Another sample of the carbodiimide, wt. 0.0337 g., was hydrolyzed by heating with 1 ml. of 85% orthophosphoric acid for two hours at 170–180°. Dilution of the cooled mixture with water gave a precipitate of 1-phenanthrylamine phosphate,[5] which was washed with sodium hydroxide solution and then water to convert it to 0.0286 g. of 1-phenanthrylamine, m.p. 142–144° (reported[34] 145–146°), undepressed when mixed with an authentic sample. Alkalizing the acidic mother liquor gave a second crop, wt. 0.0012 g., making the total yield 90%.

The benzene mother liquor from the crude carbodiimide was evaporated to a sirupy semi-solid, from which by trituration with absolute alcohol containing a little benzene a small amount of crude phenyl-1-phenanthrylcarbodiimide was obtained, m.p. 105–110°, raised to 111–113° when mixed with an authentic sample. The mother liquors yielded more solid on concentration, showing the characteristics of impure diphenylcarbodiimide polymer.

ANN ARBOR, MICH.

(34) W. E. Bachmann and C. H. Boatner, THIS JOURNAL, **58**, 2099 (1936).

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT "PLIVA" PHARMACEUTICAL AND CHEMICAL WORKS, AND DEPARTMENT OF BIOCHEMISTRY INSTITUTE "RUDJER BOŠKOVIĆ"]

Debenzylation of S-Benzyl-N-phthaloyl-L-cysteinyl Chloride with Aluminum Halides. Preparation of L-α-Phthalimido-β-propiothiolactone[1]

BY DRAGUTIN FLEŠ, ANICA MARKOVAC-PRPIC AND VILIM TOMAŠIC

RECEIVED MARCH 10, 1958

S-Benzyl-N-phthaloyl-L-cysteinyl chloride gave with two moles of aluminum halide L-α-phthalimido-β-propiothiolactone (I), while with one mole of aluminum halide a polythioester II was obtained. The structure of I and II was proved by conversion into cystine and cysteine derivatives. A mechanism for the formation of I and II is suggested.

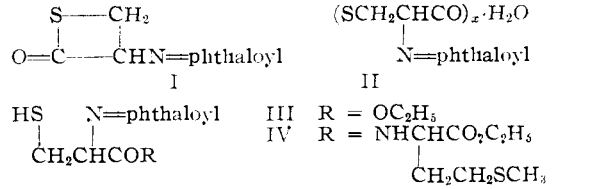
In the course of our studies on the configuration of chloramphenicol[2] and nor-pseudo-ephedrine,[3] we have correlated the configurations of these compounds with D-serine and D-alanine by means of chemical interconversion. The key intermediates in these syntheses were the optically active α-phthalimido-β- substituted propiophenones, prepared from the corresponding phthalimido-propionyl chlorides using the usual Friedel–Crafts reaction.

Incidental to this investigation it was observed that formation of propiophenone *via* Friedel–Crafts reaction largely depended on the character of the β-substituent. When O-ethyl-N-phthaloyl-DL-seryl chloride was subjected to the Friedel–Crafts reaction, the corresponding ketone was obtained in a 25% yield,[4] while the same reaction gave a yield of 63% with O-methyl-N-phthaloyl-DL-seryl chloride[5] and a yield of over 90% with N-phthaloyl-L-alanyl chloride.[3]

In continuation of this work with the idea of correlating the configuration of cysteine to chloramphenicol and nor-pseudo-ephedrine, we subjected S-benzyl-N-phthaloyl-L-cysteinyl chloride to the usual Friedel–Crafts reaction. At first it was expected that S-benzyl-N-phthaloyl-L-cysteinyl chloride would react in the normal way giving the corresponding propiophenone which upon debenzylation with aluminum halide would give rise to a ketone related to cysteine or cystine. During attempts to purify the reaction products, it was observed that no ketonic material was present in the reaction mixture. However, two different products could be obtained depending on the amount of aluminum halide used in the reaction. With two moles of aluminum halide the α-phthalimido-β-propiothiolactone(I) was obtained, while with one mole of aluminum halide a polymeric compound II was isolated. In a series of experiments it was found that the formation of I was independent of the concentration of aluminum halide as long as at least two moles was used.

