The X-ray diffraction pattern of this material (Table II) was identical with that of streptamine sulfate prepared by the alkaline degradation of streptidine according to the procedure of Folkers and co-workers.[8]

Streptidine Sulfate Monohydrate (XVIII) from Streptamine Sulfate (XVII).[32]—Streptamine sulfate (1.61 g., 5.8 millimoles) was suspended in 25.0 ml. of 0.46 *N* barium hydroxide (5.8 millimoles). The mixture was digested on the steam-bath for two minutes, filtered and washed with hot water. The filtrate and washings were concentrated under reduced pressure to about 5 ml. This solution was heated to 70–80° and maintained at this temperature during the addition of methylisothiourea sulfate. An amount of 1.02 g. of this reagent was added initially, 0.50 g. after twenty-four hours and 0.09 g. (total 1.61 g., 5.8 millimoles) after thirty-six hours. Heating was maintained for an additional twelve hours. The solution was then cooled to 0° and acetone added to incipient turbidity. The separated crystalline product was triturated with three 2-ml. portions of *N* ammonium hydroxide and the crystalline residue of streptidine sulfate monohydrate was washed with water and acetone and dried under reduced pressure (*ca.* 0.5 mm.) at 100°; yield 24 mg. The material was identified as streptidine sulfate monohydrate by its powder X-ray diffraction pattern (identical with the published[12] data), nitrogen analysis (calcd., 22.2; found, 22.1) and octaacetyl derivative. The latter was prepared from 5 mg. of the product by refluxing for one hour with sodium acetate (5 mg.) and acetic anhydride (1 ml.). The acetic anhydride was evaporated in a stream of dry air, the residue was triturated with water and the insoluble portion was crystallized from ethanol-water; m. p. 259–261°, unchanged on admixture with an authentic specimen of octaacetylstreptidine of like melting point. Folkers and co-workers[7] record the value 260–262° (micro-block) for this substance.

Summary

Reaction of N-acetyl-D-glucosamine diethyl thioacetal (II) with mercuric chloride in the presence of mercuric oxide gave ethyl 2-acetamido-2-desoxy-α-D-glucothiofuranoside (III). The furanoside structure of III was established by the fact that on periodate oxidation it yielded one mole (per mole of III) of formaldehyde with the consumption of one mole of oxidant. Oxidation of III with lead tetraacetate yielded a dialdehyde derivative (VII, not characterized) which on alkaline condensation with nitromethane gave ethyl 2-acetamido-6-nitro-2,6-didesoxy-α-D-glucothiofuranoside and ethyl 2-acetamido-6-nitro-2,6-didesoxy-β-L-idothiofuranoside (VIII and IX). The configuration of C_5 in each of the two isomers (m. p. 114–115° and 190–193° with dec.) was not determined. The thioethoxy group was hydrolyzed, in the presence of mercuric chloride, from the higher melting isomer and the resultant nitro sugar (X, not characterized) was cyclized by alkali and the product (XI and XII) was hydrogenated in acid solution with Raney nickel catalyst. The resultant mixture of diamino compounds (XIII and XV) was separated through the hexaacetyl derivatives. One of these (XIV) was identical with hexaacetylstreptamine from streptomycin.

Deacetylation of hexaacetylstreptamine (XIV) yielded streptamine, isolated as the sulfate (XVII), which produced streptidine, isolated as the sulfate monohydrate (XVIII), on reaction with methylisothiourea.

This synthesis of streptidine, a *meso* compound, from D-glucosamine rigorously establishes as all-*trans* the configuration of five of the asymmetric centers in streptidine and streptamine. From the known nature of the cyclization reactions, it is highly probable that the remaining center (C_1, XVII) is *trans* with respect to the adjacent nitrogen atoms. On this basis, streptidine and streptamine appear to possess the all-*trans* configurations.

It is probable that the other isolated product of the cyclization reaction has the structure and configuration shown in XVI.

The anomeric forms of ethyl 2-acetamido-2-desoxytriacetyl-D-glucothiofuranoside (IV and VI) are described.

