TABLE V

PREPARATION OF N^2-BENZYL-N-ALKYL-*dl*-α-ASPARAGINES

All substances were recrystallized from ethanol.

Substance, N^2-benzyl-N-alkyl-*dl*-α-asparagine	Yield, %	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Ethyl	72	159	$C_{13}H_{18}O_3N_2$	62.4	62.2	7.2	7.4	11.2	11.0
n-Propyl	50	163	$C_{14}H_{20}O_3N_2$	63.6	63.2	7.6	7.6	10.6	10.3
Isopropyl	70	182	$C_{14}H_{20}O_3N_2$	63.6	63.3	7.6	7.0	10.6	10.2
Allyl	55	153	$C_{14}H_{18}O_3N_2$	64.1	64.2	6.9	6.9	10.7	10.7
n-Butyl	60	161	$C_{15}H_{22}O_3N_2$	64.7	64.2	7.9	7.9	10.0	9.8
n-Hexyl	55	167	$C_{17}H_{26}O_3N_2$	66.6	67.0	8.5	8.6	9.1	9.0
Cyclohexyl	70	188	$C_{17}H_{24}O_3N_2$	67.0	66.2	7.9	7.9	9.2	9.0

TABLE VI

PREPARATION OF N-ALKYL-*dl*-α-ASPARAGINES

Yields were almost quantitative in all cases

Substance, N-alkyl-*dl*-α-asparagine	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Ethyl[a]	197	$C_6H_{12}O_3N_2$	45.0	45.0	7.5	7.5	17.5	16.9
n-Propyl[b]	222	$C_7H_{14}O_3N_2$	48.3	47.6	8.1	8.1	16.1	16.1
n-Propyl[b] (*via* allyl)	221	$C_7H_{14}O_3N_2$	48.3	48.2	8.1	8.4	16.1	16.0
Isopropyl[b]	233	$C_7H_{14}O_3N_2$	48.3	48.1	8.1	8.0	16.1	16.0
n-Butyl[b]	226	$C_8H_{16}O_3N_2$	51.0	51.0	8.5	8.4	14.9	14.7
n-Hexyl[c]	223	$C_{10}H_{20}O_3N_2$	55.5	55.5	9.3	9.0	12.9	13.2
Cyclohexyl[c]	247	$C_{10}H_{18}O_3N_2$	56.0	56.2	8.4	8.3	13.1	12.7

[a] Purified by trituration with hot ethanol. [b] Recrystallized from aqueous ethanol. [c] Recrystallized from water.

Preparation of N^2-Alkyl-*dl*-asparagines.—Maleamic acid (0.025 mole) and the alkylamine (0.025 mole) in 15 ml. of pyridine was heated under reflux (for reaction times see Table IV). After cooling, the precipitate was filtered off and washed with acetone.

Preparation of N^2-Benzyl-N-alkyl-*dl*-α-asparagines.—To a cooled solution of the mixed anhydride of N-benzyl-*dl*-aspartic acid and chlorocarbonic acid,[5] freshly prepared from 9 g. of N-benzyl-*dl*-aspartic acid in 150 ml. of dry dioxane, was added 0.1 mole of the amine.[8] After having stood overnight at room temperature, the formed precipitate which contained various amounts of the amide in addition to amine-hydrochloride, was filtered off, washed with ether and dried. Separation of the amine-salt was then effected by trituration with acetone. The dioxane solution was evaporated *in vacuo* to dryness and the residue redissolved in acetone and left in a refrigerator overnight (or sometimes longer) until the amide crystallized out. Both fractions of the substance were finally recrystallized as indicated in Table I.

Preparation of N-Alkyl-*dl*-α-asparagines.—These substances were obtained in the same way as their corresponding β-isomers.

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(8) An excess of the amine must be taken to bind any free hydrochloric acid already present in the reaction mixture. This depends on the purity of the phosgene which besides greatly influences the formation of the mixed anhydride and consequently the yield of the amide. Therefore, only first grade quality phosgene should be used.

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, "PLIVA" PHARMACEUTICAL AND CHEMICAL WORKS, ZAGREB, YUGOSLAVIA]

The Correlation of Configurations of Chloroamphenicol and D-Serine

BY DRAGUTIN FLEŠ AND BRANKO BALENOVIĆ

RECEIVED NOVEMBER 21, 1955

Chloroamphenicol (D-*threo*-1-*p*-nitrophenyl-2-dichloroacetamido-1,3-propanediol) (I) was prepared from O-methyl-N-phthaloyl-D-serine (III) through the intermediate α-phthalimido-β-methoxy-D-propiophenone (V). The configurational correlation between I and D-serine was thus established.