The propiothiolactone ring was first prepared by Lin'kova, Kil'disheva and Knunyants[6] in 1955. By condensing the ethylchloro carbonate with the triethylamine salt of β-mercaptoisovaline the Russian authors prepared β,β-dimethylpropiothiolactone. In a similar way N-formyl-, N-acetyl-, N-phenacetyl- and N-phenaceturyl-β,β-dimethylpropiothiolactones also were prepared.[7]

The structure of the compound I was proved by several routes. Hydrolysis of I with hydriodic acid in glacial acetic acid afforded L-cystine and phthalic acid. The acid-catalyzed reaction with ethanol gave an almost quantitative yield of N-phthaloyl-L-cysteine ethyl ester (III). It is interesting to note that in the propiolactone series the acid-catalyzed reaction with strong acids afforded ether rather than ester.[8] The ester III is an oily product which can be distilled in high vacuum without decomposition and significant racemization. Upon hydrolysis with hydriodic acid, the ester III gave cystine and phthalic acid. Benzyla-



(1) Presented before XVIth International Congress of Pure and Applied Chemistry, Paris, July, 1957; Congress Handbook (Division of Organic Chemistry), p. 41.

(2) D. Fleš and B. Balenović, THIS JOURNAL, **78**, 3072 (1956).

(3) D. Fleš and A. Markovac-Prpić, *Croat. Chem. Acta*, **29**, 183 (1957).

(4) D. Fleš, B. Balenović, R. Marušić and N. Manger, *Arhiv kem.*, **27**, 1 (1955).

(5) D. Fleš and B. Balenović, *ibid.*, **27**, 149 (1955).

(6) M. G. Lin'kova, O. V. Kil'disheva and I. L. Knunyants, *Izvest. Akad. Nauk S.S.S.R., Otdel. Khim. Nauk*, 569 (1955).

(7) I. L. Knunyants, O. V. Kil'disheva and E. Petrova, *ibid.*, 689 (1955).

(8) T. L. Gresham, J. E. Jansen, F. W. Shaver, J. T. Gregory and W. L. Beears, THIS JOURNAL, **70**, 1004 (1948); F. A. Long and M. Purchase, *ibid.*, **72**, 3267 (1950).

tion of the ester III with benzyl bromide gave S-benzyl-N-phthaloyl-L-cysteine ethyl ester which was converted to S-benzyl-L-cysteine by hydrolysis with hydriodic acid. Desulfuration of propiothiolactone I with Raney nickel gave a low yield of phthalimidopropionaldehyde which was characterized as 2,4-dinitrophenylhydrazone and semicarbazone. The low yield of aldehyde was probably due to the formation of alcohol which is the normal product of the reduction of thiolester with Raney nickel.[9] The proposed structure was also proved by molecular weight determination (cryoscopically in dioxane).

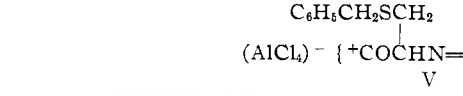
Propiothiolactone I can be crystallized from ethanol without decomposition, but it reacts very readily with amines, giving the corresponding amides. With L-methionine ethyl ester, for example, propiothiolactone I gave in dioxane at room temperature the N-phthaloyl-L-cysteinyl-L-methionine ethyl ester (IV). The remarkable acylating properties of propiothiolactone represent a valuable tool for the preparation of cysteine polypeptides.

The polymeric nature of compound II was proved by molecular weight determination (cryoscopically in *m*-dinitrobenzene) which corresponded to a value of about 1500. Hydrolysis of II with hydriodic acid gave L-cystine and phthalic acid. On the basis of analogy with propiolactone, which gave a polyester when polymerized thermally,[10] we consider the most probable structure of compound II to be the one corresponding to a linear polythioester, although a cyclic structure is not excluded. The detailed investigation necessary to prove the correct structure of polymer II is outside the scope of this paper.

It is worthwhile to note that in all cases, regardless of the amount of aluminum halide used in the reactions, diphenylmethane was isolated in a 50% yield.

An attempt was made to learn something of the mechanism of formation of I and II. In these experiments aluminum bromide was used because of its solubility in benzene. But in a series of experiments it was found that formation of I at a temperature of 14° was almost instantaneous, and that the maximal yield of 50% obtained after 5 minutes dropped to 20% after 24 hours. For this reason we failed to obtain any conclusive kinetic results.

