THE SYNTHESIS OF DL-β,β-DIETHYLCYSTEINE AND DL-β-ETHYL-β-METHYLCYSTEINE

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The various penicillins of natural origin that have so far been isolated (1-4) differ from one another only in the nature of the R group, as illustrated in the general formula (I) based on the β-lactam structure for penicillin. However, the results of chemical studies in this and other laboratories (5, 6) indicate that several penicillins in which various groups have been substituted at R' and R" (I) have been synthesized in minute yield, although as yet these substances have not been isolated in pure form. These penicillins were prepared by the condensation of an oxazolone (II) with the appropriate α-amino-β-mercapto acid (III).

For a study (7) of further variations of the penicillin molecule at R' and R", it was desired to prepare additional α-amino-β-mercapto acids for condensation with an appropriate oxazolone. The synthesis of two such compounds, namely DL-β,β-dicetylcysteine (IIIa) and DL-β-ethyl-β-methylcysteine (IIIb), is the subject of the present paper. During the
war-time studies on penicillin, the investigators at the Abbott Laboratories reported the preparation of \( \beta \)-ethyl-\( \beta \)-methylcysteine (IIIb) (8), but did not fully characterize the final product. Consequently the details of its preparation and isolation as the hydrochloride monohydrate (VIIIb) are included here.

The series of reactions used for the synthesis of these two \( \alpha \)-amino-\( \beta \)-mercapto acids was similar to that already developed for the synthesis of DL-penicillamine (IIIc) (8).

\[
\begin{align*}
\text{R'} & \quad \text{CHCHCOOH} & \quad \text{ClCH}_2\text{COCl} & \quad 4\text{NNaOH} & \quad \text{R'} & \quad \text{CHCHCOOH} & \quad \text{Ac}_2\text{O} \\
\text{R''} & \quad \text{NH}_2 & & & & \text{R''} & \quad \text{NH—COCH}_2\text{Cl} \\
\text{(IVA,b)} & & & & & & \\
\text{R'} & \quad \text{C} & \quad \text{C} & \quad \text{O} & \quad \text{NH} & \quad \text{COCH}_3 & \quad \text{C} & \quad \text{C} & \quad \text{O} & \quad \text{SH} & \quad \text{NHCOCH}_3 \\
\text{R''} & \quad \text{N} & \quad \text{O} & \quad \text{C} & \quad \text{CH}_3 & & & & & & \\
\text{(VIIA,b)} & & & & & & \\
\text{R'} & \quad \text{C} & \quad \text{CH—COOH} & \quad \text{SH} & \quad \text{NH}_2\cdot\text{HCl}\cdot\text{H}_2\text{O} & \quad \text{R''} & \quad \text{C} & \quad \text{CH—COOH} & \quad \text{SH} & \quad \text{NH}_2\cdot\text{HCl}\cdot\text{H}_2\text{O} & \quad \text{(VIIIa,b)} \\
\text{(a) (R' = R'' = C}_2\text{H}_5); & \text{(b) (R' = C}_2\text{H}_5, \text{R'' = CH}_3) \\
\end{align*}
\]

In the above reactions the commercially available \( \text{DL-Isoleucine (IVb)} \) served as a starting compound for the synthesis of \( \text{DL-\( \beta \)-ethyl-\( \beta \)-methylcysteine hydrochloride monohydrate (VIIIb)} \). However, the \( \text{DL-\( \beta \),\( \beta \)-diethylalanine (IVa)} \) needed as a starting compound for the preparation of \( \text{DL-\( \beta \),\( \beta \)-diethylcysteine hydrochloride monohydrate (VIIIa)} \) had not been prepared previously. The method devised for the synthesis of the \( \text{DL-\( \beta \),\( \beta \)-diethylalanine} \) is outlined in the accompanying equations.

\[
\begin{align*}
\text{C}_2\text{H}_4\text{OCH}&&\text{C(COOCC}_2\text{H}_4)\text{2} & \quad \text{C}_2\text{H}_2\text{MgBr} & \quad \text{Then H}^+ & \quad \text{CHCH(COOCC}_2\text{H}_4)\text{2} & \quad \text{C}_2\text{H}_5 \\
\text{(IX)} & & & & & & \text{(X)}
\end{align*}
\]
Diethyl (1-ethylpropyl)-malonate (X) was prepared in 80 per cent yield by the action of ethyl magnesium bromide with ethoxymethyleneenamalic ester (IX) according to the procedure of Reynolds (9). The malonic ester derivative (X) was converted to β, β-diethylalanine (IVa) by the malonic ester synthesis of amino acids (10). In this series of reactions the saponification of the malonic ester, bromination of the resulting acid, and decarboxylation of the α-bromo acid proceeded smoothly. However, when aqueous ammonia was used to aminate the α-bromo-β-ethylvaleric acid, which was not obtained in pure state, difficulty was encountered in the isolation of the amino acid from the reaction mixture. This difficulty was overcome by carrying out the amination in ethanolic ammonia.