The configurational correlation between chloramphenicol (D-*threo*-1-*p*-nitrophenyl-2-dichloroacetamido-1,3-propanediol) and (−)-nor-*pseudo*-ephedrine, based on optical rotation data of chloramphenicol and its derivatives, was first suggested by Rebstock, *et al.*[1] The correctness of this assumption was later confirmed by several investigators. Fodor, *et al.*,[2] established the configurational relation of chloramphenicol to nor-*pseudo*-ephedrine by means of chemical interconversion. Miyamoto[3] showed that DL-*erythro*-1-phenyl-1-methoxy-2-benzamido-3-hydroxypropane was related to nor-ephedrine, through appropriate transformations. Honjo[4] synthesized the L-*threo*-1-*p*-nitrophenyl-2-amino-1,3-propanediol from D-*threo*-phenylserine, correlating in this way the configuration of chloramphenicol with the L-*threo*-phenylserine.

In the course of our studies on the configuration

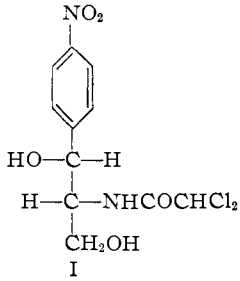
(1) M. C. Rebstock, H. M. Crooks, Jr., J. Controulis and Q. R. Bartz, THIS JOURNAL, **71**, 2458 (1949).

(2) G. Fodor, J. Kiss and I. Sallay, *J. Chem. Soc.*, 1858 (1951).

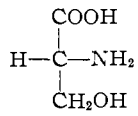
(3) M. Miyamoto, *J. Pharm. Soc. Japan*, **72**, 677 (1952); *C. A.*, **47**, 6373 (1953).

(4) M. Honjo, *J. Pharm. Soc. Japan*, **73**, 368 (1953); *C. A.*, **48**, 2642 (1954).

of chloramphenicol, we tried to correlate the configuration of chloramphenicol with that of the amino acid serine. We therefore outlined a synthesis of chloramphenicol from optically active serine, which through the intermediate propiophenone gives phenylserinol and subsequently the chloramphenicol of known configuration. In preliminary experiments we used the inactive intermediates, and DL-*threo*-1-*p*-nitrophenyl-2-dichloroacetamido-1,3-propanediol was thus obtained.[5] This paper deals with the preparation of the D-*threo*-isomer (I) from O-methyl-N-phthaloyl-D-serine (III). The racemic III was resolved by the fractional crystallization of the brucine salt, and its configuration was established by converting III to D-serine (II) with hydriodic acid.



I



II

When O-methyl-N-phthaloyl-D-seryl chloride (IV) was condensed with benzene using the usual Friedel–Crafts reaction, the optically active propiophenone V with a specific rotation of $[\alpha]^{20}D$ +226° was obtained. However the yield was only 19.1% and some inactive ketone was recovered from the mother liquor. The separation of optically active V was easily accomplished, because the racemic modification is much more soluble in ethyl acetate than the optically active modification. Reduction of ketone V with aluminum isopropoxide gave a mixture of diastereomers from which the D-*threo*-1-phenyl-1-hydroxy-2-phthalimido-3-methoxypropane (VI) was easily separated, being less soluble in ethanol than its diastereomer. The carbinol VI was then converted to chloramphenicol using reactions similar to those reported by Rebstock[6] in a paper describing the synthesis of DL-*threo*-1-*p*-nitrophenyl-2-dichloroacetamido-1,3-propanediol which also involved the use of the intermediate DL-α-phthalimido-β-methoxypropiophenone. The *threo*-carbinol VI was first acetylated with acetic anhydride in pyridine and then nitrated. After the protective groups were removed, the optically active base IX was obtained. The dichloroacetamide of this base was in all properties identical with the antibiotic, chloramphenicol.

Since the reactions used in this synthesis do not involve the asymmetric carbon atoms of chloramphenicol, we consider that this synthesis presents additional support for the configuration assigned to the antibiotic. It is proved that the configuration of the carbon atom on which the dichloroacetamido group of chloramphenicol is attached is the same as that of the natural amino acids.

