TABLE I

PEPTIDE FRAGMENTS FROM N-TERMINUS OF CORTICOTROPIN-A

Peptide no.	Origin of peptide[a]	R_f values[b] Partridge	2-But.–NH_3	Amino acids detected after complete acid hydrolysis[c]	N-Terminal amino acid as detd. by DNFB[d]	C-Terminal work with carboxypeptidase[e]	Sequence
1	Chymotrypsin (24 hr.)	0.42	Pro	Ser, Tyr	Ser	Abt. 25% split into Ser Tyr after 24 hr. with 1% enzyme	Ser·Tyr·
2	Chymotrypsin (24 hr.)	.36	Glu+	Ser,Met,Glu,His, Phe	Ser	Only Phe split off after 20 hr. with 1% enzyme	Ser·(Met,Glu,His)·Phe
3	Pepsin (24 hr.) followed by chymotrypsin (24 hr.)	.42	Ileu	His,Phe	Not tried	Almost completely split in 24 hr. with 5% enzyme	His·Phe
4	Pepsin (24 hr.) followed by trypsin (2 hr.)	.42	Met+	His,Phe,Arg	His	Not tried	His·(Phe,Arg)
5	Pepsin (24 hr.)	.65	Glu+	Ser,Tyr,Met,Glu	Ser	Almost completely split into constituent amino acids in 24 hr.	Ser·(Tyr,Ser,Met,Glu)
6	Chymotrypsin (24 hr.) on peptide #5		Glu–	Ser,Met,Glu	Not tried	Only Glu split off after 20 hr. with 1% enzyme	(Ser,Met)·Glu
7	Trypsin (2 hr.)	.38	Pro	Ser,Tyr,Met,Glu, His,Phe,Arg	Ser	Not tried	Ser·Tyr·Ser·Met·Glu·His·Phe·Arg

[a] In all cases crystalline enzymes prepared by Armour Laboratories were used. Tryptic and chymotryptic reactions were done at *p*H 7.5 in 0.1 *N* ammonium acetate; peptic reactions in 0.1 *N* formic acid (*p*H 2.2). Digestions were done at a substrate concentration of 10 mg. per ml. and an enzyme concentration of 0.1 mg. per ml. A temperature of 37° was used. [b] Details of the use of the 2-butanol:NH_3 system are given in: J. F. Roland and A. M. Gross, *Anal. Chem.*, **26**, 502 (1954). Since this solvent system was allowed to run into a pad fixed to the bottom of the sheet, R_f values are given in terms of the nearest reference amino acid. No suffix means that the peptide ran at a rate equal to the amino acid, "+" indicates slightly faster than, "–" indicates slightly slower than. All separations were made on Whatman #3 paper by means of two unidimensional chromatograms. [c] Hydrolyses were carried out in redistilled 6 *N* HCl in sealed capillary tubes at 105° for 16 hours. Severe losses of methionine occurred, but enough remained for detection. [d] The reaction was carried out according to Sanger and Thompson, *Biochem. J.*, **53**, 353 (1953). The DNP-amino acids were detected by paper chromatography using the system *n*-butyl alcohol:ethanol:water (40:10:50). P. W. Kent, G. Lauson and A. Senior, *Science*, **113**, 354 (1951). Where serine was involved, the identification was confirmed in the system: phenol:isoamyl alcohol:water (1:1:1) of G. Biserte and R. Osteux, *Bull. soc. chim. biol.*, **33**, 50 (1951). This was necessary because methionine gives rise to a yellow derivative which interferes with DNP-serine in the first system. [e] Carboxypeptidase 6 times crystallized (Armour Laboratories) was used at *p*H 7.5 in 0.1 *N* ammonium acetate. The enzyme was treated with diisopropylfluorophosphate before use.

