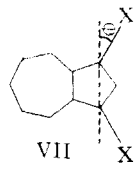
viation of which from reality is negligible for the present purpose, the angle θ has a value of 18°.



VII

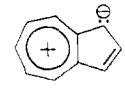
then

$$\mu_{total} = 2 \sin \theta \text{ (bond moment}_{C-Cl}) + \mu \text{ azulene}$$

and

$$\text{bond moment}_{C-Cl} = \frac{\mu_{total} - \mu_{azulene}}{2 \sin \theta} = 2.2 \text{ D.}$$

The calculated moments for the corresponding carbon–bromine and carbon–iodine bonds are 2.3 and 2.2 D., respectively.[33] It was to be expected that the carbon–chlorine bond moment would be greater in the 1-chloro- than in the 2-chloroazulene. This follows from consideration of the contribution to the ground state resonance hybrid of structures represented by VIII which provide electrons for release by induction (and resonance in the case of groups such as nitro) to the substituent. Structures having the negative charge at the 2-position are energetically less favored. The calculated ground state electron densities for the 1-position (1.0960) and the 2-position (0.9787)[15] are in agreement with this interpretation.



VIII

(33) A similar treatment of the unsymmetrical 1-nitroazulene cannot be made since the moment axis and the trans-annular axis are not coincident. The high dipole moment of this compound probably indicates a high moment with respect to the 1-position and the nitro group.

Acknowledgments.—The authors are indebted to Prof. A. C. Huitric for generous and valuable aid in the determination of the dipole moments, and to Profs. W. M. Schubert and W. T. Simpson for their interest and helpful suggestions.

SEATTLE 5, WASH.

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

The Absolute Configuration of the C_1 Atom in Retronecanone (1-Methyl-7-oxopyrrolizidine)

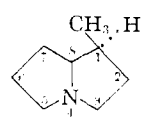
BY ROGER ADAMS AND D. FLEŠ

RECEIVED MARCH 11, 1959

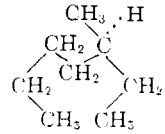
(+)-2-Methyl-4-aminobutyric acid was converted *via* an Arndt–Eistert synthesis to (−)-3-methyl-5-aminovaleric acid which had previously been used for synthesis of (−)-retronecanone. Reduction of (−)-2-methyl-4-aminobutyric acid with lithium aluminum hydride gave (+)-2-methyl-4-amino-1-butanol. The antipode of this amino alcohol was prepared by the reduction of (−)-β-carbomethoxy-*n*-butyramide with lithium aluminum hydride. Hydrolysis of the corresponding (−)-β-carboxy-*n*-butyramide gave (−)-methylsuccinic acid. Since the absolute configuration of (−)-methylsuccinic acid is known to be S, these experiments establish the absolute configuration of the C_1 atom in (−)-retronecanone as S and hence the C_1 atom in (−)-retronecanol from which the retronecanone was prepared by oxidation as S.

Many papers and several monographs have dealt with the relative configuration of the asymmetric carbon atoms in pyrrolizidine bases.[1] Most of the structural correlations are based on the chemical interconversion and degradations of retronecine, platynecine and retronecanol. The absolute configuration of the C_1, C_7 and C_8 atoms in various natural-occurring substituted pyrrolizidines still requires clarification and a research with this objective is now under way in this Laboratory.

The conclusions of Leonard[2] regarding the absolute configuration of the C_1 atom in 1-methylpyrrolizidine (I)[3] based on the correlation between the molecular-rotational shifts of the isoretronecanol and (−)-lupinine have been experimentally tested by Warren and Klemperer.[4] Through the appropriate degradation of heliotridane (I), they prepared (+)-3-methylheptane (II) of known absolute configuration and proved in this way that the C_1 atom of heliotridane has the S-configuration[5] which is the opposite of that deduced by Leonard.



I



II

Adams and Leonard[6] synthesized (−)-retronecanone (III) from (−)-3-methyl-5-aminovaleric acid (IV). The *m*-nitrobenzoyl derivative of IV was brominated in the 2-position, cyclized to the corresponding methylproline, hydrolyzed, and condensed with ethyl acrylate. The product then was subjected to a Dieckmann condensation, hydrolysis

(1) F. L. Warren, in "Progress in the Chemistry of Organic Natural Products," Vol. XII, p. 198, Springer, Wien, 1955; R. Adams and M. Giannurco, *Angew. Chem.*, **69**, 5 (1957); R. Adams, *ibid.*, **65**, 433 (1953); N. J. Leonard, in R. H. F. Manske and H. L. Holmes, "The Alkaloids," Vol. I, Academic Press, Inc., New York, N. Y., 1950, p. 107.

(2) N. J. Leonard, *Chemistry & Industry*, 1455 (1957).

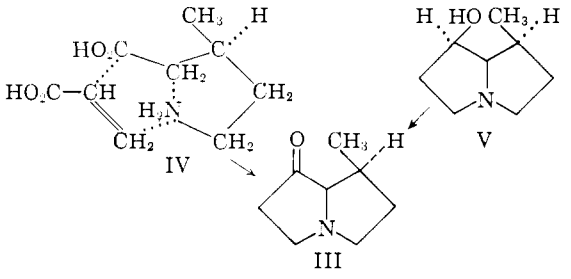
(3) The structural formulas of the pyrrolizidine bases are drawn in such a way that the  bond is in the plane of the paper, while the two rings are inclined on the carbon–nitrogen axis toward each other above the plane of the paper.

(4) F. L. Warren and M. E. von Klemperer, *J. Chem. Soc.*, 4574 (1958).

(5) The symbolism presented in R. S. Cahn, C. K. Ingold and V. Prelog, *Experientia*, **12**, 81 (1956).

