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Studies on Local Anesthetics XV *

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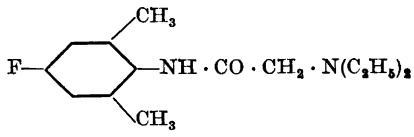
The thermodynamic ionization constants, the apparent molar refractions and the ultraviolet absorption spectra of α-diethylamino-4-fluoro-2,6-dimethylacetanilide and of xylocaine have been determined. The electronic effect induced by the fluorine atom is discussed, and finally, a possible explanation is offered for the fact that α-diethylamino-4-fluoro-2,6-dimethylacetanilide as compared with xylocaine has such a low local anesthetic potency.

In a previous work [2] some xylocaine analogues, each with a fluorine atom in the benzene nucleus, were synthesized and studied for their local anesthetic activity. In comparison with xylocaine, the fluoro derivatives were found to have a lower potency, and contrary to xylocaine they exercised an irritant action.

One of these compounds, α-diethylamino-4-fluoro-2,6-dimethylacetanilide, with the formula



had a duration on rabbit cornea which was about one tenth of that of xylocaine. As this compound is so closely related to xylocaine, (the non-fluorinated derivative), the low potency is rather astonishing.

Löfgren [3], Fischer and Löfgren [4] investigated some physico-chemical properties of xylocaine and closely related compounds. In making a comparison between xylocaine and its nuclear isomers and homologues, xylocaine was found to have: (a) a very low exaltation in its molar refraction, (b) a low pK_a value, (c) the ultraviolet absorption bands displaced towards shorter wave lengths, and (d) the intensities of the bands appreciably lowered. Now, it seemed to be of interest to us to study how the fluorine atom in the

* For paper XIV of this series see Lüning [1].

4-position affects these properties of xylocaine. The fluorinated compound and also xylocaine have therefore been subjected to measurements of thermodynamic ionization constants in water at 25°, apparent molar refractions in benzene solutions at 20° and ultraviolet absorption spectra in hexane solution.

Determination of the thermodynamic ionization constant. For the thermodynamic ionization constant, K_a, we have

$$pK_a = pH - \log \frac{C_B}{C_{BH^+}} - pf_{BH^+} \quad (1)$$

C_B is the concentration of the uncharged base B, C_{BH^+} is the concentration of the acid BH^+, $pf_{BH^+} = -\log f_{BH^+}$, and f_{BH^+} is the activity coefficient of BH^+. The pH values were measured with the aid of a cell of the type:

(—) "Glass electrode" / H^+ (aq) / / KCl (satd.) / Hg_2Cl_2, Hg (+)

For this cell we have

$$pH = \frac{E - E'}{k} \quad (2)$$

where E is the e.m.f. of the cell in mV and E' is the standard potential of the cell including the liquid junction potential (assumed to be constant). At 25°, k is 59.16 mV. Kielland [5] has calculated activity coefficients for a large number of ions at different concentrations in water at 25° by use of the Debye-Hückel formula:

$$-\log f_i = pf_i = \frac{0.358 \cdot z_i^2 \cdot \Gamma^{1/2}}{1 + 10^8 \cdot d_i \cdot 0.2325 \cdot \Gamma^{1/2}} \quad (3)$$

where f_i is the activity coefficient of the ith ion with valence z_i and effective diameter d_i. Γ is the ionic concentration, given by $\Gamma = \Sigma C_i z_i^2$ with C_i in moles per litre.

According to measurements by Löfgren [3] the effective diameter for the BH^+ ions were assumed to be 8×10^{-8} cm. The values of f_i for ions with this effective diameter were logarithmed, and pf_{BH^+} was plotted against $\frac{1}{2}\Gamma = C_{salt}$ in a diagram from which pf_{BH^+} could be read off directly at any concentration.

Since it is difficult to obtain a clear survey of the systematic errors, (above all, errors caused by liquid junction potentials) we have confined ourselves to an accuracy of two decimals for pK_a. For mutual comparison, however, the values can be given with greater accuracy; the error here seldom exceeds $\pm$ 0.005.

The experimental data from the measurements of the ionization constants are compiled in Table 1. The double e.m.f. values arise from the two glass electrodes used. From Table 1 it is seen that the fluorinated compound ($pK_a = 7.78_1$) is a weaker base than xylocaine ($pK_a = 7.84_8$; previously found (Löfgren [3]) 7.85_5).

