acid appears to be more available to the organism as a precursor than the hydroxy derivative.

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF WISCONSIN
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The Molecular Size and Shape of Botulinus Toxin[1]

BY GERSON KEGELES[2]

Type A Botulinus toxin has been prepared in crystalline form by two independent groups of workers.[3,4] Concurrent with fractionation studies by these groups, the physicochemical properties of the toxin were investigated.[5] Electrophoretic investigations are reported elsewhere.[4] This communication reports conclusions as to the size and shape of the toxin molecule, based on studies of diffusion, apparent specific volume, and viscosity performed on crystalline materials.

The diffusion measurements were carried out in 0.06 molar sodium acetate buffer at *p*H 4.48 in the Tiselius electrophoresis apparatus[6] as described by Longsworth.[7] The results appear in Table I, where the diffusion constants have been averaged from both channels.

TABLE I

Da is calculated from height and area; *Dm* calculated by method of moments; $D_{20,w}$ calculated from average of *Da* and *Dm*.

Source	C-50 (Lamanna)[3]	XII (Abrams)[4]	XII (Abrams)[4]
Protein concn., %	0.47	0.98	0.50
Temp., °C.	1.0	1.0	20.0
$Da(10)^7$ cm.²/sec.	0.93	1.04	1.99
$Dm(10)^7$ cm.²/sec.	0.95	1.22	2.00
$D_{20,w}(10)^7$ cm.²/sec.	1.79	2.16	2.10

The lower value observed for the electrophoretically homogeneous fraction C-50 may be due, possibly, to partial denaturation resulting from the use of chloroform in its purification.

Density measurements on fractions prepared by both methods were made in an uncapped pycnometer at 30°. Since the protein becomes insoluble when dried, solutions for density determinations were prepared by dialysis against the buffers used as reference solvents. Protein concentrations in these solutions were determined by Kjeldahl nitrogen, using 14.2% as the best available figure for nitrogen content.[3,4] Apparent specific volumes in 0.06 molar sodium acetate buffer at *p*H 4.48 varied somewhat with concentration. On the assumption that the variation was a charge effect, the density measurement was repeated nearer to the isoelectric point[4] in the presence of a large excess of salt. The apparent specific volume obtained for a 0.59% solution of the salt-fractionated protein[4] in 0.2 molar sodium chloride–0.02 molar sodium acetate buffer at *p*H 5.38 was 0.76. The value of the partial specific volume corrected[8] to 20° is taken as $V_{20} = 0.75_5$.

Viscosity measurements at seven protein concentrations from 0.1 to 0.8 per cent. by weight in 0.06 molar sodium acetate buffer at *p*H 4.48 were made with an Ostwald viscometer, using fractions prepared by both methods. Although subsequent examination revealed extensive electrophoretic inhomogeneity in the crystalline chloroform-treated protein fraction studied,[3] satisfactory agreement in the viscosity data was obtained, giving an intrinsic viscosity of 10.6. This corresponds to an axial ratio of 8.3 according to the Simha theory[9] for elongated ellipsoids and a frictional ratio f/f_0 of 1.45 from the Perrin theory.[10] The isoelectric point[4] is 5.60 and further addition of neutral salt would suppress charge effects, giving a lower value for the frictional ratio.

The molecular weight *M* is 1,130,000 as calculated from the diffusion constant $D_{20,w} = 2.10\ (10)^{-7}$ cm²/sec., the partial specific volume $V_{20} = 0.75_5$ and the frictional ratio $f/f_0 = 1.45$ with the equation[11]

$$MV_{20} = [2.89(10)^{-5}/D_{20,w}(f/f_0)]^3$$

This must be regarded as a lower limit, because the error in the frictional ratio is tripled by this method of calculation. It is hoped that future ultracentrifuge studies which were not possible at the time of this investigation will improve the accuracy of the data. The large size of the molecule is particularly surprising in view of previous studies on bacterial toxins.[12]

(1) Investigations conducted at Camp Detrick, Frederick, Maryland, from July through October, 1945, by Gerson Kegeles, 1st Lt., CWS.

(2) Present address: Chemistry Department, University of Wisconsin, Madison, Wisconsin.

(3) Lamanna, McElroy and Ecklund, *Science*, **103**, 613 (1946).

(4) Abrams, Kegeles and Hottle, *J. Biol. Chem.*, **164**, 63 (1946).

(5) The author is indebted to the senior author of each group for the materials for these studies.

