tive, a ring of precipitate of the split product formed under the inlet tube. When all the hydriodic acid was added to the acetic anhydride, the remaining hydrogen iodide was forced over by heating the hydrogen iodide generator with a small flame. The reaction was considered complete when no more precipitate formed in the reaction flask. After the reaction mixture was cool and the precipitation complete, the supernatant liquid was poured off and the 5-iodo-2-thiouracil was washed on the Buchner funnel with peroxide-free ether to remove the residual iodine. It was then twice extracted with hot glacial acetic acid to remove unreacted material, then washed alternately with water and alcohol to remove the acetic acid. The almost pure product was purified by dissolving in dilute sodium hydroxide with gentle warming; the addition of acetic acid precipitated 27 g. of 5-iodo-2-thiouracil (57%).

The supernatant liquid from the reaction mixture was concentrated *in vacuo* and 7.4 g. of unreacted 5-iodo-2-benzylthiouracil was recovered.

When the 5-iodo-2-thiouracil was heated in a capillary the product became discernibly yellow at 190°; slowly darkened to brown at 210°; and decomposed to a black tar at 214–215°. On the Dennis melting point bar it decomposed slowly with melting at 231–236° and melted instantaneously with decomposition at 278–280°.

Anal. Calcd. for $C_4H_3N_2OSI$: I, 49.95; N, 11.03; S, 12.62. Found: I, 50.08; N, 10.9; S, 12.77.

5-Iodo-2-thiouracil and 5-iodo-2-benzylthiouracil are light sensitive and are best dried over phosphorus pentoxide *in vacuo*.

6-Methyl-5-iodo-2-benzylthiouracil.—Twelve grams of 6-methyl-2-benzylthiouracil was iodinated as described above but, since some of the material escaped iodination, the product was washed thoroughly with water, and re-iodinated. Eleven grams (58%) of 6-methyl-5-iodo-2-benzylthiouracil was obtained, m. p. 180–181° with decomposition.

Anal. Calcd. for $C_{12}H_{11}N_2OSI$: I, 35.44. Found: I, 35.65.

6-Methyl-5-iodo-2-thiouracil.—Ten grams of 6-methyl-2-benzylthiouracil was split as described for the preparation of 5-iodo-2-thiouracil. The yield was 3 g. (40%). On the Dennis melting point bar it decomposed slowly above 220°; it melted instantly at 285–289° with decomposition. When heated slowly in the capillary 6-methyl-5-iodo-2-thiouracil began to darken at 175°, progressively decomposed with loss of iodine, and decomposed completely at 195° without melting.

Anal. Calcd. for $C_5H_5N_2OSI$: I, 47.34. Found: I, 47.10.

Acknowledgment.—The authors wish to thank Dr. Noel E. Foss of the Calco Chemical Division of the American Cyanamid Company for generous supplies of thiouracil.

Summary

Methods are described for the preparation of 5-iodo-, 5-bromo- and 5-chloro-2-thiouracil, and the 5-iodo-, 5-bromo-, and 5-chloro-6-methyl-2-thiouracil. These compounds were prepared by the direct halogenation of either the S-methyl or S-benzyl derivatives followed by splitting with anhydrous hydrogen iodide.

An apparatus is illustrated for the convenient preparation of anhydrous hydrogen iodide.

BOULDER, COLORADO RECEIVED DECEMBER 27, 1947

[CONTRIBUTION FROM THE LABORATORY FOR THE STUDY OF HEREDITARY AND METABOLIC DISORDERS, AND THE DEPARTMENTS OF BIOLOGICAL CHEMISTRY AND MEDICINE, UNIVERSITY OF UTAH SCHOOL OF MEDICINE]

The Preparation of D- and L-Homoserine[1]

BY MARVIN D. ARMSTRONG

In the course of the synthesis of some biologically interesting compounds, it became necessary to prepare a considerable amount of pure L-homoserine (α-amino-γ-hydroxybutyric acid). A review of the literature revealed that little work had been accomplished on homoserine since its first preparation by Fischer and Blumenthal[1a] in 1907. Kitagawa's discovery of canavanine[2] and his demonstration that it was α-amino-γ-guanidinoxy-*n*-butyric acid[3–6] was the beginning of an increasing number of references to homoserine in the later literature. The main emphasis in such reports has been in connection with both syntheses and degradations of methionine.[7–12]

The previously reported O-phenylhomoserine[1a] provided a suitable intermediate for the preparation of the optically active homoserines. The N-formyl derivative was easily prepared and was found to give a crystalline strychnine salt; the use of 50% aqueous methanol as a solvent gave a good separation of the two diastereoisomers in one step, the salt of the D-isomer being more insoluble.

