Studies on Amino Acids. IX.

On the Synthesis of \(\alpha\)-Keto Acid Analogue of Methionine*  

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The synthetic procedures for the preparation of \(\alpha\)-keto acid analogue of methionine, \(\alpha\)-keto-\(\gamma\)-methylmercaptobutyric acid, were investigated. As a result, the procedure consisting of the ester condensation between methyl \(\beta\)-methylmercaptopropionate and methyl oxalate, and subsequent hydrolysis and decarboxylation was found to be a satisfactory route.

In regard to amino acid metabolism, \(\alpha\)-keto acids are considered very interesting substances. On the keto acid derived from methionine, \(\alpha\)-keto-\(\gamma\)-methylmercaptobutyric acid (VI), several reports have been published. For instance, Cahill and Rudolph\(^1\) observed that \(\alpha\)-keto acid analogue of methionine resembles \(\text{L}\)-methionine itself, effective in promoting the growth of rats fed with a diet low of this amino acid. Handler and Bernheim\(^2\), reported that the \(\alpha\)-keto derivative was as active as the natural amino acid in creatine synthesis by rat liver slices. Lampen et al.\(^3\), also reported that the growth rate of a methionineless mutant of \(E.\) coli was approximately the same as that in the case of \(L\)-methionine and the inhibition of growth of the strain caused by norvaline was restored by the \(\alpha\)-keto acid analogue of methionine.

On the preparation of the \(\alpha\)-keto acid, biochemical procedures such as oxidation of methionine by liver slices\(^4\) and by purified amino acid oxidase\(^5\), have mainly been employed, but no satisfactory synthetic procedure has yet been published. For the purpose to obtain this substance in a larger amount, the synthetic chemical procedure is desirable. Therefore, in order to establish a convenient method of preparation the authors attempted the synthetic route, which had been employed for the preparation of the keto acids, as follows:

\[
\begin{align*}
\text{CH}_3\text{COCH}_2\text{COOC}_2\text{H}_5 + \text{CH}_3\text{SCH}_2\text{CH}_2\text{Cl} & \xrightarrow{\text{NaOCH}_3} \text{C}_2\text{H}_5\text{OH} \\
\text{H} & \xrightarrow{\text{C}_2\text{H}_4\text{ONO}} \\
\text{CH}_3\text{SCH}_2\text{CH}_2\cdot\text{C} \cdot \text{COOC}_2\text{H}_5 & \xrightarrow{\text{CH}_3\text{SCH}_2\text{CH}_2\cdot\text{C} \cdot \text{COOC}_2\text{H}_5} \xrightarrow{\text{NO} \cdot \text{HSO}_3} \\
& \text{CH}_3\text{SCH}_2\text{CH}_2\cdot\text{C} \cdot \text{COOC}_2\text{H}_5 & \xrightarrow{\text{CH}_3\text{SCH}_2\text{CH}_2\cdot\text{C} \cdot \text{COOC}_2\text{H}_5} \xrightarrow{\text{NO} \cdot \text{HSO}_3} \\
& \text{CH}_3\text{SCH}_2\text{CH}_2\cdot\text{C} \cdot \text{COOC}_2\text{H}_5 & \xrightarrow{\text{CH}_3\text{SCH}_2\text{CH}_2\cdot\text{C} \cdot \text{COOC}_2\text{H}_5} \xrightarrow{\text{NO} \cdot \text{HSO}_3}
\end{align*}
\]

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2) P. Handler and M.L.C. Bernheim, J. Biol. Chem., 150, 335 (1943).
although, the yield of the reaction from isonitroso compound (IV) to the \( \alpha \)-keto ester (V) was poor. Consequently, the route was not suitable for practical purposes.