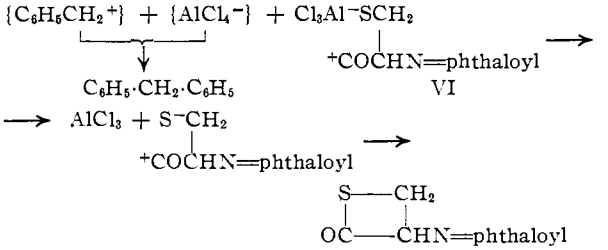
In view of the fact that with one mole of aluminum halide the polymeric thioester II and with two moles the propiothiolactone I were obtained, we interpret the reaction mechanism as follows: When one mole of aluminum halide is used, it ionizes the covalent carbon–chlorine bond and forms an ion-pair V in which for steric reasons the CO^+ residue cannot get close enough to its own sulfur to debenzylate it as easily as it can approach



V

and debenzylate the sulfur of another molecule. As a result of this reaction a linear polymer of the type II will be formed.

With two moles of aluminum halide, the first molecule forms the same ion-pair as shown in formula V, while the second molecule forms a complex with thioether and debenzylates the sulfur, forming an ion-pair (benzylcarbonium ion and aluminum halide ion) and a betaine structure VI. The mechanism of debenzylation is probably similar to the one described by Harnish and Tarbell[11] for debenzylation of benzyl phenyl sulfide with aluminum bromide. In molecule VI the zwitterion character of the betaine structure leads to a close internal approach of the reactive centers and thus to the formation of propiothiolactone



We consider that the reaction used in this work for the preparation of α-phthalimido-β-propiothiolactone represents a general and convenient method for the preparation of propiothiolactone and derivatives, and we hope that by using simpler intermediates we will be able to study the reaction mechanism more closely.

Experimental[12]

L-α-Phthalimido-β-propiothiolactone (I).—A mixture of 55 ml. of benzene and 10 g. (0.075 mole) of anhydrous aluminum chloride was heated to 65° and with rapid stirring 9 g. (0.025 mole) of L-α-phthalimido-β-benzylthiopropionyl chloride dissolved in 55 ml. of benzene was added in the course of 20 minutes. The reaction mixture was then kept for an additional three hours at a temperature of 65–70°, cooled and hydrolyzed with 60 g. of ice and 10 ml. of concentrated hydrochloric acid. The water layer was separated and extracted with two 20-ml. portions of benzene, the combined benzene layers were extracted with two 30-ml. portions of water, followed by two 30-ml. portions of a saturated sodium bicarbonate solution and dried over magnesium sulfate. The benzene was removed under reduced pressure and the residue (9 g.) recrystallized from 12 ml. of ethyl acetate to give 3.1 g. of thiolactone crystallizing in prisms, m.p. 135–137°. The crude thiolactone was recrystallized from 10 ml. of ethyl acetate and a yield of 2.7 g. (46.3%) of white prisms was obtained; m.p. 140–141°, $[\alpha]^{20}D$ −152° (*c* 2.510 in benzene). An analytical sample was recrystallized from ethyl acetate and finally from ethanol in very fine needles melting at 141–143° (Kofler microscopic method), $[\alpha]^{20}D$ −162° (*c* 0.690 in benzene) and $[\alpha]^{20}D$ −106° (*c* 1.415 in dioxane). The propiothiolactone I is orthorhombic with cell dimensions: a = 5.35 A., b = 20.70 A., c = 9.30 Å. The number of molecules per unit cell is Z = 4 (the density measured pycnometrically is 1.55 g. cm.$^{-3}$). The space group is D^4_2–$P2_12_12_1$ and was determined from the systematic absence of the reflections on the oscillation photographs (Ni-filtered CuK-radiation was used).[13]

Anal. Calcd. for $C_{11}H_7NO_3S$: C, 56.64; H, 3.03; N, 6.00; S, 13.75; mol. wt., 233.2. Found: C, 56.59; H, 2.94; N, 6.02; S, 13.94; mol. wt., 235 (cryoscopic in dioxane).

(9) E. Mosettig in "Organic Reactions," Vol. VIII, John Wiley and Sons, Inc., New York, N. Y., 1954, p. 230.

(10) T. L. Gresham and J. E. Jansen, U. S. Patent 2,526,554, Oct. 17, 1950; T. L. Gresham, J. E. Jansen and F. W. Shaver, THIS JOURNAL, **70**, 998 (1948).

(11) D. P. Harnish and D. S. Tarbell, *ibid.*, **70**, 4123 (1948).