That the more soluble strychnine salt was of the L-configuration was shown by an application of the rule of Lutz and Jirgensons[13] to the crude (+)-O-phenylhomoserine obtained by decomposition of the mother liquors from the first crystallization of the strychnine salt. A definite negative maxi-

(1) This research was supported by a grant from the United States Public Health Service. Presented in part before the Division of Biological Chemistry at the 112th meeting of the American Chemical Society, New York, September 16, 1947.

(1a) E. Fischer and H. Blumenthal, *Ber.*, **40**, 106 (1907).

(2) M. Kitagawa and S. Monobe, *J. Biochem. Japan*, **18**, 333 (1933); *C. A.*, **28**, 1021[9] (1934).

(3) M. Kitagawa and S. Monobe, *J. Agr. Chem. Soc. Japan*, **9**, 845 (1933); *C. A.*, **28**, 2678[7] (1934).

(4) M. Kitagawa, *ibid.*, **12**, 871 (1937); *C. A.*, **31**, 1362[2] (1937).

(5) M. Kitagawa and A. Takani, *J. Biochem. Japan*, **23**, 181 (1936); *C. A.*, **30**, 4818[2] (1936).

(6) M. Kitagawa, *ibid.*, **24**, 107 (1936); *C. A.*, **30**, 8162[4] (1936).

(7) L. W. Butz and V. du Vigneaud, *J. Biol. Chem.*, **99**, 135 (1932).

(8) E. M. Hill and W. Robson, *Biochem. J.*, **30**, 248 (1936).

(9) H. R. Snyder, J. H. Andreen, G. W. Cannon and C. F. Peters, THIS JOURNAL, **64**, 2082 (1942).

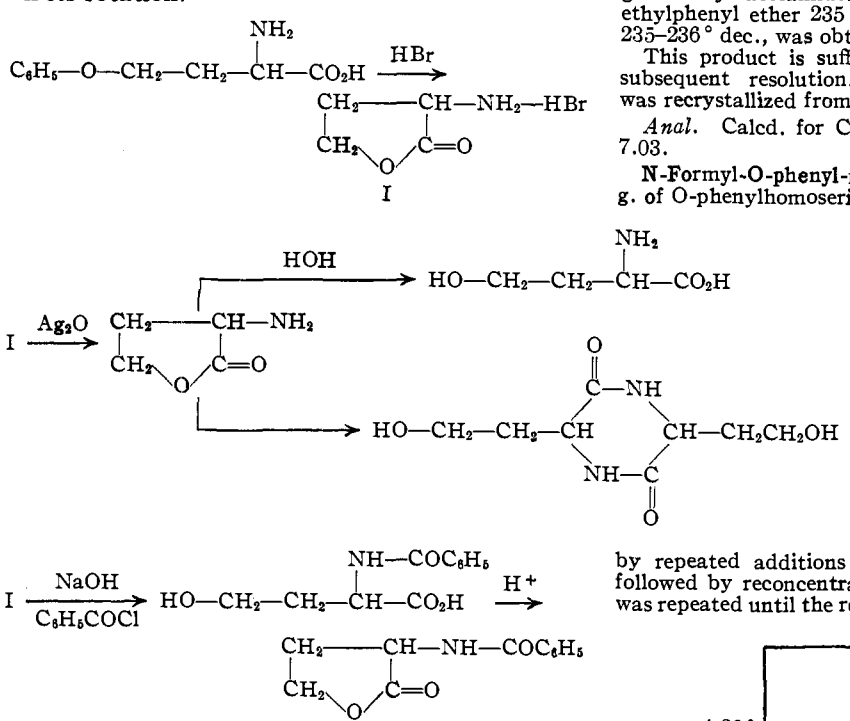
(10) J. E. Livak, E. C. Britton, J. C. VanderWeele and M. F. Murray, *ibid.*, **67**, 2218 (1945).