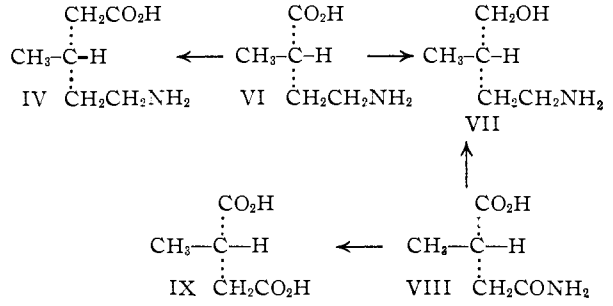
(6) R. Adams and N. J. Leonard, THIS JOURNAL, **66**, 257 (1944).

and decarboxylation to give (−)-retronecanone. This was identical with the product obtained by oxidation of natural retronecanol (V) with aluminum *t*-butoxide.[7]



In this communication the (−)-3-methyl-5-aminovaleric acid (IV) has been correlated with (−)-methylsuccinic acid (IX) of which the absolute configuration is known to be S.[8] The absolute configuration of the C_1 atom in (−)-retronecanone and hence in (−)-retronecanol is thus shown to be S, the same configuration as found by Warren and Klemperer[4] for the C_1 atom in heliotridane. This work therefore constitutes an independent confirmation of the findings of Warren and Klemperer,[4] because (−)-retronecanol may be converted either to (−)-retronecanone or (−)- heliotridane.[9]

The key compound in this research was optically active 2-methyl-4-aminobutyric acid (VI) which was correlated first with the corresponding 3-methyl-5-aminovaleric acid (IV) and second with optically active methylsuccinic acid (IX).



The starting material was (±)-2-methyl-4-phthalimidobutyric acid (X) made by either of two methods but preferably the second. The first method involved the condensation of β-phthalimidopropionyl chloride and diazoethane followed by rearrangement of the resulting diazophthalimidopentanone by the procedure of Eistert and Balenović, *et al.*[10] The anilide then was hydrolyzed and phthaloylated to give 2-methyl-4-phthalimidobutyric acid (X). The second method consisted in the reaction of 2-methylbutyrolactone[9b] with potassium phthalimide following the general procedure of Talbot, *et al.*[11]

(7) R. Adams and K. E. Hamlin, Jr., THIS JOURNAL, **64**, 2597 (1942).

(8) A. Fredga, *Arkiv. Kemi*, **14B**, No. 27 (1941); **15B**, No. 23 (1942); K. Freudenberg and W. Hohmann, *Ann.*, **584**, 54 (1953).

(9) R. A. Konovalova and A. Orekhov, *Bull. soc. chim. France*, [5] **4**, 1285 (1937); (b) R. Adams and E. F. Rogers, THIS JOURNAL, **63**, 228 (1941).

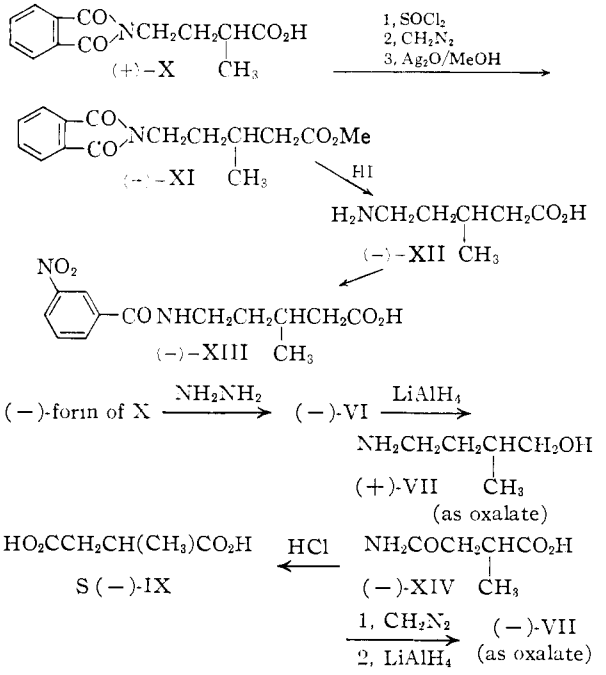
(10) (a) B. Eistert, *Angew. Chem.*, **54**, 124 (1941); (b) K. Balenović, I. Jambrešić and I. Ranogajec, *Croat. Chem. Acta*, **29**, 87 (1957).

(11) (a) G. Talbot, R. Gaudry and L. Berlinguet, *Can. J. Chem.*, **36**, 593 (1958). (b) 2-Methyl-4-phthalimidobutyric acid also has been prepared by condensing β-bromoethylphthalimide with diethyl methylmalonate; R. Robinson and H. Suginome, *J. Chem. Soc.*, 304 (1932).

After resolution of compound X, the (+)-form was converted to the corresponding acid chloride, then with diazomethane to the diazoketone which was rearranged in methanol to methyl (−)-3-methyl-5-phthalimidovalerate (XI). Hydrolysis with hydriodic acid provided (−)-3-methyl-5-aminovaleric acid (XII) which was *m*-nitrobenzoylated to an amide identical in rotation and all other properties with the product used by Adams and Leonard[6] in synthesis of (−)-retronecanone.

The (−)-2-methyl-4-aminobutyric acid (VI) was reduced with lithium aluminum hydride in tetrahydrofuran in low yield (11%) to (+)-2-methyl-4-amino-1-butanol (VII), isolated as the crystalline normal oxalate. The yield did not improve appreciably when heating under reflux was prolonged for 36 hours. The same procedure, however, was reported to give yields ranging from 70–80% with other amino acids.[12]

CHART I



Racemic β-carboxy-*n*-butyramide (XIV), prepared by reduction of β-carboxycrotonamide,[13] was resolved through the fractional crystallization of the brucine salts. In order to correlate the configuration of β-carboxy-*n*-butyramide with 2-methyl-4-aminobutyric acid, (−)-XIV was converted to the methyl ester with diazomethane and then reduced with lithium aluminum hydride to give a 48.5% yield of (−)-2-methyl-4-aminobutanol (VII), which proved to be the mirror image of the same butanol obtained by reduction of (−)-2-methyl-4-aminobutyric acid. The configuration of (−)-β-carboxy-*n*-butyramide was proved by hydrolysis to (−)-methylsuccinic acid (IX) of which the absolute configuration has been established.[8]

The configuration of all the intermediates which correlate the configuration of (−)-methylsuccinic acid with (−)-retronecanone have thus been re-

(12) O. Vogl and M. Pöhm, *Monatsh.*, **83**, 541 (1952).

