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

THE RESOLUTION OF SYNTHETIC METHIONINE

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The question of the structure of methionine, the sulfur-containing amino acid first isolated from protein by Mueller,[1] was rather conclusively settled by the work of Barger and Coyne.[2] They synthesized γ-methylthiol-α-amino-*n*-butyric acid and showed that this substance was undoubtedly the racemic form of natural methionine. The new method of synthesis[3] of this amino acid which has recently been described has made available a considerable quantity of the racemic product and has made it possible to accomplish the resolution.

The resolution has been accomplished by the general method which was developed by Fischer[4] for other α-amino acids. The formyl derivative was obtained by his general method and the salts with *d*-α-phenylethylamine, strychnine and brucine were prepared. The brucine salt was used for the resolution. It is interesting to note that although the brucine salts of *d*- and *l*-formylmethionine differed considerably in solubilities and melting points, they had almost identical optical rotations.

Mueller[1] has recorded the rotation of the methionine which he isolated as $-7.2°$. A product isolated by his procedure in this Laboratory[5] had a rotation of $-6.87°$ ($\pm 0.5°$). The rotations obtained on the two isomers of the amino acid isolated by this resolution were $+8.7$ and $-8.1°$, respectively.

Odake[6] has reported a rotation of $-11.77°$ on a sample of methionine isolated from the products of hydrolysis of yeast. No evidence of such high rotatory power has been obtained in this investigation. It is interesting to note that the direction of the rotation of *d*-methionine changes in either acid or alkaline solution.

Further proof of the identity of the synthetic *l*-isomer with natural methionine was obtained by comparisons of the formyl, α-naphthylurea and *p*-tolylurea derivatives of these two products.

Experimental Part

***dl*-Formylmethionine.**—A mixture of 25 g. of *dl*-methionine and 38 g. of absolute formic acid was heated for three hours on a steam-bath in a small flask fitted with a short

[1] Mueller, *J. Biol. Chem.*, **56**, 157 (1923).

[2] Barger and Coyne, *Biochem. J.*, **22**, 1417 (1928).

[3] Windus and Marvel, THIS JOURNAL, **52**, 2575 (1930). The procedure described in this reference has now been improved in some of the details. A complete description of the preparation of the amino acid has been submitted to "Organic Syntheses" for publication.

[4] Fischer, *Ber.*, **38**, 3997 (1905).

[5] We are indebted to Professor W. C. Rose and Mrs. Harriett King Klabunde for the natural methionine which we used in this work.

[6] Odake, *Biochem. Z.*, **161**, 446 (1925).

reflux condenser and protected from the air by a calcium chloride tube. After this heating period, the excess formic acid and water were removed by distillation under reduced pressure. The residue was again heated for three hours with 25 g. of absolute formic acid. The solution was then evaporated under diminished pressure and the treatment with a fresh 25-g. portion of absolute formic acid again repeated. After removing the excess formic acid and water, the hot residue was dissolved in about 400 cc. of hot ethyl acetate and the solution was filtered. If the filtrate was not completely clear, it was heated and again filtered. On cooling in an ice-bath, the formylmethionine crystallized. The crystals were collected on a filter, the filtrate was concentrated to about 100 cc. and a second fraction was obtained. The yield at this point was 21.5 g. of a product which melted at 99–100°. By careful evaporation of the mother liquor and recrystallization of the material thus obtained, another 2 g. of pure formyl derivative was isolated. The total yield of pure product reached 23.5 g. (79% of the theoretical amount).

Anal. (Parr bomb). Subs., 0.4168: $BaSO_4$, 0.5503. Calcd. for $C_6H_{11}O_3NS$; S, 18.10. Found: S, 18.13. Subs., 0.1875: 10.26 cc. of 0.1015 *N* NaOH. Calcd. for $C_6H_{11}O_3NS$: neutral equivalent, 177. Found: 180.

Ethyl acetate did not dissolve the unreacted amino acid and from the material insoluble in this solvent about 1.5 g. of pure *dl*-methionine was recovered by dissolving the crude product in water, adding a little (2–3 cc.) pyridine and precipitating the amino acid by the addition of two volumes of alcohol.

