sym-N,N'-**Dimethylguanidine Hydrobromide.**—The preparation of this compound was patterned after the procedure of Schenck.[13] This involved the reaction of 45 ml. of a 10% solution of methylamine (0.14 mole) in absolute ethanol with 5.2 g. of cyanogen bromide[14] (0.05 mole) at room temperature for 48 hours in a tightly stoppered flask. Evaporation of the contents of the flask *in vacuo* yielded the crude hydrobromide, m.p. 142°. After four recrystallizations from a 1:1 ether–absolute ethanol mixture at 6°, the purified product melted at 144°.

Anal. Calcd. for $C_3H_{10}N_3Br$: C, 21.44; H, 5.99. Found: C, 21.53, 21.42; H, 5.88, 5.95.

The picrate was prepared, m.p. 177° (lit. m.p. 177.0, 177.5°,[7] 178°[13]).

sym-N,N'-**Diethylguanidine Picrate.**—*sym*-N,N'-Diethylguanidine hydrobromide was prepared from ethylamine and cyanogen bromide in a manner similar to that described above for the corresponding dimethyl compound. This compound failed to crystallize when treated in the usual manner and was converted to the picrate, m.p. 143–144° (lit. m.p. 143–144°,[7] 141°[15]).

Anal. Calcd. for $C_{11}H_{16}N_6O_7$: C, 38.37; H, 4.68. Found: C, 38.13, 38.18; H, 4.79, 4.55.

sym-N,N'-**Dibutylguanidine Picrate.**—*sym*-N,N'-Dibutylguanidine hydrobromide was prepared from *n*-butylamine and cyanogen bromide in a manner similar to that described for the corresponding dimethyl compound. This compound failed to crystallize when treated in the usual manner and was converted to the picrate, m.p. 120–122° (lit. m.p. 122.5°[16]).

Anal. Calcd. for $C_{15}H_{24}N_6O_7$: C, 44.99; H, 6.04. Found: C, 44.91; H, 5.94.

sym-N,N',N''-**Triethylguanidine Hydroiodide.**—This compound was prepared by a procedure adapted from the method of Angyal and Warburton[8] for the preparation of the symmetrical trimethyl compound. One mole of ethyl isothiocyanate (Eastman Kodak Co.) was treated with 1.2 moles of ethylamine in ether solution to give N,N'-diethylthiourea, m.p. 77° (lit. m.p. 77°). This compound (1 mole) was treated with ethyl iodide (1 mole) to give S-ethyl N,N'-diethylthiouronium iodide which was isolated by evaporation *in vacuo* and allowed to react with 2 moles of ethylamine in aqueous solution for 24 hours on the water-bath and a further 24 hours under reflux. The product, *sym*-N,N',N''-triethylguanidine hydroiodide, was isolated by evaporating the solution to dryness *in vacuo*. Purification was accomplished by crystallization from ethanol–ether solution or by crystallization from water at 5°. The purified product melted at 139°.

Anal. Calcd. for $C_7H_{18}N_3I$: C, 31.00; H, 6.69. Found: C, 31.90, 31.06; H, 6.54, 6.37.

Acknowledgment.—We are indebted to Dr. Benjamin Warshowsky for his most helpful cooperation.

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The Synthesis of α,γ-Dihydroxy-β-amino-*n*-butyric Acid

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The structural formula of the base sphingosine is now known in sufficient detail to recognize that the degradation of this base *via* ozonolysis should give as one of the degradation products an α,γ-dihydroxy-β-amino-*n*-butyric acid instead of an α-amino-β,γ-dihydroxy-*n*-butyric acid as was originally claimed by Klenk and Diebold.[3,4] Although it was shown by Niemann and Nichols,[5] through the synthesis of D-*erythro*- and D-*threo*-α-amino-β,γ-dihydroxy-*n*-butyric acid, that Klenk and Diebold's acid could not possess the structure claimed for it there appears to be no record of an attempt to synthesize the acid which Klenk and Diebold isolated. Therefore we wish to describe in this communication a synthesis of an optically inactive α,γ-dihydroxy-β-amino-*n*-butyric acid by a method which should be capable of application to the synthesis of the various optically active forms.