(11) G. Toennies and J. J. Kolb, *ibid.*, **67**, 1141 (1945).

(12) W. H. Stein and S. Moore, *J. Org. Chem.*, **11**, 681 (1946).

(13) O. Lutz and B. Jirgensons, *Ber.*, **63**, 448 (1930); **64**, 1221 (1931).

mum of rotation at the isoelectric point was shown when its rotation was measured in solutions containing different concentrations of acid and alkali (Fig. 1). Further confirmation was found when the free homoserine was obtained by hydrolysis of this isomer; its properties checked those reported by Kitagawa[3,4] for the homoserine obtained upon degradation of canavanine. Canavanine, itself, had earlier been shown to possess the L configuration by a study of the effect of acid concentration on its rotation.[14]



As indicated in the equations the active O-phenylhomoserines were readily converted to the active lactone hydrobromides of homoserine; other derivatives were prepared in the manner described by Fischer and Blumenthal in their original description of DL-homoserine.

Relatively poor yields of the active lactone hydrobromides were obtained as compared with the yield of the racemic compound. This was due to a significant amount of racemization (10–20%) under the conditions employed for the hydrolysis of the active O-phenylhomoserines. Fortunately the optically active derivatives could be easily obtained by recrystallization of the crude product from the hydrolysis; usually one recrystallization from aqueous ethanol sufficed to produce an optically pure compound.

Unsatisfactory yields were also obtained in the conversion of the isomers of α-aminobutyrolactone hydrobromide to the corresponding isomers of homoserine. However, by evaporating the mother liquors from the first recrystallization of the free homoserine to dryness, refluxing the residue for a short time with 48% hydrobromic acid and reworking the solution, most of the lost homoserine could be reisolated as the lactone hydrobromide. It is probable that formation of the diketopiperazines under the conditions for the preparation of homoserine caused the low yields.

Experimental

O-Phenyl-DL-homoserine.—This compound was prepared according to the method of Painter.[15] From 340 g. of ethyl acetamidomalonate and 350 g. of β-bromoethylphenyl ether 235 g. (76% yield) of product, m. p. 235–236° dec., was obtained.

This product is sufficiently pure for formylation and subsequent resolution. For analysis a small sample was recrystallized from hot water, m. p. 236–237° dec.

Anal. Calcd. for $C_{10}H_{13}O_3N$: N, 7.17. Found: N, 7.03.

N-Formyl-O-phenyl-DL-homoserine.—A solution of 200 g. of O-phenylhomoserine in 1700 ml. of 88% formic acid was warmed to 50° and 600 ml. of acetic anhydride was added dropwise at such a rate that the temperature remained at 50–60°. After the addition was completed the solution was allowed to stand for six hours at room temperature, at the end of which time 500 ml. of water was added and the reaction mixture was allowed to stand overnight. It was then concentrated to dryness *in vacuo*, keeping the bath temperature below 50°, and the last traces of water, formic acid and acetic acid were removed by repeated additions of 500 ml. portions of benzene followed by reconcentration to dryness. This treatment was repeated until the residue was dry and almost odorless.

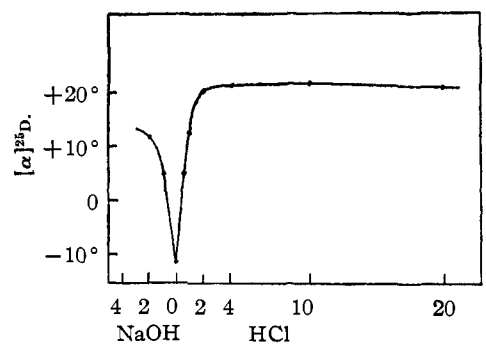


Fig. 1.—The effect of acid and alkali on the rotation of (+)-O-phenylhomoserine. The abscissa gives the ratio of the number of moles of acid and alkali, respectively, to the number of moles of amino acid in solution. A 1% aq. solution of the compound was used.

