

## UNITED STATES PATENT OFFICE

2,556,791

ALPHA-AMINO-ALPHA-METHYLOL  
MALONIC ACID

John H. Billman, Bloomington, Ind., and Earl E. Parker, Milwaukee, Wis.; said Parker assignor to said Billman

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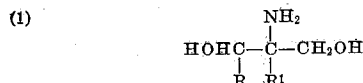
1 Claim. (Cl. 260-534)

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Our invention relates to the method of making  $\beta$ -hydroxy- $\alpha$ -amino acids, and to certain new  $\beta$ -hydroxy- $\alpha$ -amino acids produced by that method.

Among the amino acids which have value as foods are certain  $\beta$ -hydroxy- $\alpha$ -amino acids. It is commonly accepted that at least one of these  $\beta$ -hydroxy- $\alpha$ -amino acids (threonine) is included in the so-called "essential amino acids" which are necessary for growth if not for the maintenance of life; and others have food value.

In carrying out our invention, we start with a  $\beta$ -amino-polyhydroxy-alcohol, either dihydroxy or trihydroxy but preferably dihydroxy, of the following general formula:

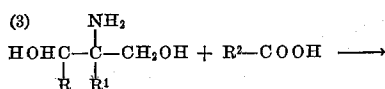
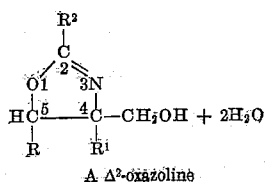


in which R represents a member of the class consisting of hydrogen and the lower alkyl groups, and R<sup>1</sup> represents a member of the class consisting of hydrogen, the lower alkyl groups, and the methylol group. A number of such alcohols are commercially available and are relatively cheap; and these are effective to yield some of the most desirable of the final products obtainable by our invention.

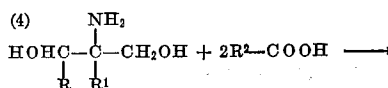
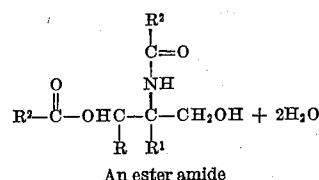
We first acylate this initial  $\beta$ -amino-polyhydroxy alcohol to protect both the amino group and a  $\gamma$ -hydroxy group, while leaving unprotected at least one methylol group; by treating the alcohol with an acyl-containing compound of the class consisting of carboxylic acids of the following general formula:



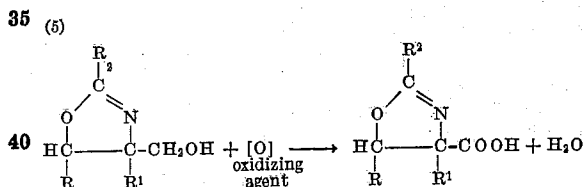
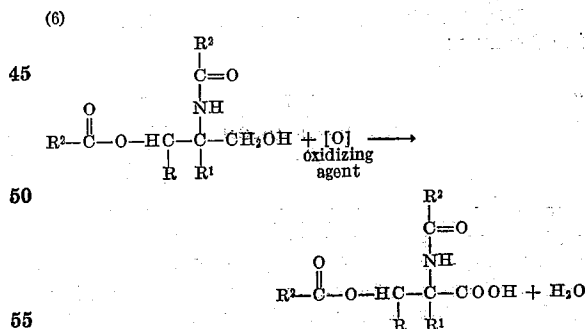
in which R<sup>2</sup> represents a member of the class consisting of lower alkyl groups, the phenyl group, and the tolyl group; and their acid anhydrides and acid chlorides. According to whether one or two molecular equivalents of the acylating compound is used, we produce either a  $\Delta^2$ -oxazoline, by going one step beyond simple acylation and splitting out water to produce a ring closure, or an ester amide, in accordance with one or the other of the following two reactions:



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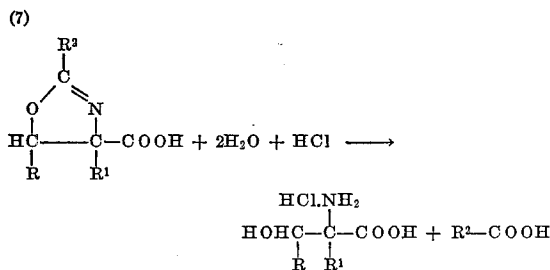
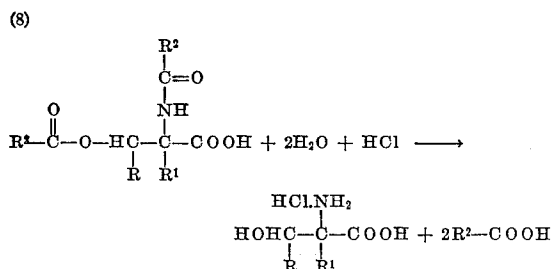
Next we oxidize the  $\Delta^2$ -oxazoline or ester amide so produced by treating it with an oxidizing agent lying between ferric chloride and persulfuric acid inclusive in the series of oxidation-reduction potentials. Such oxidizing agents include: metallic permanganates, for example an alkali-metal permanganate, an alkaline-earth permanganate, or zinc permanganate, in an aqueous medium of any reaction, whether alkaline, acid, or neutral; chromic oxidizing agents, including chromic acid CrO<sub>3</sub>, metallic dichromates, and metallic chromates, such as sodium dichromate, in an acid medium; oxidizing acids, including, in addition to the chromic acid already named, persulfuric acid, chromic acid, and nitric acid; lead peroxide; and ferric chloride. (For instance, see "Oxidation Potentials," by Latimer, published in New York by Prentiss Hall Inc., 1938, pp. 293 et seq.) This converts any methylol group in the oxazoline or ester amide to a carboxyl group, by a reaction as shown by one or the other of the following two formulas:



In these equations, if R<sup>1</sup> is the methylol group it is at the same time changed to the carboxyl group; but otherwise it is unchanged.

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After this oxidation to convert the methylol group or groups to a carboxyl group or groups, the acid produced is hydrolyzed to deacylate it; by heating in an aqueous solution of a mineral acid, such as sulfuric acid or hydrochloric acid or hydrobromic acid. The reaction removes an acyl group (after first opening the ring in the case of the oxazoline) and adds two molecular equivalents of water, and forms the hydrolyzing-acid salt of the desired  $\beta$ -hydroxy- $\alpha$ -amino acid, illustrated by the following two equations using hydrochloric acid as the hydrolyzing acid:



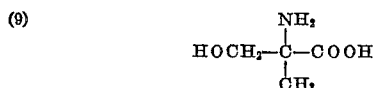
If initially  $R^1$  was the methylol group, so that in Equations 5 and 6 it was changed to the carboxyl group, then in Equations 7 and 8 it remains the carboxyl group. In that case, this second carboxyl group may be removed by heating the product to drive off carbon dioxide and leave hydrogen where  $R^1$  was. This heating may be done before or after the final step, now to be described, or (if desired) before the deacylation step.

The final step is to remove the hydrochloric acid, and thus convert the mineral-acid salt of the  $\beta$ -hydroxy- $\alpha$ -amino acid into the free  $\beta$ -hydroxy- $\alpha$ -amino acid. This may be done by treating the salt with any convenient base, such as sodium hydroxide, ammonium hydroxide, pyridine, or aniline.