Determination of the apparent molar refraction. For a mixture, the following equation is valid

$$\frac{n^2 - 1}{n^2 + 2} \cdot \frac{m_0 + m_1}{d} = \frac{n_0^2 - 1}{n_0^2 + 2} \cdot \frac{m_0}{d_0} + (r_1)_{app.} \cdot m_1 \quad (4)$$

Table 1. Determination of the ionization constants. NaOH-solution = 0.0265_0 M; test solution = 100 ml. $AHNO_3$ = xylocaine hydronitrate; $BHNO_3$ = α-diethylamino-4-fluoro-2,6-dimethylacetanilide hydronitrate.

Compound	salt weighed (g)	NaOH added (ml)	$\log \frac{C_B}{C_{BH^+}}$	pf_{BH^+}	$-E'$ (mV)	$+E$ (mV)	pH	pK_a	pK_a average
	0.1315	9.05	+0.073	0.029	135.1 120.0	335.1 350.0	7.948	7.846	
	0.1439	9.15	+0.002	0.031	135.1 120.0	331.2 346.1	7.882	7.849	
$AHNO_3$	0.1622	9.33	−0.082	0.032	135.1 120.0	326.6 341.3	7.802	7.852	7.84_8
	0.2142	9.14	−0.296	0.037	135.1 120.0	313.6 328.8	7.586	7.845	
	0.1426	9.20	+0.068	0.030	135.1 119.9	331.0 346.2	7.880	7.782	
	0.1552	9.10	−0.018	0.031	135.1 119.9	326.4 341.2	7.795	7.782	
$BHNO_3$	0.1710	9.26	−0.083	0.032	135.1 119.9	321.9 337.3	7.727	7.778	7.78_1
	0.2203	9.14	−0.275	0.035	135.1 119.9	311.2 326.2	7.542	7.782	

where n is the refractive index, d is the absolute density, m is the amount weighed and $(r_1)_{app.}$ is the apparent specific refraction of the solute; quantities referring to the solution are designated by no index, those referring to the solvent by the index 0 and those referring to the solute by the index 1. The apparent molar refraction is obtained from $(r_1)_{app.}$

$$(R_1)_{app.} = (r_1)_{app.} \cdot M_1 \tag{5}$$

The figures from the measurements of the molar refractions are compiled in Table 2. For the computation of the molar refraction from atomic refractions, the usual published values have been employed; see Table 3. Whereas xylocaine has a small exaltation = 0.16 (previously [3] found to be 0.24), the fluorinated compound has a small depression = 0.17.

Calculation of the errors in the molar refractions. According to Kohner and Johnson (see Weissberger [6]) an expression for the maximum error is

$$(\nabla R_1)_{app.} = \frac{1\,000}{C}\left[\frac{6n_0}{(n_0^2+2)^2}\cdot\nabla(\Delta n) + \frac{\Phi_0}{d_0}\cdot\nabla(\Delta d)\right] \tag{6}$$

where C is the concentration in moles per litre,

$$\Delta n = n - n_0,\ \Delta d = d - d_0 \text{ and } \Phi_0 = \frac{n_0^2 - 1}{n_0^2 + 2}$$

The "Pulfrich" used gave a maximum error in reading = ± 30″. This means that $(\nabla n)_a = \pm 4 \times 10^{-5}$. For a temperature change = 0.02° we obtain for

Table 2. Determination of apparent molar refractions [a]). A = xylocaine; B = α-diethylamino-4-fluoro-2,6-dimethylacetanilide.

Compound	Weighed amount (g) of		d_{20}	$(n_D^{20})^2$	$(R_1)_{app.}$	$(R_1)_{app.}$ average	Exalt.
	compound	benzene					
A	2.17328	7.29727	0.90513	2.26646	71.13		
	2.22808	7.56269	0.90498	2.26646	71.13	71.14	+0.16
	2.17145	7.40230	0.90483	2.26646	71.16		
B	2.24338	7.08697	0.91698	2.25695	70.72		
	2.27927	7.59943	0.91529	2.25639	70.67	70.71	−0.17
	2.26824	7.44430	0.91587	2.25695	70.73		

[a] The calculations are based on the refractive index $n_D^{20} = 1.50110$ for benzene. If, however, one should use another recorded value [20] ($n_D^{20} = 1.50144$) for benzene, the mean values of the apparent molar refractions will be 71.18 and 70.75 for A and B, respectively.