The dry residue was suspended in 800 ml. of boiling 95% ethanol and the suspension was filtered while hot. The residue was resuspended in 400 ml. of hot ethanol and filtered; the residue of recovered impure O-phenylhomoserine (48 g., m. p. 195–199° dec.) is suitable for reformylation. The combined filtrates were cooled overnight in a refrigerator and filtered; 95 g., m. p. 137–154° dec., of crude formyl derivative was obtained. The filtrate was concentrated to dryness *in vacuo* and the residue was recrystallized from 130 ml. of hot ethanol

(14) J. F. Cadden, *Proc. Soc. Exptl. Biol. Med.*, **45**, 224 (1940).

(15) E. P. Painter, THIS JOURNAL, **69**, 233 (1947).

(5 g. of crude O-phenylhomoserine was separated by filtering the hot solution); an additional 57 g. of crude N-formyl derivative was obtained. The crude N-formyl-O-phenylhomoserine (152 g.) was recrystallized from 200 ml. of hot 95% ethanol; 126 g., m. p. 135–143°, suitable for resolution, was obtained.

By concentrating the combined mother liquors to dryness and refluxing the residue with 1 *N* HCl an almost quantitative recovery of unformylated O-phenylhomoserine may be made.

For analysis a sample of the N-formyl-O-phenylhomoserine was recrystallized two times from aqueous ethanol; m. p. 137–137.5°.

Anal. Calcd. for $C_{11}H_{13}O_4N$: N, 6.27. Found: N, 6.45.

Resolution of N-Formyl-O-phenyl-DL-homoserine.—To a dry mixture of 112 g. (0.5 mole) of N-formyl-O-phenylhomoserine and 170 g. (0.5 mole) of powdered strychnine was added 5 liters of hot 50% methanol and the suspension was swirled and heated in a water-bath until almost all of the solids had dissolved. The solution was filtered while hot and was allowed to stand overnight at room temperature. The crystalline strychnine salt of N-formyl-O-phenyl-D-homoserine was collected on a filter, washed with a small amount of cold water, and dried; yield, 152 g.; $[\alpha]^{27}D$ −28° (1% in HOAc). One recrystallization from 4 liters of hot 50% methanol yielded 128 g.; $[\alpha]^{25}D$ −27° (1% in HOAc). Four more recrystallizations of the salt from aqueous methanol produced a pure strychnine salt, $[\alpha]^{27}D$ −25° (1% in HOAc), but in practice the best method of obtaining the pure isomer proved to be recrystallization of the free O-phenyl-D-homoserine resulting from the decomposition of the once recrystallized strychnine salt.

O-Phenyl-L-homoserine.—The original mother liquors from the crystalline strychnine salt were concentrated to a volume of approximately 2.5 liters, made alkaline by the addition of 20 ml. of concd. ammonia, cooled and filtered; the strychnine may be dried and reused. The filtrate was concentrated to a volume of about 1500 ml., made 1 *N* in hydrochloric acid by the addition of the proper amount of concd. hydrochloric acid and refluxed for two hours. The solution was then concentrated to dryness under reduced pressure and the residue was dissolved in 200 ml. of hot water; the hot solution was made neutral to congo red by the careful addition of concd. sodium hydroxide solution, the suspension was cooled and filtered. The residue was recrystallized from 500 ml. of hot water; yield, 35 g., m. p. 210–211° dec., $[\alpha]^{26}D$ +21.5° (1% in 1 *N* HCl). The combined mother liquors were concentrated to a volume of approximately 500 ml., cooled and filtered, yielding an additional 13 g. of impure product; m. p. 196–204° dec.; $[\alpha]^{25}D$ +5° (1% in 1 *N* HCl).

Two more recrystallizations of the pure derivative from 400-ml. portions of hot water yielded 22.0 g. of pure O-phenyl-L-homoserine; m. p. 241–242° dec., $[\alpha]^{26}D$ +23.5° (1% in 1 *N* HCl).