**EXPERIMENTAL**

*Diethyl (1-Ethylpropyl)-malonate*—Ethyl magnesium bromide was prepared by the addition of 272 gm. of ethyl bromide in 300 cc. of dry ether to 61 gm. of magnesium in 400 cc. of dry ether. After the addition of the ethyl bromide had been completed, the reaction mixture was heated under gentle reflux. Then 216 gm. of ethoxymethyleneenamalic ester in 150 cc. of ether were added over the period of 2 hours. Cooling of the reaction mixture in a water bath was necessary during this addition. After the reaction mixture had cooled to room temperature, it was poured slowly onto a mixture of 215 cc. of 12 N HCl and 1 kilo of ice. The ether layer was separated, and the aqueous layer was shaken with three 200 cc. portions of ether. After the combined ether layers had been dried over anhydrous MgSO₄, the ether was removed and the residue was distilled. The diethyl (1-ethylpropyl)-malonate distilled at 112–113° and amounted to 183 gm.

1 All the melting points are corrected and are capillary melting points unless otherwise specified.
or 80 per cent of the theoretical amount based on ethoxymethylene-malonic ester.

\( \alpha \)-Bromo-\( \beta \)-ethyvaleric Acid—171 gm. of KOH were dissolved in 150 cc. of water and the solution was heated to 100°. To this solution, diethyl (1-ethylpropyl)-malonate (183 gm.) was added dropwise at first and then more rapidly as the reaction got under way. After the addition was complete, the reaction mixture was heated at 100° with stirring for 5 hours.

The contents of the flask were transferred to a beaker, cooled to 15°, and acidified by the addition of 274 cc. of 12 N HCl. A precipitate which formed after the addition of about 170 cc. of acid disappeared upon addition of the rest of the acid. The aqueous solution was shaken with three 200 cc. portions of ether and the combined ether layers were dried over CaCl₂. The ether solution was concentrated to a volume of about 400 cc. and bromine (37.8 cc., 113 gm.) was added. The first 3 to 5 cc. of bromine were added in one portion and the mixture was stirred until the color had disappeared. The remainder of the bromine was added dropwise over a period of about 1 hour. After the addition of bromine was complete, 140 cc. of water were added slowly so as not to produce foaming. The ether layer was separated and the aqueous layer was shaken with a 100 cc. portion of ether. The ether layers were combined, the ether was removed, and the residue was subjected to decarboxylation by heating under a reflux at 140° for 2 hours. The residue was distilled at 5 mm. and the crude \( \alpha \)-bromo-\( \beta \)-ethylvaleric acid was collected in two fractions (b.p. 106–125°, 30.1 gm., and b.p. 125–141°, 93.7 gm.). The total weight of these two fractions corresponded to 74 per cent of the theoretical amount based on diethyl (1-ethylpropyl)-malonate.

\( DL-\alpha,\beta \)-Diethylalanine—A mixture of 30 gm. of the crude \( \alpha \)-bromo-\( \beta \)-ethylvaleric acid (b.p. 125–141°) and 75 cc. of absolute ethanol was cooled to -70° and 45 gm. of liquid ammonia were added. The mixture was heated in an autoclave to 80° over a period of 5 hours. The ethanol and ammonia were then removed by a stream of air and the residue was washed with ether. Although the product consisted of a mixture of the desired amino acid and \( NH_3Br \), it was suitable for our use without further purification. The weight of the mixture amounted to 34.8 gm. When the fraction of \( \alpha \)-bromo-\( \beta \)-ethylvaleric acid boiling at 106–125° at 5 mm. was used, a lower yield of product was obtained.