COLUMBUS, OHIO RECEIVED JUNE 11, 1949

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, NO. 1313, PASADENA, CALIFORNIA]

A New Series of Substrates for the Evaluation of Chymotrypsin Activity[1]

BY B. M. ISELIN, H. T. HUANG, R. V. MACALLISTER AND CARL NIEMANN

Bergmann and Fruton[2] have recommended the use of glycyl-L-phenylalaninamide as a substrate for the estimation of chymotrypsin activity and more recently Kaufman, Neurath and Schwert[3–5] have suggested the use of benzoyl- and acetyl-L-tyrosinamide for the same purpose. While the above substrates may be satisfactory for some purposes all three possess characteristics which limit their general usefulness, *i. e.*, the first is relatively unstable,[6,7] the second is too insoluble in aqueous media[8] and the third, in common with acetyl-L-phenylalaninamide (*cf.* Table I), is hydrolyzed at too slow a rate. In contrast it has

(1) Supported in part by a grant from Eli Lilly and Co. Request for information relative to this article should be addressed to Dr. C. Niemann.

(2) M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **145**, 253 (1942).

(3) S. Kaufman, H. Neurath and G. W. Schwert, *J. Biol. Chem.*, **177**, 793 (1949).

(4) S. Kaufman and H. Neurath, *Arch. Biochem.*, **21**, 245 (1949).

(5) *Idem., J. Biol. Chem.*, **180**, 181 (1949).

(6) H. T. Huang and Carl Niemann, THIS JOURNAL, in press.

(7) It is probable that the "spontaneous hydrolysis" of the analogous glycyl-L-tyrosinamide acetate noted by Kaufman, *et al.*,[3] is actually spontaneous conversion into the corresponding diketopiperazine.

(8) The practice of using aqueous methanol systems[3] does not appear to be as attractive as the use of water soluble substrates principally because of the introduction of an additional variable in the former case.

been observed that the nicotinyl-phenylalanin-, tyrosin-, tryptophan- and methionin-amides are easily prepared, are relatively stable compounds, are sufficiently soluble in water to permit the preparation of at least 0.02 formal solutions, and the L-isomers of the first three compounds are hydrolyzed by chymotrypsin at a rate sufficiently rapid to make them useful and practical substrates. The advantage of having three substrates possessing the above properties and differing only in regard to side chain is too well known to require elaboration.

The mode of hydrolysis of the above substrates, *i. e.*, formation of acylated amino acid and ammonia, was anticipated from previous studies[2-5,9,10] as was the observed side chain specificity.[10,11] A detailed analysis of the kinetics of hydrolysis of the above substrates will be given in subsequent communications.

Experimental[12,13]

Acetyl-DL-phenylalaninamide.—Esterification of 28 g. of acetyl-DL-phenylalanine with methanolic hydrogen chloride gave 25.5 g. of acetyl-DL-phenylalanine methyl ester, m. p. 60–61° after recrystallization from a 30–60° ligroin–ether mixture.

Anal. Calcd. for $C_{12}H_{15}O_3N$ (221): C, 65.1; H, 6.8; N, 6.3. Found: C, 65.2; H, 6.8; N, 6.2.

Ammonolysis of the above ester gave N-acetyl-DL-phenylalaninamide, m. p. 165–166°.[14]

Benzoyl-DL-phenylalaninamide.—Esterification of benzoyl-DL-phenylalanine in the usual manner gave benzoyl-DL-phenylalanine methyl ester, m. p. 100–101° after recrystallization from ether; lit.,[15,16] m. p. 86.5–87.5° and 90°.

Anal. Calcd. for $C_{17}H_{17}O_3N$ (283): C, 72.1; H, 6.1; N, 4.9. Found: C, 72.1; H, 6.1; N, 5.0.

Ammonolysis of the above ester gave benzoyl-DL-phenylalaninamide, m. p. 197–198°.[17]

Nicotinyl-DL-phenylalaninamide.—To an ethyl acetate solution (50 ml.) of DL-phenylalaninemethyl ester prepared from 13 g. of the hydrochloride, was added 13 g. (1.5 mole) of nicotinyl azide,[18] the mixture allowed to stand overnight, washed with cold 1 *F* hydrochloric acid and water, the aqueous phases made alkaline with sodium bicarbonate, extracted with ethyl acetate, the combined ethyl acetate phases dried and the solvent removed by distillation *in vacuo*. The residual sirup upon extraction with hot 30–60° ligroin and standing at 0° gave 12.9 g. of nicotinyl-DL-phenylalanine methyl ester, m. p. 63–64° after recrystallization from a mixture of 30–60° ligroin and ether.