zene and detection of DNP-serine after hydrolysis. The final problem thus became the fixing of positions four and five. This was accomplished by treating peptide no. 5 with chymotrypsin. Two new fragments were produced, one having an R_f value identical with that of seryltyrosine and the other with a rate somewhat slower than that of glutamic acid. A quantity of the latter fragment (no. 6) was separated in the 2-butanol:ammonia system and was treated with carboxypeptidase. Now, apparently due to the fact that the residue (serylmethionine) was a dipeptide,[5] the rate of splitting of glutamic acid was higher than that of the next amino acid and the test chromatogram clearly showed glutamic acid as the C-terminal amino acid of peptide no. 6. Thus glutamic acid became the fifth amino acid in the sequence of corticotropin-A and, by difference, methionine became the fourth.

Table II shows the points of enzymatic attack on the N-terminal sequence of corticotropin-A. Following the notation of Sanger, the solid arrows indicate sites of rapid enzyme action, while the broken arrows indicate sites of slower enzyme action.

TABLE II

N-TERMINAL SEQUENCE OF CORTICOTROPIN-A, SHOWING POINTS OF ENZYMATIC ATTACK

N-Terminal sequence of corticotropin-A: Ser·Tyr·Ser·Met·Glu·His·Phe·Arg.....

Bond split by pepsin ↕

Bonds split by chymotrypsin ↑ ↑

Bond split by trypsin ↑

(5) S. S. Yanari and M. A. Mitz, *Federation Proc.*, **13**, 326 (1954), have shown that the proteolytic coefficient for dipeptides is of the order of 1/200 that of the N-substituted derivative.

Acknowledgment.—The authors wish to acknowledge the technical assistance of Mr. A. Gross.

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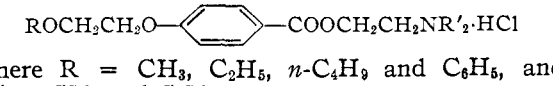
Dialkylaminoethyl Esters of Some Alkoxybenzoic Acids[1]

BY MELDRUM B. WINSTEAD, SAUL H. WISHNOFF AND R. W. BOST[2]

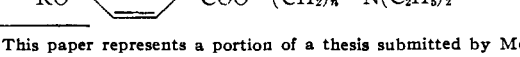
RECEIVED JUNE 14, 1954

A previous communication[3] described the preparation and characterization of a number of new alkoxybenzoic acids prepared as part of an investigation of the relationship of position isomerism to surface activity. The present paper illustrates the preparation of the hydrochlorides of some dialkylaminoethyl esters of these alkoxybenzoic acids having the type structure



where R = CH_3, C_2H_5, n-C_4H_9 and C_6H_5, and R′ = CH_3 and C_2H_5.

Rohmann and Scheurle[4] found that in a homologous series of esters of the type



(1) This paper represents a portion of a thesis submitted by Meldrum B. Winstead in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of North Carolina, June, 1952.

(2) Deceased, Sept. 22, 1951.

(3) R. W. Bost and M. B. Winstead, THIS JOURNAL, **74**, 1821 (1952).

(4) C. Rohmann and B. Scheurle, *Arch. Pharm.*, **274**, 110 (1936).