A sample was purified for analysis by recrystallization from 70 per cent ethanol. On the hot stage the crystals of \( DL-\alpha,\beta \)-diethylalanine changed from prisms to needles at 170–185° and melted at 245–249° (micro).
**N-Chloroacetyl-DL-β, β-diethylalanine**—14.4 gm. of crude β, β-diethylalanine were dissolved in 16 cc. of 4 N NaOH and 30 cc. of water. While the solution was cooled in an ice bath, 14.3 gm. of chloroacetyl chloride and 55 cc. of 4 N NaOH were added dropwise with stirring. Then the solution was acidified with 6.3 cc. of 12 N HCl, causing the precipitation of the product. The crude N-chloroacetyl-DL-β, β-diethylalanine (m.p. 122–123.5°) weighed 8.2 gm.

27.0 gm. from several runs were dissolved in 100 cc. of ethanol and 250 cc. of water. The hot solution was treated with 1 gm. of norit and filtered. The N-chloroacetyl-DL-β, β-diethylalanine crystallized from the cooled solution, m.p. 127–129°.

\[ \text{C}_{22} \text{H}_{20} \text{O}_{\text{CIN}} \] Calculated. C 48.8, H 7.28, N 6.32

211.7 Found. “ 48.9, “ 7.50, “ 6.36

**2-Methyl-4-(1'-ethylpropylidene)-5(4)-oxazolone**—27.2 gm. of recrystallized N-chloroacetyl-β, β-diethylalanine and 40 cc. of acetic anhydride were heated in an oil bath at 60–70° for a period of 2 hours. At the end of this time the acetic anhydride was removed under reduced pressure (water pump) at 60° and the oxazolone was distilled at 54–55° at 0.01 to 0.05 mm. The yield was 11.1 gm. or 54 per cent of the theoretical amount.

For purposes of characterization the oxazolone was converted to α-acetamido-β, β-diethylacrylic acid by heating the oxazolone in an excess of water. The acrylic acid derivative was recrystallized from ethyl acetate to give prisms, m.p. 178–178.5° (with decomposition).

\[ \text{C}_{9} \text{H}_{15} \text{O}_{3} \text{N} \] Calculated. C 58.4, H 8.16, N 7.57


**α-Acetamido-β, β-diethylacrylamide** was also readily obtained by dissolving 0.2 gm. of the oxazolone in 10 cc. of 10 per cent NH₄OH. After the solution had cooled a crystalline precipitate formed, m.p. 198–214°. This was recrystallized from ethanol, m.p. 220–227°.

\[ \text{C}_{9} \text{H}_{16} \text{O}_{2} \text{N} \] (184.2). Calculated, N 15.21; found, N 15.33

**N-Acetyl-DL-β, β-diethylcysteine**—To 48.5 cc. of methanol was added 0.3 gm. of sodium and the resulting solution was saturated with H₂S. 11.0 gm. of 2-methyl-4-(1'-ethylpropylidene)-5(4)-oxazolone were added and H₂S was passed through the solution for 12 hours. The reaction mixture was acidified with 1.1 cc. of 12 N HCl and the methanol was removed by distillation under reduced pressure. The crystalline residue was dissolved in a mixture of 55 cc. of methanol and 225 cc. of water and treated with 1 gm. of charcoal (norit). The N-acetyl-DL-β, β-diethylcysteine (m.p. 158–161°) recovered from the solution weighed 8.9 gm. or 62 per cent of
the theoretical amount. After a sample of the crude compound had been recrystallized twice from aqueous ethanol, it possessed a melting point of 167–168°.

\[
\text{C}_9\text{H}_{17}\text{O}_{2}\text{NS}. \quad \text{Calculated.} \quad \text{C} 49.3, \quad \text{H} 7.82, \quad \text{S} 14.62 \\
\text{219.3} \quad \text{Found.} \quad \text{"} 49.3, \quad \text{"} 7.80, \quad \text{"} 14.03
\]

**DL-β,β-Diethylcysteine Hydrochloride Monohydrate**—7.2 gm. of the N-acetyl-DL-β,β-diethylcysteine were heated under a reflux with 100 cc. of 2 N HCl for 16 hours. After the reaction mixture had been concentrated to a volume of about 30 cc., a crystalline precipitate formed which weighed 2.68 gm., representing 35 per cent of the theoretical amount. A 100 mg. sample was recrystallized twice from 5 cc. of 12 N HCl. The twice recrystallized material was dissolved in 0.2 cc. of absolute ethanol and to this were added 2 cc. of absolute ether. The crystals thus obtained had a capillary melting point of 126–127°. However, when the crystals were heated slowly on the hot stage, they changed from prisms to needles at 152° and melted at 176–177° (micro).