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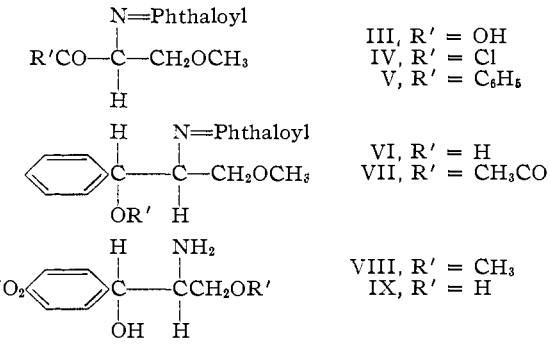
(6) M. C. Rebstock, *J. Org. Chem.*, **19**, 851 (1954).



III, R′ = OH
IV, R′ = Cl
V, R′ = C_6H_5

VI, R′ = H
VII, R′ = CH_3CO

VIII, R′ = CH_3
IX, R′ = H

Acknowledgments.—The authors are grateful to Professor K. Balenović of the University of Zagreb, for suggesting this work. Thanks are due to Mr. R. Marušić and Mrs. M. Milohnoja for their capable technical assistance.

Experimental[7]

D- and L-O-Methyl-N-phthaloylserine.—A mixture of 95 g. of O-methyl-N-phthaloyl-DL-serine and 149 g. of anhydrous brucine was dissolved under reflux in 5.5 l. of acetone and left overnight in a refrigerator. The crystalline product, predominantly brucine-salt of the D-form, was removed by filtration under suction, and crystallized seven times from hot acetone to give 26 g. of a product with a rotation of $[\alpha]^{20}D$ +29° (*c* 2.00% in ethanol). All mother liquors giving a residue with positive rotation, after evaporation of acetone, were combined and recrystallized from acetone to a rotation of +29°. A crop of 18 g. of the D-salt was obtained. The repetition of the same procedure afforded an additional 10 g. of the D-salt. A total of 54 g. of the D-salt was obtained. The mother liquors having a negative rotation after the evaporation of acetone were crystallized from water (3 ml. of water per gram of the residue) to a rotation of $[\alpha]^{20}D$ −50° (*c* 2.00% in ethanol). A yield of 30 g. of the L-brucine salt was obtained.

The brucine salts were then converted to the respective enantiomorphs in the following manner: Fifty grams of the D-salt was dissolved in 150 ml. of hot water and 75 ml. of 10% hydrochloric acid was added. The oily precipitate was extracted with four 75-ml. portions of benzene, dried over magnesium sulfate and the solvent evaporated at reduced pressure. The oily residue (20 g.) was dissolved in 170 ml. of a saturated sodium bicarbonate solution at 35°, then treated with charcoal and acidified with 10% hydrochloric acid. The oily precipitate solidified very soon and, after standing overnight in a refrigerator, a yield of 17 g. of prismatic crystals was obtained. The crude III was recrystallized from 100 ml. of ethanol–water (1:2) to give 13.2 g. of a product melting at 99–101°. An analytical sample was recrystallized from benzene–petroleum ether (1:2) and finally from ethanol–water (1:2); m.p. 101–102.5°, $[\alpha]^{20}D$ +46.9° (*c* 1.82% in ethanol).

Anal. Calcd. for $C_{12}H_{11}NO_5$: C, 57.83; H, 4.45; N, 5.62. Found: C, 57.69; H, 4.37; N, 5.63.

For O-Methyl-N-phthaloyl-L-serine, m.p. 101–102°, $[\alpha]^{20}D$ −48.1° (*c* 2.015% in ethanol). *Anal.* Found: C, 57.94; H, 4.19; N, 5.76.

Conversion of O-Methyl-N-phthaloyl-L-serine to L-Serine.—O-Methyl-N-phthaloyl-L-serine (7.5 g., 0.03 mole) was refluxed for 4 hours in a mixture of 45 ml. of 47% hydriodic acid and 22.5 ml. of glacial acetic acid. The reaction mixture was cooled and the separated phthalic acid removed by filtration under suction and washed with two 10-ml. portions of acetic acid. The filtrate and washings were evaporated *in vacuo* and the residue repeatedly treated with water to remove traces of hydriodic acid. The crude hydriodide was dissolved in 750 ml. of water and passed through a column of Amberlite IR-4B. The column was washed with water until the ninhydrin reaction was negative (500 ml.). The water was evaporated under reduced pressure and the residue (2.1 g.) dissolved in 15 ml. of water, treated with charcoal and crystallized by the addition of 15 ml. of ethanol

(7) Melting points are uncorrected. Microanalyses were carried out by Mr. N. Manger and Miss E. Jaeger.

to yield 1.6 g. (50.6%) of colorless prisms, m.p. 210° dec. After three crystallizations from water–ethanol (1:1), the product melted at 220° dec., $[\alpha]^{22}D$ +13.4° (*c* 10.57% in *N* hydrochloric acid), $[\alpha]^{22}D$ −7.6° (*c* 10.585% in water); reported m.p. 223°,[8] $[\alpha]^{26}D$ +13.9° (*c* 10% in *N* hydrochloric acid), $[\alpha]^{26}D$ −6.8° (*c* 10% in water).[9]

Anal. Calcd. for $C_3H_7NO_3$: C, 34.28; H, 6.71; N, 13.33. Found: C, 34.20; H, 6.74; N, 13.44.