(6) Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

(7) Longsworth, *Ann. N. Y. Acad. Sci.*, **41**, 267 (1941).

(8) Svedberg and Pedersen, "The Ultracentrifuge," Oxford Press, 1940, Appendix II.

(9) Simha, *J. Phys. Chem.*, **44**, 25 (1940).

(10) Perrin, *J. phys. rad.* **VII**, **7**, 1 (1936).

(11) Reference 8, equation (70a).

(12) Krejci, Stock, Sanigar and Kraemer, *J. Biol. Chem.*, **142**, 735 (1942).

LABORATORIES, TECHNICAL DEPARTMENT
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Preparation of *p*-Alkylbenzyl Chlorides

BY G. M. KOSOLAPOFF

The preparation of *p*-alkylbenzyl chlorides has been effected usually by the method of Blanc[1] or by minor variations thereof. Such procedures utilize the catalytic effect of zinc chloride, which necessitates rather strict temperature control to avoid resinification and, generally, polysubstitu-

(1) Blanc, *Bull. soc. chim.*, (4) **33**, 313 (1923).

tion. Furthermore, the zinc chloride has to be removed with scrupulous and time-consuming aqueous washing in order to avoid decomposition of the products on distillation. It was found that much of the tedium of these preparations can be avoided by the use of a procedure which is in essence that of Cambron.[2] With elimination of the zinc chloride the usually troublesome emulsion formation on washing is avoided, the necessity for close temperature control is eliminated and the yields on reacted hydrocarbon are ample for most purposes. The following examples illustrate the procedure.

Experimental

***p*-Ethylbenzyl Chloride.**—238 g. of ethylbenzene, 90 g. of paraformaldehyde, 250 g. of glacial acetic acid, 280 cc. of concentrated hydrochloric acid and 135 cc. of 85% phosphoric acid were agitated at 100° (steam-bath) for four and one-half hours. On cooling the organic layer was separated, washed three times with cold water and distilled to yield 114 g. of recovered ethylbenzene, b. p. 42° at 28 mm., and 132 g. of *p*-ethylbenzyl chloride, b. p. 111° at 26 mm., $n^{25}D$ 1.5290, for 38% conversion and 73% yield.

***p*-Butylbenzyl Chloride.**—96 g. of *n*-butylbenzene, 29 g. of paraformaldehyde, 79 g. of glacial acetic acid, 88 cc. of concentrated hydrochloric acid and 43 cc. of 85% phosphoric acid were stirred under reflux for sixteen hours. On cooling, the organic layer was separated and washed three times with cold water. On distillation there was obtained 69.5 g. of recovered *n*-butylbenzene and 24 g. of *p*-butylbenzyl chloride, b. p. 142–146° at 27 mm., $n^{25}D$ 1.5159, for 27.5% conversion and 67% yield.

(2) Cambron, *Can. J. Research*, **17B**, 10 (1939).

MONSANTO CHEMICAL COMPANY
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The Toxicity of 3-Fluoro-*d*(+)- and *l*(−)-tyrosine

BY CARL NIEMANN AND M. M. RAPPORT

The toxicity and physiological action of 3-fluoro-*dl*-tyrosine have been the subject of numerous investigations[1] and in view of the interest shown in this substance it appeared desirable to compare the toxicity of the *d*- and *l*-isomers with that of the *dl*-mixture.[2] The *d*- and *l*-isomers were obtained from the *dl*-mixture by adoption of the method of enzymatic resolution originally developed by Bergmann and co-workers.[3]

In the rat the toxicity of each antipode was found to be identical with that of the *dl*-mixture and one may conclude that antipodal specificity with reference to the amino side chain is not a critical factor in the toxic action of 3-fluorotyrosine. One cannot conclude that the amino acid side chain is without effect, as *o*-fluorophenol is much less toxic than is 3-fluorotyrosine.[4] It appears that the presence of the amino acid side chain denies to 3-fluorotyrosine the detoxification routes ordinarily available to phenols with the result that the rat is forced to metabolize 3-fluorotyrosine with the concomitant formation of toxic end products other than fluoride ion.[1a] It appears from the work of Niedner[1b] that 3-fluorotyrosine behaves similarly in the mouse, for the LD_{50} for the mouse is apparently equal to that for the rat.

Experimental

N-Benzoyl-3-fluoro-*dl*-tyrosine.—3-Fluoro-*dl*-tyrosine[2] (20 g.) was benzoylated following the procedure of Carter and Stevens[5] to give 20.1 g. (66%) of N-benzoyl-3-fluoro-*dl*-tyrosine, m. p. 178–179° after recrystallization from a mixture of ethyl acetate and ligroin.