Formylmethionine was also prepared by refluxing the solution of methionine in absolute formic acid. However, the product was less pure and the yield was lower. The scheme of formylating by distilling a mixture of toluene, formic acid and methionine[7] was also tried but was not as satisfactory as the method already described.

Resolution of *dl*-Formylmethionine.—To a solution of 30 g. of formyl derivative in 5000 cc. of hot absolute alcohol was added 48 g. of recrystallized anhydrous brucine (slightly more than one-half the theoretical amount to form the neutral salt). The solution was allowed to stand at about 0° for four days and then filtered. The first crop of crystals weighed 51.5 g. (Fraction 1) and melted at 144–146°.

Rotation. 0.2000 g. made up to 25 cc. with water at 20°, 2-dm. tube: α_D −0.34° (±0.01°); $[\alpha]_D^{25}$ −21.25° (±0.5°).

Fraction 1 was recrystallized from 1500 cc. of absolute alcohol. On boiling, the salt did not go completely into solution. On cooling the solution for one day at 0° and filtering there was obtained 47 g. of salt, m. p. 144–145°.

Rotation. 0.2001 g. made up to 25 cc. with water at 20°, 2-dm. tube: α_D −0.38° (±0.01°); $[\alpha]_D^{25}$ −23.74° (±0.5°).

Anal. (Parr bomb). Subs., 0.4011: $BaSO_4$, 0.1601. Calcd. for $C_{29}H_{37}O_7N_3S$: S, 5.60. Found: S, 5.48.

The filtrate from this recrystallization was added to the original filtrate from Fraction 1 and 50 g. of brucine was added. After standing at 0° for about twenty-four hours, the solution was filtered and 10.5 g. (Fraction 2) of salt was obtained. This salt softened at about 140° and melted with decomposition at 185–190°. It was not used in further work.

The filtrate from Fraction 2 was evaporated to a volume of about 3 liters and allowed to stand at 0° for about twenty-four hours. The precipitate was collected on a filter and after air-drying weighed 29.5 g. (Fraction 3). It softened at 192° and melted at 194–196°.

The filtrate from Fraction 3 was evaporated to about 300 cc. and on cooling to 0°

[7] Steiger, *J. Biol. Chem.*, **86**, 695 (1930).

for about twenty-four hours an additional 7 g. (Fraction 4) of salt was obtained. This fraction softened slightly at 155° and melted at 185–187°. Fractions 3 and 4 were combined.

Rotation. 0.2000 g. made up to 25 cc. with water at 20°, 2-dm. tube: α_D −0.32° (±0.01°); $[\alpha]_D^{25}$ −20.00 (±0.5°).

The strychnine salt was prepared from formylmethionine in absolute alcohol solution. No fractionation was obtained from this solvent because the salt was too soluble. From ethyl acetate a crystalline product melting at 138–140° with a rotation of −20.57° was obtained. The more soluble fraction was obtained as a solid melting at 60–63° with a rotation of −16.25°. These products were analyzed for sulfur and found to be the desired strychnine salts. However the separation was not satisfactory and further work was abandoned in favor of the brucine salt.

The *d*-α-phenylethylamine salt of formylmethionine was formed in ethyl acetate solution and a product melting at 89–90° which analyzed correctly was isolated. However, the rotations were extremely low and no fractionation was obtained.

Isolation of *d*- and *l*-Formylmethionine.—To a solution of 47 g. of the least soluble brucine salt in 100 cc. of water was added 500 cc. of a saturated solution of barium hydroxide. The solution was chilled for thirty minutes in an ice–salt mixture and filtered. The filtrate was extracted with three 150-cc. portions of chloroform and one 150-cc. portion of ether. The solution was then exactly neutralized with 1 *N* sulfuric acid so that it gave no test for barium or sulfate ions. After removal of the barium sulfate by filtration, the water was evaporated under reduced pressure until the volume was about 40 cc. The residue was extracted with two 100-cc. portions of hot ethyl acetate. The ethyl acetate solution was filtered and concentrated to about 25 cc. on a hot-plate. It was then further concentrated by drawing a current of air over the solution at ordinary temperatures. Crystals separated and were collected on a filter. The first fraction amounted to 5.8 g. of a product which melted at 99–100°. Two grams of less pure material was obtained by evaporation of the mother liquors.