O-Methyl-N-phthaloyl-D-serine was converted in the same way to **D-serine**, m.p. 222° dec., $[\alpha]^{20}D$ +6.7° (*c* 3.52% in water); reported $[\alpha]^{25}D$ +6.87° (*c* 10.5% in water).[8]

α-Phthalimido-β-methoxy-D-propionyl Chloride (IV).—α-Phthalimido-β-methoxy-D-propionic acid (III, 10.7 g., 0.043 mole) was refluxed for half an hour with 10.7 ml. of thionyl chloride. The excess of thionyl chloride was removed *in vacuo*, the residue repeatedly treated with benzene and dried over potassium hydroxide in a vacuum desiccator. This product was directly used in the next conversion. A sample was purified for analysis by crystallization from benzene–petroleum ether (1:3); m.p. 97–99°, $[\alpha]^{19}D$ +123° (*c* 2.666% in benzene).

Anal. Calcd. for $C_{12}H_{10}ClNO_4$: C, 53.86; H, 3.76. Found: C, 53.98; H, 3.81.

α-Phthalimido-β-methoxy-D-propiophenone (V).—A mixture of 60 ml. of benzene and 15.7 g. (0.12 mole) of anhydrous aluminum chloride was placed in a 500-ml. three-necked flask, equipped with a mechanical stirrer, a dropping funnel and a reflux condenser. The reaction mixture was heated to 70°, and with rapid stirring, 11.1 g. (0.041 mole) of α-phthalimido-β-methoxy-D-propionyl chloride (IV), dissolved in 55 ml. of benzene, was added at such rate as to maintain constant refluxing. The reaction mixture was refluxed for an additional three hours, cooled and hydrolyzed with 45 g. of ice and 9 ml. of concentrated hydrochloric acid. The water layer was separated and extracted with two 100-ml. portions of benzene, followed by two 20-ml. portions of a saturated sodium bicarbonate solution, then decolorized with charcoal and dried over magnesium sulfate. The benzene was removed under reduced pressure and the residue (12 g.) was crystallized from 22 ml. of ethanol to give 6.0 g. of ketone, m.p. 117–127°. The crude ketone was dissolved in 15 ml. of ethyl acetate, treated with charcoal and crystallized by cooling. A crop of 2.45 g., representing a 19.1% yield was obtained, m.p. 130–131°, $[\alpha]^{20}D$ +212.5° (*c* 2.00% in ethyl acetate). An analytical sample was recrystallized from ethyl acetate and finally from ethanol; m.p. 132.5–133.5°, $[\alpha]^{20}D$ +226° (*c* 1.031% in ethyl acetate).

Anal. Calcd. for $C_{18}H_{15}NO_4$: C, 69.98; H, 4.89; N, 4.53. Found: C, 69.75; H, 4.63; N, 4.64.

D-*threo*-1-Phenyl-1-hydroxy-2-phthalimido-3-methoxypropane (VI).—In a 100-ml. round-bottomed flask were placed 2.33 g. (0.008 mole) of ketone V, 4.4 g. (0.022 mole) of distilled aluminum isopropoxide and 44 ml. of dry isopropyl alcohol. A Hahn partial condenser was attached and the reaction mixture heated at such rate as to maintain the slow distillation of acetone. When the theoretical amount of acetone was obtained (six hours), the isopropyl alcohol was removed *in vacuo*, and the residue hydrolyzed with a solution of 30 g. of tartaric acid in 50 ml. of water in the presence of 20 ml. of benzene. The water layer was removed and extracted with three 10-ml. portions of benzene. The combined benzene solutions were dried, the benzene removed *in vacuo*, and the residue (2.5 g.) dissolved in 8 ml. of ether. The addition of 16 ml. of petroleum ether caused an oily precipitate to separate. The mother liquor was decanted and the residue crystallized from 10 ml. of ethanol to give 1.2 g. (50%) of a product melting at 126–127°. An analytical sample was recrystallized from ethanol and finally from a mixture of benzene–petroleum ether (1:1); m.p. 128–130°, $[\alpha]^{21}D$ −25.2° (*c* 8.34% in ethyl acetate).

