Preparation of the Enantiomers of *threo*- and *erythro*-2-Amino-3-mercaptobutyric Acid

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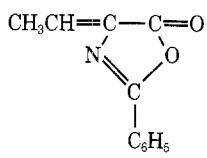
In a recent paper[1] we reported the synthesis of bisnorpenicillin V from D-cysteine. In view of a similar synthesis of the two norpenicillins, the D isomers of *threo*- and *erythro*-2-amino-3-mercaptobutyric acids (**1a** and **1b**) were needed. The synthesis of the two racemic forms of these amino acids has been described by Carter, *et al.*[2] The synthesis consists in the addition of benzyl mercaptan to 2-phenyl-4-ethylidene-5-oxazolone (**2**), hydrolysis of the reaction product, and separation of the two diastereoisomeric 2-benzamido-3-benzylmercapto-DL-butyric acids (**3a** and **3b**). The isomer of **3**, melting at 145–147°, which was soluble in benzene was designated as **a**, and the higher melting (181–184°) isomer as **b**. Removal of the *N*-benzoyl and *S*-benzyl groups afforded the two amino acids **1a** and **1b**.



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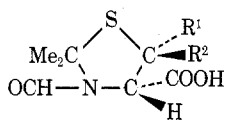
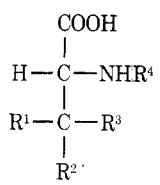


D enantiomer

4a, $R^1 = H; R^2 = Me$

b, $R^1 = Me; R^2 = H$

5, $R^1 = R^2 = Me$



D enantiomer

1a, $R^1 = SH; R^2 = Me; R^3 = R^4 = H$

b, $R^1 = R^4 = H; R^2 = Me; R^3 = SH$

3a, $R^1 = SBz; R^2 = Me; R^3 = H; R^4 = COC_6H_5$

b, $R^1 = H; R^2 = Me; R^3 = SBz; R^4 = COC_6H_5$

6, $R^1 = R^2 = Me; R^3 = SH; R^4 = H$

7a, $R^1 = OH; R^2 = Me; R^3 = R^4 = H$

b, $R^1 = R^4 = H; R^2 = Me; R^3 = OH$

8a, $R^1 = SBz; R^2 = Me; R^3 = H; R^4 = Ac$

b, $R^1 = H; R^2 = Me; R^3 = SBz; R^4 = Ac$

9a, $R^1 = SBz; R^2 = Me; R^3 = R^4 = H$

b, $R^1 = R^4 = H; R^2 = Me; R^3 = SBz$

10, $R^1 = R^2 = H; R^3 = SBz; R^4 = Ac$

11, $R^1 = R^2 = Me; R^3 = SBz; R^4 = Ac$

Evidence for a threo configuration for isomer **a** and an erythro configuration for isomer **b** was obtained by the study of the nmr spectra of the *N*-formyl-4-carboxythiazolidines **4a**, **4b**, and **5** obtained by reaction of DL-**1a**, DL-**1b**, and D-penicillamine (**6**) with acetone, followed by N-formylation. The assignment of the relative configuration was based on the comparison of the chemical shift values (δ) of the C-5 methyl protons. The nmr spectra of the three *N*-formylthiazolidines recorded in DMSO-d_6 showed the presence of two conformational isomers with a low rate of interconversion.[3] The C-5 methyl protons of **4a** (δ 1.44 ppm) appeared at a lower field than those of **4b** (δ 1.28 ppm). Similar shift differences for these methyl protons were observed in the spectrum of **5** (δ 1.58 and 1.38 ppm). Assignment of the methyl resonances of the latter was obtained by an intramolecular nuclear Overhauser effect (NOE).[4] Irradiation of the low-field methyl protons (δ 1.58 ppm) gave a NOE of the C-4 methine proton (25%), whereas a negligible increase of the intensity of this proton was observed upon irradiation of the high-field methyl protons (δ 1.38 ppm). This indicates that the high-field resonance signal (δ 1.38 ppm) can be assigned to the methyl group in the cis position to the C-4 carboxyl group (and consequently the low-field signal to the methyl group in the trans position).[5] Comparison of these data with shift differences observed for **4a** and **4b** suggest a trans relationship of the C-5 methyl and the C-4 carboxyl group for the **a** isomer and a cis relationship for the **b** isomer. Thus the threo configuration can be assigned to the amino acid **1a** and the erythro configuration to **1b**. Additional nmr data and a detailed discussion of the conformations of these thiazolidines are given in a separate paper.[6] Arnstein[7] proposed a threo configuration for **1b**. His assignment was based on the conversion of DL-threonine (**7a**) and DL-allothreonine (**7b**) into DL-**1b** *via* a thiazoline derivative. Since DL-**1b** was isolated from both DL-**7a** and DL-**7b**, he suggested that inversion at C-2 occurred during ring closure of the *N*-thiobenzoyl ester of **7b** and inversion at C-1 and C-2 during the conversion of **7a** into **1b**. Since no arguments are given for these inversions, we feel that no conclusions should be drawn from these inversions regarding the relative configuration of **1a** and **1b**.