Table 3. Atomic refraction (D-line) of element or structural feature.

C	H	F	O in carbonyl group	Double bond	N in tert. amine	N in sec. amide
2.418	1.100	0.997 [a]	2.211	1.733	2.840	2.676

[a] Value according to Schiemann [21] (*cf.* also v. Auwers [22]).

benzene [7] $(\nabla n)_t = \pm 1.3 \times 10^{-5}$ and for aniline [7] the change is about the same. For these reasons we take ∇n for our substances to be $\pm 5 \times 10^{-5}$. For the same inaccuracy of temperature $(\nabla d)_t$ is estimated [8] to 2×10^{-5} and $(\nabla d)_{weight}$ to 5×10^{-5}. The total error ∇d is therefore estimated to be 1×10^{-4}.

For the following values

$$\nabla(\Delta d) = \pm (|\nabla d| + |\nabla d_0|) = \pm 2 \times 10^{-4}$$

$$\nabla(\Delta n) = \pm (|\nabla n| + |\nabla n_0|) = \pm 1 \times 10^{-4}$$

$$n_0 \sim 1.50,\ C \sim 1.0,\ d_0 \sim 0.88$$

is obtained $(\nabla R_1)_{app.} = \pm 0.1$.

The apparent molar refraction has therefore been given to two decimals.

In a previous paper [2], the nitration of 3,5-dimethylfluorobenzene yielding two mononitro derivatives was described. One of them was proved to be 4-nitro-3,5-dimethylfluorobenzene. The other nitro derivative was consequently considered to consist of 2-nitro-3,5-dimethylfluorobenzene. This assumption has now been proved experimentally. By heating 2-nitro-3,5-dimethylfluoro-

benzene with KOH in methanolic solution, the fluorine atom was replaced by the methoxy group and the resulting methoxy compound was identified as 2-nitro-3,5-dimethylanisole.

DISCUSSION

The strengths of aromatic bases are influenced by methyl groups in the *ortho*-position in a way which could not be predicted on the basis of the inductive and mesomeric effects. Thus *o*-toluidine and 2,6-xylidine are weaker bases than aniline (Thomson [9]). This means that the methyl groups decrease the electron-availability of the nitrogen atom. "In o-$CH_3C_6H_4NH_2$ the proton from the methyl group may go to the unshared pair of electrons of the amino group" (Branch and Calvin [10]), *i. e.* in this compound it is supposed that there is a hydrogen bond between the amine nitrogen and the methyl group. This type of hydrogen bond should, of course, be more pronounced in the di-*ortho*-methylated compound.

From measurements of ionization constants, Löfgren [3] suggests that the same type of hydrogen bond occurs in α-diethylamino-2,6-dimethylacetanilide ("xylocaine"). This compound, when compared with α-diethylaminoacetanilide, has a pK_a value about 0.2 units lower. It is particularly interesting to note that the *ortho*-effect is so strongly emphasized throughout the relatively long chain. From investigations on molar refractions and spectra, Löfgren concludes that the mobility in the resonance system is suppressed. Further, the steric conditions in this molecule indicate strongly that the interaction between the unshared electron pair of the amide nitrogen and the π-electronic system of the nucleus is suppressed. In other words, the electron-attracting capacity of the benzene nucleus is smaller in the 2,6-xylidide than in the anilide. One would then think that the pK_a value of the xylidide should be greater than that of the anilide. Supposing, however, that the unshared electron pair of the amide nitrogen is not only prevented from interfering with the nucleus but is also so influenced (by the *o*-methyl groups) that it becomes "bound", this would influence the electronic state at the carbonyl group. In the anilide, for instance, the key-oxygen-atom may have its electronic density increased by a mesomeric effect from the unshared electron pair of the amide nitrogen, but also by an inductive attraction which also affects the unshared electron pair of the diethylamino group at the end of the molecule. Through the *ortho*-effect described above, the mesomeric effect of the amide group on the carbonyl group in the 2,6-xylidide may be considered reduced, and therefore the inductive electron-attraction of the key-atom is more strongly pronounced. This means a weaker proton-binding capacity and appears in a low pK_a value.