Anal. Calcd. for $C_{10}H_{13}O_3N$: N, 7.17. Found: N, 7.21.

O-Phenyl-D-homoserine.—A solution of 125 g. of the strychnine salt of N-formyl-O-phenyl-D-homoserine in 4 liters of hot 50% methanol was made alkaline by the addition of 20 ml. of concd. ammonia. The solution was cooled overnight in a refrigerator, the strychnine was removed by filtration, and the crude O-phenyl-D-homoserine was prepared in the same manner as previously described for the L-isomer; yield 38.5 g., m. p. 216–219° dec., $[\alpha]^{24}D$ −19° (1% in 1 *N* HCl). By reworking the mother liquors 6 g. of impure compound was obtained; m. p. 214–216° dec., $[\alpha]^{26}D$ −10° (1% in 1 *N* HCl).

Two recrystallizations of the first crop from 300 ml. portions of hot water yielded 26.5 g., m. p. 218–220° dec., $[\alpha]^{26}D$ −22.0° (1% in 1 *N* HCl). One more recrystallization from 500 ml. of hot water yielded 22 g., m. p. 241° dec., $[\alpha]^{24}D$ −23.5° (1% in 1 *N* HCl).

Anal. Calcd. for $C_{10}H_{13}O_3N$: N, 7.17. Found: N, 7.22.

In spite of its low solubility the D isomer possesses a definitely sweet taste, whereas the L isomer is tasteless or nearly so.

Preparation of DL-α-Aminobutyrolactone Hydrobromide.—A solution of 10 g. of O-phenyl-DL-homoserine in 100 ml. of 48% hydrobromic acid was refluxed for twenty hours[16] and was then concentrated to dryness *in vacuo*. The contents of the flask were dissolved in 50 ml. of distilled water and the solution was heated to boiling, treated with Norit, and filtered. The filtrate was concentrated to dryness *in vacuo* and the residue was suspended in 20 ml. of cold absolute ethanol and filtered; the solid residue was washed once with a 10 ml. portion of cold alcohol. The combined alcoholic filtrates were again concentrated to dryness, and the procedure was repeated. The weight of pure white α-aminobutyrolactone hydrobromide obtained was 7.5 g. (81% yield), m. p. 225–228° dec. It was recrystallized by dissolving it in a mixture of 3 ml. of water and 3 ml. of absolute ethanol, the hot solution was diluted with 54 ml. of warm absolute alcohol and allowed to stand in a refrigerator overnight. Only 65–70% recovery can be made upon recrystallization of the compound but the remainder can be obtained by reworking the mother liquors; m. p. 226–228° dec. (F. and B., 227° dec.).

Anal. Calcd. for $C_4H_8O_2NBr$: N, 7.69. Found: N, 7.89.

L-α-Aminobutyrolactone Hydrobromide.—A solution of 10 g. of O-phenyl-L-homoserine ($[\alpha]^{25}D$ +23.5°) in 100 ml. of 48% hydrobromic acid was refluxed for twenty hours and was worked up as described for the DL-α-aminobutyrolactone hydrobromide. After one recrystallization, 4.1 g. (44% yield) was obtained; m. p. 242–244° dec., $[\alpha]^{27}D$ −21.0° (1% in water).

Anal. Calcd. for $C_4H_8O_2NBr$: N, 7.69. Found: N, 7.63.

D-α-Aminobutyrolactone Hydrobromide.—Ten grams of O-phenyl-D-homoserine ($[\alpha]^{25}D$ −23.5°) was hydrolyzed and worked up in the same manner as described for the L compound; yield, 2.8 g. (41% yield); m. p. 242–244° dec., $[\alpha]^{27}D$ +21.0° (1% in water).

Anal. Calcd. for $C_4H_8O_2NBr$: N, 7.69. Found: N, 7.68.

N-Benzoyl-DL-homoserine.—Prepared from α-aminobutyrolactone hydrobromide according to the directions of Fischer and Blumenthal[16]; m. p. 126–127° (F. and B., 121°).