Another route described in the following scheme was investigated, and a satisfactory result was obtained.

\[
\begin{align*}
\text{CH}_3\text{SH} + \text{CH}_2=\text{CHCOOCH}_3 & \quad \rightarrow \quad \text{CH}_3\text{SCH}_2\text{CH}_2\text{COOCH}_3 \\
\text{COOCH}_3 & \quad \rightarrow \quad \text{H} \quad \rightarrow \quad \text{CH}_3\text{SCH}_2\text{CH}_2\text{COOCH}_3 \\
\text{KOCH}_3 & \quad \rightarrow \quad \text{HCl} \quad \rightarrow \quad \text{CH}_3\text{SCH}_2\text{CH}_2\text{COOCOOH} \\
\text{(VII)} & \quad \rightarrow \quad \text{CH}_3\text{SCH}_2\text{CH}_2\text{COOCH}_3 \\
\text{(VIII)} & \quad \rightarrow \quad \text{COOCH}_3 \\
\text{(or NaOCH}_3) & \quad \rightarrow \quad \text{CH}_3\text{SCH}_2\text{CH}_2\text{COOH} \\
\text{(X)} & \quad \rightarrow \quad \text{CH}_3\text{SCH}_2\text{CH}_2\text{COOH} \\
\text{(XI)} & \quad \rightarrow \quad \text{NH}_2
\end{align*}
\]

Addition of methylmercaptan (VII) to methyl acrylate (VIII) gave methyl \( \beta \)-methylmercapto-propionate (IX), which was then condensed with methyl oxalate in the presence of potassium (or sodium) methoxide to give methyl \( \alpha \)-keto-\( \beta \)-carbomethoxy-\( \gamma \)-methylmercaptobutyrate (X). This substance was a new compound and the potassium salt was obtained as a crystalline form, while the free ester was a viscous oily substance, which gave the 2,4-dinitrophenylhydrazone in yellow prisms, m.p. 115–117°.

As for the hydrolysis and decomposition of the derivatives of oxaloacetate to obtain keto acid, a number of procedures such as heating with hydrochloric acid-acetic acid, with hydrobromic acid-acetic acid\(^6,7\), and boiling with dilute hydrochloric acid\(^8\), have been reported. Under these conditions, however, the compound (X) decomposed, accompanying the evolution of methylmercaptan and the desired product was not obtained. Therefore the conditions of hydrolysis and decarboxylation were examined. As a result, it was found that heating of compound (X) with 5% hydrochloric acid at 70–75° accomplished the reaction. A mixture of reactants which at first formed two layers became homogeneous and the evolution of carbon dioxide was observed with proceeding of the reaction. The \( \alpha \)-keto acid (VI) thus obtained, was an oily substance of which an aqueous solution was neutralized with sodium hydroxide, and acetone was added. The separated precipitate was recrystallized from water and methyl alcohol to yield the crystalline sodium salt, of which analysis agreed with calculated values. For further confirmation of the obtained \( \alpha \)-keto acid (IV), 2,4-dinitrophenylhydrazone was prepared from the hydrochloric acid solution and paperchromatography and elemental analysis were performed. Furthermore, the 2,4-dinitrophenylhydrazone hydrogenated by tin in hydrochloric acid and the product was identified as being DL-methionine. When 2,4-dinitrophenylhydrazone was prepared from the methanol solution in the presence of sulfuric acid, 2,4-dinitrophenylhydrazone of the \( \alpha \)-keto acid methyl ester was obtained.

Thus, the procedure described above seemed to be convenient for the preparation of \( \alpha \)-keto-\( \gamma \)-methylmercaptobutyric acid.

**EXPERIMENTAL**

*Ethyl \( \alpha \)-Acetyl-\( \gamma \)-Methylmercaptobutyrate (III)*\(^9,10,11\)

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Metallic sodium (3.3 g) was dissolved in absolute alcohol (90 ml) and ethyl acetoacetate (I) (30 g) was added. To the above mixture, $\beta$-methylmercaptoethylchloride (II) (26 g) was added dropwise with stirring at 30-35°, and boiled on a water-bath for 3.5 hours. After standing overnight the separated sodium chloride was filtered off. The filtrate was concentrated and extracted with ether. The ethereal extract was dried over anhydrous sodium sulfate, evaporated, and the residue was distilled in vacuo to yield 26.4 g (56.1%) of III, b.p. 127-128°/5 mm.

