recrystallized from chloroform (25 ml.) and carbon tetrachloride (225 ml.); wt. 13.3 g., m.p. 136.5–138°.

Material with this melting point (37.4 g.) was recrystallized again from chloroform (50 ml.) and carbon tetrachloride (500 ml.); wt. 35.2 g., m.p. 138.5–139.5°, $[\alpha]^{22}_{D}$ −56.5° (*c* 1, ethanol), $[\alpha]^{22}_{D}$ −63.0° (*c* 5, methanol). Reported,[4] m.p. 118.5–119.5°, $[\alpha]_D$ −62.7° (methanol).[5]

Anal. Calcd. for $C_{19}H_{26}O_5N_2$: C, 63.0; H, 7.23; N, 7.73. Found: C, 62.8; H, 7.38; N, 7.72.

Method B. Methyl carbobenzoxy-L-prolyl-L-leucinate (3.8 g.) was dissolved in methanol (15 ml.). Normal sodium hydroxide (10.5 ml.) was added and the resulting solution was stirred at room temperature for 2 hr. The solution was then acidified by the slow addition of concentrated hydrochloric acid. Methanol was removed *in vacuo*. The crystalline product was filtered off and was washed with water; wt. 3.6 g., m.p. 136–138°. This material was recrystallized from chloroform (7 ml.) and carbon tetrachloride (50 ml.); wt. 3.2 g., m.p. 138.5–139.5°, $[\alpha]^{22}_{D}$ −57.5° (*c* 1, ethanol).

Anal. Found: C, 63.0; H, 7.31; N, 7.74.

Ethyl carbobenzoxy-L-prolyl-L-leucylglycinate. Carbobenzoxy-L-prolyl-L-leucine (1.45 g.) was dissolved in tetrahydrofuran (20 ml.). Triethylamine (0.56 ml.) was added and the solution was cooled to −10°. Isobutyl chloroformate (0.55 g.) in tetrahydrofuran (10 ml.) was added. The mixture was stirred for 20 min. at −10°. Then a solution of ethyl glycinate hydrochloride (0.67 g.) and triethylamine (0.70 ml.) in water (5 ml.) was added. Stirring was continued without further cooling for 90 min. Water (25 ml.) was added and the mixture was acidified by slow addition of concentrated hydrochloric acid. Tetrahydrofuran was removed *in vacuo*. The resulting solid was filtered off and washed successively with 30-ml. portions of *N* hydrochloric acid, water, 5% sodium bicarbonate, and water; wt. 1.6 g., m.p. 148–150°. The product was recrystallized from ethanol (15 ml.); wt. 1.3 g., m.p. 150–152°, $[\alpha]^{21}_{D}$ −83.2 (*c* 2.5, ethanol). Reported, m.p. 148–149°,[1a] 148–149.5°,[1e] 150–151°,[1d] 151–152°,[1f] $[\alpha]_D$ −79.8°,[1a] −81.2°,[1e] −82.6°,[1f] (ethanol).

Carbobenzoxy-L-prolyl-L-leucylglycinamide. Carbobenzoxy-L-prolyl-L-leucine (3.6 g.) was dissolved in tetrahydrofuran (25 ml.). Triethylamine (1.5 ml.) was added and the solution was cooled to −10°. Isobutyl chloroformate (1.4 g.) in tetrahydrofuran (20 ml.) was added. The solution was stirred at −10° for 20 min. Then a solution of glycinamide hydrochloride (1.2 g.) and triethylamine (1.6 ml.) in water (10 ml.) was added. Stirring was continued without further cooling for 90 min. Water (25 ml.) was added and the reaction mixture was acidified by the slow addition of concentrated hydrochloric acid. Tetrahydrofuran was removed *in vacuo*. The product was filtered off and was washed successively with 30-ml. portions of *N* hydrochloric acid, water, 10% sodium bicarbonate, and water. After drying, the product was washed by trituration with ethyl acetate (30 ml.); wt. 3.4 g., m.p. 159–161°.

Material with this melting point (6.8 g.) was purified further by stirring in boiling water (125 ml.). After cooling, the product was filtered off; wt. 6.3 g., m.p. 161–163°, $[\alpha]^{22}_{D}$ −73.3° (*c* 2, 95% ethanol). Reported, m.p. 163–163.5°,[1a] 163°,[1c] 162–163°,[1d] 162–164°,[1f] $[\alpha]_D$ −73.3°[1a] (95% ethanol).

