—38°, *cf.* the synthetic part compound II:a). When tested on the rabbit cornea, the compound gave a relative activity of 18 (xylocaine used as a standard, *cf.* above). At the same time it was observed that the compound is a pronounced irritant, causing conjunctivitis and corneitis. As mentioned above, Büchi *et al.* also performed their measurements on the rabbit cornea but they have not reported any irritating property of the compound *. We also tested the butoxy compound in injection anesthesia on man, comparing it with xylocaine in subcutaneous wheals and in block anesthesia of the fingers.

For the tests in subcutaneous wheals, 1.5 % hydrochloride solutions of both compounds were compared. Each solution had a physiological NaCl content and an epinephrine concentration of 1:80 000; the pH value of the solutions was 4.5. On each of six persons, 1 ml of the two solutions was injected subcutaneously (dorsal side of the *brachium*). During injections, the compound of Büchi *et al.* caused marked pain. The average duration of the compound was 7.2 hr whereas xylocaine gave a corresponding value of 7.3 hr. The compound of Büchi *et al.* injured strongly the tissues in the centre of the infiltrated area and owing to this damage the centre was anesthesized for a long period, in some cases up to two weeks ("irreversibel" anesthesia).

For the experiments in block anesthesia of the fingers, a 1 % xylocaine · HCl solution was compared with a 1.5 % solution of the hydrochloride of the butoxy compound. (Both solutions had the same pH value, NaCl content, and epinephrine concentration as the solutions used for the subcutaneous wheals.) In each of four persons, 3 ml of the xylocaine solution were injected at the base of a third finger and the same operation was made with the butoxy compound on the other third finger. The xylocaine solution gave an average duration of 5.7 hr whereas the corresponding value of the solution of the butoxy compound was 5.6 hr. The latent period of the xylocaine solution was 3.8 minutes; the corresponding period for the other solution was 9 minutes. The extent of anesthesia obtained with the xylocaine solution was greater than that of the solution of the butoxy compound. Further it was observed that the compound of Büchi *et al.* injured the tissues, so that sore swellings persisted for several days.

In a later paper, Büchi[18] reports: "Die Verbindung übertrifft das Pantocain als Oberflächenanästheticum und das Novocain und das Xylocain als Infiltrationsanalgeticum".** He says nothing about the irritating properties of the compound. — To judge from our experiments on man, the compound of Büchi *et al.* is not superior to xylocaine in infiltration anesthesia. Furthermore, our experiments show definitely that the compound is useless as a clinical anesthetic.

Toxicity measurements, as carried out by subcutaneous injections in white mice, gave the same LD_{50} value of I and II, *viz.* 0.7 g/kg. The LD_{50} value of III was found to be 0.9 g/kg. The corresponding value for xylocaine is 0.34 g/kg.

* In their paper, Büchi *et al.* have graduated most of their compounds according to a certain scale of irritant power.

** In what way these measurements were carried out is not stated. — In the same paper, Büchi has misinterpreted Löfgren's discussions[5] on the relationsships between structure and local anesthetic action.

Compounds IV, V, and VI, belonging to the other group of compounds, gave relative activities 0.5 (approx.), 2.3, and 2.7, respectively. Compound VI showed a slight irritation of the cornea. The toxicity of the compounds was found to be low, the LD_{50} values (white mouse) for all three compounds being > 2 g/kg.

Compound V which has an activity 2.3 times that of xylocaine on the rabbit cornea was also tested subcutaneously on man and compared with xylocaine In this experiment the compound was found to be strongly inferior to xylocaine.

For the test on man, a 0.5 % hydrochloride solution with a physiological NaCl content and with an epinephrine concentration of 1:50 000, was prepared (pH of the solution = 5). Of this solution, four subcutaneous injections, each of one ml, were made on the volar side of the forearm. During injections strong pain was observed. The average duration of the anesthesia was 12 minutes. A solution of xylocaine · HCl as weak as 0.2 % tested under the same conditions, gave a duration of at least four hours.

The experiments with compound V show that a compound may have a higher activity than xylocaine on the rabbit cornea but a lower activity than xylocaine in subcutaneous anesthesia on man (*cf.* also the measurements on the compound of Büchi *et al.* as described above). We have often observed this discrepancy previously between the two methods *.

All six compounds were tested for their spasmolytic and histaminolytic power. No appreciable effects were found.