(13) W. Cocker and A. K. Fateen, *J. Chem. Soc.*, 2630 (1951).

corded. Since the reactions used in this investigation do not involve the asymmetric carbon center and the Wolff rearrangement in the Arndt–Eistert synthesis is known to proceed with retention of configuration,[14] the absolute configuration of the C_1-atom in retronecanone may be accepted as unequivocally proved to be S.

The resolution of 2-methyl-4-phthalimidobutyric acid (X) was difficult. By use of quinine, which was the most satisfactory alkaloid, only partial separation of the two salts was effected even after repeated crystallizations. However, it was observed that an optically active form of the 2-methyl-4-phthalimidobutyric acid was more soluble in benzene than the racemic form. Hence, mixed acids from the partially separated salts were dissolved in benzene and inoculated with the (±)-form of the acid. This caused the racemic form to crystallize and in the filtrate an active form with rotation $[\alpha]_D$ + or −20° was isolated. The highest rotation obtainable after many fractional crystallizations of the salts, followed by hydrolysis, was about $[\alpha]_D$ −15°.

Worthy of mention is the observation that 2-methyl-4-aminobutyramide readily cyclizes to 3-methyl-2-pyrrolidone. The reduction of the optically active form of this pyrrolidone would provide a very elegant way to obtain the absolute configuration of the resulting 3-methylpyrrolidine.

Acknowledgment.—The authors thank the A. P. Sloan Foundation for the financial help which made this investigation possible. The authors are indebted to Mr. J. Nemeth, Miss C. Higham and Miss Jane Liu for the microanalyses and to Mr. P. E. McMahon and Mr. B. A. Shoulders for the determination of the infrared spectra.

Experimental

2-Diazo-5-phthalimido-3-pentanone.—To 1 l. of cold ethereal solution of diazoethane prepared from 45 g. of nitrosoethylurea was added gradually with stirring 15 g. of finely powdered β-phthalimidopropionyl chloride. The reaction mixture was kept overnight in a refrigerator, the ether evaporated *in vacuo* to about 100 ml., the crystalline precipitate separated by filtration and washed with two 20-ml. portions of ether. Further evaporation of the ether furnished more of the ketone. The total yield of crude diazoethyl ketone was 5.6 g. (34.5%), which after two recrystallizations from a mixture of ethyl acetate and petroleum ether (b.p. 40–60°) (1:1) melted at 93–94° dec.

Anal. Calcd. for $C_{13}H_{11}N_3O_3$: C, 60.69; H, 4.31; N, 16.34. Found: C, 61.01; H, 4.37; N, 16.09.

(±)-2-Methyl-4-phthalimidobutyranilide.—To a solution of 12 g. of diazoethyl ketone in 100 ml. of dry aniline heated to 90° was added an aniline suspension of silver oxide.[10b] The temperature was raised to 115° when a vigorous evolution of nitrogen occurred. The reaction mixture was kept for 10 minutes at 120° under occasional addition of silver oxide. The solution was cooled and poured on 330 g. of cracked ice and 160 ml. of concentrated hydrochloric acid. The precipitate was separated by filtration, washed with 1 l. of cold water and dried in air. The crude product which weighed 9.2 g. was dissolved in 50 ml. of acetone, the solution filtered and the acetone evaporated under diminished pressure. The crystalline residue was then recrystallized from 20 ml. of ethanol, yielding 5.7 g. (38%) of white needles, m.p. 149–150°. Complete purification was achieved by two recrystallizations from ethanol and finally from a mixture of ethyl acetate and petroleum ether (b.p. 40–60°), m.p. 150–151°.

Anal. Calcd. for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.78; H, 5.88; N, 8.78.

(14) J. F. Lane and E. S. Wallis, THIS JOURNAL, **63**, 1674 (1941).

(±)-2-Methyl-4-aminobutyric Acid.—A solution of 5.7 g. of anilide in 35 ml. of acetic acid and 17.5 ml. of 47% hydriodic acid was refluxed for 10 hours. After standing overnight in a refrigerator, 2.6 g. of phthalic acid was filtered, washed with 5 ml. of acetic acid and the filtrate evaporated *in vacuo*. The residue was treated with water and again evaporated *in vacuo* until most of the acids were removed. This process was repeated. The residue then was dissolved in 80 ml. of water, the phthalic acid extracted with ether, and water evaporated *in vacuo*. The residue was dissolved in 200 ml. of water, treated with charcoal, washed with water and the filtrate and washings diluted to 1.5 l. The solution was passed through a column of Amberlite IR-4B (2.2 × 52 cm.), the resin washed with 1 l. of water and the water evaporated *in vacuo*. White, crystalline amino acid weighing 2.16 g. resulted, m.p. 183–185°. To purify, the crude acid was suspended in 1 ml. of hot water, 9 ml. of absolute ethanol was added and the mixture cooled overnight in a refrigerator. The yield was 1.53 g. (74%). A second recrystallization gave white cubes, m.p. 203–204°.

Anal. Calcd. for $C_5H_{11}NO_2$: C, 51.26; H, 9.46; N, 11.96. Found: C, 51.44; H, 9.60; N, 11.87.