Anal. Calcd. for $C_{11}H_{13}O_4N$: N, 6.27. Found: N, 6.18.

DL-α-Benzamidobutyrolactone.—The benzoyl derivative was dissolved in a small amount of hot water containing a trace of hydrochloric acid, the solution was heated a few minutes and was then cooled. The α-benzamidobutyrolactone that crystallized was collected and was recrystallized from hot water; m. p. 140–141° (F. and B., 142°).

Anal. Calcd. for $C_{11}H_{17}O_4N$: N, 6.82. Found: N, 6.96.

L-α-Benzamidobutyrolactone.—A solution of 1.0 g. of L-α-aminobutyrolactone hydrobromide in 11 ml. of 1 *N* NaOH was cooled to 0° and benzoylation was carried out in the customary manner using 0.85 g. of benzoyl chloride and 2 ml. of 3 *N* NaOH. The reaction mixture was worked up by making the solution just acid to congo red, extracting the excess benzoic acid with ether, and recrystallizing the precipitate of crude N-benzoyl-L-homoserine by warming the mixture just enough to dissolve the solid and then

(16) Fischer and Blumenthal report complete hydrolysis after seven hours refluxing. This laboratory is located at an elevation of 5000 ft. with a usual barometric perssure of approximately 630 mm. of mercury, hence many of the reaction times in refluxing solutions or amounts of solvents needed for recrystallization may vary considerably at ordinary elevations from those reported herein. In this particular experiment it was found that fifteen hours of refluxing was not sufficient for complete hydrolysis.

quickly cooling the solution. It was collected on a filter, washed with water and dried: N-benzoyl-L-homoserine[17]; m. p. 139–141°.

The N-benzoyl-L-homoserine obtained was dissolved in 20 ml. of hot water containing two drops of concd. hydrochloric acid, the solution was boiled for two minutes and cooled. Beautifully formed needles of L-α-benzamidobutyrolactone crystallized and were collected; m. p. 139°, $[\alpha]^{20}D$ −21.5° (1.25% in 95% EtOH).[18]

Anal. Calcd. for $C_{11}H_{11}O_3N$: N, 6.82. Found: N, 6.80.

D-α-Benzamidobutyrolactone.—Prepared from D-α-aminobutyrolactone hydrobromide in the same manner as described for the L compound; N-benzoyl-D-homoserine, m. p. 139–141°; D-α-benzamidobutyrolactone m. p. 139–140°, $[\alpha]^{23}D$ +22.5° (1% in 95% EtOH).[18a]

Anal. Calcd. for $C_{11}H_{11}O_3N$: N, 6.82. Found: N, 6.93.

DL-Homoserine.—To a solution of 1.0 g. of DL-α-aminobutyrolactone hydrobromide in 5 ml. of water was added 0.7 g. of silver oxide and the suspension was shaken at room temperature for five minutes. The silver bromide was removed at the centrifuge, the clear supernatant solution was treated with hydrogen sulfide, again centrifuged, and the clear colorless solution was evaporated to dryness on a steam-bath. The residue was dissolved in 2 ml. of water, filtered, and the filtrate was diluted with 10 ml. of warm absolute ethanol and allowed to stand overnight in a refrigerator. The crystalline product was collected on a filter, washed with 95% ethanol and dried; yield 0.30 g., (46% yield); m. p. 186–187° dec.

Anal. Calcd. for $C_4H_9O_3N$: N, 11.76. Found: N, 11.98.

(17) Kitagawa and Monobe, refs. 3, 4, reported the following physical properties for homoserine and its derivatives as obtained by the degradation of canavanine: (1) homoserine, m. p., 201–202° dec.; $[\alpha]^{14}D$ −8.20, (2) N-benzoylhomoserine, m. p., 140–144°, (3) α-benzamidobuytrolactone, m. p. 139°, $[\alpha]^{17}D$ −27.99° (in EtOH).

(18) $[\alpha]^{20}D$ −27.0° (1% w/v in 95% EtOH).

(18a) $[\alpha]^{23}D$ +28.0° (1% w./v. in 95% EtOH).