DESCRIPTION OF SYNTHESES **

ω-Diethylamino-4-hydroxy-2,6-dimethylacetanilide (I). 4-Hydroxy-2,6-dimethylaniline was obtained by nitrosating 3,5-dimethylphenol and subsequent reduction of the nitroso derivative with ammonium sulphide. As regards the details, the directions given by Kremers *et al.*[19] for the preparation of aminothymol were followed. The compound was recrystallized from water; colourless crystals melting at 179—182° (decomp.), in agreement with the recorded melting points [12,18]; yield 22 %, calculation based on xylenol.

For the preparation of ω-chloro-4-hydroxy-2,6-dimethylacetanilide, the following method was used: 35 g (0.26 mole) of 4-hydroxy-2,6-dimethylaniline were dissolved in 21 ml of 12.2 *N* hydrochloric acid and 120 ml of water. The solution was cooled to 17°. An acetate solution was prepared by dissolving 74 g of AcONa · $3H_2O$ in 120 ml of water, and cooled to 2°. To the solution of the aminophenol · HCl were first added 32 g (0.28 mole) of chloroacetyl chloride and then, without any delay, the acetate solution. The mixture was then immediately agitated by vigorous shaking which was carried on for five minutes, and was thereafter cooled in an ice-bath. The precipitate was filtered off, washed with ice-cold 1 *N* hydrochloric acid, then with water of the same temperature and finally dried in a desiccator over silica gel. The yield of the crude product was 38 g (0.18 mole), *i. e.* 69 %. The substance was purified by recrystallization from glacial acetic acid and colourless crystals were obtained.

From the chloro derivative, ω-diethylamino-4-hydroxy-2,6-dimethylacetanilide was prepared in the following manner. In a flask fitted with a reflux condenser, a mixture of 26 g (0.12 mole) of the purified 4-hydroxy-2,6-dimethylacetanilide, 20 g (0.27 mole) of diethylamine, and 200 ml of benzene was boiled for five hours. The precipitated diethylammonium chloride was filtered off from the hot solution by suction and then washed with a small quantity of hot benzene. The filtrate and the washings were combined. As the temperature dropped, the ω-diethylamino-4-hydroxy-2,6-dimethylacetanilide

* *Cf.* for instance Löfgren and Takman [7].

** All melting points and boiling points are uncorrected unless otherwise stated.

crystallized from the solution. The product was filtered from the cold solution by suction, dried, and washed with ice-cold water. The substance was then dried in a vacuum desiccator over silica gel and finally recrystallized from benzene with the addition of some methyl ethyl ketone. Colourless needles of m. p. 181—182° (corr.) were obtained.

Anal. Calcd. for $C_{14}H_{22}N_2O_2$ (250.3): C 67.2, H 8.86. Found: C 67.2, H 8.88.

Hydrochloride. When recrystallized from *n*-propanol with the addition of some methanol, the salt was obtained as colourless laths. It melted at 260—266° (corr.) under decomp.

Anal. Calcd. for $C_{14}H_{23}ClN_2O_2$ (286.8): C 58.6, H 8.08, N 9.77. Found: C 58.4, H 8.06, N 9.96.

ω-Diehylamino-4-methoxy-2,6-dimethylacetanilide (II). 4-Methoxy-2,6-dimethylazobenzene was prepared according to Saunders and Watson [14]. From this compound, 4-methoxy-2,6-dimethylaniline was obtained by reduction with sodium hydrosulphite in the following way: in a flask fitted with a reflux condenser, 45.0 g (0.185 mole) of 4-methoxy-2,6-dimethylazobenzene were dissolved in 900 ml of 50 % ethanol by heating to boiling, and then 120 g (0.55 mole) of sodium hydrosulphite were added portion by portion under continued boiling. When the red colour had disappeared most of the alcohol was removed by distillation under reduced pressure. The residue was made slightly alkaline and then extracted with ether. The ethereal solution was dried (Na_2SO_4), the ether driven off, and the residue was fractionated by distillation under reduced pressure. The 4-methoxy-2,6-dimethylaniline was collected as an almost colourless oil at 130—135°/15 mm. The oil soon solidified and recrystallization from light petroleum gave crystals melting at 42°, in agreement with the recorded values of the melting point [14,20]. (Owing to an accident we are unable to state the yield.)

The 4-methoxy-2,6-dimethylaniline was chloroacetylated, using Löfgren's acetate buffer method [1,5]. The yield of the crude ω-chloro-4-methoxy-2,6-dimethylacetanilide was 88 %. On recrystallization from toluene, colourless crystals were obtained; m. p. 179—180°.

Anal. Calcd. for $C_{11}H_{14}ClNO_2$ (227.7): C 58.0, H 6.20. Found: C 58.1, H 6.13.