For the separation of the enantiomers, the 2-acetamido-3-benzylmercapto-DL-butyric acid **8a** was incubated with acylase. The rate of hydrolysis was extremely slow, even in the presence of Co^{2+}. Only 7.4% of the theoretical amount of L-**9a** could be isolated after a 400-hr incubation.[8] It should be noted that *N*-acetyl-*S*-benzyl-DL-cysteine (**10**) could be resolved with this method,[9] but that *N*-acetyl-*S*-benzyl-L-penicillamine (**11**) is almost resistant to the action of acylase.[10]

Subsequently the fractional crystallization of amine salts of *N*-formylthiazolidine carboxylic acids, a method which has been used for the separation of the enantiomers of penicillamine,[11] was applied. Fractional crystallization of the brucine salt of DL-*threo*-*N*-formyl-2,2,5-trimethyl-4-carboxythiazolidine (**4a**) from water yielded two fractions. The less soluble fraction afforded 33.5% (calculated on DL-**4a**) of D-**4a** with $[\alpha]^{25}D$ +81°, the other 32.5% of L-**4a** with $[\alpha]^{25}D$ −78.5°. When a similar experiment was carried out on DL-**4b**, thiazolidines with low specific rotation were obtained. Therefore another optically active base was chosen for the **b** isomer. Fractional crystallization of the dehydroabietylamine salt of DL-**4b** gave a less soluble fraction, from which 37.8% (calculated on DL-**4b**) of the thiazolidine D-**4b** with $[\alpha]^{25}D$ +89° was isolated. The more soluble fraction yielded 25.7% of the L enantiomer of **4b** with $[\alpha]^{25}D$ −88.5°. Hydrolysis of the enantiomers of **4a** and **4b** afforded the hydrochlorides of **1a** and **1b**, which were converted into their neutral forms. Optical rotations of the enantiomers of the amino acids (neutral forms) measured in water and in 1 *N* hydrochloric acid are summarized in Table I. The values obtained for the amino acids recovered from the less soluble brucine or dehydroabietylamine salts of **4a** and **4b** are given under the heading M, those obtained from the more soluble salts under the heading N. It can be seen from this table that acidification causes the molecular rotations to become more negative in the M series and more positive in the N series. According to the Clough–Lutz–Jirgensons rule[12] the M series thus has the D configuration, the N series the L configuration. A similar shift of the rotation induced by acidification was observed for the structurally related

Table I
Optical Rotations of 2-Amino-3-mercaptobutyric Acids

Compd	Solvent	M series[a] $[\alpha]^{25}D$[c]	M series[a] $[M]D$	N series[b] $[\alpha]^{25}D$[c]	N series[b] $[M]D$
1a					
Hydrochloride	1 *N* HCl	+13.5°	+2316	−13°	−2230
Neutral form	H_2O	+36°	+4866	−35°	−4731
Neutral form	1 *N* HCl	+17.5°	+2366	−17°	−2298
1b					
Hydrochloride	1 *N* HCl	−35°	−6004	+35.5°	+6090
Neutral form	H_2O	−17°	−2298	+17°	+2298
Neutral form	1 *N* HCl	−45°	−6083	+45°	+6083
D-penicillamine (**6**)[d]					
Hydrochloride	1 *N* HCl	+1.6°	+297		
Neutral form	H_2O	+25.3°	+3580		

[a] Amino acids recovered from less soluble salts of **4** with optically active bases. [b] Amino acids recovered from more soluble salts of **4** with optically active bases. [c] Measured at *c* 1. [d] Determined on a sample of D-**6** HCl with $[\alpha]D$ −42.2° (*c* 1, 1 *N* NaOH) (−55.3° calculated for the neutral form).