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(4) G. W. Anderson and F. M. Callahan, *J. Am. Chem. Soc.*, **82**, 3359 (1960).

(5) The discrepancy in the observed melting point and that reported by Anderson and Callahan is apparently due to dimorphism. When first prepared in this laboratory, the compound melted at 119.5–120.5°. However, since the first recrystallization from acetic acid and water, the higher melting material has always been obtained. F. M. Callahan kindly agreed to recrystallize a sample of his product using seed crystals of our higher melting form. He obtained the higher melting form. When a sample of the lower melting form furnished by F. M. Callahan was recrystallized in this laboratory without the use of seed crystals, the higher melting form was obtained. Attempts in both laboratories to convert the higher melting into the lower melting form have not been successful.

Potential Growth Antagonists. II.[1] A Route to Alkyl Substituted Homoserines[2]

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In connection with the work on substituted glycines as metabolic antagonists,[1] an attempt was made to prepare DL-2-amino-4-hydroxy-2-methylbutyric acid (α-methylhomoserine) from 4-hydroxy-2-butanone (I) *via* the hydantoin (II), followed by hydrolysis to the amino acid (IVa). Unfortunately, the initial reaction of the hydroxy ketone with ammonium carbonate and sodium cyanide did not give the expected hydantoin, but led to an intractable oil. The reaction product also had the characteristic odor of methyl vinyl ketone, which suggests that the basicity of the reaction medium caused dehydration of the hydroxy ketone, followed by polymerization. Hays,[3] in the preparation of 4-hydroxy-2-butanone, has reported the decomposition of the ketone in the presence of base.

In the second attempt to prepare the amino acid, the commercially available 4-acetoxy-2-butanone (I. R = CH_3CO-; R_1 = H) was treated with sodium cyanide and ammonium carbonate in the hope that the rate of ammonolysis of the ester would be slower than the rate of hydantoin formation. However, only a trace of crystalline material was isolated and it had an infrared spectrum suggesting the presence of the unsaturated lactone:

CH_2—CH=C(CH_3)—CO (ring closed through —O— between CH_2 and CO); λ^{Nujol}_{max} 5.67 μ (C=O)

(1) L. H. Goodson, I. L. Honigberg, J. J. Lehman, and W. H. Burton, *J. Org. Chem.*, Paper I, **25**, 1920(1960).

(2) This research was supported by Contract No. SA-43-ph-2394 with the Cancer Chemotherapy National Service Center, National Cancer Institute of the National Institutes of Health, Bethesda, Md.

(3) J. T. Hays, G. F. Hager, H. M. Engelmann, and H. M. Spurlin, *J. Am. Chem. Soc.*, **73**, 5369 (1951).