α-Bromo-β-ethoxypropionic acid (I) was prepared essentially as described by Wood and du Vigneaud.[6] Ammonolysis of I gave α-amino-β-ethoxypropionic acid (II) which was condensed with phthalic anhydride to give α-phthalimido-β-ethoxypropionic acid (III). All attempts to prepare an ester of III by the direct condensation of an ester of I with potassium phthalimide were unsuccessful. The reaction of III with thionyl chloride gave the corresponding acid chloride IV which was allowed to react with diazomethane to give α-phthalimido-β-ethoxyethyl diazomethyl ketone (V). Rearrangement of V with silver oxide in methanol gave methyl β-phthalimido-γ-ethoxy-*n*-butyrate (VI) which was hydrolyzed to the corresponding acid VII. Bromination of VII, in the presence of red phosphorus, gave a mixture of isomeric α-bromo-β-phthalimido-γ-butyrolactones (VIIIa and VIIIb) which were separated on the basis of their different solubilities in ethanol. When the less soluble and higher melting isomer VIIIb was heated under refluxing conditions with either silver or sodium acetate in acetic acid VIIIb was recovered unchanged. After preliminary experiments had shown that α-bromo-β-phthalimidopropionic acid (IX) could be converted into isoserine by heating IX under refluxing conditions with an aqueous suspension of barium carbonate, VIIIb was similarly treated to give a DL-mixture of one of the diastereoisomeric forms of α,γ-dihydroxy-β-amino-*n*-butyric acid.

Experimental[7,8]

α-**Amino-β-ethoxypropionic Acid (II).**—Ethyl α-bromo-β-ethoxypropionate[6] (60 g.) was converted into II essentially as described by Wood and du Vigneaud.[6] Evaporation of the ammoniacal reaction mixture to dryness followed by extraction of the resulting residue with a large volume of boiling ethanol gave 19.35 g. of II, colorless platelets, m.p. 238–240° with decomposition.

Anal. Calcd. for $C_5H_{11}O_3N$ (133.2): C, 45.1; H, 8.3; N, 10.5. Found: C, 45.2; H, 8.3; N, 10.4.

α-**Phthalimido-β-ethoxypropionic Acid (III).**—An intimate mixture of 19.3 g. of II and 21.5 g. of phthalic anhydride was heated to 150–160°. When the reaction had subsided the reaction mixture was cooled, dissolved in hot benzene, the solution cooled, the brown crystalline precipitate recovered, dissolved in hot aqueous ethanol, the hot solution decolorized with Norite, and the solution cooled to give 19.7 g. of III, m.p. 136–138°.

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Anal. Calcd. for $C_{12}H_{13}O_5N$ (263.2): C, 59.3; H, 5.0; N, 5.3. Found: C, 59.4; H, 5.1; N, 5.3.

α-Phthalimido-β-ethoxypropionyl Chloride (IV).—III, 14.4 g., was heated under refluxing conditions for 1 hour with 30 ml. of thionyl chloride, the reaction mixture freed of excess thionyl chloride by distillation and repeated co-distillation with benzene, and the residue recrystallized from a mixture of 60–80° ligroin and benzene to give 10.0 g. of IV, m.p. 70–72°.

Anal. Calcd. for $C_{13}H_{12}O_4NCl$ (281.7): C, 55.4; H, 4.3; Cl, 12.6. Found: C, 55.5; H, 4.3; Cl, 12.6.

α-Phthalimido-β-ethoxyethyl Diazomethyl Ketone (V).—A solution of 13.75 g. of IV in 400 ml. of dry ether was slowly added, at 0–5°, to 500 ml. of an ethereal solution containing *ca.* 7 mole equivalents of diazomethane, the reaction mixture maintained at 0–5° for 2 hours and then allowed to reach room temperature before the excess diazomethane and solvent was removed by distillation. The residue was recrystallized from a mixture of ethyl acetate and 60–80° ligroin to give 9.7 g. of V, yellow prisms, m.p. 87.5–89°.

Anal. Calcd. for $C_{14}H_{13}O_4N_3$ (287.3): C, 58.5; H, 4.6; N, 14.6. Found: C, 58.6; H, 4.7; N, 14.5.

Methyl β-Phthalimido-γ-ethoxy-*n*-butyrate (VI).—To a solution of 10 g. of V in 100 ml. of methanol was added a small amount of freshly prepared silver oxide suspended in methanol and the reaction mixture heated under refluxing conditions. When the evolution of nitrogen had subsided a further quantity of the silver oxide suspension was added and the process repeated until no further reaction was observed. The precipitate present in the reaction mixture was removed by centrifugation, washed with methanol and the combined supernatant and washings freed of solvent. The residual oil was dissolved in chloroform, the solution filtered, the filtrate evaporated to dryness and the residue distilled to give 8.3 g. of a yellow viscous oil, b.p. *ca.* 170° (0.1 mm.), which crystallized upon standing to give VI, m.p. 66–67°, after two recrystallizations from methanol.

Anal. Calcd. for $C_{15}H_{17}O_5N$ (291.3): C, 61.8; H, 5.9; N, 4.8. Found: C, 62.3; H, 5.9; N, 4.6.

β-Phthalimido-γ-ethoxy-*n*-butyric Acid (VII).—A mixture of 3.25 g. of VI, 7 ml. of concentrated hydrochloric acid and 150 ml. of water was heated under refluxing conditions for 3 hours, the hydrolysate cooled, extracted with benzene, the extract freed of solvent and the residue recrystallized first from aqueous ethanol and then from a mixture of benzene and 60–80° ligroin to give 2.3 g. of VII, m.p. 104–106°.