Concerning *p*-substituted fluorine, it is known to induce a weak electron-attractive effect; in some cases *p*-F is even weakly electron-repulsive. *m*-F, however, exerts throughout clearly an electron-attractive effect (Watson [11], Hammet [12]). For our investigations it is of special value to recognize the influence of *p*-substituted fluorine on the amino group. From measurements of ionization constants (Bennet *et al.*[13], *cf.* also Watson [11]) of aniline and of *p*-fluoroaniline it is evident that there is an approximate equality between the

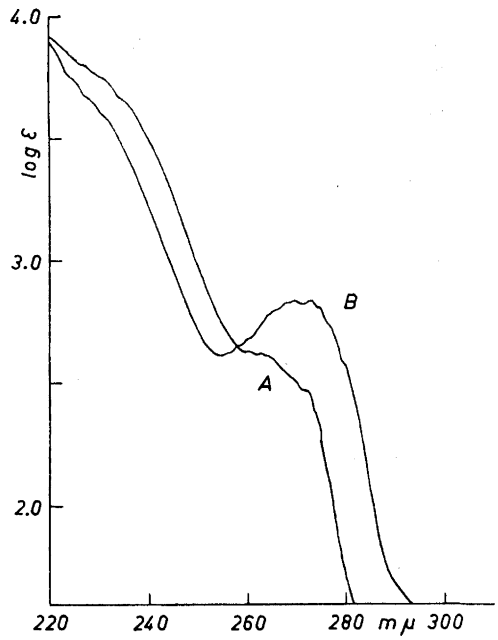


Fig. 1. Ultraviolet absorption spectra of hexane solutions of xylocaine (A) and α-diethylamino-4-fluoro-2,6-dimethylacetanilide (B)

constants of the two bases. Hence, we consider that *p*-F has very little influence on the electron pair of the amine nitrogen. On account of the steric conditions, the influence of the fluorine atom *para* to the nitrogen in 4-fluoro-2,6-dimethylaniline must be still less than in *p*-fluoroaniline.

We therefore conclude that the fluorine atom in α-diethylamino-4-fluoro-2,6-dimethylacetanilide has a negligible effect on the unshared electron pair of the amide nitrogen, but that the fluorine atom must exert a certain electron-attractive effect on the methyl groups (*cf.* above concerning *m*-substituted fluorine), *i. e.* the methyl groups must to a certain degree be "protonized". This should favour chelation of hydrogen bond type methyl group — amide nitrogen. The electron pair of the amide nitrogen in the fluorinated compound is therefore supposed to exert less interference with the carbonyl group than in the non-fluorinated compound, and for that reason (*cf.* above) the fluorinated compound has a lower pK_a value than the non-fluorinated compound, *i. e.* xylocaine. — To sum up, we put forward as a theory that the lower basicity of the fluorinated compound as compared with xylocaine is due to a stronger hydrogen bonding methyl group — amide nitrogen.

The differences in the molar refractions and absorption spectra are too small to permit any assumptions as regards the difference in the electronic states of the actual molecules. However, the findings from those measurements

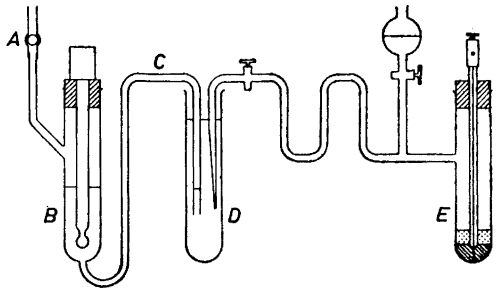


Fig. 2. Diagram of the cell. A = rubber tube with glass pellet (valve). B = glass electrode vessel. C = junction tube. D = junction vessel. E = satd. KCl calomel electrode.

do not in any way contradict what has been said above concerning the electronic effect of fluorine.

Finally, we want to make a possible explanation of the fact that the fluorinated compound, in comparison with xylocaine, has such a low local anesthetic potency. Generally it may be stated that the introduction of a fluorine atom in a physiologically active molecule changes to a marked degree its physiological activity, and this change in activity is probably due to the introduction of a strongly polarized C—F bond (*cf.* Burger *). According to Löfgren [3,15], there is a general rule for anilides of this type, *viz.* that the introduction of a strongly polar group in *p*-position to the amide nitrogen results in a low local anesthetic potency.

EXPERIMENTAL

Benzene, "Merck pro anal." was subjected to the usual purifying procedure, stored over sodium wire and distilled immediately before use; $d^{20} = 0.87902$; $n_D^{20} = 1.50110$ (recorded value [16]).