Anal. Calcd. for $C_{16}H_{14}O_4NF$ (303); C, 63.4; H, 4.7; N, 4.6. Found: C, 63.5; H, 4.4; N, 4.5.

3-Fluoro-*l*(−)-tyrosine.—N-Benzoyl-3-fluoro-*dl*-tyrosine (8.75 g.) was dissolved in 35 ml. of *N* sodium hydroxide and 44 ml. of 2 *M* sodium acetate and the solution filtered prior to the addition of 88 ml. of 0.1 *M* citrate buffer (*p*H 5.0), 0.65 g. of cysteine hydrochloride, 5.25 ml. of aniline, 88 ml. of a filtered papain solution prepared by dissolving 0.9 g. of purified papain[3a] in 100 ml. of 0.05 *M* citrate buffer (*p*H 5.0) and 170 ml. of water. After the addition of 1 ml. of 50% acetic acid the solution (*p*H 5.8) was incubated at 40° for seven days adding 1 ml. of 50% acetic acid on the second and third days. The precipitate was collected, washed with cold water and 50% aqueous ethanol and dried to give 3.25 g. of crude N-benzoyl-3-fluoro-*l*(−)-tyrosylanilide, m. p. 194–197° dec. The filtrate obtained after the removal of the precipitated anilide was adjusted to *p*H 5.5 with 50% acetic acid and incubated at 40° for another week. A second crop of 1.70 g. of anilide, m. p. 192–196°, was obtained to give a total yield of 4.95 g. or 91% of the theoretical quantity. A suspension of 4.95 g. of the above anilide in 200 ml. of 10% hydrochloric acid was refluxed for eighteen hours, the hydrolysate cooled to 25°, filtered and the filtrate extracted with ether. The aqueous phase was concentrated *in vacuo* to 50 ml. and neutralized by the addition of sodium acetate. The addition of ether to the solution induced crystallization whereupon the ethereal phase was decanted, the product collected and recrystallized twice from water to give 1.2 g. (48%) of 3-fluoro-*l*(−)-tyrosine, m. p. 278–279° with decomposition starting at 265° when heated at the rate of 5°/min.

Anal. Calcd. for $C_9H_{10}O_3NF$ (199): C, 54.3; H, 5.1; N, 7.0. Found: C, 54.5; H, 5.2; N, 6.9: $[\alpha]^{26}D = \frac{-0.29 \times 1.95}{1 \times 0.100} = -5.7°$ (in 4% hydrochloric acid).

3-Fluoro-*d*(+)-tyrosine.—The filtrate remaining after the removal of the second crop of N-benzoyl-3-fluoro-*l*(−)-tyrosylanilide was acidified with concd. hydrochloric acid to *p*H 1–2 and exhaustively extracted with ethyl acetate. The ethyl acetate phase was dried over sodium sulfate, the solvent removed and the residual oil refluxed with 200 ml. of 10% hydrochloric acid for eighteen hours. The hydrolysate was treated as described above and 2.2 g. of a mixture of 25% of 3-fluoro-*l*(−)-tyrosine and 75% of 3-fluoro-*d*(+)-tyrosine was obtained. This product was dissolved in the minimum quantity of hot water, the solution cooled, the precipitate discarded and the filtrate evaporated to dryness. The residue was recrystallized from water to give 0.9 g. of 3-fluoro-*d*(+)-tyrosine, m. p. 279–

(1) See for example (a) P. Boyer, R. Evans and P. Phillips, *J. Pharmacol. Exptl. Therap.*, **73**, 176 (1941); (b) K. Niedner, *Z. Krebsforsch.*, **51**, 159 (1941).

(2) C. Niemann, A. A. Benson and J. F. Mead, THIS JOURNAL, **63**, 2204 (1941).

(3) (a) M. Bergmann and H. Fraenkel-Conrat, *J. Biol. Chem.*, **119**, 707 (1937); (b) C. Niemann and P. L. Nichols, Jr., *ibid.*, **143**, 191 (1942).

(4) Unpublished experiments have shown that the LD_{50} of *o*-fluorophenol in the rat is greater than 100 mg./kg. when administered subcutaneously.

(5) H. E. Carter and C. M. Stevens, *J. Biol. Chem.*, **138**, 628 (1941).