Anal. Calcd. for $C_{14}H_{15}O_5N$ (277.3): N, 5.0. Found: N, 4.7.

α-Bromo-β-phthalimido-γ-butyrolactone (VIII).—To an intimate mixture of 2.0 g. of VII and 0.1 g. of red phosphorus was added dropwise and with cooling 1.2 ml. of bromine. After all of the bromine was added the reaction mixture was heated on a steam-bath for 6 hours. Water was then added, the excess bromine removed by heating, and the colorless aqueous solution cooled to give a crystalline precipitate of VIII which was suspended in 15 ml. of boiling ethanol. The hot ethanol solution was decanted from the undissolved portion of the precipitate and cooled to give 0.4 g. of product m.p. 143–167°. This product was alternately recrystallized from methanol and from ethanol to give the more soluble and lower melting diastereoisomeric mixture (VIIIa) of VIII, m.p. 185°.

Anal. Calcd. for $C_{12}H_{10}O_4NBr$ (310.1): C, 46.5; H, 2.6; N, 4.5; Br, 25.8. Found: C, 46.5; H, 2.6; N, 4.5; Br, 25.9.

The crystalline residue remaining after the initial extraction with a limited amount of ethanol was dissolved in a relatively large volume of boiling ethanol, and the solution cooled to give 0.5 g. of the less soluble and higher melting diastereoisomeric mixture (VIIIb) of VIII, m.p. 196–201° with decomposition after one additional recrystallization from ethanol.

Anal. Calcd. for $C_{12}H_8O_4NBr$ (310.1): C, 46.5; H, 2.6; N, 4.5; Br, 25.8. Found: C, 46.8; H, 2.5; N, 4.7; Br, 26.1.

DL-Isoserine.—A mixture of 4.0 g. of α-bromo-β-phthalimidopropionic acid (IX), 5.2 g. of barium carbonate and 18 ml. of water was heated under refluxing conditions for 1 hour. The reaction mixture was cooled, the excess barium carbonate collected and thoroughly washed with hot water, the combined filtrate and washings freed of barium ion with sulfuric acid, the aqueous solution evaporated to a small volume, cooled, the precipitated phthalic acid collected, the filtrate made alkaline with ammonium hydroxide and evaporated to dryness *in vacuo*. The residue was dissolved in water, the solution filtered, evaporated to a small volume, and cooled to give 0.55 g. of DL-isoserine, m.p. 237° with decomposition.

α,γ-Dihydroxy-β-amino-*n*-butyric Acid (X).—VIIIb, 0.8 g., was dissolved in a solution of 0.95 g. of barium oxide in 40 ml. of water, the solution saturated with carbon dioxide and then heated under refluxing conditions for *ca.* 1 hour. To the cooled reaction mixture was added 0.8 g. of concentrated sulfuric acid in 3 ml. of water, the precipitate collected and washed with hot water, and the combined filtrate and washings evaporated *in vacuo* to a small volume. The phthalic acid which separated upon cooling was collected, the filtrate freed of barium ion by the careful addition of dilute sulfuric acid, the filtrate again concentrated *in vacuo* and a second crop of phthalic acid collected. To the filtrate was added 1 g. of silver carbonate, the mixture heated to boiling, the precipitate removed by centrifugation, the supernatant liquid again concentrated *in vacuo*, the precipitate removed by centrifugation, the supernatant liquid saturated with hydrogen sulfide, the silver sulfide collected and the supernatant liquid evaporated *in vacuo* to give X, granular crystals, m.p. 214° with decomposition.

Anal. Calcd. for $C_4H_9O_4N$ (135.1): C, 35.6; H, 6.7; N, 10.4. Found: C, 35.7; H, 6.5; N, 10.1.

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Substrate and Cosubstrate Requirements for Enzymatic Transfructosidation[1]

BY JOHN H. PAZUR

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Transfructosidation action of enzymes from yeasts[2–4] and molds[5,6] on fructosyl oligosaccharides results in the synthesis of new carbohydrates. Sucrose and raffinose have been found to function as substrates and cosubstrates (acceptor molecules) for the transfructosidase of *Aspergillus oryzae* while fructose and inulobiosylglucose function as cosubstrates only. The enzyme is without action on turanose and melezitose.[5,6] In the studies being reported, three new fructosyl compounds (inulobiose,[7] stachyose[8] and planteose[9]) have been tested as potential substrates for the transfructosidase of *Aspergillus oryzae*. The possibility that these oligosaccharides may function as acceptor molecules in the transfer of fructose units of sucrose has also been investigated.

The transfructosidase was allowed to act on solutions of the fructosyl compounds at room temperature. Aliquots of the digestion mixtures were removed at various time intervals and heated at 100° for 5 minutes to arrest enzyme action. The quali-

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