Anal. Calcd. for $C_{16}H_{16}O_3N_2$ (284): C, 67.6; H, 5.7; N, 9.9. Found: C, 67.7; H, 5.7; N, 10.0.

Ammonolysis of 12.9 g. of the above ester in methanol solution gave 10.2 g. of nicotinyl-DL-phenylalaninamide, m. p. 193–194° after recrystallization from water.

Anal. Calcd. for $C_{15}H_{15}O_2N_3$ (269): C, 66.9; H, 5.6; N, 15.6. Found: C, 66.9; H, 5.6; N, 15.5.

Nicotinyl-L-phenylalaninamide.—To a solution of L-phenylalanine methyl ester, prepared from 5 g. of L-phenylalanine, $[\alpha]^{25}D$ $-34 \pm 1°$ (C = 1.2% in water), in 50 ml. of ethyl acetate was added 4.5 g. of recrystallized nicotinyl azide and the sirupy nicotinyl-D-phenylalanine methyl ester ammonolyzed to give 5.3 g. of nicotinyl-L-phenylalaninamide, m. p. 185° after recrystallization from methanol–ether.

Anal. Calcd. for $C_{15}H_{15}O_2N_3$ (269): C, 66.9; H, 5.6; N, 15.6. Found: C, 66.9; H, 5.6; N, 15.5.

Nicotinyl-L-tyrosinamide.—Acylation of 4 g. of L-tyrosine ethyl ester with 2.5 g. of nicotinyl azide gave 2.5 g. of nicotinyl-L-tyrosine ethyl ester, m. p. 147–149° after recrystallization from ethyl acetate.

Anal. Calcd. for $C_{17}H_{18}O_4N_2$ (314): C, 65.0; H, 5.8; N, 8.9. Found: C, 65.2; H, 6.0; N, 8.9.

Ammonolysis of 2 g. of the above ester gave 1.5 g. of nicotinyl-L-tyrosinamide, m. p. 226–227° after two recrystallizations from water.

Anal. Calcd. for $C_{15}H_{15}O_3N_3$ (285): C, 63.2; H, 5.3; N, 14.7. Found: C, 63.0; H, 5.6; N, 14.8.

Nicotinyl-*p*-chloro-DL-phenylalaninamide.—Esterification of 7.2 g. of *p*-chloro-DL-phenylalanine[19] with methanolic hydrogen chloride gave 8.4 g. of *p*-chloro-DL-phenylalanine methyl ester hydrochloride, m. p. 185–186° after recrystallization from methanol–ether.

Anal. Calcd. for $C_{10}H_{13}O_2NCl_2$ (250): C, 48.0; H, 5.2; N, 5.6. Found: C, 47.8; H, 5.1; N, 5.5.

Acylation of *p*-chloro-DL-phenylalanine methyl ester prepared from 7 g. of the above hydrochloride with 1.2 moles of nicotinyl azide gave 5.3 g. of nicotinyl-*p*-chloro-DL-phenylalanine methyl ester, m. p. 89–90° after two recrystallizations from a 30–60° ligroin–ether mixture.

Anal. Calcd. for $C_{16}H_{15}O_3N_2Cl$ (319): C, 60.3; H, 4.8; N, 8.8. Found: C, 60.6; H, 4.7; N, 8.9.

Ammonolysis of 4.2 g. of the above ester gave 3.4 g. of nicotinyl-*p*-chloro-DL-phenylalaninamide, m. p. 220–221° after recrystallization from methanol.

Anal. Calcd. for $C_{15}H_{14}O_2N_3Cl$ (304): C, 59.3; H, 4.7; N, 13.8. Found: C, 59.2; H, 4.7; N, 13.6.