The following are examples which illustrate our invention:

#### Example 1.— $\alpha$ -Methyl serine

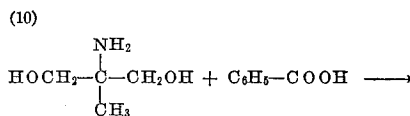
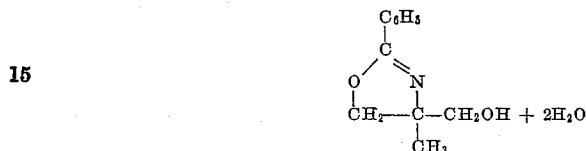
$\alpha$ -Methyl serine is represented by the following formula:



To produce  $\alpha$ -methyl serine, we start with 2-amino-2-methyl-1,3-propanediol. We place 189 g. (1.8 mols) of it, 219.8 g. (1.8 mols) of benzoic acid, and about 1500 cc. of xylene in a suitable flask, desirably equipped with a moisture trap and a water condenser. This mixture is refluxed at about 170° to 180° C. until no more water collects in the trap; which usually requires about 96 hours for the quantities given; although with

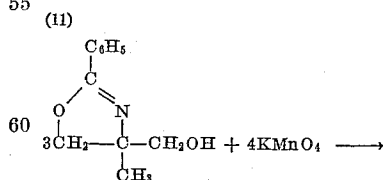
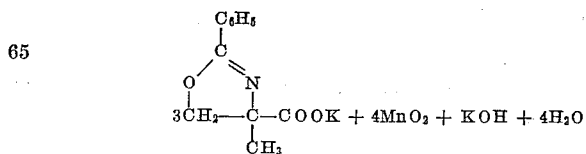
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smaller quantities it is possible to reduce this time of reaction. The heating for the refluxing is conveniently done in an electrically heated oil bath, but this is incidental. During the refluxing a reaction occurs as follows:



20 The reaction product thus obtained is 2-phenyl-4-methyl-4-methylol- $\Delta^2$ -oxazoline. It may be recovered in any suitable manner; for instance, by chilling the mixture after the refluxing, to crystallize the reaction product, and recovering that crystallized material by filtering. For more complete recovery, the filtrate obtained may be reduced in volume, by evaporation under reduced pressure of the xylene used as a solvent, and repeating the chilling and filtering. The yield of the crude oxazoline is about 80% of the calculated amount. This crude oxazoline melts in the range from 86° to 101° C. To purify it, the oxazoline may be recrystallized from a solution containing 100 cc. of 95% alcohol and 240 cc. of water per 100 g. of the crude oxazoline; by which recrystallization a purified oxazoline is obtained melting at 103–104° C. The yield of this purified oxazoline is about 67–69%. On analysis the oxazoline showed 7.43% nitrogen, as against 7.33% calculated nitrogen. This oxazoline is believed to be new with us.

The oxazoline thus obtained is now oxidized. To do this, we place 57.3 g. of the purified 2-phenyl-4-methyl-4-methylol- $\Delta^2$ -oxazoline, 8 g. of sodium hydroxide, and 900 cc. of water in a suitable flask, and while vigorously stirring the mixture we add 63.0 g. of potassium permanganate in small portions at a sufficiently slow rate to prevent the temperature from exceeding 40° C. It is usually takes about 90 minutes for the reaction to be complete. The reaction yields 2-phenyl-4-methyl-4-carboxy- $\Delta^2$ -oxazoline, and is an oxidation shown by the following equation:

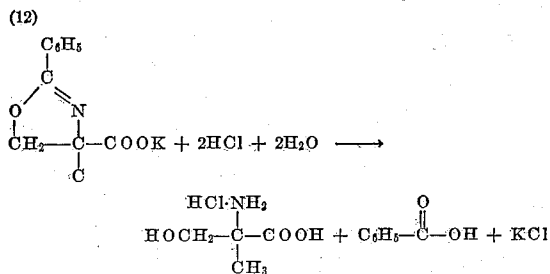


70 The manganese dioxide, obtained as a precipitate in the mixture resulting from the reaction, is filtered off.

75 Then the filtrate is acidified with aqueous hydrochloric acid, and the acidified mixture is re-

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fluxed for 9 hours. A hydrolysis occurs as shown by the following equation:



The resultant mixture is now cooled to room temperature, and filtered. The filtrate is subjected to evaporation under reduced pressure, to leave a solid residue. This residue is twice taken up in 20 cc. portions of water, and the water then removed by evaporation under reduced pressure, in order to eliminate excess hydrochloric acid. Then the residue is taken up several times, conveniently about three times, in 20 cc. portions of absolute alcohol, and the alcohol removed by evaporation under reduced pressure, to eliminate any water. The residue remaining is the hydrochloride shown in Equation 12, with some potassium chloride mixed with it; and is in dry form.

