stable silanols such as dimethylsilanediol. The latter compound was prepared by Hyde using a cold hydrolysis of dimethyldiethoxysilane and by Kantor using a hot hydrolysis of dimethyldimethoxysilane. Kantor hydrolyzed phenyltrimethoxysilane with distilled water at reflux temperature to obtain a low polymer phenylpolysiloxane.

Prior to Kantor's publication it was found in this Laboratory that the hydrolysis of phenyltrimethoxysilane at lower temperatures gave a good yield of phenylsilanetriol. The hydrolysis is accomplished best at temperatures near 10° although temperatures as high as 40° can be used. An acid catalyst facilitates the hydrolysis at low temperatures and 0.5% acetic acid was found quite satisfactory. The hydrolysis can be performed more slowly using distilled water.

As would be expected the compound is not very stable. It decomposes on heating and is sensitive to alkali and acid catalysts. On standing in sealed containers some samples have gradually resinified with loss of water although a few samples have been stored for over a year with no outward signs of decomposition and with only a slight drop in melting point. The melting point (or decomposition point) was difficult to determine and appeared to be a function of time *vs.* temperature. Determining the melting points in Pyrex capillary tubes using a rate of heating of 3° a minute gave consistent results on freshly prepared samples. The compound melted 128–130°.

Phenylsilanetriol crystallizes during its preparation in the form of flat, shiny, white platelets. It is slightly soluble in water, is somewhat more soluble in methanol and acetone, and is insoluble in toluene and petroleum ether. It is very unstable in solution. Dilute aqueous solutions become milky in less than an hour and all attempts to purify the compound by recrystallization led to complete loss of the material due to condensation or resulted in a product with a lower melting point and a more pronounced tendency to decompose on standing. The triol decomposes on melting with loss of water to give a resinous product which is toluene soluble. If heated further this resin condenses to an insoluble, hard gel.

Experimental

Hydrolysis of Phenyltrimethoxysilane.—Into a one-liter, three-necked flask was placed 198 g. (1.0 mole) of phenyltrimethoxysilane (b.p. 108° (20 mm.), $n^{25}D$ 1.4701, d^{25}_{25} 1.067). To this was added 108 g. of 0.5% acetic acid. The mixture was cooled to 10° and agitated thoroughly for four hours. During this period a white, crystalline material precipitated gradually and the liquid phase became homogeneous. The mixture was cooled to −20° and then filtered. The crystals which were obtained were washed once with distilled water and allowed to dry overnight at room temperature. The product weighed 117 g. (0.75 mole), a 75% yield of phenylsilanetriol.

Anal. Calcd. for $SiC_6H_8O_3$: Si, 17.9; OH, 32.7. Found: Si, 17.56, 17.55; OH, 32.5, 32.9.

In determining the hydroxyl content it was found that the Zerewitinoff technique gave erratic results due to gel formation. The analyses were made by condensing the triol in xylene at reflux temperature using a trace of KOH as catalyst. The water evolved was measured by collection in a Dean–Stark water trap. Fairly large (50 g.) samples were used for maximum accuracy.

The infrared spectrum of phenylsilanetriol shows absorption maxima at wave lengths of 3.15, 7.01, 8.82, 11.03, 13.50 and 14.35 μ. Bands at 6.3, 7.0, 8.8, 13.5 and 14.3 μ are characteristic of a single phenyl group on silicon. The 3.15 band is the stretching mode of the strongly associated hydroxyl and the OH bending vibrations lie between 10.5 and 12 μ. The sample was run using the KBr pressed pellet technique.

The author wishes to thank Dr. J. F. Hyde for suggestions and advice and Dr. A. L. Smith for the infrared spectrum and analysis.

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Studies on Pituitary Adrenocorticotropin. X. Further Sequences Near the N-Terminus of Corticotropin-A

BY W. F. WHITE AND W. A. LANDMANN

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In a previous communication[1] it was reported that serine and tyrosine comprise the first two amino acids at the N-terminus of corticotropin-A. A systematic study of the fragments produced by enzymatic hydrolysis of corticotropin-A has led to the recognition of the seven peptide fragments listed in Table I. The cumulative evidence obtained from these peptides (as shown in the table) is best explained by the sequence: Ser·Tyr·Ser·Met·Glu·His·Phe·Arg. . . .[2,3]

Peptides no. 1, 2, 5 and 7 are primary ones, being formed by direct treatment of corticotropin-A with crystalline enzymes. Of the remaining peptides, no. 3 and 4 are formed by secondary hydrolysis of the large fragment[4] remaining after long-term peptic hydrolysis of corticotropin-A. Present evidence indicates that such peptic hydrolysis removes a total of eleven amino acid residues from the carboxyl end of corticotropin-A, in addition to the five amino acid residues from the amino end comprising peptide no. 5.

The first two positions in the amino acid chain of corticotropin-A were clear from early work on the intact molecule[1] and the sixth, seventh and eighth positions were obtained by a consideration of peptides no. 2, 3, 4, 5 and 7. An attempt was made to fix the third, fourth and fifth positions by treatment of peptide no. 5 with carboxypeptidase. However, the rate of splitting of glutamic acid proved to be the limiting factor in the reaction. As a consequence, it was not possible to determine the sequence even though the peptide was almost completely split into its constituent amino acids after 24 hours.

The third position was finally determined by treatment of peptide No. 2 with dinitrofluoroben-

(1) W. A. Landmann, M. P. Drake and W. F. White, THIS JOURNAL, **75**, 4370 (1953).

(2) With one omission this sequence was proposed tentatively in a paper by E. E. Hays and W. F. White presented at the Laurentian Hormone Conference, Sept. 9, 1953. Most of the work up to that time had been done with the peptic and tryptic fragments, and the second serine was not discovered until the chymotryptic fragments were studied in detail. The Laurentian paper is published in "Recent Progress in Hormone Research," V. IX, Academic Press, New York, N. Y., 1954.

(3) In a recent paper (THIS JOURNAL, **76**, 3607 (1954)), Harris and Li have shown that the first seven amino acids of α-corticotropin (sheep origin) are the same as we have found for corticotropin-A (hog origin).

(4) W. F. White, THIS JOURNAL, **76**, 4194 (1954).

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.

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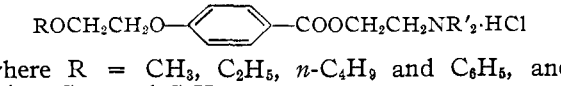
Dialkylaminoethyl Esters of Some Alkoxybenzoic Acids[1]

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

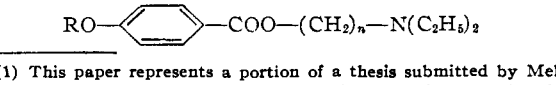
RECEIVED JUNE 14, 1954

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).