(12) Melting points are uncorrected. Microanalyses were performed by Mrs. E. Stevčevski-Jaeger.

(13) The crystallographic data were determined by Prof. D. Grdenić and Mr. Z. Despotović from the Institute "Rudjer Bošković."

The ethyl acetate mother liquor from which 3.1 g. of crude thiolactone was obtained, gave, on distillation at 50–52° at 0.02 mm., 2.2 g. (52%) of diphenylmethane, m.p. and mixed m.p. 26–27°, $n^{20}D$ 1.5771; reported m.p. 26–27°, $n^{20}D$ 1.5768,[14] b.p. 68° at 0.05 mm.[15]

Anal. Calcd. for $C_{13}H_{12}$: C, 92.81; H, 7.19. Found: C, 92.81; H, 7.24.

DL-α-**Phthalimido-β-propiothiolactone** was prepared in the same manner from DL-α-phthalimido-β-benzylthiopropionyl chloride and aluminum chloride, m.p. 138–139° (needles from ethanol).

Anal. Found: C, 56.71; H, 3.16; N, 5.94.

DL-α-**Phthalimido-β-propiothiolactone** also was prepared from DL-α-phthalimido-β-benzylthiopropionyl chloride and aluminum bromide in the following manner: A mixture of 100 ml. of benzene and 4.5 g. (0.0169 mole) of aluminum bromide was treated with a solution of 2.7 g. (0.0075 mole) of DL-α-phthalimido-β-benzylthiopropionyl chloride in 30 ml. of benzene. The reaction mixture was kept for two hours at room temperature (20°), hydrolyzed with 20 g. of ice and 4 ml. of concentrated hydrochloric acid, and processed in the same way as described for the preparation of I with aluminum chloride; yield 0.86 g. (49.1%), m.p. 138.5–139°.

Anal. Found: C, 56.57; H, 3.20.

From the mother liquors 0.8 g. (63.4%) of diphenylmethane was obtained.

Conversion of the Thiolactone I to L-Cystine.—The thiolactone I (1.8 g., 0.0077 mole) was refluxed for four hours with 5.4 ml. of glacial acetic acid and 12.6 ml. of 47% hydriodic acid and the reaction mixture was kept overnight at room temperature. The phthalic acid was filtered off, washed with two 3-ml. portions of water and the filtrate evaporated to dryness under reduced pressure. The excess of acids was removed by successive treatment with water, the residue dissolved in 5 ml. of water, extracted with ether and the water layer adjusted to *p*H 4.5 with a saturated sodium acetate solution. The solution was exposed to air and after several days 0.35 g. (38%) of L-cystine separated, m.p. 250° dec., $[\alpha]^{20}D$ −210° (*c* 0.5 in *N* HCl); reported m.p. 258–261° dec.,[16] $[\alpha]^{24.91}D$ −212.9° (*c* 0.9947 in 1.02*N* HCl).[17] On paper chromatography the compound gave the same spot with ninhydrin as the authentic specimen of L-cystine.

Anal. Calcd. for $C_6H_{12}N_2O_4S_2$: N, 11.64. Found: N, 11.76.

Conversion of the Thiolactone I to S-Benzyl-L-cysteine. (a) **N-Phthaloyl-L-cysteine Ethyl Ester (III).**—The thiolactone I (1 g.) was refluxed for 1 hour with 10 ml. of a saturated solution of hydrochloric acid in absolute ethanol, the solvent evaporated *in vacuo*, the residue dissolved in 10 ml. of ethanol, the insoluble part was filtered off, and the solvent was evaporated to give 1.1 g. (92%) of white viscous oil which was used directly in the next step. A sample was purified for analysis by distillation at 130–140° at a pressure of 0.03 mm., $[\alpha]^{20}D$ −56° (*c* 1.05% in benzene), $n^{25}D$ 1.5621.

Anal. Calcd. for $C_{13}H_{13}NO_4S$: C, 55.91; H, 4.70; N, 5.02; mol. wt., 279.24. Found: C, 56.04; H, 4.54; N, 5.29; mol. wt., 267 (cryoscopic in benzene).