TABLE I

DIALKYLAMINOETHYL ALKOXYBENZOATE HYDROCHLORIDES, R—C$_6$H$_4$—COOCH$_2$CH$_2$NR$_2$'·HCl

R	R'	Yield, %	M.p., °C.	Formula	Nitrogen, % Calcd.	Nitrogen, % Found	Chlorine, % Calcd.	Chlorine, % Found
p-(2-Methoxyethoxy)-	C_2H_5	78	114–115.5	$C_{16}H_{26}ClNO_4$	4.22	4.28	10.69	10.72
p-(2-Methoxyethoxy)-	CH_3	69	132–133	$C_{14}H_{22}ClNO_4$	4.61	4.73	11.67	11.62
m-(2-Methoxyethoxy)-	C_2H_5	55	104.5–105.1	$C_{16}H_{26}ClNO_4$	4.22	4.20	10.69	10.80
p-(2-Ethoxyethoxy)-[5,6a]	C_2H_5	61	106–107.5	$C_{17}H_{28}ClNO_4$	..	..	10.25	10.33
p-(2-Ethoxyethoxy)-	CH_3	93	121.5–122.5	$C_{15}H_{24}ClNO_4$	4.41	4.50	11.16	11.11
m-(2-Ethoxyethoxy)-	CH_3	63	111.5–113.5	$C_{15}H_{24}ClNO_4$	4.41	4.26	11.16	11.15
o-(2-Ethoxyethoxy)-	C_2H_5	78	71.5–73	$C_{17}H_{28}ClNO_4$	4.05	4.04	...	...
p-(2-Butoxyethoxy)-	C_2H_5	60	104–105.7	$C_{19}H_{32}ClNO_4$	3.74	3.87	9.48	9.47
p-(2-Butoxyethoxy)-	CH_3	81	104–106	$C_{17}H_{28}ClNO_4$	4.05	4.16	10.25	10.13
o-(2-Butoxyethoxy)-	C_2H_5	20	96–97	$C_{19}H_{32}ClNO_4$	3.74	3.66	...	...
p-(2-Phenoxyethoxy)-	C_2H_5	62	154–156	$C_{21}H_{28}ClNO_4$	3.56	3.45	9.00	9.05
m-(2-Phenoxyethoxy)-	C_2H_5	40	192–193.5	$C_{21}H_{28}ClNO_4$	3 56	3.40	9.00	8.89
o-(2-Phenoxyethoxy)-	C_2H_5	52	113–115	$C_{21}H_{28}ClNO_4$	3.56	3.49	9.00	8.96
Morpholinoethyl *p*-(2-ethoxyethoxy)-benzoate hydrochloride								
		81	154–155.5	$C_{17}H_{26}ClNO_5$	3.89	3.76	9.85	9.85
Piperidinoethyl *p*-(2-ethoxyethoxy)-benzoate hydrochloride								
		71	130–131	$C_{18}H_{28}ClNO_4$	3.91	3.89	9.91	9 88

the anesthetic potency increases as R becomes larger, and normal alkyls are more effective than branched-chain alkyls. An extensive study concerning the relationship of chemical structure and local anesthetic activity has been made by Christiansen, Harris and co-workers.[5-7] It was found that diethylaminoethyl *p*-methoxybenzoate was considerably less active than diethylaminoethyl *p*-aminobenzoate (Procaine), but that diethylaminoethyl *p*-ethoxybenzoate was very much more active than diethylaminoethyl *p*-methoxybenzoate and somewhat more active than diethylaminoethyl *p*-aminobenzoate.

The purpose of the present investigation was to prepare several new dialkylaminoethyl alkoxybenzoate hydrochlorides, to evaluate their local anesthetic activity, and to study the effect of the introduction of one or two additional ether linkages into the alkoxy substituent of the molecule upon the local anesthetic activity of the compounds. Dimethylaminoethanol and diethylaminoethanol were selected as the aminoalcohols to be used in this investigation. In two of the experiments N-(2-hydroxyethyl)-morpholine and N-(2-hydroxyethyl)-piperidine were used as the aminoalcohol.

One general procedure was used for preparing the esters. This involved, first, the preparation of the alkoxybenzoyl chloride from the corresponding alkoxybenzoic acid and excess thionyl chloride, and, second, reaction of the alkoxybenzoyl chloride with the dialkylaminoethanol. A major portion of the esters herein reported consist of the *p*-alkoxybenzoates, although several *o*- and *m*-alkoxybenzoates have been prepared for comparison purposes. Previous investigators[5] showed that the anesthetic activity of diethylaminoethyl *o*-ethoxybenzoate and diethylaminoethyl *m*-ethoxybenzoate was considerably less than that of diethylaminoethyl *p*-ethoxybenzoate.

