[CONTRIBUTION FROM THE INSTITUTE FOR CANCER RESEARCH AND LANKENAU HOSPITAL RESEARCH INSTITUTE]

Synthesis of Certain Hydroxycarboxylic Acids Related to Isocitric Acid[1]

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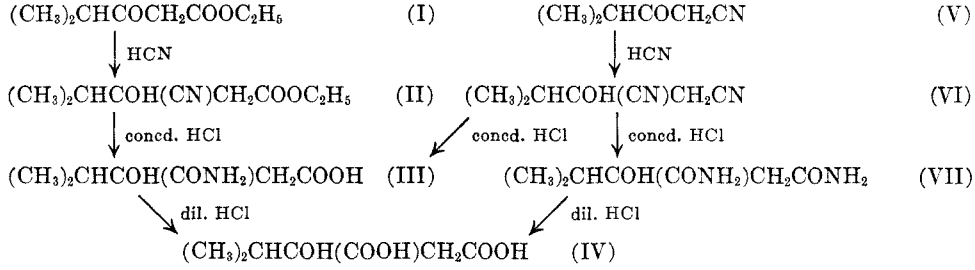
Syntheses of 2-hydroxy-2-isopropylsuccinic acid and 2-hydroxy-3-carboxyadipic acid are described.

2-Hydroxy-2-isopropylsuccinic acid and 2-hydroxy-3-carboxyadipic acid were proposed by Strassman *et al.*[3,4] as intermediates in the biosynthesis of leucine and lysine, respectively. These hydroxy acids were presumed to result from enzymatic condensation reactions analogous to citric acid formation from oxalacetic acid and acetyl coenzyme A. In order to appraise this hypothesis it was desirable to test these substances for biological activity,[5] and their synthesis is reported in the present communication.

2-Hydroxy-2-isopropylsuccinic acid. Ssemenow[6] reported the synthesis of this compound, having obtained a product melting at 165–166° by hydrolysis of 2-bromo-2-isopropylsuccinic acid. Darzens and Sejourné,[7] however, obtained a melting point of 139° for what they believed to be the same compound. Their method of preparation involved the hydrolysis of diethyl 2-isopropylsuccinate, prepared by condensation of ethyl 4,4-dimethyl-



glycidate with ethyl bromoacetate in the presence of zinc.

This compound was prepared by two straightforward procedures outlined below. In the first, ethyl isobutyrylacetate (I) was treated with hydrogen cyanide and, without isolation, the oily cyanhydrin (II) was hydrolyzed with concentrated hydrochloric acid. The product, 2-hydroxy-2-isopropylsuccinamic acid (III), m.p. 156–157°, was refluxed with dilute hydrochloric acid to yield 2-hydroxy-2-isopropylsuccinic acid (IV). An overall yield of 33% was obtained. The product, after repeated crystallization from ethyl acetate and petroleum ether, melted at 145–147°.

In the second procedure, isobutyrylacetonitrile (V), prepared by a modification of the method of Kroeker and McElvain,[8] was treated with hydrogen cyanide, and the resultant cyanohydrin (VI) was hydrolyzed with concentrated hydrochloric acid to yield 2-hydroxy-2-isopropylsuccinamide (VII), m.p. 195–197°, accompanied by small amounts of 2-hydroxy-2-isopropylsuccinamic acid (III). The diamide (VII) was hydrolyzed with dilute hydrochloric acid at 100° giving a 60% yield of 2-hydroxy-2-isopropylsuccinic acid (IV), m.p. 145–147° (not lowered by admixture with a sample prepared by the first method).

2-Hydroxy-3-carboxyadipic acid. Substances having this composition were described previously by Perlmutter,[9] in the form of a non-crystalline lactone, obtained by reduction and cleavage of quinolinic acid; and by Freudenberg and Geiger[10] who obtained the L-lactone melting at 110–111° by oxidation of methyl 3-acetyldihydroshikimate. In the present study, the hydroxy ester (XI) was obtained by reduction of triethyl 2-oxaloglutarate (X), prepared by condensation of diethyl glutarate (IX) with diethyl oxalate (VIII) according to a modification of the procedure of Gault.[11] On saponification, the free acid (XII) melting at 127–129° was obtained.

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(3) M. Strassman, L. A. Nocke, A. J. Thomas, and S. Weinhouse, *J. Am. Chem. Soc.*, **78**, 1599 (1956).

(4) M. Strassman and S. Weinhouse, *J. Am. Chem. Soc.*, **75**, 1680 (1953).

(5) The biological activities of these two compounds will be reported separately.

(6) I. Ssemenow, *Chem. Zentr.*, **I**, 1205 (1899).