D-penicillamine. Another argument for this assignment was obtained by S-benzylation of L-**1a**. The reaction product showed $[\alpha]^{25}D$ +72° (*c* 1, 1 *N* HCl), which is in agreement with the value determined for this compound, obtained by incubation of DL-**8a** with acylase.

Experimental Section

Melting points were taken in open capillary tubes and are uncorrected. Solvents were evaporated under reduced pressure. Compound **5** was prepared as reported by Leach, *et al.*[11b] Hog kidney acylase (1175 μ/mg) was obtained from Sigma Chemical Co. Dehydroabietylamine was isolated from "Amine D" (Hercules Powder Co.) as described by Gottstein, *et al.*[13] Mass spectra were recorded on an AEI MS 12 spectrometer, ir spectra (KBr pellets) with a Perkin-Elmer 257 spectrometer. Nmr spectra were obtained on a Varian XL 100-15 spectrometer. Chemical shifts (δ) are reported in parts per million downfield from TMS.

2-Amino-3-mercapto-DL-butyric acid (DL-1). The condensation of acetaldehyde with hippuric acid in acetic anhydride in the presence of sodium acetate[14] gave an 85% yield of 2-phenyl-4-ethylidene-5-oxazolone (**2**). The crude product with mp 78–90° (the unsharp melting point is probably due to a mixture of cis and trans isomers, which can be differentiated by tlc) gave the same yield in the next step as the purified product[14] with mp 90–92°. Addition of benzyl mercaptan and hydrolysis gave the two diastereoisomeric 2-amino-3-benzylmercaptobutyric acids DL-**9a** and DL-**9b**. The benzyl group was removed by reaction with sodium in liquid ammonia.[2]

DL-*N*-Formyl-2,2,5-trimethyl-4-carboxythiazolidine. A. Threo Isomer (DL-4a). Crude DL-**1a** (27.04 g, 200 mmol) was suspended in 1000 ml of acetone containing 22.5 ml of concentrated HCl and refluxed for 6 hr under a nitrogen atmosphere. The hot suspension was filtered off and the residue was extracted twice with 250 ml of boiling absolute EtOH. The residue obtained on evaporation of the EtOH extracts was dried and taken up in a mixture of 300 ml of HCOOH (98–100%) and 13 g of HCOONa. Acetic anhydride (100 ml) was added dropwise in 3 hr to the cooled mixture. The solution was kept overnight in the refrigerator and evaporated to dryness after addition of 200 ml of water. The residue was recrystallized from boiling water, yielding 24.7 g (60.7%) of DL-**4a**: mp 163–167°; *m/e* 203; nmr[3] (TMS, DMSO-d_6) δ 1.44 (d, C-5 Me), 1.76 and 1.86 (s, C-2 Me), 3.76 (m, H-5), 4.38 (dd, H-4), 8.42 (d, CHO).

B. Erythro Isomer (DL-4b). This compound, mp 199–203°, *m/e* 203, nmr[3] (TMS, DMSO-d_6) δ 1.28 (d, C-5 Me), 1.79 and 1.84 (s, C-2 Me), 4 (m, H-5), 4.68 (dd, H-4), 8.38 (d, CHO), was prepared (69.9%) in an identical way.

Resolution of DL-*threo*-*N*-Formyl-2,2,5-trimethyl-4-carboxythiazolidine. DL-**4a** (65.6 g, 321.5 mmol) and 142 g (330 mmol) of brucine dihydrate were dissolved by gentle heating in 800 ml of water. The solution was filtered warm and kept for 48 hr at room temperature. The needle-like crystals (32 g) of the brucine salt of D-**4a** were collected. Evaporation of the filtrate yielded three other fractions (43.7 g) of the D enantiomer. The mother liquid of the preceding operation, containing the L enantiomer, was evaporated to an oil which was taken up in 500 ml of water. Crystallization at room temperature afforded 76.5 g of the crude brucine salt of L-**4a**. Concentration of the filtrate yielded a second fraction (41.7 g) of the L enantiomer. Both D- and L-brucine salts were purified by fractional crystallization from water, yielding 73.6 g of the D and 65 g of the L enantiomer.

Resolution of DL-*erythro*-*N*-Formyl-2,2,5-trimethyl-4-carboxythiazolidine. To a stirred solution of 25.37 g (125 mmol) of DL-**4b** and 35.62 g (125 mmol) of dehydroabietylamine in 300 ml of methanol was added 250 ml of water. The mixture was kept overnight in a cool place and the crude dehydroabietylamine salt of D-**4b** (27.9 g) was isolated. The residue obtained on evaporation of the mother liquid was crystallized from benzene–petroleum ether (bp 30–60°) (1:20) yielding 23.4 g of the pure dehydroabietylamine salt of L-**4b**. The crude salt of the D isomer was recrystallized from methanol–water (1:1), yielding 25.1 g.