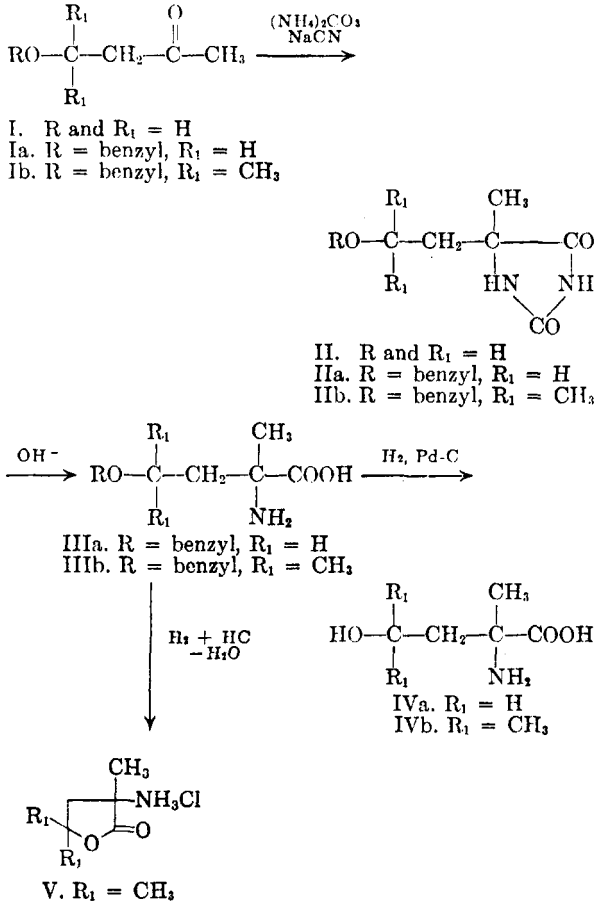

Fig. 1. Preparation of α-methylhomoserine, IVa, and α, γ, γ-trimethylhomoserine, IVb

compared to 5.70μ (C=O) for the saturated amino lactone (V) and no appreciable absorption in the 2.5–3.5μ range (—OH or —NH).

The third attempt to circumvent these difficulties involved the preparation of the *O*-benzyl derivative of α-methylhomoserine from the appropriate ketone and hydantoin, followed by catalytic hydrogenolysis of the benzyl group. This route not only proved successful for this amino acid but was easily adaptable to the preparation of the higher homolog, 4-hydroxy-2-methylleucine (α,γ,-γ-trimethylhomoserine, IVb). The steps are outlined in Fig. 1 (I→IV) for α-methylhomoserine (series a) and for α,γ,γ-trimethylhomoserine (series b).

The preparation of the starting benzyloxy ketones gave the poorest yields in the over-all synthetic route. The methods used for the preparation and hydrolysis of the intermediate hydantoins have been described in the first paper of this series.[1] The hydrogenolysis of the benzyl groups with 15% palladium-charcoal in alcoholic hydrochloric acid gave a good yield of DL-α-methylhomoserine (IVa); however, this procedure led to lactone formation in the hydrogenolysis of the *O*-benzyltrimethylhomoserine derivative (IIIb). The free amino acid, in the latter case, was obtained by hydrogenolysis in aqueous ammonia.

The hydantoins and the amino acids were submitted to the Cancer Chemotherapy National Service Center of the National Cancer Institute for screening. Completed screening results of these compounds will be published by that organization at a later date. Preliminary results on compounds IIa, IIIa, and IVa show no tumor inhibitory effect on mice inoculated with Sarcoma-180, Carcinoma-755 or Leukemia-1210.

EXPERIMENTAL[4]

4-Benzyloxy-2-butanone (Ia). Seventy grams (0.66 mole) of 4-chloro-2-butanone[5] in 60 ml. of benzyl alcohol was maintained at a temperature of 25° during the addition of (0.6 mole) of freshly prepared sodium benzyloxide in 260 ml. of benzyl alcohol. The reaction mixture was then stirred and warmed to 35° for an additional 3 hr. at which time the reaction tested neutral to litmus. The mixture was cooled, diluted with 500 ml. of benzene and the benzene solution washed with three 50-ml. portions of water. The benzene solution was dried over calcium chloride for 24 hr., filtered and the benzene removed under reduced pressure. The residue was fractionated at 0.05–0.07 mm. through a 30 × 1.8 cm. Vigreux column. The fraction boiling 78–82°/0.05–0.07 mm., weighed 35 g.; n_D^{20} 1.5080.

Anal. Calcd. for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 74.27; H, 7.73.

DL-*5-(2-Benzyloxyethyl)-5-methylhydantoin* (IIa). The hydantoin was prepared by treating 30 g. (0.17 mole) of 4-benzyloxy-2-butanone with 9 g. (0.18 mole) of sodium cyanide and 65 g. of ammonium carbonate according to Method A previously described.[1] The hydantoin was collected by suction filtration, washed with water, dried and then washed with petroleum ether (b.p. 60–70°). Recrystallization from ethanol-water gave 40 g. of product; m.p. 146.5–147.5°.

Anal. Calcd. for $C_{13}H_{16}N_2O_3$: C, 62.88; H, 6.50; N, 11.29. Found: C, 63.01; H, 6.41; N, 11.30.