(±)-2-Methyl-4-phthalimidobutyric Acid. A. From (±)-2-Methyl-4-aminobutyric Acid.—A finely powdered mixture of 1 g. (1 mole equiv.) of 2-methyl-4-aminobutyric acid and 1.34 g. (1.05 mole equiv.) of phthalic anhydride was placed in a round-bottom flask and heated in an oil-bath with a thermometer immersed in the reaction mixture. A vigorous evolution of water started at 130°. The heating was continued for 25 minutes at a temperature of 130–140°. The water formed during the reaction was removed *in vacuo*, and the residue dissolved in 5 ml. of benzene, filtered from insoluble material and washed with two 1-ml. portions of benzene. To the combined benzene solution 3 ml. of petroleum ether (b.p. 40–60°) was added and the mixture was allowed to stand overnight in a refrigerator. The phthalimido acid crystallized in small needles; yield 1.55 g. (74%), m.p. 114–115° which did not change on recrystallization; lit.[11b] m.p. 112–113°.

Anal. Calcd. for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.12; H, 5.15; N, 5.86.

B. **From 2-Methylbutyrolactone.**—To a solution of 11.6 g. of 2-methylbutyrolactone in 50 ml. of purified dimethylformamide was added 18.5 g. of potassium phthalimide. The reaction mixture was refluxed for 10 hours, after which time a clear, slightly yellow solution was obtained. The solution was poured on a mixture of 50 g. of ice, 200 ml. of water and 30 ml. of glacial acetic acid. After standing overnight at room temperature, the crystalline precipitate was filtered, washed with water and dried in air. The crude product was recrystallized from a mixture of benzene–petroleum ether (b.p. 40–60°) (2 ml. of benzene and 1.5 ml. of petroleum ether per 1 g. of solid), to yield 20 g. (70%) of phthalimido acid, m.p. 114–115°, unchanged on recrystallization. The product was identical with a sample of 2-methyl-4-phthalimidobutyric acid prepared *via* the Arndt–Eistert synthesis, as shown by a melting point of the mixture.

Anal. Found: C, 62.92; H, 5.23; N, 5.73.

Resolution of 2-Methyl-4-phthalimidobutyric Acid.—A solution of 24.7 g. of (±)-phthalimido acid and 32.4 g. of quinine in 1150 ml. of ethyl acetate was left overnight at a temperature of 15°. The crystalline product was separated by filtration and recrystallized from ethyl acetate (20 ml. of solvent per 1 g. of salt). The procedure was repeated until 28 g. (50%) of the crystalline salt was obtained (three to four crystallizations). This salt (fraction A) melted at 143–145° and had a rotation $[\alpha]^{25}_D$ −97° (*c* 2.398% in ethanol). The ethyl acetate mother liquors were combined and the solvent evaporated to yield 28 g. of crystalline product (fraction B).

Fraction A.—In a separatory funnel 28 g. of salt was treated with 70 ml. of 10% hydrochloric acid and 250 ml. of benzene. The aqueous layer was removed and extracted with 20 ml. of benzene, the combined benzene solutions were washed with three 20-ml. portions of water, the washings were extracted with 20 ml. of benzene and combined benzene extracts washed once more with 20 ml. of water. After drying, the solvent was evaporated. The crystalline residue which weighed 11.9 g., $[\alpha]^{23}_D$ −5.1° (*c* 2.558 % in benzene), was dissolved in 84 ml. of benzene, the solution cooled to room temperature, inoculated with racemic 2-methyl-4-phthalimidobutyric acid and allowed to stand overnight at

room temperature (18°). The crystalline precipitate which weighed 8.1 g. was separated by filtration. It proved to be racemic 2-methyl-4-phthalimidobutyric acid ($[\alpha]^{24}D$ −0.14 ± 0.1° (*c* 3.46% in benzene)). On evaporation of the mother liquor 3.6 g. of crystalline product separated, $[\alpha]^{24}D$ −16.3° (*c* 4.84% in benzene). This product was dissolved in 24 ml. of benzene, inoculated with racemic phthalimido acid and kept at room temperature until a sample of mother liquor after evaporation of solvent gave a product with specific rotation higher than 18°. The crystalline portion, weighing 0.7 g., $[\alpha]^{24}D$ −3° (*c* 2.506% in benzene), was removed by filtration, and the filtrate evaporated to afford 2.9 g. of crystalline phthalimido acid. This product was dissolved in 10 ml. of benzene, and 10 ml. of petroleum ether (b.p. 40–60°) was added slowly until the solution became slightly turbid. 2-Methyl-4-phthalimidobutyric acid crystallized in fine needles; yield 2.8 g. (22.6% or 65% if calculated on the basis of recovered ±-acid), $[\alpha]^{24}D$ −18.4° (*c* 2.28% in benzene). After two recrystallizations from a mixture of benzene–petroleum ether (b.p. 40–60°) (1:1) the product had a m.p. 103–104° (softening at 102°); rotation, 0.0875 g. made up to 5 ml. with benzene at 24° gave αD −0.75°, *l* 2; $[\alpha]^{24}D$ −21.5 ± 0.4°.

Anal. Found: C, 63.12; H, 5.01; N, 5.80.