Nicotinyl-L-tryptophanamide.—Acylation of a solution of L-tryptophan methyl ester,[20] prepared from 10 g. of hydrochloride, with 5 g. of nicotinyl azide and subsequent ammonolysis of the sirupy nicotinyl-L-tryptophan methyl ester gave 9.2 g. of nicotinyl-L-tryptophanamide, m. p. 180–181° after recrystallization from aqueous methanol.

Anal. Calcd. for $C_{17}H_{16}O_2N_4$ (308): C, 66.2; H, 5.2; N, 18.2. Found: C, 66.1; H, 5.2; N, 18.3.

Nicotinyl-DL-methioninamide.—Acylation of 7.3 g. of DL-methionine methyl ester, prepared from the hydrochloride,[21] in 30 ml. of ethyl acetate with 6.6 g. (1 mole) of nicotinyl azide gave 8.3 g. of nicotinyl-DL-methionine methyl ester, m. p. 60–61° after recrystallization from a 30–60° ligroin–ether mixture.

Anal. Calcd. for $C_{12}H_{16}O_3N_2S$ (268): C, 53.7; H, 6.0; N, 10.4. Found: C, 53.8; H, 6.1; N, 10.6.

Ammonolysis of 7 g. of the above ester gave 5.8 g. of nicotinyl-DL-methioninamide m. p. 146–147° after recrystallization from ethanol.

Anal. Calcd. for $C_{11}H_{15}O_2N_3S$ (253): C, 52.2; H, 6.01; N, 16.6. Found: C, 52.2; H, 5.9; N, 16.5.

Nicotinyl-L-histidinamide.—Acylation of 4.3 g. of L-histidine methyl ester[22] in 50 ml. of ethanol-free chloroform with 3.8 g. (1 mole) of nicotinyl azide and subsequent ammonolysis of the sirupy nicotinyl-L-histidine methyl ester gave 1.85 g. of nicotinyl-L-histidinamide m. p. 237–

(9) J. E. Snoke and H. Neurath, *Arch. Biochem.*, **21**, 351 (1949).

(10) S. Kaufman and H. Neurath, *ibid.*, **21**, 437 (1949).

(11) M. Brenner, E. Sailer and V. Kocher, *Helv. Chim. Acta*, **31**, 1908 (1948).

(12) All melting points are corrected.

(13) Microanalyses by Dr. A. Elek.

(14) M. Bergmann, F. Stern and C. Witte, *Ann.*, **449**, 277 (1926).

(15) J. Max, *ibid.*, **369**, 276 (1909).

(16) M. Bergmann and L. Zervas, *J. Biol. Chem.*, **113**, 341 (1936).

(17) E. Mohr and F. Stroschein, *Ber.*, **42**, 2521 (1909).

(18) T. Curtius and E. Mohr, *ibid.*, **31**, 2493 (1898).

(19) J. C. Nevenzel, W. E. Shelberg and C. Niemann, THIS JOURNAL, **71**, 3024 (1949).

(20) E. Abderhalden and M. Kempe, *Z. physiol. Chem.*, **52**, 214 (1907).

(21) G. Barger and T. Weichselbaum, *Biochem. J.*, **25**, 997 (1931).

(22) J. P. Greenstein and F. W. Klemperer, *J. Biol. Chem.*, **128**, 245 (1939).

238° after recrystallization from methanol and then from water.

Anal. Calcd. for $C_{12}H_{13}O_2N_5$ (259): C, 55.6; H, 5.1; N, 27.0. Found: C, 55.5; H, 4.9; N, 26.9.

Enzyme Studies.—The data given in Table I are self-explanatory though it should be noted that in some cases the values given for % hydrolysis were interpolated from a smooth curve drawn through the experimentally determined points when such points did not coincide with the time interval given in Table I. The extent of hydrolysis was determined by a modified formal titration based upon coincident use of primary or secondary amine buffers. The advantages of this new analytical method will be described in a subsequent communication.