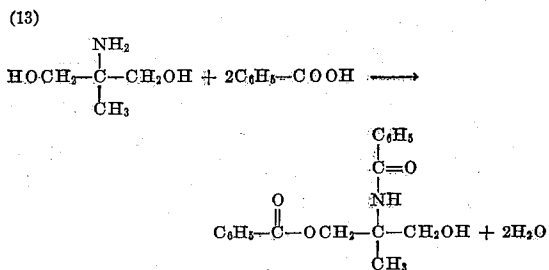
This dry residue is now extracted first with a 200 cc. portion, and then with a 100 cc. portion, of hot 95% alcohol, to take up the hydrochloride but to leave the potassium chloride behind. The two extracts are combined, cooled to room temperature, and filtered. To the filtrate is now added a suitable base, to produce neutrality or slight basicity; and for this we conveniently use about 30 cc. of freshly distilled aniline. The aniline (or other base) changes the hydrochloride to the desired free  $\beta$ -hydroxy- $\alpha$ -amino acid, which in this case is  $\alpha$ -methyl serine.

This  $\alpha$ -methyl serine is suitably recovered from the filtrate. This is conveniently done by letting the filtrate stand for 24 hours in a refrigerator, to cause the  $\alpha$ -methyl serine to crystallize; whereupon it is recovered by filtration and washed with several small portions of absolute alcohol. An additional amount may be recovered by allowing the combined filtrates to stand in the refrigerator for several days longer.

The total yield, with the quantities given, is approximately 23 g. (or about 64.5% of the theoretical). On analysis it shows 11.69% nitrogen; as against 11.67% calculated.

#### Example 2.— $\alpha$ -Methyl serine

Example 1 is repeated, save that double the amount of benzoic acid is used. Instead of getting the 2-phenyl-4-methyl-4-methylol- $\Delta^2$ -oxazoline as the intermediate, as shown in Equation 10, the reaction in this case yields an ester amide, as shown by the following formula:



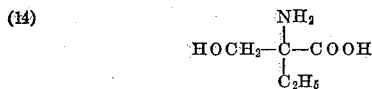
The ester amide thus produced is treated in the same way the oxazoline is treated in Example 1,

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and yields as a final product the same  $\alpha$ -methyl serine.

#### Example 3.— $\alpha$ -Ethyl serine

5  $\alpha$ -Ethyl serine is represented by the following formula:



10 Example 1 is repeated, save that instead of using 2-amino-2-methyl-1,3-propanediol we use a molecularly equivalent amount of 2-amino-2-ethyl-1,3-propanediol. This produces 2-phenyl-4-ethyl-4-methylol- $\Delta^2$ -oxazoline as an intermediate, with a yield of about 72%; and with an analysis of 6.85% nitrogen, which agrees with the calculated value.

This 2-phenyl-4-methyl-4-methylol- $\Delta^2$ -oxazoline is now oxidized, in the same way that the 2-phenyl-4-methyl-4-methylol- $\Delta^2$ -oxazoline is oxidized in Example 1, and gives 2-phenyl-4-ethyl-4-carboxy- $\Delta^2$ -oxazoline. This last-named compound is then deacylated, in the same way that the 2-phenyl-4-methyl-4-carboxy- $\Delta^2$ -oxazoline is deacylated in Example 1, to give as a final product the desired  $\alpha$ -ethyl serine, of Formula 14.

The purified  $\alpha$ -ethyl serine melts with decomposition at about 265° C.; it shows about 10.45% nitrogen on analysis, as against 10.52% calculated; and it is obtained with a yield of about 75%.