Fraction B.—This fraction of salt was treated with 10% hydrochloric acid and benzene as described for the fraction A and 12 g. of crude (+)-2-methyl-4-phthalimidobutyric acid was obtained upon evaporation of the benzene. The crude acid was dissolved in 84 ml. of benzene, inoculated with racemic acid and crystallized overnight at room temperature without shaking. The 6.5 g. of crystalline portion was racemic, while evaporation of the benzene gave 5.07 g. of crystalline product, $[\alpha]^{24}D$ +11° (*c* 3.73% in benzene). This product was dissolved in 40 ml. of benzene, inoculated with racemic acid and kept at room temperature as long as the rotation of a sample separated from the benzene solution reached a rotation of $[\alpha]^{24}D$ +17.3° (*c* 3.692% in benzene); the yield was 2.9 g. of slightly yellow crystalline product. The crude acid was dissolved in 50 ml. of a saturated aqueous sodium bicarbonate solution, treated with charcoal and immediately acidified with 10% hydrochloric acid. The crystalline precipitate was extracted with 70 ml. of benzene, followed by three 15-ml. portions of benzene, the combined benzene solutions were dried and the solvent evaporated. A yield of 2.4 g. of acid was obtained which after crystallization from 20 ml. of benzene and 20 ml. of petroleum ether (b.p. 40–60°) gave 2.2 g. of pure (+)-2-methyl-4-phthalimidobutyric acid in the form of shiny white needles $[\alpha]^{25}D$ +19.3° (*c* 1.662% in benzene) (17.9% or 37.5% on the basis of recovered (±)-acid). One more crystallization from a mixture of benzene–petroleum ether (b.p. 40–60°) (1:1) gave a product, m.p. 102–103.5°, rotation, 0.1144 g. made up to 5 ml. with benzene at 23° gave αD +0.93°, *l* 2; $[\alpha]^{23}D$ +20.3 ± 0.4°.

Anal. Found: C, 63.33; H, 5.18; N, 5.61.

(−)-2-Methyl-4-aminobutyric Acid.—A mixture of 2.8 g. of (−)-2-methyl-4-phthalimidobutyric acid ($[\alpha]D$ −19.4°) and 14.6 ml. of M-hydrazine hydrate solution in ethanol was refluxed for one hour. The residue after evaporation of the ethanol was treated with 50 ml. of water, adjusted to *p*H 5 with acetic acid and allowed to stand 10 min. at 50° and one hour at room temperature. The phthalyl hydrazide weighing 1.6 g. (88%), was filtered and washed with four 5-ml. portions of water. The combined water and washings were evaporated *in vacuo*, the traces of water removed by repeated evaporation with absolute ethanol, 15 ml. of ethanol and 5 ml. of ether were added and left overnight in a refrigerator. The crystalline precipitate after separation by filtration and washing with ethanol, weighed 1.21 g. (91%), m.p. 192–193°, $[\alpha]^{24}D$ −6.5° (*c* 5.1% in water). On purification by two recrystallizations from a mixture of water and ethanol (1:30), the m.p. was 196–197° dec.; rotation, 0.1385 g. made up to 5 ml. with water at 24° gave αD −0.37°, *l* 2; $[\alpha]^{24}D$ −6.7 ±0.3°.

Anal. Calcd. for $C_5H_{11}NO_2$: C, 51.26; H, 9.46; N, 11.96. Found: C, 51.50; H, 9.59; N, 12.13.

A sample of 141 mg. of (−)-2-methyl-4-aminobutyric acid was heated for 20 minutes at 135–140° with 205 mg. of phthalic anhydride. The reaction mixture was worked up as described for the inactive product. The yield was 150 mg. of (−)-2-methyl-4-phthalimidobutyric acid, m.p. 103–104°, rotation, 0.1419 g. made up to 5 ml. with benzene at 25° gave αD −1.02°, *l* 2; $[\alpha]^{25}D$ −18°.

(±)-2-Methyl-4-amino-1-butanol Oxalate.—To 39 ml. of a 2.9% solution of lithium aluminum hydride in tetrahydrofuran and 100 ml. of tetrahydrofuran, 1.2 g. of (−)-2-methyl-4-aminobutyric acid was added gradually. The reaction mixture was stirred with a magnetic stirrer while refluxed for 6 hours after which 100 ml. of dry ether was added, followed by 80 ml. of wet ether and 2 ml. of water. The hydroxides were separated by suction filtration, washed with ether, and extracted twice with 100 ml. of boiling absolute ethanol. The total filtrates were evaporated *in vacuo* and the residue distilled at 1.8 mm. and 80° (bath temperature) as a colorless oil with a typical amine odor. The amino alcohol, weighing 0.12 g. (11.3%) ($[\alpha]^{25}D$ + 11.8° (*c* 1.782% in ethanol)), was dissolved in 2 ml. of ethanol and 3 ml. of an ethanolic solution of 0.8 g. of oxalic acid was added. The crystalline salt was dissolved by heating the solution, and crystallization was effected by addition of 20 ml. of ether. The solution was cooled for half an hour in a refrigerator and filtered, yielding 0.22 g. of shining plates, m.p. 70°. The crude oxalate was dissolved in 2 ml. of absolute ethanol and crystallized by the addition of 5 ml. of ether; m.p. 75°, yield 0.175 g. After two crystallizations from a mixture of 95% ethanol and ether (3:5) the melting point rose to 178–179° and the product was no longer soluble in absolute ethanol. The product was recrystallized from 90% ethanol to give shining needles, m.p. 181–182°; rotation, 0.0446 g. made up to 3 ml. with 50% ethanol at 26° gave αD +0.39°, *l* 2; $[\alpha]^{25}D$ +13.2 ± 0.5°.

Anal. Calcd. for $(C_5H_{13}NO)_2 \cdot C_2H_2O_4$: C, 48.64; H, 9.52; N, 9.45. Found: C, 49.04; H, 9.62; N, 9.50.

In the same way (±)-2-methyl-4-amino-1-butanol oxalate was prepared, m.p. 184–186°.

Anal. Found: C, 48.88; H, 9.61; N, 9.49.

(+)-2-Methyl-4-phthalimidobutyryl Chloride.—A mixture of 2.2 g. of (+)-2-methyl-4-phthalimidobutyric acid and 10 ml. of thionyl chloride was heated for one hour with the bath temperature not allowed to rise above 80°. The excess of thionyl chloride was distilled *in vacuo*, the residue dried overnight in a desiccator over potassium hydroxide, and the oily residue was extracted with four 30-ml. portions of warm petroleum ether (b.p. 40–60°). Evaporation of the petroleum ether gave 2.36 g. (100%) of colorless oil, $[\alpha]^{21}D$ +10.5° (*c* 2.528% in benzene). A 120-mg. sample was distilled, b.p. 170–175° at 0.02 mm., while the rest of the chloride was used without further purification in the next step.