L-Homoserine.—Prepared from 1.0 g. of L-α-aminobutyrolactone hydrobromide as described above for the DL compound; yield, 0.28 g. (43% yield); m. p. 203° dec., $[\alpha]^{23}D$ −8.0° (1% in water).

Anal. Calcd. for $C_4H_9O_3N$: N, 11.76. Found: N, 11.98.

D-Homoserine.—Prepared from 1.0 g. of D-α-aminobutyrolactone hydrobromide as described above; yield 0.30 g. (46% yield); m. p. 203° dec., $[\alpha]^{23}D$ +8.0° (1% in water).

Anal. Calcd. for $C_4H_9O_3N$: N, 11.76. Found: N, 12.00.

3,6-bis-(β-Hydroxyethyl)-2,5-diketopiperazine.—Prepared from 3.34 g. of DL-α-aminobutyrolactone hydrobromide according to the directions of Livak, *et al.*[10]; yield, 1.10 g. (60% yield); m. p. 189–191° dec.

Anal. Calcd. for $C_8H_{14}O_4N_2$: N, 13.86. Found: N, 14.22.

L-3,6-bis-(β-Hydroxyethyl)-2,5-diketopiperazine.—The above reaction was repeated using 3.34 g. of L-α-aminobutyrolactone hydrobromide; yield, 1.25 g. (67% yield); m. p. 190.5–191° dec.; $[\alpha]^{27}D$ −30.0° (1% in water).

Anal. Calcd. for $C_8H_{14}O_4N_2$: N, 13.86. Found: N, 13.79.

Acknowledgment.—The author wishes to thank Marie S. Hanson for performing the nitrogen analyses reported in this paper.

Summary

D- and L-homoserine have been prepared by the acid hydrolysis of the corresponding O-phenylhomoserines. The properties of L-homoserine were shown to agree with those reported for the optically active α-amino-γ-hydroxybutyric acid obtained by the degradation of canavanine.

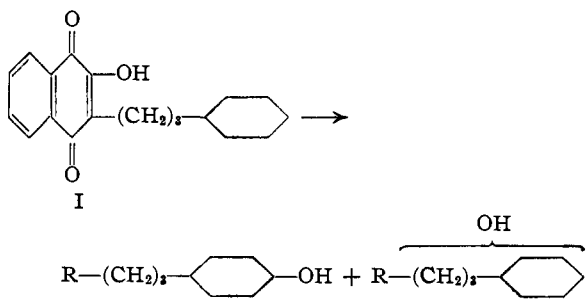
SALT LAKE CITY, UTAH RECEIVED OCTOBER 18, 1947

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

The Synthesis of 2-Hydroxy-3-[3′-*cis*-(4-hydroxycyclohexyl)-propyl]-1,4-naphthoquinone

BY WILLIAM G. DAUBEN AND RAYLENE E. ADAMS

It has recently been reported by Fieser[1] that various [2-hydroxy-3-alkyl-1,4-naphthoquinones when administered to humans undergo degradation. It was found that when the alkyl group was 3-cyclohexylpropyl (I), two hydroxylated quinones (II and III) could be isolated. Compound II, which melts at 155°, was shown to be 2-hydroxy-3-[3′-(4-hydroxycyclohexyl)-propyl]-1,4-naphthoquinone by synthesis from γ-(*p*-hydroxycyclohexyl)-butyric acid (V). This series of compounds can be assumed to be of the *trans* configuration since the starting acid (V) was obtained by the hydrogenation of γ-(*p*-hydroxyphenyl)-butyric acid (IV) in basic solution over Raney nickel catalyst.[2] Compound III was shown to be optically inactive, to contain a secondary hydroxyl group, and to melt at 112°. In view of these facts it was thought that this degradation product might be the *cis*-isomer of compound II and the synthesis of this isomer is reported in this paper.



R = 2-Hydroxy-1,4-naphthoquinone-3-yl

(1) Fieser and co-workers, THIS JOURNAL, in preparation.

(2) (a) Macbeth and Mills, *J. Chem. Soc.*, 709 (1945); (b) Skita, *Ber.*, **53**, 1792 (1920), and later papers.