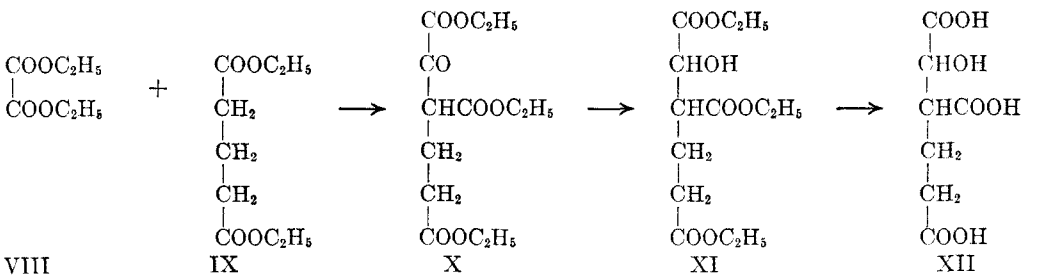
(7) G. Darzens and J. Sejourné, *Compt. rend.*, **152**, 1105 (1911).

(8) E. H. Kroeker and S. M. McElvain, *J. Am. Chem. Soc.*, **56**, 1172 (1934).

(9) A. Perlmutter, *Monatsh.*, **13**, 842 (1892).

(10) K. Freudenberg and J. Geiger, *Ann.*, **575**, 145 (1952).

(11) M. H. Gault, *Compt. rend.*, **148**, 1113 (1909).



VIII IX X XI XII

EXPERIMENTAL

2-Hydroxy-2-isopropylsuccinamic acid (III) *from ethyl isobutyrylacetate* (I). One and five-tenths g. (0.01 mole) of ethyl isobutyrylacetate,[12] and 0.98 g. (0.02 mole) of powdered sodium cyanide were stirred mechanically and cooled with ice while 1.7 ml. of concentrated hydrochloric acid was added dropwise. After stirring for 2 hr. at 0° the reaction mixture was extracted with ether, and the ether was removed by evaporation. The remaining oil (II) was added to 2 volumes of concentrated hydrochloric acid and the mixture was allowed to stand 48 hr. at room temperature. The reaction mixture was diluted with 2 volumes of water and extracted with ether continuously for 48 hr. Removal of the ether yielded 1 g. of crystals which on recrystallization from ethyl acetate, melted at 156–157°.

Anal. Calcd. for $C_7H_{13}NO_4$: C, 47.99; H, 7.48; N, 8.00; neut. equiv., 175.18. Found: C, 48.08; H, 7.70; N, 7.95; neut. equiv., 174.

2-Hydroxy-2-isopropylsuccinic acid (IV) *from 2-hydroxy-2-isopropylsuccinamic acid* (III). One g. of 2-hydroxy-2-isopropylsuccinamic acid was refluxed in 6 ml. of *N* HCl for 3 hr. The product was evaporated to dryness under reduced pressure and extracted with ethyl acetate. The ethyl acetate solution was concentrated to a small volume, petroleum ether was added until the beginning of turbidity, and the mixture placed overnight in the refrigerator. The white crystals which deposited were recrystallized from ethyl acetate and petroleum ether; m.p. 145–147°, yield 0.5 g.

Anal. Calcd. for $C_7H_{12}O_5$: C, 47.72; H, 6.87; neut. equiv., 176.17. Found: C, 47.74; H, 6.89; neut. equiv., 176.

Isobutyrylacetonitrile (V) *from methyl isobutyrate and acetonitrile.* To 12.6 g. (0.2 mole) of sodium ethoxide was added a mixture of 23 ml. (0.2 mole) of methyl isobutyrate and 15 ml. (0.2 mole) of acetonitrile, and the mixture was heated for about 4 hr. at 115–120° with stirring, while the alcohol formed in the reaction was distilled off. The unchanged reactants were separated by fractional distillation and returned to the reaction flask, for additional refluxing for 4 hr., the alcohol being removed as before. This process was repeated until alcohol was no longer formed; total elapsed time was about 15 hr. The mixture was then acidified with ice water containing a slight excess of acetic acid and extracted with ether. The ether layer was washed with 5% sodium bicarbonate solution, dried over anhydrous sodium sulfate, the ether was evaporated, and the residual oil was distilled under diminished pressure, yielding 9 g. (45% of theory) of a light yellow oil, b.p. 102–104° at 12 mm.

2-Hydroxy-2-isopropylsuccinamide (VII) *and 2-hydroxy-2-isopropylsuccinamic acid* (III) *from isobutyrylacetonitrile* (V). To a mixture of 4.5 g. (0.04 mole) of isobutyrylacetonitrile and 3.9 g. (0.08 mole) of powdered sodium cyanide at 0° there was added dropwise 6.5 ml. of concentrated hydrochloric acid with stirring, and stirring was continued for 2 hr. at the same temperature after the addition of hydrochloric acid was completed. The reaction mixture was extracted with ether, washed with water, and the ether was removed by evaporation; the residual oil was added to 2 volumes of concentrated hydrochloric acid, and allowed to stand 48 hr. at room temperature. After addition of 2 volumes of water the hydrochloric acid solution was extracted with ether continuously for 80 hr. Evaporation of the ether yielded 2.5 g. of crystals, which after recrystallization from water, melted at 195–197°.