Anal. Calcd. for $C_{13}H_{12}ClNO_3$: N, 5.27. Found: N, 5.14.

(+)-1-Diazo-3-methyl-5-phthalimido-2-pentanone.—A solution of 2.16 g. of (+)-2-methyl-4-phthalimidobutyryl chloride in 50 ml. of dry ether was added slowly to 600 ml. of an ethereal solution of diazomethane prepared from 24 g. of nitrosomethylurea. The reaction mixture was left overnight in a refrigerator, filtered from any precipitate and the ether evaporated. The residue which crystallized on scratching was purified by recrystallization from a mixture of 6 ml. of ethyl acetate and 9 ml. of petroleum ether (b.p. 40–60°). The prismatic crystals were crystallized twice from ethyl acetate–petroleum ether (b.p. 40–60°). The yield was 1 g. (45%) of pure product, m.p. 94.5–95.5°; rotation, 0.1476 g. made up to 5 ml. with ethyl acetate at 25° gave αD +5.02°, *l* 2; $[\alpha]^{25}D$ +85 ± 0.3°.

Anal. Calcd. for $C_{14}H_{13}N_3O_3$: C, 61.98; H, 4.83; O, 17.69. Found: C, 62.11; H, 4.97; O, 17.56.

The racemic diazomethyl ketone was prepared in the same way, m.p. 71–72°.

Anal. Found: C, 62.14; H, 4.70.

Methyl (−)-3-Methyl-5-phthalimidovalerate.—To 1.8 g. of diazomethyl ketone ($[\alpha]^{23}D$ +63°) in 30 ml. of boiling methanol was added gradually a methanolic suspension of freshly prepared silver oxide until the evolution of nitrogen ceased (4 hours). The silver oxide was removed by filtration, washed with two 20-ml. portions of methanol, the solvent evaporated and the residue dissolved in 30 ml. of ether. After filtering from the precipitated silver oxide, the ether was evaporated and the 1.6-g. residue was distilled, b.p. 160–180° (bath temperature) at 0.02 mm., yielding 0.7 g. (38.5%) of slightly yellow oil, $[\alpha]^{24}D$ −10.7° (*c* 2.056% in benzene). Redistillation indicated a b.p. of 160° at 0.02

mm.; rotation, 0.0394 g. made up to 3 ml. with benzene at 24° gave α_D −0.28°, *l* 2; $[\alpha]^{24}D$ −10.7 ± 0.2°.

Anal. Calcd. for $C_{15}H_{17}NO_4$: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.33; H, 6.20; N, 5.38.

(−)-3-Methyl-5-aminovaleric Acid.—A mixture of 0.7 g. of distilled ester, 4.2 ml. of glacial acetic acid and 2.1 ml. of hydriodic acid (47%) was refluxed for 10 hours and then allowed to stand overnight in a refrigerator. Phthalic acid was separated by filtration, washed with 2 ml. of acetic acid, and the filtrate was evaporated under diminished pressure. The traces of acids were removed by repeated addition and evaporation of water. The residue was dissolved in 10 ml. of water, extracted with ether, and the solvent again evaporated to dryness. Evaporation of the ether gave further amounts of phthalic acid. The crude iodide was dried overnight *in vacuo* over potassium hydroxide. The 0.7 g. of red oil was dissolved in 50 ml. of water, treated with charcoal, filtered and charcoal washed with 50 ml. of water. The solution was made up to 500 ml. with water and passed through a column of Amberlite IR-4B (26 × 2.2 cm.) and the column was washed with 500 ml. of water. The effluent was evaporated *in vacuo* and the residue was dissolved repeatedly in absolute ethanol and the alcohol evaporated until the product crystallized. It was finally dried *in vacuo*. The white crystalline product, which weighed 0.2 g., was boiled for a few minutes with 5 ml. of absolute ethanol and left overnight in a refrigerator. The product weighed 0.150 g. (45%). The amino acid was twice recrystallized from 1 ml. of 80% ethanol and 6 ml. of absolute ethanol; fine needles, m.p. 172–173°; rotation, 0.0419 g. made up to 3 ml. with water gave α_D −0.36°, *l* 2; $[\alpha]^{24}D$ −12.9 ± 0.2°.

Anal. Calcd. for $C_6H_{13}NO_2$: C, 54.94; H, 9.99; N, 10.68. Found: C, 54.76; H, 9.95; N, 10.76.

(−)-3-Methyl-5-*m*-nitrobenzoylaminovaleric Acid.—A mixture of 0.1 g. of (−)-3-methyl-5-aminovaleric acid, 0.131 g. of *m*-nitrobenzoyl chloride and 71 mg. of sodium hydroxide in 1.2 ml. of water was vigorously shaken for 30 minutes at 30–35°. After cooling, the reaction mixture was acidified with 10% hydrochloric acid, extracted with three 20-ml. portions of ether, the extract solution dried over magnesium sulfate and the solvent evaporated. The colorless oil which weighed 0.17 g. crystallized gradually and was purified from 1 ml. of benzene and 2.5 ml. of petroleum ether (b.p. 40–60°). The crude acid was recrystallized twice from 2.5 ml. of chloroform and 0.5 ml. of petroleum ether (b.p. 40–60°), and finally from aqueous ethanol; white needles, m.p. 113–114°; rotation, 0.0583 g. made up to 3 ml. with ethanol at 24° gave α_D −0.24°, *l* 2; $[\alpha]^{24}D$ −6.2 ± 0.5°; reported[6] rotation $[\alpha]^{30}D$ −5.0°.

