not all of these unreactive groups can be liberated in the presence of urea. When both heat and urea are used as denaturing agents, all tyrosine groups become available for iodination. (4) From the kinetic data, a computation has been made of the number of free tyrosine groups in pepsin and in serum albumin.

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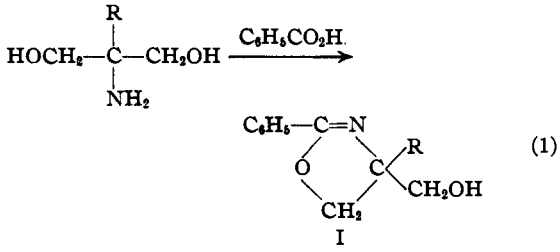
[CONTRIBUTION OF THE CHEMICAL LABORATORY OF INDIANA UNIVERSITY]

Amino Acids. IV. Amino Acids Related to Serine

BY JOHN H. BILLMAN AND EARL E. PARKER

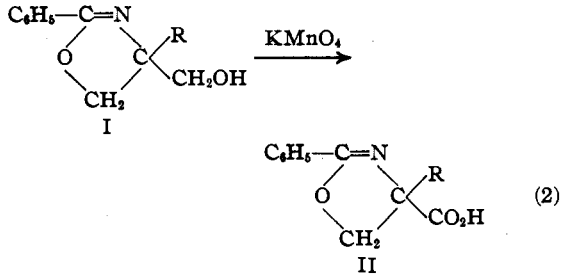
A new method[1] for synthesizing amino acids has now been extended to include the conversion of dihydric and trihydric amino alcohols to β-hydroxy-α-amino acids. The amino alcohols were first allowed to react with benzoic acid in xylene to yield the corresponding oxazolines (I).



(1)

The group R stands for $-CH_3$, $-C_2H_5$, or $-CH_2OH$.

The oxazolines were then oxidized to the corresponding oxazoline carboxylic acids II with alkaline permanganate.



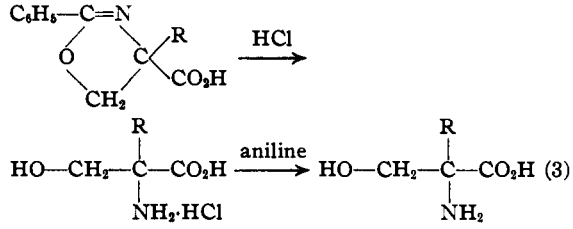
(2)

R stands for $-CH_3$, $-C_2H_5$, or $-COOH$.

When R was ethyl or methyl, the acid II proved to be difficult to isolate and for this reason the mixtures were hydrolyzed without isolating the carboxylic acids. When R was —COOH the dibasic acid was isolated in the pure state but again it was more convenient to hydrolyze the entire oxidation mixture.

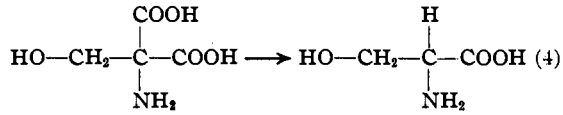
The oxazolines were hydrolyzed with hydrochloric acid to the corresponding β-hydroxy-α-amino acid hydrochloride and benzoic acid. Treatment of these amino acid hydrochlorides with aniline produced β-hydroxy-α-amino acids that were free of halogen.

(1) Billman and Parker, THIS JOURNAL, 66, 538 (1944).



(3)

In the case when R = COOH it was hoped that this compound could be decarboxylated to yield serine.



(4)

Numerous attempts to decarboxylate this compound or its hydrochloride failed to produce pure serine.

Likewise 2-phenyl-Δ²-oxazoline-4,4-dicarboxylic acid did not decarboxylate satisfactorily. However, further attempts are being made to prepare pure serine.

Experimental

2-Phenyl-4-methyl-4-hydroxymethyl-Δ²-oxazoline.—In a 3-liter round-bottomed flask were placed 1500 cc. of xylene, 219.6 g. (1.8 moles) of benzoic acid, and 189 g. (1.8 moles, m. p. 105–107°) of 2-amino-2-methyl-1,3-propanediol. The flask was equipped with a moisture trap and a water condenser. The contents were refluxed by means of an electrically heated oil-bath maintained at 170–180° until approximately the theoretical amount of water was collected. The time required for this reaction was ninety-six hours while a 0.3 mole reaction required only twenty-four hours.

The reaction product crystallized on being chilled in the refrigerator. The solid was filtered off and the filtrate was concentrated to 750 cc. and then to 150 cc. to obtain additional amounts of crude material. When 100 g. of this material was recrystallized from a solution containing 120 cc. of 95% alcohol and 240 cc. of water 83.5–86.5 g. of pure product melting at 103–104° was obtained; yield 67–69%. *Anal.* Calcd. for $C_{11}H_{13}O_2N$: N, 7.33. Found: N, 7.43.

α-Methylserine.—In a two-liter 3-necked flask were placed 900 cc. of water, 8 g. of sodium hydroxide, and 57.3 g. of pulverized 2-phenyl-4-methyl-4-hydroxymethyl-Δ²-oxazoline. To this vigorously stirred mixture was added 63 g. of potassium permanganate in small portions at such a rate that the temperature never exceeded 40°. The reaction was complete in ninety minutes. The manganese dioxide was filtered off and the filtrate acidified with hydrochloric acid and the mixture refluxed nine hours.

The mixture was cooled to room temperature and filtered. The filtrate was evaporated to dryness on a steam cone under reduced pressure. Two 20-cc. portions of water were added and removed under reduced pressure. The process was repeated with three 20-cc. portions of absolute alcohol.