(b) **S-Benzyl-N-phthaloyl-L-cysteine Ethyl Ester.**—A mixture of 1.08 g. (0.0039 mole) of the ester III, 0.67 g. (0.0039 mole) of benzyl bromide, 0.78 g. (0.0078 mole) of triethylamine and 10 ml. of dioxane was refluxed for 2 hours, cooled, diluted with 20 ml. of water, extracted with three 15-ml. portions of ether, the ether layer was washed with water and dried over magnesium sulfate. Evaporation of ether gave a crop of 1.21 g. (85%) of a slightly yellow oil with a specific rotation of $[\alpha]^{20}D$ −90° (*c* 1.86 in benzene). An analytical sample was distilled twice at 220–230° at a pressure of 0.045 mm., $[\alpha]^{20}D$ −88° (*c* 1.595 in benzene).

Anal. Calcd. for $C_{20}H_{19}NO_4S$: C, 65.03; H, 5.19. Found: C, 64.90; H, 4.95.

(c) **S-Benzyl-L-cysteine.**—S-Benzyl-N-phthaloyl-L-cysteine ethyl ester (1 g., 0.0027 mole) was refluxed for 4 hours with 2 ml. of glacial acetic acid and 5 ml. of hydriodic acid (47%) and the reaction mixture was kept overnight in a refrigerator. Phthalic acid (300 mg.) was filtered off, the acids removed *in vacuo*, the residue repeatedly treated with water, dissolved in 20 ml. of water, extracted with two 10-ml. portions of ether, the water layer concentrated to about 10 ml. and adjusted to *p*H 4.5 with concentrated ammonia. Characteristic plates of S-benzyl-L-cysteine separated and were removed by filtration and washed with 3 ml. of water. A yield of 0.3 g. (52.5%) was obtained. A sample for analysis was crystallized twice from water; m.p. and mixed m.p. 206–208°, $[\alpha]^{20}D$ +27.5° (*c* 1.75 in *N* NaOH); reported[18] m.p. 208–211° and $[\alpha]^{20}D$ +29° (*c* 2 in *N* NaOH).

Anal. Calcd. for $C_{10}H_{13}NO_2S$: N, 6.63. Found: N, 6.88.

Desulfuration of the Thiolactone I.—DL-α-Phthalimido-β-propiothiolactone (2 g., 0.0086 mole) was heated under reflux with 4 g. of Raney nickel C[19] in 120 ml. of absolute ethanol and left overnight at room temperature. Nickel was removed by filtration, washed with two 20-ml. portions of absolute ethanol and the combined filtrate and washings were evaporated *in vacuo* to give 1.2 g. of an oily product.

The crude oily product (0.6 g.) was dissolved in 2.5 ml. of ethanol and treated with 0.130 g. of a methanolic solution of 2,4-dinitrophenylhydrazine. After standing overnight, a crystalline product separated. More of the same was obtained upon addition of 2.5 ml. of water. Total yield was 0.3 g. The analytical sample was crystallized from 90% ethanol to a melting point of 186–188°.

Anal. Calcd. for $C_{17}H_{13}N_5O_6$: C, 53.26; H, 3.42; N, 18.27. Found: C, 53.18; H, 3.25; N, 18.37.

Another portion of the crude aldehyde (0.6 g.) was treated with a methanolic semicarbazide acetate solution. Semicarbazone which separated was recrystallized from methanol and had a melting point of 220°. The m.p. of this product was undepressed upon admixture with an authentic sample of DL-phthalimidopropionaldehyde semicarbazone.[20]

Anal. Calcd. for $C_{12}H_{12}N_4O_3$: N, 21.53. Found: N, 21.56.

N-Phthaloyl-L-cysteinyl-L-methionine Ethyl Ester (IV).—A solution of 2 g. (0.0113 mole) of L-methionine ethyl ester[21] and 2 g. (0.0086 mole) of thiolactone I in 20 ml. of dioxane was kept for 3 days at room temperature. The solvent was evaporated under reduced pressure and the residue dissolved in 50 ml. of ethyl acetate, washed with two 10-ml. portions of 10% hydrochloric acid, followed by 10 ml. of water, dried over magnesium sulfate, and the solvent evaporated *in vacuo*. The yellow oily residue (3 g.) was dissolved in 3 ml. of benzene and crystallized by addition of 2 ml. of petroleum ether. A crop of 1.09 g. (31.5%) of white needles was obtained, m.p. 95–102°, $[\alpha]^{22}D$ −35.7° (*c* 1.8 in benzene). For analysis a sample was recrystallized twice from a mixture of benzene–petroleum ether (3:2), m.p. 108–109°, $[\alpha]^{24}D$ −42.3° (*c* 0.59 in benzene).