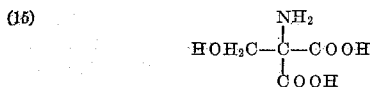
Both intermediate oxazolines and the final  $\alpha$ -ethyl serine produced by this example are believed to be new with us.

#### Example 4.— $\alpha$ -Ethyl serine

Example 3 is repeated, save that double the quantity of benzoic acid is used; in the same way that Example 2 repeats Example 1. This gives the same final product as in Example 3,  $\alpha$ -ethyl serine; but instead of an oxazoline as an intermediate, it gives an ester amide as an intermediate, corresponding to that referred to in Example 2 and shown in Equation 13 save that the ethyl group replaces the methyl group.

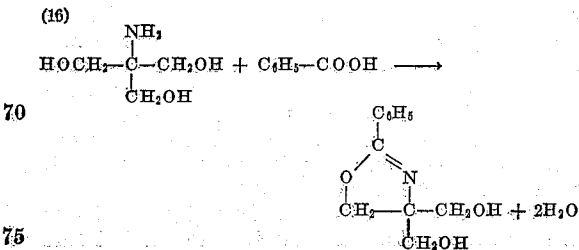
#### Example 5.— $\alpha$ -Amino- $\alpha$ -methylol malonic acid

This  $\alpha$ -amino- $\alpha$ -methylol malonic acid has the following formula:



55 To produce it we start with trishydroxymethyl-amino methane. We place 24.2 g. of this, 24.4 g. of benzoic acid, and 100 cc. of xylene, in a suitable flask, desirably provided with a moisture trap; and reflux the mixture, desirably over an open flame but with the flask protected by wire gauze, for about 18 hours, by the end of which time the moisture has usually ceased to collect in the moisture trap.

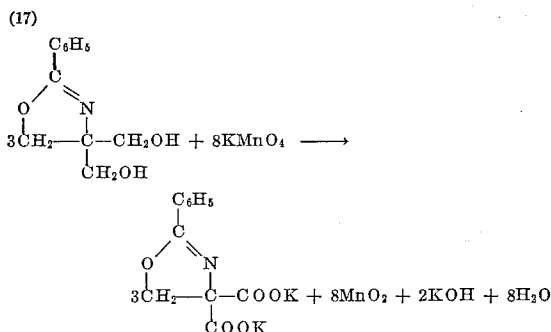
Upon this refluxing a reaction occurs as follows:



The reaction product thus obtained is 2-phenyl-4,4-dimethylol- $\Delta^2$ -oxazoline. It may be recovered by chilling the mixture after the refluxing is completed, filtering, and saving the precipitate, which is the oxazoline; and, if desired, increasing the amount of oxazoline recovered by reducing the filtrate in volume to about 25 cc., by evaporation, and filtering again to recover more precipitate. The yield of crude material is about 37-39 g., which is about 89% to 94% of the theoretical. The crude material thus obtained melts at 125-128° C.

Purification may be obtained by recrystallizing two or more times from acetone, and decolorizing with activated charcoal. The yield after the second recrystallization is about 25-27 g., with an observed nitrogen value of 64.7%, which agrees with the calculated value. The purified oxazoline, which we believe is new with us, has a melting point of about 131° C.

This oxazoline is now oxidized. To do this, about 20.7 g. of the purified 2-phenyl-4,4-dimethylol- $\Delta^2$ -oxazoline, about 3 g. of sodium hydroxide, and about 300 cc. of water are placed in a suitable condenser, and while stirring it vigorously about 42 g. of potassium permanganate is slowly added—sufficiently slowly that the temperature is never allowed to exceed about 40° C. It usually takes about 4 hours to complete the reaction, which yields 2-phenyl-4,4'-dicarboxy- $\Delta^2$ -oxazoline, or rather its dipotassium salt, and is as follows:



The manganese dioxide, obtained as a solid in this reaction, is removed by filtration; and the filtrate is subjected to evaporation to reduce its volume to about 150 cc. To this reduced-volume filtrate we now add about 100 cc. of concentrated aqueous hydrochloric acid, and reflux the mixture for about 8 days. After this refluxing we cool the mixture to room temperature, and filter; and evaporate the filtrate to dryness under reduced pressure. To remove excess hydrochloric acid we take the residue up two or more times in 20 cc. portions of water, and remove the water by evaporation under reduced pressure; and to remove any residual water we treat the residue successively three or more times with 20 cc. portions of absolute alcohol, and remove that by evaporation under reduced pressure.