Titration. Subs., 0.1499: 7.79 cc. of 0.1070 *N* NaOH. Calcd. for $C_6H_4O_3NS$. Neutral equivalent, 177. Found: 179.8.

Anal. Subs., 0.1013: $BaSO_4$, 0.1334. Calcd. for $C_6H_{11}O_3NS$: S, 18.18. Found: S, 18.13.

Rotation. 0.2002 g. made up to 25 cc. with water at 20°, 2-dm. tube: α_D +0.17 (±0.01°); $[\alpha]_D^{25}$ +10.62° (±0.5°).

l-Formylmethionine was obtained by approximately the same procedure. From 36 g. of the more soluble brucine salt, 4.2 g. of product which melted at 99–100° was obtained. In order to obtain the *l*-isomer in the crystalline form, the ethyl acetate solution was evaporated to about 25 cc. and then ether was added to the hot solution until it became turbid. On cooling the formyl derivative separated.

Rotation. 0.2000 g. made up to 25 cc. with water 20°, 2-dm. tube: α_D −0.16 (±0.01°); $[\alpha]_D^{25}$ −10.00 (±0.5°).

Some of the *l*-formyl derivative remained in the water solution and was not extracted by the ethyl acetate. This water solution was boiled with a little hydrochloric acid and the derivative hydrolyzed as described below to give a sample of *l*-methionine of the highest rotatory power which was any sample of this resolution.

***d*- and *l*-Methionine.**—A solution of 1 g. of *d*-formylmethionine in 10 cc. of 10% hydrochloric acid was heated on the steam cone for one hour. The solution was concentrated to one-half of its original volume under reduced pressure, and to it was added 5 cc. of pyridine. The methionine was precipitated by adding three volumes of alcohol. After thoroughly chilling the solution, the *d*-methionine was collected on a filter. The yield was 0.450 g.

Rotation. 0.1997 g. made up to 25 cc. with water, 2-dm. tube: α_D +0.14 (±0.01°); $[\alpha]_D^{25}$ +8.76° (±0.5°).

This sample was recrystallized by the same procedure and gave 0.4 g. of product.

Rotation. 0.2000 g. made up to 25 cc. with water, 2-dm. tube: α_D +0.13° (±0.01°); $[\alpha]_D^{25}$ +8.12° (±0.5°). 0.2004 g. made up to 25 cc. with 5% sodium bicarbonate at 20°, 2-dm. tube: α_D −0.12° (±0.01°); $[\alpha]_D^{25}$ −7.47° (±0.5°). 0.2006 g. made up to 25 cc. with 0.2001 *N* hydrochloric acid at 20°, 2-dm. tube: α_D −0.34° (±0.01°); $[\alpha]_D^{25}$ −21.18° (±0.5°).

Anal. Subs., 0.1086: $BaSO_4$, 0.1705. Subs., 3.069 mg.: 0.263 cc. of N_2 at 27° and 750 mm.[8] Calcd. for $C_5H_{11}O_2NS$: S, 21.50; N, 9.40. Found: S, 21.53; N, 9.54.

l-Methionine was obtained by the same procedure. From 1 g. of *l*-formylmethionine there was obtained 0.5 g. of product.

Rotation. 0.2000 g. made up to 25 cc. with water at 20°, 2-dm. tube: α_D −0.12° (±0.01°); $[\alpha]_D^{25}$ −7.5° (±0.5°).

Anal. Subs., 3.130 mg.: 0.273 cc. of N_2 at 28° and 748 mm. Calcd. for $C_5H_{11}O_2NS$: N, 9.40. Found: N, 9.73.

The water solution of *l*-formylmethionine left after the extraction with ethyl acetate was hydrolyzed as mentioned above and the *l*-methionine was isolated by the usual procedure. This yielded 0.3 g. of *l*-methionine.

Rotation. 0.2004 g. made up to 25 cc. with water at 20°, 2-dm. tube: α_D −0.13° (±0.01°); $[\alpha]_D^{25}$ −8.11 (±0.5°).

Comparison of Natural Methionine with the *l*-Isomer of Synthetic Methionine.—A sample of natural methionine was isolated from hydrolyzed casein by the procedure described by Mueller.[1]

Rotation. Subs., 0.2000 g. made up 25 cc. with water at 20°, 2-dm. tube: α_D −0.11° (±0.01°); $[\alpha]_D^{25}$ −6.87° (±0.5°).