α-Diethylamino-2,6-dimethylacetanilide (xylocaine), prepared according to Löfgren [3], was recrystallized six times from petroleum ether; m. p. 67°. — The hydronitrate was prepared according to Löfgren [3], and then recrystallized three times from absolute ethanol; m. p. 133° (decomp.).

α-Diethylamino-4-fluoro-2,6-dimethylacetanilide, prepared according to Löfgren *et al.*[2], was recrystallized six times from petroleum ether; m. p. 56°. — The hydronitrate was obtained as colourless, thin needles from methyl ethyl ketone; m. p. 98°. (Found: C 53.2; H 6.99. Calc. for $C_{14}H_{22}FN_3O_4$ (315.3): C 53.3; H 7.03).

Solutions for pK_a determinations were made by dissolving the hydronitrates in water, neutralizing to 30—60 % with sodium hydroxide and diluting to definite volume.

The sodium hydroxide solution, practically carbonate free, was standardized against potassium hydrogen phthalate using phenolphthalein as an indicator. The distilled water had a specific conductivity of about 6×10^{-6} ohm^{-1} cm^{-1}.

The complete cell (see Fig. 2) was placed in a thermostat at 25° ± 0.05 °C. The vessel containing the glass electrode was filled by sucking up the solution through the junction

* According to Burger [14] fluorinated compounds appear to be much less physiologically active than derivatives containing other halogens, and even less than the corresponding non-fluorinated substances.

tube. To be able to measure the test solution with two different glass electrodes, the junction vessel was connected with two glass electrode vessels. Before use, each glass electrode vessel, with its electrode in place, was rinsed several times with distilled water and three times with the test solution. The boundary surface, originally at the end of the junction tube, was automatically raised 1.5 cm into the tube when the tube was lowered into the saturated potassium chloride solution. After ten minutes, readings were taken every other minute on each electrode until they became constant to within 0.1 mV (15—20 min.). With this apparatus the constancy and reproducibility of the measurements were very good.

The electromotive force was measured with an accuracy of 0.1 mV by a valve potentiometer (type PHM 3a made by Radiometer, Copenhagen). The standard cell of the potentiometer was checked against a Weston cell from the Cambridge Instrumental Company.

As a standard for the pH measurements, the buffer solutions recommended by Hitchcock and Taylor [17] were used.

The E' values of the glass electrodes (type G102A, Radiometer) were based on measurements with two freshly prepared different buffers, both before and after each day's work. The glass electrodes were also tested with the borate buffer (pH = 9.180) and no alkaline errors could be found.

The indices of refraction for the sodium D line, n_D, were determined with a Zeiss-Pulfrich refractometer at a temperature of 20° ± 0.02 °C. Repeated readings were taken in order to get a mean value. The refractive index of the prism was determined by means of carefully purified benzene. In this determination two different benzene preparations, "Merck pro anal." and "Stockholms Gasverk pro anal.", were used. Both benzene preparations showed the same angle of emergence.

The *absolute* density, d^{20}, of benzene and the benzene solutions were determined in Sprengel-Ostwald pyknometers, the weight being reduced to vacuum. The same thermostat and the same calibrated thermometer (graduated in 0.1°) were used in the measurements of the indices of refraction and the density. The density of benzene was determined by six separate measurements and the mean value agreed with a recorded value [16].

The ultraviolet absorption spectra were measured on a Beckman quartz spectrophotometer with a hydrogen lamp as a light source. The solvent was spectroscopically pure hexane and the concentrations were about 3×10^{-4} M. The interval between two readings was 1 mμ, except in the more interesting regions where the interval was 1/4 mμ

Proof of the position of the nitro group in 2-nitro-3,5-dimethylfluorobenzene. The nitro compound was treated with KOH in methanol in the same manner as described previously [2] for the determination of the position of the nitro group in the 4-nitro derivative. The melting point of the crude product was found to be 43—44°; yield 93 % (assuming the product to be a nitro-3,5-dimethylanisole). The substance was recrystallized from methanol. Pale yellow crystals melting at 44—45° were obtained; yield 75 % (*cf.* above). This melting point agrees with that of 2-nitro-3,5-dimethylanisole [18,19]. 2-Nitro-3,5-dimethylanisole was prepared according to the method of Rowe *et al.*[18] (nitration of 3,5-dimethylphenol and subsequent methylation of the 2-nitro derivative). The mixed m. p. showed no depression, and therefore the nitro compound must be 2-nitro-3,5-dimethylfluorobenzene.

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