In most cases the alkoxybenzoyl chlorides were obtained as oils and were purified by vacuum distillation. However, the isomeric 2-phenoxyethoxybenzoyl chlorides were obtained as solids and were used directly for preparation of the ester hydrochlorides. The reaction between the alkoxybenzoyl chlorides and the dialkylaminoethanol took place smoothly in all cases. The resulting dialkylaminoethyl alkoxybenzoates were obtained as oils and were converted directly into the corresponding hydrochloride. The dialkylaminoethyl alkoxybenzoate hydrochlorides were obtained as white, crystalline compounds which were recrystallized from acetone and petroleum ether mixed solvent.

The local anesthetic action of morpholinoethyl *p*-(2-ethoxyethoxy)-benzoate hydrochloride (compound I), piperidinoethyl *p*-(2-ethoxyethoxy)-benzoate hydrochloride (compound II) and diethylaminoethyl *p*-(2-phenoxyethoxy)-benzoate hydrochloride (compound III) was determined by the infiltration technique in hamsters with Procaine as a control compound. Varying amounts ranging from 0.5 to 1 cc. of a 2% aqueous solution of the hydrochloride of the test substance were injected into the muscular posterior portion of the thigh of the right hind leg of the hamster. Duration of local anesthesia was determined by the animal's response to a stimulus at frequent intervals. Under these conditions compound I showed no local anesthetic activity. Compound II produced a local anesthetic activity of 30–50 minutes duration. Compound III produced effects which lasted well over six weeks. During this period the animal was completely insensitive to stimuli imposed upon the injected area. In addition voluntary muscular control over this area was lost.

Length of visceral insensitivity was determined by intraperitoneal injections of the animal, and the time required to produce a response to a stimulus was observed. Using 1 cc. of a 2% test solution a nearly uniform response with all test compounds

(5) W. G. Christiansen, S. E. Harris and W. A. Lott, *J. Am. Pharm. Assoc.*, **27**, 661 (1938).

(6) W. G. Christiansen and S. E. Harris (to E. R. Squibb and Sons). (a) U. S. Patent 2,404,691 (July 24, 1946); (b) U. S. Patent 2,414,966 (December 24, 1946).

(7) W. G. Christiansen and G. O. Chase (to E. R. Squibb and Sons), U. S. Patent 2,444,395 (June 29, 1948).

was obtained, visceral insensitivity lasting 15–25 minutes. Procaine produced an effect lasting 35–50 minutes.

Experimental[8]

The following procedure was used to prepare the dialkylaminoethyl alkoxybenzoate hydrochlorides reported in this paper.

Dialkylaminoethyl Alkoxybenzoate Hydrochloride.—Into a small round-bottomed flask was placed 0.1 mole of the alkoxybenzoic acid.[3] Four equivalents of thionyl chloride was added and the mixture was refluxed gently for one hour or until no more hydrogen chloride was evolved. After the removal of the excess thionyl chloride by distillation under reduced pressure the resulting alkoxybenzoyl chloride was vacuum distilled.

The alkoxybenzoyl chloride was dissolved in 50 cc. of dry benzene, and a benzene solution containing two equivalents of the dialkylaminoethanol was slowly added with shaking. Dialkylaminoethanol hydrochloride precipitated in the benzene solution, and the reaction was completed by refluxing the solution for one hour. The solution was chilled and then filtered, and the dialkylaminoethanol hydrochloride was discarded. Following the removal of the benzene solvent under reduced pressure the dialkylaminoethyl alkoxybenzoate, which was obtained as an oil, was dissolved in 500 cc. of absolute ether, and this ethereal solution was transferred to a 1-l. three-necked flask fitted with a stirrer. In a few cases it was necessary to add a small amount of acetone to the ethereal solution in order to obtain a homogeneous solution. Dry hydrogen chloride was passed directly into the ethereal solution with stirring until the precipitation of the dialkylaminoethyl alkoxybenzoate hydrochloride was completed. The solution was filtered and the precipitate was washed with absolute ether and dried over phosphorus pentoxide. The product was recrystallized twice from a mixture of acetone and petroleum ether (60–90°). Table I lists the dialkylaminoethyl alkoxybenzoate hydrochlorides that were prepared.