The chloro derivative was then treated with diethylamine in benzene solution as described in the reaction between ω-chloro-4-hydroxy-2,6-dimethylacetanilide and diethylamine (see under the preparation of compound I). After having boiled the reaction mixture for four hours, the precipitated diethylammonium chloride was filtered off and washed with some hot benzene. The filtrate and the washings were combined and the benzene solution was extracted exhaustively with 3 *N* HCl. The aqueous solution was made alkaline and the base taken up in ether. The ethereal solution was dried (Na_2SO_4), the solvent removed, and the residue then fractionated by distillation under reduced pressure. The ω-diethylamino-4-methoxy-2,6-dimethylacetanilide was collected as a colourless oil boiling at 144—145°/0.02 mm; $n_D^{20} = 1.5257$; yield 57 %.

Anal. Calcd. for $C_{15}H_{24}N_2O_2$ (264.4): C 68.1, H 9.15. Found: C 68.6, H 9.13.

*ω-Diethylamino-4-*n*-butoxy-2,6-dimethylacetanilide* (II:a). This compound has earlier been prepared by Büchi *et al.*[17] (synthesis of 4-*n*-butoxy-2,6-dimethylaniline, chloroacetylation of this amine, and subsequent treatment of the chloro derivative with diethylamine). Büchi *et al.* describe the compound as being an oil at ordinary room temperature. We synthesized this compound following the directions given by Büchi *et al.* and found the compound to be a solid which could be recrystallized from petroleum ether.

The 4-*n*-butoxy-2,6-dimethylaniline, as obtained by us, had the same b. p. as that stated by Büchi *et al.* We also prepared the hydrochloride of this amine *; colourless small crystals from absolute ethanol with the addition of some absolute ether; m. p. 208—210° (decomp.).

Anal. Calcd. for $C_{12}H_{19}NO$ (229.75): C 62.7, H 8.77, N 6.10. Found: C 62.4, H 8.72, N 5.92.

For ω-chloro-4-*n*-butoxy-2,6-dimethylacetanilide we obtained the same m. p. (120—121°) as that given by Büchi *et al.*

The ω-diethylamino-4-*n*-butoxy-2,6-dimethylacetanilide (II:a) was collected as an oil boiling at 166—167°/0.03 mm (Büchi *et al.* state 158°/0.1 mm). The oil soon solidified and after recrystallization from light petroleum the compound showed a m. p. of 37—38°; $n_D^{20} = 1.5137$ (determined on the *supercooled* liquid compound).

* Not prepared by Büchi *et al.*

Anal. Calcd. for $C_{18}H_{30}N_2O_2$ (306.4): C 70.6, H 9.87, Equiv.w. 306. Found: C 70.4, H 9.80, Equiv.w. 305 *.

ω-Diethylamino-4-butyroxy-2,6-dimethylacetanilide hydrochloride (III). In a flask, fitted with a calcium chloride tube, a solution of 4.0 g (0.016 mole) of ω-diethylamino-4-hydroxy-2,6-dimethylacetanilide (compound I) in 50 ml of dry dioxan was mixed with 1.7 g (0.016 mole) of *n*-butyryl chloride under vigorous shaking. The mixture was allowed to stand at room temperature over-night. 150 ml of absolute ether were then added and after half an hour the hydrochloride of ω-diethylamino-4-butyroxy-2,6-dimethylacetanilide was filtered off; colourless crystals of m. p. 103—104°; yield 3.5 g (0.010 mole), *i. e.* 63 %.

Anal. Calcd. for $C_{18}H_{29}ClN_2O_2$ (356.9): C 60.6, H 8.19. Found: C 60.3, H 8.11.

ω-(N-Ethyl-carbethoxymethylamino)-2,6-dimethylacetanilide (IV). ω-Ethylamino-2,6-dimethylacetanilide was prepared as is described by Löfgren and Widmark [4]. To a solution of 10.5 g (0.051 mole) of this compound in 50 ml of dry benzene were added 4.3 g (0.026 mole) of ethyl bromoacetate under vigorous stirring. The mixture was then heated for 48 hours at 50° in a sealed bottle. The separated hydrobromide of ω-ethylamino-2,6-dimethylacetanilide was filtered off and washed with some hot benzene. From the combined filtrate and washings the solvent was driven off. The residue, a viscous oil, on distillation gave an almost colourless oil; b. p. 180—181°/0.5 mm; $n_D^{20} = 1.5162$; yield 4.0 g (0.014 mole), *i. e.* 54 %.

Anal. Calcd. for $C_{16}H_{24}N_2O_3$ (292.4): C 65.7, H 8.27. Found: C 66.0, H 8.42.