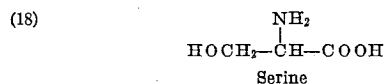
The residue then remaining is extracted successively with at least two 75 cc. portions of hot 95% alcohol, and the extracts are combined. The combined extract is cooled to room temperature, and filtered. We now add a base to make the filtrate neutral or basic; as by adding 10 cc. of freshly distilled aniline. This produces the desired free  $\alpha$ -amino- $\alpha$ -methylol malonic acid; which we believe is new with us.

This is recovered in crude form by permitting the mixture to stand in a refrigerator for 24 hours

after the addition of the aniline, and then filtering it and washing the precipitate with several small portions of absolute alcohol. This gives a yield of about 5 g. of the crude malonic acid (about 34%).

To purify this, we dissolve it in about 50 cc. of water, filter it, and add 100 cc. of methyl alcohol to the filtrate; which yields about 3.5 g. of a white material, which is the purified  $\alpha$ -amino- $\alpha$ -methylol malonic acid. Analysis shows 9.51% nitrogen, as against a calculated value of 9.40% on the basis of  $\text{C}_6\text{H}_7\text{O}_5\text{N}$ . This malonic acid gives a strongly positive ninhydrin test; and decomposes at about 247° C.

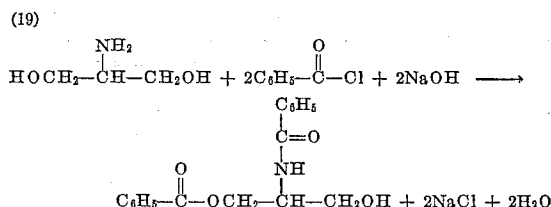
This  $\alpha$ -amino- $\alpha$ -methylol malonic acid probably has value as a food in itself; but its main present utility appears to be as an intermediate for the formation of the highly valuable serine. While we have not yet worked out the most effective method of producing and isolating serine from it, we have produced products which contain serine by heating the  $\alpha$ -amino- $\alpha$ -methylol malonic acid to a point where carbon dioxide is evolved; which indicates decarboxylation by the removal of carbon dioxide from one of the carboxyl groups to give the desired serine, which has the following formula:



#### Example 6.—Serine

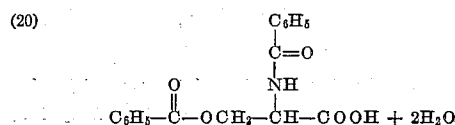
Serine is represented by Formula 18 just above.

To a mixture of 18.2 g. (0.2 mol) of 2-amino-1,3-propanediol in 200 ml. of water containing 20.0 g. (0.5 mol) of sodium hydroxide is slowly added, with stirring, 56.0 g. (0.4 mol) of benzoyl chloride. A reaction occurs, to produce the ester amide, as follows:



The ester amide is suitably recovered when the reaction is complete, as in earlier examples, and may be used (with or without purification as by recrystallization from alcohol) in the subsequent oxidation step.

This oxidation step, to oxidize the methylol group of the ester amide to the carboxyl group, is carried out by placing the full amount recovered of the crude (or the purified) ester amide in about 300 ml. of water, and adding 125 ml. of concentrated nitric acid (which is a slight excess of nitric acid) sufficiently slowly that the reaction proceeds without violence—desirably at a temperature between 50° and 90° C. It is sometimes necessary to warm the reaction mixture to start the reaction. After all the nitric acid has been added, the mixture is heated in order to complete the reaction and to remove most of the excess nitric acid. The oxidation by the nitric acid produces N,O-dibenzoylserine, which has the following formula:

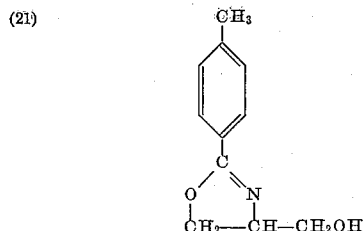


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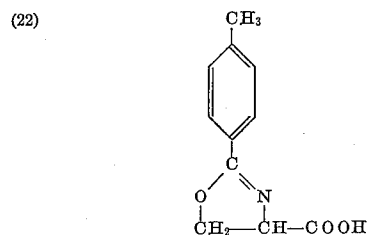
Then 100 ml. of concentrated hydrochloric acid is added, and the mixture is refluxed for about five hours; which hydrolyzes off the two benzoyl groups, and gives serine hydrochloride. The resulting mixture is cooled to room temperature, and filtered; and the filtrate is evaporated to dryness. The residue remaining, which is the serine hydrochloride, is twice taken up in water, and the water is removed by evaporation, preferably under reduced pressure. The residue is now practically free from hydrochloric acid. This residue is dissolved in the minimum amount of 99% methyl alcohol. To the solution thus produced is added sufficient pyridine to change the serine hydrochloride to the free serine. On standing, the serine precipitates out, and may be recovered by filtration.

#### Example 7.—Serine

Example 6 is repeated, save that one mol of p-tolyl chloride is used instead of two mols of benzoyl-chloride. The p-tolyl-amide of 2-amino-1,3-propanediol is first produced, and is suitably recovered, by separating it from the co-present water. This p-tolyl-amide is mixed with several parts of xylene, and the mixture is refluxed at about 170–180° C. in a suitable flask, desirably equipped with a moisture trap, until no more water collects in the trap. During the refluxing a reaction occurs to give 2-p-tolyl-4-methylol- $\Delta^2$ -oxazoline, which has the following formula:



This oxazoline is now oxidized with nitric acid by a procedure similar to the oxidation described in Example 6, but desirably with the addition of a trace of a vanadium compound (such for instance as ammonium meta-vanadate) as a catalyst. The oxidation produces 2-p-tolyl-4-carboxy- $\Delta^2$ -oxazoline, which has the following formula:

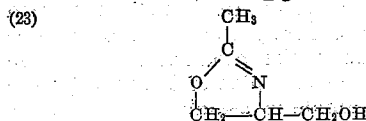


This is now suitably hydrolyzed, as with hydrobromic acid, to produce serine hydrobromide; from which the free serine may be obtained by treating with aniline.

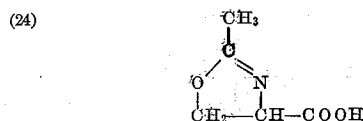
#### Example 8.—Serine

Example 7 is repeated, save that acetic anhydride in acetic acid is used as the acylating agent instead of p-tolyl chloride in sodium hydroxide. The acylated product obtained after the refluxing and the removal of water is 2-methyl-4-methylol- $\Delta^2$ -oxazoline, which has the following formula:

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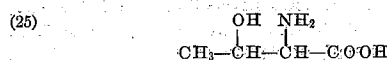
This oxazoline is oxidized, conveniently with nitric acid in the same way the oxazoline in Example 7 was oxidized, to yield 2-methyl-4-carboxy- $\Delta^2$ -oxazoline, which has the following formula:



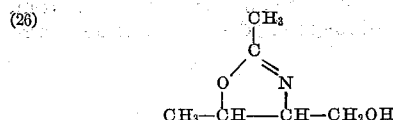
This oxazoline is hydrolyzed in the same way as the hydrolysis was obtained in Example 7; to give serine as the final product.