Anal. Calcd. for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.50; N, 4.50. Found: C, 69.38; H, 5.48; N, 4.66.

D-*threo*-1-Phenyl-1-acetoxy-2-phthalimido-3-methoxypropane (VII).—The carbinol VI (1.1 g., 0.0035 mole) was dissolved in 3.9 ml. of pyridine and acetylated with 3.9 ml. (0.0037 mole) of acetic anhydride. The reaction mixture was cooled in ice for half an hour, then left at room temperature overnight, and poured on 30 g. of ice. The crystalline precipitate was extracted with three 20-ml. portions of ethyl acetate, washed with 2% sulfuric acid, neutralized with a saturated sodium bicarbonate solution, and dried over magnesium sulfate. The removal of the benzene and crystallization from 5 ml. of absolute ethanol, gave 1.1 g. (88.1%) of needles, melting at 117–118.5°. A sample for analysis was recrystallized from ethanol; m.p. 118–119.5°, $[\alpha]D$ −39.0° (*c* 6.0% in ethyl acetate).

Anal. Calcd. for $C_{20}H_{19}NO_5$: C, 67.89; H, 5.42; N, 3.96. Found: C, 67.90; H, 5.35; N, 4.24.

D-*threo*-1-*p*-Nitrophenyl-1-hydroxy-2-amino-3-methoxypropane (VIII).—The acetylated product VII (2.4 g., 0.007 mole) was nitrated with 10 ml. of fuming nitric acid at −20° according to the procedure described by Rebstock for the racemic compound.[6] The crude nitrated product (2.9 g.) was used directly in the next conversion.

The nitrate product (2.9 g.) was refluxed for 2.5 hours with 20 ml. of ethanol and 7.5 ml. (0.0075 mole) of *N* hydrazine hydrate in ethanol. The solvent was removed *in vacuo*, and the residue treated for 10 minutes at 50° with 17 ml. of 5% hydrochloric acid, then kept for an hour at room temperature, and phthalyl hydrazide was removed by filtration under suction. The filtrate was heated for 2.5 hours on a water-bath and the hydrochloric acid removed *in vacuo*. The residue (2.25 g.) was taken into 8 ml. of water and the solution adjusted to *p*H 10 with ammonium hydroxide. The crystalline precipitate was collected by filtration and washed with 2 ml. of water. A yield of 1 g. was obtained, m.p. 128–132°. The crude product was crystallized from 8 ml. of ethylene dichloride to give 0.5 g. (32.5% based on VII), m.p. 141–142°. A sample was purified for analysis by sublimation at 135–140° at a pressure of 0.025 mm., m.p. 141.5–142.5°, $[\alpha]^{20}D$ −8.6° (*c* 1.728% in methanol).

Anal. Calcd. for $C_{10}H_{14}N_2O_4$: C, 53.09; H, 6.24; N, 12.38. Found: C, 52.88; H, 6.07; N, 12.45.

D-*threo*-1-*p*-Nitrophenyl-2-dichloroacetamido-1,3-propanediol (X).—The methyl ether VIII (0.5 g., 0.002 mole) was converted to the chloramphenicol base (IX) by heating in a sealed tube with 7 ml. of 48% hydrobromic acid, following the method previously published.[6] A yield of 355 mg. (75.7%) of the base IX was obtained, m.p. 161–162°, $[\alpha]^{20}D$ −23.7° (*c* 1.218% in methanol). The product was undepressed upon admixture with an authentic sample of chloramphenicol base, melting at 161–162°.

The base IX was converted to chloramphenicol according to the method described by Long and Jenesel.[10] The product melted at 150–151°, gave no depression of melting point when mixed with an authentic sample of chloramphenicol and had a specific rotation of $[\alpha]^{19}D$ +17.9° (*c* 2.889% in ethanol). The antibacterial activity against *Shigella paradysenteriae* (Sonnei) was the same as that of an authentic sample of chloramphenicol; reported m.p. 150–151°,[1] $[\alpha]^{27}D$ +18.6° (*c* 4.86% in ethanol).[11]

Anal. Calcd. for $C_{11}H_{12}Cl_2N_2O_5$: C, 40.88; H, 3.74; N, 8.68. Found: C, 40.89; H, 3.53; N, 8.55.

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