Ethyl $\alpha$-Isonitroso-$\gamma$-Methylmercaptobutyrate (IV)$^{9,12}$

Metallic sodium (2.9 g) was dissolved in absolute alcohol (500 ml) and III (2.6 g) was added. The mixture was cooled to 10° and with stirring, n-butyl nitrite (14 g) was added dropwise for 20 minutes. Stirring was further continued for 2.5 hours at the same temperature, and the reaction mixture was then concentrated in vacuo. To the residue, a small amount of ice water was added and acidified with sulfuric acid. The separated oily substance was extracted with ether, and the ethereal layer was dried over anhydrous sodium sulfate. After evaporation of the solvent, the residue was distilled in vacuo to yield 18 g (75.0%) of IV, b.p. 143-145°/1.5 mm.

Ethyl $\alpha$-Keto-$\gamma$-Methylmercaptobutyrate (V)$^{9}$

To a solution of IV (18 g) dissolved in 83% formic acid (40 ml), nitrosylhydrosulfate (13 g) was added over the period of 50 minutes with stirring, while the temperature was kept below 3° during the addition. After the addition stirring was further continued until the temperature of the reaction mixture came to room temperature. At the end of the reaction, the dark red mixture turned to pale yellow. The reaction mixture was poured into ice water (100 ml) and extracted with ether. The ethereal layer was dried over anhydrous sodium sulfate, and evaporated. From the residue formic acid was removed by distillation under reduced pressure (20 mm), and the residue was then extracted with ether. The ethereal extract was washed with aqueous sodium bicarbonate, dried over anhydrous sodium sulfate. After evaporation of the solvent, the residue was distilled in vacuo to give 1.5 g (8.8%) of the product, b.p. 83-86°/2 mm.

2,4-Dinitrophenylhydrazone: Yellow prisms, m.p. 115-117°. Anal. Calcd. for C_{13}H_{18}O_{6}N_{4}S: C, 43.0; H, 4.03; N, 14.00. Found: C, 43.02; N, 4.24; N, 14.33. In place of potassium, sodium could be used in this reaction.

$\alpha$-Keto-$\beta$-Methyl mercaptobutyric Acid (VI)

i) From X—A mixture of X (23.5 g), cone. hydrochloric acid (47 ml) and water (230 ml) was stirred at 70-75° for 7 hours. The reaction mixture which formed two layers at the initial stage of the reaction turned to a clear pale yellow solution at the end of the reaction. Sodium chloride was added to the reaction mixture which was extracted with ether. The ethereal layer was washed with aqueous sodium bicar-
bonate to dissolve the acidic substance. The aqueous
layer was washed with ether, acidified with hydro-
chloric acid, salted out with sodium chloride, and
extracted with ether. The ether extract was washed
with water and dried over anhydrous sodium sulfate.
Evaporation of ether left viscous oil of VI; yield 9.6 g
(60.8%).

Sodium salt: VI was dissolved in water and ad-
justed to pH 6.8 with sodium hydroxide. After the
solution was allowed to stand overnight, acetone was
then added, and the separated precipitate was collected
by filtration. Recrystallization from water, and sub-
sequent recrystallization from methanol yielded color-

2,4-Dinitrophenylhydrazone: The substance from
the dilute hydrochloric acid solution was recrystallized
from methanol-acetic acid mixture to give yellow
needles; m.p. 150-151 °. Anal. Calcd. for C₁₅H₁₄N₄O₆S: C, 40.25; H, 3.69; N, 17.07. Found: C,
40.15; H, 3.94; N, 16.82.

Paperchromatography of the 2,4-dinitrophenylhy-
drazone was carried out by the ascending method
employing n-butanol saturated with 3% aqueous
ammonia as a developing solvent. The obtained RF
value was 0.62 (13°, Toyo filter paper No. 50) which
agrees with the value reported15).

When 2,4-dinitrophenylhydrazone was prepared from
the methanol-sulfuric acid solution, esterification oc-
curred and 2,4-dinitrophenylhydrazone of methyl a-
keto-γ-methylmercaptobutyrate was obtained as yellow
41.95; H, 4.11; N, 16.04.

15) D. Cavallini, N. Fronta I and C. Toschi, Nature, 163, 568
(1949).

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