\[
\text{C}_9\text{H}_{19}\text{O}_{2}\text{NS-HCl-H}_2\text{O}. \quad \text{Calculated.} \quad \text{C} 36.3, \quad \text{H} 7.83, \quad \text{Cl} 15.32 \\
\text{231.7} \quad \text{Found.} \quad \text{"} 36.4, \quad \text{"} 7.97, \quad \text{"} 15.38
\]

**2-Methyl-4-(sec-butylidene)-5(4)-oxazolone**—A mixture of 69.6 gm. of N-chloroacetyl-DL-isoleucine (11) and 110 cc. of acetic anhydride was agitated by a stream of nitrogen while being heated at 55–60° for 2 hours. After the acetic anhydride had been removed by distillation under reduced pressure (water pump), the residue was distilled at 0.01 to 0.2 mm. and the product distilling at 50–61° was collected. The yield of oxazolone was 41.0 gm. or 79 per cent of the theoretical amount. It was found advisable to use this material immediately for the preparation of N-acetyl-DL-β-ethyl-β-methylcysteine.

The oxazolone was characterized by conversion to α-acetamido-β-ethyl-β-methylacrylic acid (m.p. 174–175°) by heating the oxazolone in an excess of water.

\[
\text{C}_9\text{H}_{15}\text{O}_3\text{N}. \quad \text{Calculated.} \quad \text{C} 56.1, \quad \text{H} 7.65, \quad \text{N} 8.18 \\
\text{171.2} \quad \text{Found.} \quad \text{"} 56.2, \quad \text{"} 7.68, \quad \text{"} 8.01
\]

**N-Acetyl-DL-β-ethyl-β-methylcysteine**—1.15 gm. of sodium were dissolved in 190 cc. of methanol and the resulting solution was saturated with H₂S. 41 gm. of 2-methyl-4-(sec-butylidene)-5(4)-oxazolone were dissolved in 55 cc. of methanol and this solution was added to the sodium methyleate solution. H₂S was passed through the reaction mixture for 16 hours. The mixture was acidified with 4.5 cc. of 12 N HCl and the methanol was removed by distillation under reduced pressure. The residue was crystal-
alyzed from a mixture of 150 cc. of water and 5 cc. of methanol. This crude product (m.p. 138–138.5°) weighed 44.1 gm. or 80 per cent of the theoretical amount. After the crude product had been treated with charcoal (norit) and crystallized from water, the N-acetyl-DL-β-ethyl-β-methylcysteine possessed a melting point of 144–146.5° and was suitable for conversion to the amino acid. A sample prepared for analysis by two recrystallizations from water had a melting point of 144–145°.

\[
\text{C}_{13}\text{H}_{25}\text{O}_{3}\text{NS} \quad \text{Calculated.} \quad \text{C} \ 46.8, \ \text{H} \ 7.37, \ \text{S} \ 15.62 \\
205.3 \quad \text{Found.} \quad " \ 46.6, " \ 7.44, " \ 15.86
\]

**DL-β-Ethyl-β-methylcysteine Hydrochloride Monohydrate**—6.1 gm. of the recrystallized N-acetyl-β-ethyl-β-methylcysteine were heated under a reflux with 85 cc. of 2 N HCl for 16 hours. The volume of the reaction mixture was concentrated to about 20 cc. and the crystalline product was collected. The yield amounted to 2.41 gm. or 38 per cent of the theoretical amount. The β-ethyl-β-methylcysteine hydrochloride monohydrate was purified by recrystallization from 12 N HCl. As in the case of the β,β-diethylcysteine hydrochloride monohydrate, a difference was noted between the capillary melting point and that obtained on the hot stage. The capillary melting point was 117–119°, while that obtained on the hot stage was 169–170° (micro).

\[
\text{C}_{18}\text{H}_{31}\text{O}_{2}\text{NS}.\text{HCl}.\text{H}_{2}\text{O} \quad \text{Calculated.} \quad \text{C} \ 33.1, \ \text{H} \ 7.41, \ \text{Cl} \ 16.29 \\
217.7 \quad \text{Found.} \quad " \ 33.1, " \ 7.74, " \ 16.29
\]

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**SUMMARY**

The synthesis of DL-β,β-diethylalanine and its use in the preparation of DL β,β diethylcysteine hydrochloride monohydrate have been described. In addition, details have been presented for the synthesis of DL-β-ethyl-β-methylcysteine hydrochloride monohydrate from DL-isoleucine.

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