The product was identical with that prepared by Adams and Leonard[6] by a different procedure as shown by the infrared spectra and the undepressed melting point of a mixture.

Anal. Calcd. for $C_{13}H_{16}N_2O_5$: C, 55.71; H, 5.75; N, 10.00; O, 28.54. Found: C, 56.05; H, 5.66; N, 10.04; O, 28.47.

(±)-β-Carboxy-*n*-butyramide.—A suspension of 8 g. of β-carboxycrotonamide[13] in 200 ml. of absolute ethanol was reduced in the presence of platinum oxide catalyst at a pressure of 40 p.s.i. The theoretical amount of hydrogen was absorbed in 15 min. The catalyst was separated by filtration and the ethanol evaporated to obtain the butyramide, m.p. 124–125°. It was purified for analysis by two recrystallizations from absolute ethanol, m.p. 126–127°; lit.[13] m.p. 124–125°.

Anal. Calcd. for $C_5H_9NO_3$: C, 45.79; H, 6.92; N, 10.68. Found: C, 46.08; H, 6.88; N, 10.95.

Resolution of β-Carboxy-*n*-butyramide.—A solution of 17.4 g. of β-carboxy-*n*-butyramide and 52.2 g. of anhydrous brucine in 700 ml. of methanol was permitted to stand for 24 hours at 15°. The crystalline precipitate was separated by filtration and was recrystallized from methanol (10 ml. of methanol per 1 g. of the salt). After five crystallizations, 13.5 g. of shining prismatic crystals resulted, m.p. 181–182°; rotation, 0.2315 g. made up to 5 ml. with methanol at 20° gave α_D −2.01°, *l* 2; $[\alpha]^{20}D$ −21.7°.

No attempt was made to isolate the more soluble brucine salt from the mother liquors.

A solution of 13.5 g. of less soluble salt in 50 ml. of chloroform was treated with 40 ml. of a saturated aqueous solution of sodium bicarbonate. The aqueous layer was extracted with three 30-ml. portions of chloroform and neutralized with 10% hydrochloric acid until slightly acidic to congo. The water was removed under diminished pressure and the residue extracted with three 20-ml. portions of absolute ethanol. Evaporation of the ethanol gave 2.8 g. of crude product which was crystallized from 10 ml. of absolute ethanol (carbon). A yield of 1.4 g. resulted. Two more crystallizations from ethanol gave pure amide, m.p. 132–133.5°; rotation, 0.1044 g. made up to 5 ml. with absolute ethanol at 25° gave α_D −0.89°, *l* 2; $[\alpha]^{25}D$ −21.4 ± 0.5°.

Anal. Found: C, 45.69; H, 6.79; N, 10.53.

Hydrolysis of (−)-β-Carboxy-*n*-butyramide.—A solution of 0.4 g. of (−)-β-carboxy-*n*-butyramide in 9 ml. of concentrated hydrochloric acid was heated under reflux for 18 hours. After cooling, the methylsuccinic acid was extracted with three 50-ml. portions of ether, the solvent dried over magnesium sulfate and evaporated. The crude acid weighing 0.37 g. was purified by two crystallizations from benzene, m.p. 101–102°; lit.[15] m.p. 115°; rotation, 0.0811 g. made up to 5 ml. with absolute ethanol at 26° gave α_D −0.37°, *l* 2; $[\alpha]^{26}D$ −11.4 ± 0.3°; lit.[16] $[\alpha]^{20}D$ +16.5° (*c* 4.352% in ethanol).

Anal. Calcd. for $C_5H_8O_4$: C, 45.45; H, 6.10. Found: C, 45.69; H, 6.36.

The melting point curve for the system (+)- and (−)-methylsuccinic acid given by Berner and Leonardsen[15] indicates our compound to contain about 60% of active enantiomorph. A mixture containing 2.5 mg. of optically pure (+)-methylsuccinic acid and 3.7 mg. of partly racemic (−)-methylsuccinic acid melted at 110–111° and was undepressed upon admixture with an authentic specimen of (±)-methylsuccinic acid.

It is noteworthy that an optically pure sample of (+)-methylsuccinic acid, $[\alpha]^{25}D$ +15.9° (*c* 1.36% in ethanol), when boiled for 18 hours with concentrated hydrochloric acid was also partly racemized, $[\alpha]^{26}D$ +10.9° (*c* 1.39% in ethanol).

(−)-β-Carbomethoxy-*n*-butyramide.—To 100 ml. of an ethereal solution of diazomethane prepared from 10 g. of nitrosomethylurea was added gradually 0.8 g. of (−)-β-carboxy-*n*-butyramide. Two milliliters of methanol was added to facilitate the esterification.[17] After half an hour the evolution of nitrogen ceased and solvent was evaporated. The colorless oil, weighing 0.87 g., was twice distilled for analysis at 130–135° at 0.03 mm.; rotation, 0.0405 g. made up to 5 ml. with methanol at 25° gave α_D −0.10°, *l* 2; $[\alpha]^{25}D$ −6.2 ± 1.2°.

Anal. Calcd. for $C_6H_{11}NO_3$: N, 9.65. Found: N, 9.89.

(−)-2-Methyl-4-amino-1-butanol Oxalate.—A solution of 0.85 g. of crude (−)-β-carbomethoxy-*n*-butyramide in 60 ml. of tetrahydrofuran was added slowly with stirring to a solution of 1.17 g. of lithium aluminum hydride in 100 ml. of tetrahydrofuran. The reaction mixture was stirred while heating under reflux for 6 hours and was worked up as described for the reduction of (−)-2-methyl-4-aminobutyric acid. A yield of 0.29 g. (48.5%) of the distilled aminobutanol was obtained and was converted to the oxalate, m.p. 181–182°. The melting point of a mixture with (+)-2-methyl-4-amino-1-butanol oxalate was 183–184°. The infrared spectra of both oxalates were identical; rotation, 0.0502 g. made up to 3 ml. with 50% ethanol at 24° gave α_D −0.49°, *l* 2; $[\alpha]^{24}D$ −14.7 ± 0.3°.