*ω-(N-Ethyl-carbo-*n*-propoxymethylamino)-2,6-dimethylacetanilide* (V). This compound was synthesized by the same method as described above for the preparation of compound IV, with the only difference that *n*-propyl bromoacetate was used instead of ethyl bromoacetate. — A viscous oil was obtained; b. p. 155—156°/0.05 mm; $n_D^{20} = 1.5109$; yield 94 %.

Anal. Calcd. for $C_{17}H_{26}N_2O_3$ (306.4): C 66.6, H 8.55, Equiv.w. 306. Found: C 66.8 H 8.52, Equiv.w. 307 **.

Hydrochloride. Colourless small hygroscopic crystals from dioxan; m. p. 151—152°

Anal. Calcd. for $C_{17}H_{27}ClN_2O_3$ (342.9): Cl 10.34. Found: Cl 10.31 (Mohr).

*ω-(N-Ethyl-carbo-*n*-butoxymethylamino)-2,6-dimethylacetanilide* (VI). The compound was synthesized from *n*-butyl bromoacetate and ω-ethylamino-2,6-dimethylacetanilide by the method described above for the preparation of compound IV. — A colourless viscous oil was obtained; b. p. 151—152°/0.025 mm; $n_D^{20} = 1.5081$; yield 83 %.

Anal. Calcd. for $C_{18}H_{28}N_2O_3$ (320.4): C 67.5, H 8.81, Equiv.w. 320. Found: C 67.4, H 8.90, Equiv.w. 321 ***.

Hydrochloride. Colourless small crystals from dioxan; m. p. 133—134°.

Anal. Calcd. for $C_{18}H_{29}ClN_2O_3$ (356.9): Cl 9.94. Found: Cl 10.01 (Mohr).

SUMMARY

Six new compounds, related to xylocaine, have been synthesized and tested for their local anesthetic, spasmolytic, and histaminolytic action.

We are indebted to AB Astra, Södertälje, Sweden, for financial support and to Dr. S. Wiedling of the Biological Department of Astra for performing the pharmacological tests. Our sincere thanks are also due to Professor A. Girardet of the University of Lausanne who read through the manuscript.

* Titration of the base in 30 % ethanol with 0.1 *N* HCl; mixed indicator methylene blue-methyl red.

** Titration with perchloric acid in glacial acetic acid; indicator crystal violet.

*** The same analytical method as described under compound V.

REFERENCES

1. Löfgren, N. *Arkiv Kemi, Mineral. Geol.* **A 22** (1946) No. 18.
2. Löfgren, N. and Lundqvist, B. *Svensk Kem. Tidskr.* **58** (1946) 206.
3. Löfgren, N. and Fischer, I. *Svensk Kem. Tidskr.* **58** (1946) 219.
4. Löfgren, N. and Widmark, G. *Svensk Kem. Tidskr.* **58** (1946) 323.
5. Löfgren, N. *Studies on Local Anesthetics. Xylocaine a New Synthetic Drug.* Dissertation, Stockholm 1948.
6. Löfgren, N. and Takman, B. *Acta Chem. Scand.* **6** (1952) 1006.
7. Löfgren, N. and Takman, B. *Acta Chem. Scand.* **6** (1952) 1010.
8. Ekstrand, T. and Löfgren, N. *Acta Chem. Scand.* **6** (1952) 1016.
9. Löfgren, N. and Tegnér, C. *Acta Chem. Scand.* **8** (1954) 1802.
10. Tammelin, L. and Löfgren, N. *Acta Chem. Scand.* **1** (1947) 871.
11. Fischer, I. and Löfgren, N. *Acta Chem. Scand.* **4** (1950) 1408.
12. Bamberger, E. and Rising, A. *Ann.* **316** (1901) 292.
13. Smith, L. and Irwin, W. *J. Am. Chem. Soc.* **63** (1941) 1036.
14. Saunders, B. and Watson, G. *Biochem. J. (London)* **46** (1950) 632.
15. Wiedling, S. *Acta Pharmacol. Toxicol.* **8** (1952) 117.
16. Trevan, J. and Boock, R. *Brit. J. Exptl. Pathol.* **8** (1927) 307.
17. Büchi, J., Launer, G., Ragaz, L., Böniger, H. and Lieberherr, R. *Helv. Chim. Acta* **34** (1951) 278.
18. Büchi, J. *Arzneimittel-Forsch.* **2** (1950) 70.
19. Kremers, E., Wakeman, N. and Hixon, R. *Org. Syntheses*, Coll. Vol. **1** (1946) 511.
20. Bamberger, E. *Ber.* **36** (1903) 2028.

Received July 16, 1954.