DL-*O-Benzyl-α-methylhomoserine* (IIIa). A stainless steel reaction vessel containing 27 g. (0.1 mole) of DL-5-(2-benzyloxyethyl)-5-methylhydantoin, 56 g. (0.18 mole) of barium hydroxide octahydrate and 320 ml. of water was heated to 160° for 30 min. as described in Method B, base hydrolysis[1], of the previous communication. The reaction mixture was then treated with sulfuric acid, lead carbonate and hydrogen sulfide and the crystalline amino acid was obtained by concentration of the aqueous solution under reduced pressure. The product was recrystallized from 95% ethanol; yield 20.2 g.; m.p. 243–244° (sealed tube).

Anal. Calcd. for $C_{12}H_{17}NO_3$: C, 64.55; H, 7.68; N, 6.27. Found: C, 64.47; H, 7.77; N, 6.23.

DL-*α-Methylhomoserine* (IVa). The DL-*O*-benzyl-α-methylhomoserine, 3.4 g. (0.015 mole), was dissolved in 100 ml. of 95% ethanol containing 1.1 g. (0.03 mole) of hydrogen chloride. Two grams of 15% palladium-charcoal catalyst was added and the reaction mixture shaken in a Parr hydrogenator at 60 p.s.i. After the calculated amount of hydrogen was absorbed, the catalyst was filtered off and the amino acid hydrochloride was collected by evaporation of the alcoholic solution to dryness; yield 2.3 g. The hydrochloride was not characterized but was dissolved in 10 ml. of water and placed on a 35 × 2 cm. column of Amberlite IR-45 ion exchange resin (OH^- cycle). The chloride-free amino acid was eluted with 100 ml. of water and the solution

(4) All melting points are uncorrected.

(5) F. Sondheimer and R. B. Woodward, *J. Am. Chem. Soc.*, 75, 5438 (1953).

evaporated to dryness to give 1.5 g. of DL-α-methylhomoserine; m.p. 214–215.5° (sealed tube); reported[6] m.p. 228°.

Anal. Calcd. for $C_5H_{11}NO_3$: C, 45.10; H, 8.33; N, 10.52. Found: C, 45.29; H, 8.24; N, 10.50.

4-Benzyloxy-4-methyl-2-pentanone (Ib). This ketone was prepared according to the procedure described by Hoffman[7] in which 135 g. (1.38 moles) of mesityl oxide and 146 g. (1.35 moles) of benzyl alcohol were cooled to −30° during the addition of 7.0 g. (0.07 mole) of concd. sulfuric acid. The resulting solution was maintained at this temperature for 2 weeks and then neutralized with sodium carbonate. The dark brown reaction mixture was steam distilled until 1.2 l. of distillate was collected. The organic layer in the distillate contained the unchanged benzyl alcohol and mesityl oxide. The organic layer in the distilling flask was separated, the aqueous phase extracted with three 100-ml. portions of benzene, and the combined benzene and organic layer dried over calcium chloride for 24 hr. The benzene was removed under reduced pressure and the residue fractionated through a 30 × 1.8 cm. Vigreux column. The fraction boiling at 93°/0.05 mm., weighed 58.0 g.; n_D^{25} 1.5000.

Anal. Calcd. for $C_{13}H_{18}O_2$: C, 75.69; H, 8.79. Found: C, 75.69; H, 9.33.

Semicarbazone. m.p. 136.5–137.5°; reported[7] m.p. 138–139°.

DL-*5-(2-Benzyloxy-2-methylpropyl)-5-methylhydantoin* (IIb). The hydantoin was prepared from Ib by the same method used for the preparation of DL-5-(2-benzyloxyethyl)-5-methylhydantoin; it was recrystallized from 50% ethanol; yield 85%; m.p. 180–181.5°.

Anal. Calcd. for $C_{15}H_{20}N_2O_3$: C, 65.19; H, 7.30; N, 10.14. Found: C, 65.33; H, 7.27; N, 10.18.

DL-*O-Benzyl-4-hydroxy-2-methylleucine* (IIIb). DL-5-(2-(Benzyloxy-2-methylpropyl)-5-methylhydantoin, 41.5 g. (0.15 mole), was hydrolyzed in base by the same procedure as that used for the hydrolysis of DL-5-(2-benzyloxyethyl)-5-methylhydantoin (IIa). The water insoluble amino acid was isolated by acidification of the basic reaction mixture with sulfuric acid which precipitated most of the amino acid along with the barium sulfate. The precipitate was collected by suction filtration and was washed with 300 ml. of hot 10% sulfuric acid and then an equal volume of hot water. The filtrate from the reaction mixture and the washings were combined and the *p*H adjusted to 5 with 28% aqueous ammonia to precipitate the crude amino acid. Recrystallization of the product from 50% ethanol gave a 75% yield of pure amino acid; m.p. 239.5–240.5° (sealed tube).