TABLE I

HYDROLYSIS OF ACYLATED α-AMINO ACID AMIDES BY CHYMOTRYPSIN[a]

Acylated α-amino acid amide used as substrate	s_0[b]	E_0[c]	Hydrolysis, % 15 min.	30 min.	60 min.
Acetyl-DL-phenyl-	10	0.15		5	9
alanin-	5	.15		6	12
Benzoyl-DL-phenyl-	1.25	.15	52	80	96
alanin-[d]	1.25	.075	34	56	80
Nicotinyl-DL-phenyl-	5	.15	20	36	58
alanin-	5	.075	10	18	34
	1.25	.15	32	56	80
	1.25	.075	16	34	56
Nicotinyl-L-phenyl-	10	.15	21	40	63
alanin-	10	.075	11	18	34
	5	.15	22	44	68
	5	.075	14	26	44
Nicotinyl-L-tyrosin-	5	.075	34	58	92
	10	.075	26	50	78
	20	.075	20	38	66
Nicotinyl-*p*-chloro-	1.25	.15	44	72	92
DL-phenylalanin-	1.25	.075	20	38	64
Nicotinyl-L-trypto-	10	.15	41	69	91
phan-	10	.075	20	38	61
	5	.15	60	84	98
	5	.075	34	60	86
Nicotinyl-DL-	10	.15	26% in 24 hours		
methionin-					
Nicotinyl-L-histidin-	10	.15	none in 24 hours		

[a] At 25° and *p*H 7.8 (0.02 formal ethylenediamine–hydrochloric acid buffer). [b] Initial substrate concentration of the L-isomer in micromoles per ml. reaction mixture. [c] Initial enzyme concentration in mg. protein nitrogen per ml. reaction mixture. [d] Previously reported not to be hydrolyzed by chymotrypsin.[23]

Summary

The nicotinyl-L-phenylalanin-, L-tyrosin and L-tryptophanamides have been found to be useful and practical substrates for the evaluation of chymotrypsin activity.

(23) M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **124**, 321 (1938).

PASADENA 4, CALIFORNIA RECEIVED SEPTEMBER 2, 1949

[A COMMUNICATION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

Synthesis of Alkylcyclohexanols from Phenols

BY HOMER ADKINS,[1] ALVAN DONNAN AND ROBERT H. LEVIN

A number of mono- and di-alkylcyclohexanols have been prepared from certain phenols through the sequence of reactions: phenol → aryl ester → isomeric acylphenols (separated) → alkylphenols → alkylcyclohexanols.

This sequence of reactions has been applied to the acetates, propionates and *n*-butyrates of phenol, *o*-, *m*- and *p*-cresols and *o*-ethylphenol. The aryl esters preferably were prepared from the phenols and the appropriate acyl chloride; the Fries rearrangements of these esters and separations of the resulting acylphenols were carried out essentially as described by Miller and Hartung.[2]

The acylphenols were hydrogenated to the alkylphenols in good yields over both Raney nickel and copper–chromium oxide. The hydrogenation over W-2 Raney nickel proceeded at a lower temperature (90–130°) than over the copper–chromium oxide catalyst,[3] which requires temperatures of 150–175°. These hydrogenations usually were complete within ten to thirty minutes, but with the less pure samples of the acylphenols a longer time (one to two hours) usually was required for their completion. As Raney nickel is more active toward the benzenoid ring than is copper–chromium oxide, the latter is the preferred catalyst for the preparation of the alkylphenols; also the nickel catalyst is more susceptible to poisoning by impurities in the acylphenols.

The hydrogenation of the various alkylated phenols to the corresponding cyclohexanols usually was complete in about two hours at 175° over Raney nickel. The simpler phenols were hydrogenated more rapidly and at a lower temperature while the more substituted phenols required a slightly longer time and temperatures as high as 200°. However, the purity of the sample appears to be a more important factor in determining the rate of hydrogenation than the precise structure of the phenol. With a more active Raney nickel such as W-6, these hydrogenations doubtless could be accomplished under milder conditions.[4]

The yields, properties, analyses and certain

(1) Deceased August 10, 1949.

(2) Miller and Hartung, "Org. Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, 1943, p. 543; *cf.* also Blatt, "Organic Reactions," John Wiley and Sons, Inc., New York, 1942, Vol. I, p. 342.

(3) Connor, Folkers and Adkins, THIS JOURNAL, **54**, 1138 (1932).

(4) Adkins and Billica, *ibid.*, **70**, 695 (1948).