Anal. Calcd. for $C_7H_{14}N_2O_3$: C, 48.26; H, 8.10; N, 16.08. Found: C, 48.46; H, 8.23; N, 15.93.

Evaporation of the mother liquor yielded a small quantity of a product which, after repeated recrystallization from ethyl acetate, melted at 156–157° and did not lower the melting point of 2-hydroxy-2-isopropylsuccinamic acid obtained from ethyl isobutyrylacetate.

2-Hydroxy-2-isopropylsuccinamic acid (III) *and 2-hydroxy-2-isopropylsuccinic acid* (IV) *from 2-hydroxy-2-isopropylsuccinamide* (VII). One g. of 2-hydroxy-2-isopropylsuccinamide was refluxed 3 hr. with 6 ml. of *N* HCl and the reaction mixture was evaporated to dryness under diminished pressure. The residue was extracted with ethyl acetate and the solution concentrated. The crystals which deposited were recrystallized from ethyl acetate, yielding 0.8 g. of product which melted at 156–157° and did not depress the melting point of the monoamide described above.

One g. of 2-hydroxy-2-isopropylsuccinamide was refluxed 3 hr. with 12 ml. of *N* HCl and the reaction product was evaporated to dryness under diminished pressure. This procedure was repeated twice and the final residue was recrystallized twice from ethyl acetate and petroleum ether; the white crystals melting at 145–147°, obtained in a yield of 0.6 g., did not depress the melting point of the dicarboxylic acid obtained above.

Triethyl 2-oxaloglutarate (X) *from diethyl glutarate* (IX) *and diethyl oxalate* (VIII). To 3.4 g. (0.05 mole) of sodium ethoxide in 37 ml. of anhydrous ether there was added 7.3 g. (0.05 mole) of diethyl oxalate with stirring and cooling, and stirring was continued until most of the ethoxide dissolved; then, while cooling with ice water and stirring vigorously, 9.4 g. (0.05 mole) of diethyl glutarate was added. Stirring was continued until the solution became clear, when the color of the solution turned from yellow to red. After standing 3 days in the refrigerator, the reaction mixture was poured onto ice, the ether solution was separated, and the aqueous layer was washed with ether. The aqueous solution was acidified with a slight excess of 2*N* H_2SO_4 while cooling, and extracted with ether. The ether solution was shaken with a small amount of barium carbonate to remove a trace of sulfuric acid, dried over anhydrous sodium sulfate, filtered, and the ether was removed under reduced pressure yielding 12 g. (81% of theory) of a viscous light yellow liquid. This was undistillable, even under high vacuum (0.0003 mm.), as Gault reported.[11]

Similar results were obtained with potassium ethoxide. Following the procedure for synthesis of diethyl oxalosuccinate,[13] but using diethyl glutarate instead of diethyl succinate, the main product was 3,5-dicarbethoxycyclopentadione-1,2.[14]

2-Hydroxy-3-carboxyadipic acid (XII) *by reduction of triethyl 2-oxaloglutarate with hydrogen.* Three g. of triethyl

(12) H. Fischer, M. Goldschmidt, and W. Nüssler, *Ann.*, **486**, 31 (1931).

(13) L. Friedman and E. Kosower, *Organic Syntheses*, **26**, 42 (1946).

(14) W. Dieckmann, *Ber.*, **27**, 965 (1894).

2-oxaloglutarate, 50 ml. of 95% ethanol, and 18 mg. of platinum dioxide were shaken in hydrogen under a pressure of 45 lbs./sq. in. at room temperature. The solution was filtered, evaporated under reduced pressure, and the light yellow residual oil was dissolved in 50 ml. of ether. The solution was washed 3 times with 5 ml. of 10% potassium carbonate solution to remove any unchanged compound, dried over anhydrous sodium sulfate, and evaporated under reduced pressure, yielding 2.8 g. of a residual oil having a fruit-like odor; on distillation, there was obtained a colorless liquid boiling at 140–141° (0.005 mm.) and a reddish oily residue.

Anal. Calcd. for $C_{13}H_{22}O_7$ (XI): Sapon. equiv., 96.8. Found: Sapon. equiv., 96.54.