Anal. Calcd. for $C_{18}H_{22}N_2O_5S_2$: C, 52.68; H, 5.40; N, 6.83. Found: C, 52.64; H, 5.38; N, 6.81.

Reaction of S-Benzyl-N-phthaloyl-L-cysteinyl Chloride with One Mole of Aluminum Chloride. Preparation of Polythioester II.—A mixture of 60 ml. of benzene and 4.5 g. (0.034 mole) of anhydrous aluminum chloride was heated to 65° and a solution of 10.7 g. (0.03 mole) of S-benzyl-N-phthaloyl-L-cysteinyl chloride in 60 ml. of benzene was added at once. The temperature was then kept for an additional 3 hours at 65–70°, the reaction mixture cooled and hydrolyzed with 80 g. of ice and 18 ml. of concentrated hydrochloric acid. The insoluble precipitate (3.7 g.) was filtered off, washed with water, dried, dissolved in 10 ml. of dimethylformamide, treated with charcoal, precipitated with 60 ml. of water and the polymer filtered off and dried on air; yield 2.2 g. A sample for analysis was dissolved in dimethylformamide and precipitated with methanol, m.p. 256–260°, $[\alpha]^{20}D$ −214° (*c* 1.975 in dimethylformamide).

Anal. Calcd. for $(C_{11}H_7NO_3S)_7 \cdot H_2O$: C, 56.03; H, 3.11; N, 5.94; S, 13.60; mol. wt., 1650. For $(C_{11}H_7NO_3S)_x$: C, 56.66; H, 3.03; N, 6.01; S, 13.75. Found: C, 56.86;

(14) K. v. Auwers and A. Frühling, *Ann.*, **422**, 221 (1921).
(15) H. Staudinger and H. Freudenberger, *Ber.*, **61**, 1583 (1928).
(16) M. S. Dunn and T. W. Brophy, *J. Biol. Chem.*, **99**, 221 (1933).
(17) G. Toennies and T. F. Lavine, *ibid.*, **89**, 153 (1930).
(18) B. Hegedüs, *Helv. Chim. Acta*, **31**, 737 (1948).
(19) C. D. Hurd and B. Rudner, THIS JOURNAL, **73**, 5157 (1951).
(20) E. Rade, *Ber.*, **55**, 3177 (1922).
(21) D. Fleš and A. Markovac-Prpić, *Croat. Chem. Acta*, **29**, 79 (1957).

H, 2.80; N, 6.26; S, 13.55; mol. wt., 1539 ± 200 (cryoscopic in *m*-dinitrobenzene).

Conversion of the Polythioester II to L-Cystine.—Polythioester II (0.2 g.) was heated for 24 hours under reflux with 2 ml. of hydriodic acid and 2 ml. of glacial acetic acid. The insoluble part was removed by filtration and the filtrate evaporated under reduced pressure to dryness. The residue was dissolved in 3 ml. of water, extracted with ether and adjusted to *p*H 4.5 with a saturated sodium acetate solution. After several days L-cystine separated, m.p. 252° dec., $[\alpha]^{20}D$ −187° (*c* 0.16 in *N* HCl). The compound was in all properties identical with an authentic specimen of L-cystine.

Anal. Calcd. for $C_6H_{12}N_2O_4S_2$: C, 30.00; H, 5.04; N, 11.64. Found: C, 30.30; H, 4.75; N, 11.93.

The ether extract was evaporated and the residue sublimed *in vacuo* to give 49 mg. of phthalic anhydride, m.p. and mixed m.p. 130°.

Acknowledgments.—The authors wish to express their sincere appreciation to Professor C. K. Ingold of the University College, London, for suggesting the reaction mechanism described in this paper. It is a pleasure to acknowledge to Professor N. A. Milas of the Massachusetts Institute of Technology, Cambridge, his interest in this work, and to Mr. K. Šestanj of the Research Laboratory "Pliva" for very stimulating discussions.