The residue was extracted with 200 cc. and then 100 cc. of hot 95% alcohol. The extract was cooled to room temperature and filtered. To the filtrate was added 30 cc. of freshly distilled aniline. The α-methylserine was permitted to crystallize in the refrigerator for twenty-four hours. It was then filtered off and washed with several small portions of absolute alcohol. An additional amount was obtained by allowing the combined filtrates to stand in the refrigerator for several days longer. The total yield was 23 g. (64.5%). The material melted with decomposition at 243° (sealed capillary). *Anal.* Calcd. for $C_4H_9O_3N$: N, 11.76. Found: N, 11.69.

2-Phenyl-4-ethyl-4-hydroxymethyl-Δ²-oxazoline.—One and seven-tenths moles of 2-ethyl-2-amino-1,3-propanediol (b. p. 150–152° at 9 mm.) was allowed to react with an equal molar amount of benzoic acid under the same conditions as described for the preparation of the 2-phenyl-4-methyl-4-hydroxymethyl-Δ²-oxazoline. The time required for the reaction was 100 hours, while a 0.3-mole reaction required thirty-six hours.

The oxazoline was obtained by evaporating the filtrate to 200 ml. and filtering. A small additional amount was obtained by concentrating the filtrate to 150 ml. and adding 150 ml. of petroleum ether. The total yield of crude material was 279 g. (80%).

A mixture of 300 ml. of alcohol and 500 ml. of water was used to recrystallize 225 g. of the above solid. A total of 205 g. of pure 2-phenyl-4-ethyl-4-hydroxymethyl-Δ²-oxazoline was recovered; yield 72%. *Anal.* Calcd. for $C_{12}H_{15}O_2N$: N, 6.83. Found: N, 6.85.

α-Ethylserine.—α-Ethylserine was prepared by the same procedure as that used for α-methylserine except in the following respects: 6 g. of sodium hydroxide and 61.5 g. of pulverized 2-phenyl-4-ethyl-4-hydroxymethyl-Δ²-oxazoline were treated with 63 g. of potassium permanganate. The excess permanganate was destroyed with methyl alcohol. After removing the manganese dioxide, the filtrate was concentrated to 300 ml. and refluxed with 200 ml. of concentrated hydrochloric acid for ten hours. The pure α-ethylserine melted with decomposition at 265° and weighed 30 g.; yield 75%. *Anal.* Calcd. for $C_5H_{11}O_3N$: N, 10.52. Found: N, 10.45.

2-Phenyl-4,4′-dihydroxymethyl-Δ²-oxazoline.—In a 500-cc. round-bottomed flask were placed 100 cc. of xylene, 24.4 g. of benzoic acid, and 24.2 g. of trishydroxymethylaminomethane. The mixture was refluxed vigorously over an open flame, the flask being protected only by a wire gauze. Any deviation from this method of heating gave lower yields. Refluxing was continued for about eighteen hours, at the end of which time water had ceased to collect in the moisture trap.

The reaction product crystallized on being chilled in the refrigerator. The material was filtered off and the filtrate concentrated to 25 cc. to obtain an additional amount of crude material. The yield was 37–39 g. (89–94%) of crude material melting about 125–128°. This material was recrystallized two or more times from acetone and decolorized with activated charcoal on the first recrystallization. A yield of 25–27 g. (60–65%) was obtained. *Anal.* Calcd. for $C_{11}H_{13}O_3N$: N, 6.76. Found: N, 6.76.

Hydroxymethyl-amino-malonic Acid.—In a liter beaker were placed 300 cc. of water, 3 g. of sodium hydroxide and 20.7 g. of pure 2-phenyl-4,4′-dihydroxymethyl-Δ²-oxazoline. To this vigorously stirred mixture was added 42 g. of potassium permanganate at such a rate that the temperature never exceeded 40°. The reaction was complete in four hours. The manganese dioxide was filtered off and the filtrate reduced to 150 cc. To this was added 100 cc. of concentrated hydrochloric acid and the mixture refluxed for eight days.

The mixture was cooled to room temperature and filtered. The filtrate was evaporated to dryness on a steam cone under reduced pressure. Two 20-cc. portions of water were added and removed under reduced pressure. The process was repeated with three 20-cc. portions of absolute alcohol.

The residue was extracted with two 75-cc. portions of hot 95% alcohol. The extract was cooled to room temperature and filtered. To the filtrate were added 10 cc. of freshly distilled aniline. The product was permitted to crystallize in the refrigerator for twenty-four hours. It was then filtered off and washed with several small portions of absolute alcohol. The yield was 5 g. of crude material (34%). The material was dissolved in 50 cc. of water and filtered. To the filtrate was added 100 cc. of methyl alcohol. A yield of 3.5 g. of white material was obtained. *Anal.* Calcd. for $C_4H_7O_5N$: N, 9.40. Found: N, 9.51. This analysis corresponds to hydroxy-methyl-amino-malonic acid. The material gave a strongly positive ninhydrin test and decomposed at 247°.

All attempts to decarboxylate this compound or its hydrochloride have failed to produce serine in the pure state.

2-Phenyl-Δ²-oxazoline-4,4′-dicarboxylic Acid.—A sample of 2-phenyl-4,4′-dihydroxymethyl-Δ²-oxazoline was oxidized as described above. A portion of the filtrate containing 0.1 mole of oxidation products was concentrated to 50 cc. and carefully acidified with concentrated hydrochloric acid. On standing 5.1 g. of white material crystallized out. After several recrystallizations from water, the material decomposed at 199°. *Anal.* Calcd. for $C_{11}H_9O_6N$: N, 5.96. Found: N, 6.02. This analysis corresponds to 2-phenyl-Δ²-oxazoline-4,4′-dicarboxylic acid.

Summary

Three β-hydroxy-α-amino acids related to serine have been prepared from the corresponding amino alcohols.

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