#### Example 9.—Threonine

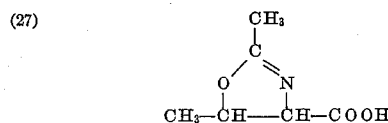
Threonine is represented by the following formula:



To prepare this, we start with 2-amino-1,3-butanediol. We first acylate this by treating 26.3 g. (0.25 mol) of it with 22.0 g. (0.28 mol) of acetyl chloride in pyridine. The acylated product is heated to remove water, as described in Example 1, to produce 2,5-dimethyl-4-methylol- $\Delta^2$ -oxazoline, which has the following formula:



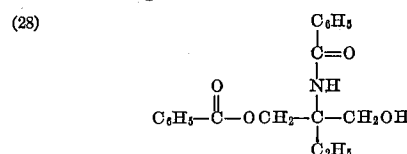
This is oxidized in the same way as the 2-phenyl-4-methyl-4-methylol- $\Delta^2$ -oxazoline was oxidized in Example 1, but in this case gives 2,5-dimethyl-4-carboxy- $\Delta^2$ -oxazoline, which has the following formula:



This latter oxazoline is hydrolyzed, in the same manner as the hydrolysis is produced in Example 1, to give threonine hydrochloride, from which the desired threonine (which is  $\alpha$ -amino- $\beta$ -hydroxy-n-butyric acid) is obtained by treatment with a suitable base, such as aniline.

#### Example 10.— $\alpha$ -Ethyl serine

As in Example 3, we start with 2-amino-2-ethyl-1,3-propanediol. To a mixture of 11.9 g. (0.1 mol) of this with 200 ml. of water containing 10 g. (0.25 mol) of sodium hydroxide is added, with stirring, 28 g. (0.2 mol) of benzoyl chloride. Reaction occurs, and after removal of the water the reaction product is an ester amide having the following formula:



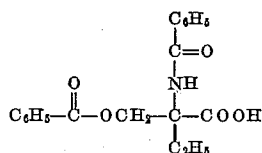
This may be called 2-ethyl-2-benzoylamino-3-benzoyloxypropanol.

This ester amide is now oxidized, to convert its methylol group to the carboxy group. This may

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be done by treating 34.3 g. (0.105 mol) of it with 14 g. (0.14 mol) of chromic acid (CrO<sub>3</sub>) in sulfuric acid solution; which produces an oxidation that converts the ester amide of the initial alcohol to the ester amide of  $\alpha$ -amino- $\beta$ -hydroxy- $\alpha$ -ethyl propionic acid—in other words, to the ester amide of  $\alpha$ -ethyl serine. This ester amide, which is O,N-dibenzoyl-ethyl-serine, has the following formula:

(20)



When the oxidation is complete, the mixture is made basic with sodium hydroxide, and filtered. The filtrate is made strongly acid with hydrochloric acid and refluxed, in order to produce a hydrolysis which removes the two benzoyl groups. When the hydrolysis is complete, the mixture is cooled and filtered, and the filtrate is evaporated to dryness under reduced pressure. The residue, which is the hydrochloride of  $\alpha$ -ethyl serine, is treated with several portions of water and then with absolute ethyl alcohol, as described in Example 1, to remove hydrochloric acid and water. Then the hydrochloride is treated with aniline, to produce the desired free  $\alpha$ -ethyl serine.

As the foregoing examples show, the oxazoline and the ester amides used always have at least one methylol (or primary-alcohol) group attached to the same carbon atom as is the nitrogen atom, and the  $\beta$ -amino-polyhydroxy alcohols used

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always have at least two alcohol groups attached to the same carbon atom as is the nitrogen atom and at least one of those alcohol groups is a methylol (or primary-alcohol) group; and so by the terms " $\beta$ -amino-polyhydroxy alcohol," "oxazoline," and "ester amide," we mean  $\beta$ -amino-polyhydroxy alcohols, oxazolines, and ester amides of that type.

The  $\beta$ -hydroxy- $\alpha$ -amino acids produced by the present invention all have at least possible value as foods. In addition, they are effective as buffer reagents in various preparations, such for instance as in cosmetic preparations.

We claim as our invention:

15  $\alpha$ -Amino- $\alpha$ -methylol malonic acid.

JOHN H. BILLMAN.  
EARL E. PARKER.

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