Natural methionine was converted to the formyl derivative by warming 0.2 g. of material with excess formic acid for one hour, and evaporating under reduced pressure. This was repeated two more times and the formyl derivative was freed from unchanged amino acid by dissolving it in ethyl acetate. The product was crystallized from an absolute alcohol and petroleum ether mixture and melted at 98–99°.

A sample of *l*-formylmethionine was similarly prepared from some of the *l*-methionine obtained by the resolution. This product melted at 99–100°. When a sample of this product was mixed with the previous material, the mixture melted at 98–99°.

A sample of synthetic *l*-methionine was treated with α-naphthyl isocyanate according to the directions of Mueller[1] and the α-naphthylurea derivative melting at 187–188° was obtained. A sample of this derivative was also made from natural methionine. This product melted at 187.5–188.5°. A mixture of the two derivatives melted at 187–188°.

In a similar manner synthetic *l*-methionine was treated with *p*-tolyl isocyanate to yield the *p*-tolylurea derivative. After recrystallization from ethyl alcohol this derivative melted at 157–158°.

Anal. Subs., 3.149 mg.: 0.280 cc. of N_2 at 28° and 748 mm. Calcd. for $C_{13}H_{18}O_3N_2S$: N, 9.93. Found: N, 9.92.

The same derivative prepared from natural methionine melted at 158° and the mixture of the derivative of the synthetic product and that of the natural product melted at 157–158°.

***dl*-Benzoylmethionine.**—To a solution of 4 g. of sodium bicarbonate and 5 g. of *dl*-methionine in 100 cc. of water there was slowly added 6 g. of benzoyl chloride. The

[8] All micro analyses reported in this paper were carried out by Mr. K. Eder.

mixture was shaken until the odor of benzoyl chloride had disappeared. The mixture was cooled and acidified to Congo paper with hydrobromic acid. On standing a sticky solid separated and was collected on a filter.

This crude product was recrystallized from dilute alcohol and the crystals thus obtained were washed repeatedly with petroleum ether to remove the benzoic acid. The final yield was 6.5 g. (80% of the theoretical amount) of a product which melted at 143–145°.

Anal. Subs., 3.610 mg.: 0.189 cc. of N_2 at 25° and 743 mm. Calcd. for $C_{12}H_{15}O_3NS$: H, 5.61. Found: N, 5.53.

Some of the *d*-α-phenylethylamine salt was prepared in ethyl acetate but it was not found useful for resolution.

Summary

Synthetic γ-methylthiol-α-amino-*n*-butyric acid has been resolved and the levo isomer has been found to be identical with the naturally occurring amino acid, methionine.

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THE HALOFORM REACTION. III. TRIHALOACETYL DERIVATIVES OF MESITYLENE, DURENE AND ISODURENE

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When it was discovered that solutions of the hypohalites converted certain di-ortho-substituted acetophenones into the corresponding trihalomethyl ketones,[1] the generality of the reaction was tested by trying it with a number of similar ketones. The present report includes the results obtained with the monoacetyl and diacetyl derivatives of mesitylene, durene and isodurene.

Derivatives of Mesitylene.—In addition to the trichloroacetylmesitylene (I) and tribromoacetylmesitylene (II) previously reported,[2] there have now been prepared the analogous di-(trichloroacetyl)-mesitylene (IV) and di-(tribromoacetyl)-mesitylene (VI). These were obtained by treating diacetylmesitylene (V) with solutions of sodium hypochlorite and sodium hypobromite, respectively. Both compounds are colorless solids which crystallize well. The hexachloro compound melts at 95–95.3° and the hexabromo compound at 160–161.5°. Di-(trichloroacetyl)-mesitylene was also prepared from di-(chloroacetyl)-mesitylene (III) by treatment with a solution of sodium hypochlorite.

Derivatives of Durene.—Acetyldurene (VIII) when treated with a solution of sodium hypobromite was slowly but completely converted into

[1] Fuson and Walker, THIS JOURNAL, **52**, 3269 (1930).

[2] Ref. 1, p. 3273.