(8) All melting points are corrected.

Acknowledgment.—One of us (M.B.W.) wishes to express his appreciation to E. I. du Pont de Nemours and Company for a fellowship grant which made part of this investigation possible. The authors wish to thank Dr. Hulda Magalhaes of the Department of Biology of Bucknell University for a generous supply of hamsters and Mr. Al George Koslin for performing the chlorine analyses necessary for this research.

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[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

The Dipole Moments and Molecular Structures of Some Highly Fluorinated Hydrocarbons and Ethers[1,2]

BY ARMAND DI GIACOMO[3] AND CHARLES P. SMYTH

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The dielectric constants of five halogenated methanes have been measured in the vapor state over a range of temperature and pressure and used to calculate the molecular dipole moments as follows: trifluoromethane, 1.62; trifluorochloromethane, 0.46; trifluorobromomethane, 0.65; trifluoroiodomethane, 0.92; difluorodibromomethane, 0.66. The moments are much larger than would be expected on the basis of the small differences between those of the four methyl halides and are explained in terms of the differences in induced charge shift resulting from the differences between the polarizabilities of the halogens. The charge shifts may also be interpreted qualitatively in terms of differences in electronegativity between the halogens. The moments of several tetrahalogenated methanes obtained by Dr. R. C. Miller from loss measurements at microwave frequencies are shown to be similarly explicable. Measurements upon the vapors of *n*-amyl bromide and several highly fluorinated compounds have yielded the following moment values: *n*-amyl bromide, 2.21; perfluorocyclobutane, 0; pentafluoroethane, 1.54; pentafluorochloroethane, 1.54; trifluorochloroethylene, 0.40; perfluorodimethyl ether, 0.54; perfluorodiethyl ether, 0.42. The moments of these highly fluorinated molecules are close to the values calculated geometrically from the moments of the halogenated methanes and those of the unsubstituted ethers.

The dipole moments of di- and trihalogenated methanes were found to be much lower than the values calculated on the basis of carbon–halogen moments equal to those of the methyl halides and acting at tetrahedral valence angles of 110° with each other. This was, at first, attributed to widening of the valence angle by mutual repulsion of the halogens, an hypothesis abandoned when electron diffraction showed very little widening in methylene chloride and chloroform. The lowering of moment by mutual induction between dipoles near each other in the same molecule offered a logical explanation of many differences between observed and calculated moments and explained semi-quantitatively the moments of several chlorofluoromethanes.[4] Differences in the moments of chloromethanes and chloroethanes, some of which could not be explained by mutual induction, were attributed[5] to differences in electron availability on the carbon atom to which the halogens were attached. The shifts of electronic charge were also described in terms of hyperconjugation and resonance.[6] Shortening of the carbon–halogen distance in several fluoromethanes and chlorofluoromethanes has been attributed[7] to resonance involving structures with positive double-bonded halogen and negative ionic halogen. Although this *ad hoc* hypothesis of structure has been used to explain the interatomic distances found in the trifluoromethyl halides,[8] its inadequacies have been pointed out, and changes in

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(3) Procter and Gamble Fellow in Chemistry.

(4) C. P. Smyth and K. B. McAlpine, *J. Chem. Phys.*, **1**, 190 (1933).

(5) A. A. Maryott, M. E. Hobbs and P. M. Gross, THIS JOURNAL, **63**, 659 (1941).

(6) E. C. Hurdis and C. P. Smyth, *ibid.*, **64**, 2829 (1942).

(7) L. Pauling, "The Nature of the Chemical Bond," 2nd ed., Cornell University Press, Ithaca, N. Y., 1940, p. 235.

(8) H. J. M. Bowen, *Trans. Faraday Soc.*, **50**, 444 (1954).