Anal. Calcd. for $C_{14}H_{21}NO_3$: C, 66.90; H, 8.42; N, 5.57. Found: C, 66.99; H, 8.14; N, 5.54.

DL-*4-Hydroxy-2-methylleucine, lactone, hydrochloride* (V). In a Parr hydrogenation bottle containing 4.5 ml. of hydrochloric acid in 100 ml. of absolute alcohol was placed 12.6 g. (0.05 mole) of DL-*O*-benzyl-4-hydroxy-2-methylleucine and 2 g. of 15% palladium-charcoal catalyst. The reaction mixture was shaken under 60 p.s.i. of hydrogen until the calculated amount of hydrogen had been absorbed. The reaction mixture was then filtered free of catalyst and evaporated to dryness. The product was redissolved in 15 ml. of hot absolute ethanol and ether added to the cloud point. The lactone crystallized on cooling to give 8 g. of crude product; m.p. 195–200°. Recrystallization from absolute ethanol afforded 6.5 g. of pure lactone; m.p. 190–191°. The infrared spectrum showed no absorption in the 2.5–3.0 μ range (—OH^-) λ_{max}^{Nujol} 3.28 μ (w.) (—NH); a series of weak bands in the 3.6–4.2 range (—NH_3^+); 5.69 μ (s.) (C=O); 9.03 μ (s.) (≡C—O—C).

Anal. Calcd. for $C_7H_{14}ClNO_2$: C, 46.80; H, 7.85; Cl, 19.74; N, 7.80. Found: C, 46.90; H, 7.87; Cl, 19.36; N, 7.62.

DL-*4-Hydroxy-2-methylleucine* (IVb). In order to avoid the cyclization which occurs during hydrogenolysis of the *O*-benzyl derivative in acid, the reduction was performed in an alkaline medium by a method previously described by Hartung.[8]

Accordingly, 2.5 g. (0.01 mole) of finely powdered DL-*O*-benzyl-4-hydroxy-2-methylleucine was suspended in a solution of 10 ml. of concd. ammonia solution and 100 ml. of water and one gram of a 15% palladium-charcoal catalyst was added. After the required amount of hydrogen was absorbed, the solution was filtered free of catalyst and the filtrate evaporated to dryness under reduced pressure. The product was redissolved in water and again evaporated to dryness to remove the last traces of ammonia. Needles were obtained from 90% ethanol; yield 1.1 g.; m.p. 218–219° (sealed tube); λ_{max}^{Nujol} 2.76 μ, 3.11 μ, 3.20 μ (—OH, —NH); 6.27–6.39 μ (CO_2^-); 8.43 μ, 8.78 μ [$(CH_3)_2$ C<, ≡C—OH].

Anal. Calcd. for $C_7H_{15}NO_3$: C, 52.15; H, 9.38; N, 8.69. Found: C, 52.23; H, 9.34; N, 8.62.

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Potential Anticancer Agents.[1] LII. *meso*-1,4-Bis(1-aziridinyl)-2,3-butanediol

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A recent note[2] from these laboratories described the synthesis of several monofunctional aziridines related to Tetramin (I) as possible anticancer agents. They were all inactive when tested on the mouse tumors Sarcoma 180, Adenocarcinoma 755, and Leukemia L-1210. In some related work, a difunctional alkylating agent, *meso*-1,4-bis(1-aziridinyl)-2,3-butanediol (IV) was prepared by the reaction of *meso*-1,2:3,4-diepoxybutane (II)[3] with ethylenimine.[4] This diaziridine (IV) showed considerable antitumor activity when tested in mice; accordingly, efforts were made to prepare a number of analogs.

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and not necessarily those of the Cancer Chemotherapy National Service Center. For the preceding paper in this series, *cf.* E. J. Reist, P. A. Hart, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 1557 (1961).

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