Anal. Calcd. for $(C_5H_{13}NO)_2 \cdot C_2H_2O_4$: C, 48.64; H, 9.52; N, 9.45. Found: C, 48.77; H, 9.47; N, 9.35.

(±)-3-Methyl-2-pyrrolidone.—A mixture of 5 g. of (±)-2-methyl-4-phthalimidobutyric acid and 26 ml. of M-hydrazine hydrate solution in ethanol was heated under reflux for one hour. The residue after evaporation of ethanol was treated with 20 ml. of 10% hydrochloric acid and allowed to stand 10 min. at 50° and one hour at room temperature. The phthalyl hydrazide, weighing 2.8 g., was filtered and washed with three 5-ml. portions of water. The combined filtrates were evaporated *in vacuo* and the residue allowed to stand 2 days at room temperature with 80 ml. of absolute ethanol saturated with hydrochloric acid. The residue, after evap-

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oration of the ethanol, was dissolved in 30 ml. of chloroform, cooled in ice and treated for 5 min. with a stream of dry ammonia. The precipitate was filtered, washed with chloroform, and the filtrate evaporated *in vacuo*. The oily residue was allowed to stand in a pressure bottle for 72 hours with 100 ml. of methanol saturated with ammonia gas at 0°. Evaporation of methanol gave 1.31 g. of crystalline product. To a solution of this in 4 ml. of ethanol was added 15 ml. of ether. Some ammonium chloride separated and was removed by filtration. The filtrate was evaporated and the product crystallized from 5 ml. of ether; large prisms, m.p. 57–58°. The yield was 0.6 g. (30%). After another crystallization from ether and sublimation at 0.03 mm. and 70–100°, the melting point was raised to 58–59.5°.

Anal. Calcd. for C_5H_9NO: C, 60.58; H, 9.15; N, 14.13. Found: C, 60.06; H, 9.20; N, 13.70.

URBANA, ILL.

[CONTRIBUTION FROM THE ROCKEFELLER INSTITUTE]

Antimetabolites of Mevalonic Acid[1]

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Antimetabolites of mevalonic acid (MVA) were envisioned as useful agents for suppression of sterol and other isoprenoid biosynthesis. A number of close relatives of MVA were synthesized by the Reformatsky reaction and tested for anti-MVA activity in bacteria, yeast and mice. Several active compounds were found, the best of which was 4-methyl MVA. The relationship of chemical structure to anti-MVA activity in bacteria was studied with a series of analogs.

Mevalonic acid (3,5-dihydroxy-3-methylvaleric acid, MVA)[2,3] has been identified as the common precursor in the biosynthesis of sterols[4] and other isoprenoid compounds.[5] This finding has made it possible to investigate systematically the modification of isoprenoid biosynthesis by applying the principles of antimetabolites, because antimetabolites of MVA would be expected to suppress specifically the biosynthesis of isoprenoids. From such interference, much might be learned of the effects of a specific deficiency of cholesterol and steroid hormones on the animal organism. Such studies also might well lead eventually to clinically useful substances.

An attempt has been made to test this hypothesis by examining a number of structural analogs of MVA for anti-MVA activity. The compounds prepared and tested were hydroxy acids which were homologs or position isomers of MVA, and derivatives of these. The compounds were prepared by condensation of the appropriate acetoxy ketones with ethyl bromoacetate or ethyl 2-bromopropionate by means of the Reformatsky reaction. The esters thus obtained were hydrolyzed to the desired acids. These compounds represented one of the general transformations which could be expected to yield antimetabolites.[6] Another method for making antimetabolites is the conversion of a carboxylic acid to a phenyl ketone. Therefore, a number of phenyl ketones bearing a formal resemblance to MVA also were prepared and assayed.

Since the object of this investigation was to influence MVA utilization in the intact mammalian organism, the plan was to examine the effect of the analogs on cholesterol synthesis by young mice. However, the analogs were first assayed for their ability to inhibit ergosterol synthesis in growing yeast, since the mouse assay was not well adapted to the rapid testing of a large number of compounds. Since the normal turnover of MVA is so great in systems synthesizing a large amount of sterol, it is apparent that either of these assay methods would reveal anti-MVA activity only if (A) the compound being tested were present in a relatively great amount, (B) the compound were extremely potent, or (C) the antagonism could not be reversed by the metabolite. Therefore, a preliminary assay was needed to detect anti-MVA activity in compounds of low potency, in order better to direct the synthetic work. Certain lactobacilli, although they do not synthesize sterol, do have a growth requirement for MVA. *Lactobacillus heterohiochii*[3] has an absolute requirement for MVA, and *L. acidophilus*[7] will grow on either MVA or acetate. Consequently, the analogs were examined for their ability to inhibit the growth of *L. acidophilus* in competition with MVA, by the method of Wright.[7]

While this work was in progress, two reports of anti-MVA activity appeared. Tamura, *et al.*,[8] by the use of *L. heterohiochii*, found 2-methyl- and 2-ethyl-MVA to have reversible anti-MVA activity. Wright[7] showed that the growth of *L. acidophilus* was inhibited by a number of compounds, the most active being the sesquiterpene, farnesinic acid, the action of which was overcome by MVA.

Experimental

All melting and boiling points are uncorrected. Melting points were determined in a capillary. Infrared spectra were measured with a Perkin–Elmer model 137 spectrophotometer and were determined on films for liquids and on Nujol mulls for solids. We thank Miss Edith Young and Monica Gallagher for technical assistance, and Mr. Theodore Bella for microanalyses.

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