ZAGREB, YUGOSLAVIA

[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, AND NEW YORK STATE PSYCHIATRIC INSTITUTE, NEW YORK CITY]

II. Synthesis of Long Chain Fatty Acid Amines of Sphingosine and Dihydrosphingosine[1]

BY BENJAMIN WEISS AND PAULA RAIZMAN

RECEIVED MARCH 12, 1958

The acyl chlorides of the even numbered fatty acids from C-12 to C-18 were allowed to react with either sphingosine or dihydrosphingosine in N,N-dimethylformamide and pyridine to yield the corresponding N-acyl derivatives. Reduction of N-stearoylsphingosine and N-stearoyldihydrosphingosine with lithium aluminum hydride gave the respective secondary amines; the olefinic bond of sphingosine in the former compound was unaffected by the reduction.

In continuation of our program of preparing various sphingosine and dihydrosphingosine derivatives for substrates in our forthcoming studies on the *in vitro* metabolism of cerebroside and sphingomyelin, it was necessary to have a selection of long chain fatty acid amides of sphingosine (ceramides) and dihydrosphingosine (dihydroceramides). The ceramide N-lignocerylsphingosine was first isolated from partially hydrolyzed sphingolipide preparations[2,3] and, later, was found free in both liver and spleen[4-6]; N-cerebronylsphingosine was prepared by partial hydrolysis of phrenosine.[7] Reichel and Thannhauser,[8] *via* an indirect route, synthesized N-stearoyl- and N-palmitoylsphingosine, by first preparing the triacylated derivative, *e. g.*, tristearoylsphingosine, and then selectively hydrolyzing the ester groups to obtain the N-acyl compound. Unsatisfactory results were obtained by us with this procedure, owing primarily to the intractable emulsions formed in the separation of the free fatty acid from the ceramide and the resulting low yields. An alternative method involving less preparative steps was sought in which the amine function was treated with the appropriate acyl chloride to give directly the N-substituted compound. A study of various solvents disclosed that of those examined, N,N-dimethylformamide was the most suitable since it appeared to suppress the nucleophilic property of the hydroxyl group while enhancing that of the amino group. This solvent had been used in the preparation of the N-palmitoyl derivative of chloramphenicol.[9] In the initial experiments, one-half the stoichiometric amount of acyl chloride was treated with sphingosine or dihydrosphingosine in order to obviate the difficulty of separating unreacted fatty acid from the product; the excess base served to neutralize the acid formed. Since this was wasteful of valuable sphingosine, pyridine was added to the reaction mixture in amounts sufficient to act as acid acceptor. When 90% of the stoichiometric amount of acyl chloride was employed with either sphingosine or dihydrosphingosine in the presence of pyridine, the yield was correspondingly increased and the product was identical with that obtained in the absence of pyridine. The ceramides and dihydroceramides formed from the C-16 and C-18 acyl chlorides were the least difficult to prepare since they precipitated out of the reaction mixture thus facilitating their isolation and purification. However, since little or no precipitate occurred with either the C-12, C-14 or oleoyl compounds, the cold reaction mixture had to be acidified, extracted with ether, and the residue, obtained after washing and concentrating the ether solution, crystallized from 95% ethanol. The N-acylsphingosine and N-acyldihydrosphingosine derivatives gave negative ester[10] and ninhydrin reactions. The melting points in the homologous series of dihydroceramides showed an increase with chain length of the acyl substituent; lauroyl, 99–101°; myristoyl, 103–104°; palmitoyl, 105–106°; and stearoyl, 106–107°. The same was true with the sphingosine homologs which had lower melting points than their corresponding saturated derivatives: lauroyl, 80–82°;

(1) This investigation was supported in part by research grant No. B-344 (C5 and C6) from the Institute of Neurological Diseases and Blindness of the National Institutes of Health, Public Health Service.

(2) P. A. Levene, *J. Biol. Chem.*, **24**, 69 (1916).

(3) S. J. Thannhauser and E. Frankel, *Z. physiol. Chem.*, **203**, 183 (1931).

(4) E. Frankel and F. Bielschowsky, *ibid.*, **213**, 58 (1932).

(5) E. Klenk and O. von Schoenebeck, *ibid.*, **209**, 112 (1932).

(6) C. Tropp and V. Wiederscheim, *ibid.*, **222**, 39 (1933).

(7) E. Klenk, *ibid.*, **153**, 74 (1926).

(8) M. Reichel and S. J. Thannhauser, *J. Biol. Chem.*, **135**, 15 (1940).

(9) W. H. Edgerton, V. H. Maddox and J. Controulis, THIS JOURNAL, **77**, 27 (1955).

(10) U. T. Hill, *Anal. Chem.*, **19**, 932 (1947).