Two and four-tenths g. of the undistilled triethyl 2-hydroxy-3-carboxyadipate was refluxed 2 hr. with 26.2 ml. (about an equivalent quantity) of 0.9*N* NaOH. The solution was acidified with a slight excess of *N* HCl, extracted continuously with ether for about 100 hr., and the ether solution was evaporated to dryness under reduced pressure. The residue was dissolved in water, and evaporated again to dryness to remove traces of hydrochloric acid. The 1.5 g. of residual sirup, after 3 days over phosphorus pentoxide in a vacuum desiccator, solidified. On repeated recrystallization from acetone and benzene, white crystals melting at 127–129° were obtained.

Anal. Calcd. for $C_7H_{10}O_7$: C, 40.78; H, 4.89; neut. equiv., 68.7. Found: C, 40.50; H, 4.88; neut. equiv., 68.3.

Reduction of triethyl 2-oxaloglutarate with sodium borohydride. To a solution of 5.1 g. of sodium borohydride in 25 ml. of water, a solution of 12 g. of triethyl 2-oxaloglutarate in 50 ml. of 70% methanol was added dropwise while stirring and cooling with ice water, and stirring was continued for 20 min. at room temperature. The reaction mixture was added to 2*N* H_2SO_4 to bring the *p*H to 3, filtered, evaporated at room temperature under vacuum, and extracted several times with ether after saturation with sodium chloride; then the ether solution was washed with 10% potassium carbonate solution until the latter gave a negative test for the carbonyl group with 2,4-dinitrophenylhydrazine, and dried over anhydrous sodium sulfate. Evaporation of the ether yielded 4.3 g. of oil. This was refluxed for 2 hr. with 17 ml. of *N* NaOH, neutralized with 17 ml. of *N* HCl with cooling, and the solution was evaporated to dryness under vacuum. The residue was extracted with acetone, the acetone solution was concentrated, and benzene was added until turbidity occurred. On standing overnight in the refrigerator crystals were precipitated which, after being recrystallized twice from acetone and benzene, melted at 127–129° and did not depress the melting point of the product obtained by catalytic reduction.

Trianilide of 2-hydroxy-3-carboxyadipic acid. Two-tenths g. of 2-hydroxy-3-carboxyadipic acid was neutralized with *N* NaOH, and the solution was evaporated to dryness and powdered. The dry sodium salt was heated 1 hr. at 150–160° with 1 ml. of aniline and 0.3 ml. of concentrated hydrochloric acid. After addition of 10 ml. of 2*N* HCl, the mixture was filtered, washed with water, and recrystallized 3 times from acetic acid; white crystals melting at 251° with decomposition were obtained in a yield of 0.15 g.

Anal. Calcd. for $C_{25}H_{25}N_3O_4$: C, 69.59; H, 5.84; N, 9.74. Found: C, 69.20; H, 5.92; N, 9.60.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, STANFORD UNIVERSITY]

Synthesis of the Racemic and Optically Active Forms of α-Amino-γ-*p*-di(β-chloroethyl)aminophenylbutyric Acid[1]

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In the continuing search for chemotherapeutic agents for the treatment of cancer, the DL-, D-, and L-α-amino-γ-*p*-di(β-chloroethyl)aminophenylbutyric acids were synthesized. Resolution of the intermediate α-acetamido-γ-*p*-nitrophenylbutyric acid as the (+)- and (−)-α-phenylethylamine salts led to the optically active isomers, the absolute configurations of which were tentatively inferred from their different degrees of biological activity; *i.e.* the L isomer caused a prompt transitory regression of a Cloudman malignant melanoma, S 91, in male mice while the D isomer caused only a barely perceptible brief regression.

Introduction. The carcinostatic and carcinolytic properties of nitrogen mustards, di(β-chloroethyl)-amino compounds, have been recognized for many years,[3] and recently the *p*-di(β-chloroethyl)amino-DL-, -D-, and -L-phenylalanines (I) have been prepared[4,5] and have displayed promising results in the treatment of certain types of tumors.[6] Interestingly, the L isomer, the absolute configuration of which was known by synthesis from L-phenylalanine,[4] showed a much greater ability to inhibit the growth of these tumors than did the D isomer.[6] This was one of the first examples of selectivity through optical isomerism with agents of this kind.

(1) A short summary of these syntheses has appeared previously (J. M. Luck, *Cancer Research*, **17**, 1071 (1957), and the compounds have also been named 2-amino-4-*p*-di(2-chloroethyl)aminophenylbutyric acids.

(2) To whom inquiries concerning this communication should be sent. Present address: Organisch-Chemisches Laboratorium der E. T. H., Zurich, Switzerland.

(3) A rather complete survey of the nitrogen mustard literature may be found in J. W. Beattie and L. H. Howells, *Quart. J. Med.*, **23**, 231 (1954).

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(6) F. Bergel and J. A. Stock, *Ann. Rep. Brit. Emp. Cancer Camp.*, **31**, 6 (1953).