Recovery of the Enantiomers of *N*-Formyl-2,2,5-trimethyl-4-carboxythiazolidine. A. Threo Isomers (D- and L-4a). The brucine salt of D-**4a** (73 g) was dissolved in 750 ml of $CHCl_3$. The solution was extracted three times with 70 ml of aqueous NH_4OH (0.5 *N*). The aqueous layer was covered with 150 ml of EtOAc and acidified to pH 1.5 with 6 *N* HCl. The EtOAc layer was dried and evaporated. The residue was taken up in 50 ml of anhydrous benzene. The solution was evaporated to dryness and triturated with petroleum ether (bp 40–60°), yielding 22.23 g of amorphous D-**4a**, $[\alpha]^{25}D$ +81° (*c* 1, EtOH). Conversion of the brucine salt of L-**4a** (65 g) into the free acid was performed in a similar way, yielding 19.8 g of pure L-**4a**, $[\alpha]^{25}D$ −78.5° (*c* 1, EtOH).

B. Erythro Isomers (D- and L-4b). The dehydroabietylamine salt of D-**4b** (25.1 g) was added to 100 ml of 1 *N* NH_4OH and extracted twice with ether. Acidification of the aqueous layer to pH 1 with 6 *N* hydrochloric acid afforded on cooling a precipitate, which was collected, washed with water, and dried, yielding 9.6 g of D-**4b**, mp 223–224° dec, $[\alpha]^{25}D$ +89° (*c* 1, EtOH). Similar treatment of the salt of the L isomer (23.4 g) gave L-**4b**, which was recrystallized from boiling water, yielding 6.6 g of L-**4b**, mp 220.5–221.5° dec, $[\alpha]^{25}D$ −88.5° (*c* 1, EtOH).

D- and L-*threo*-2-Amino-3-mercaptobutyric Acids (1a). D-**4a** (20.3 g, 113.3 mmol) was suspended in 350 ml of 2 *N* HCl and treated on a steam bath under a carbon dioxide atmosphere for 2 hr. The cooled solution was extracted twice with EtOAc. The residue obtained on evaporation of the aqueous layer was dried *in vacuo* over P_2O_5 and KOH, yielding 17 g (99%) of D-**1a** HCl, mp 147–153° dec, $[\alpha]^{25}D$ +13.5° (*c* 1, 2 *N* HCl). L-**1a** HCl prepared in an identical way (80% yield) has mp 148.5–153° dec and $[\alpha]^{25}D$ −13° (*c* 2, 2 *N* HCl).

Treatment of an ethanolic solution of the hydrochlorides of D- and L-**1a** with 1 equiv of NH_4OH yielded a precipitate of the amino acids in their neutral form. Optical rotations are given in Table I.

D- and L-*erythro*-2-Amino-3-mercaptobutyric Acids (1b). The thiazolidines **4b** were hydrolyzed for 5 hr as described for the **a** series. The physical constants are mp 206–208° dec, $[\alpha]^{25}D$ −35° (*c* 1, 1 *N* HCl) for D-**1b** HCl; mp 206–208° dec, $[\alpha]^{25}D$ +35.5° (*c* 1, 1 *N* HCl) for L-**1b** HCl.

Treatment of the hydrochlorides of both enantiomers with NH_4OH as described for the **a** series gave the amino acids in their neutral form. Optical rotations are given in Table I.

S-Benzylation of L-1a. Metallic sodium in small pieces was added to a stirred solution of L-**1a** (425 mg, 2.5 mmol) in liquid ammonia until a permanent blue color due to the excess of sodium resulted. Benzyl chloride (0.75 ml) was added slowly with continued stirring. The ammonia was evaporated, and the residue was

dried over P_2O_5 and dissolved in 25 ml of water. The solution was extracted twice with ether, and the aqueous layer was cooled and adjusted to pH 6 with HOAc. The precipitate was isolated, washed with water, and dried, yielding 244 mg of L-**9a**, mp 165–166.5° dec, $[\alpha]^{25}D$ +72° (*c* 1, 1 *N* HCl).

2-Acetamido-3-benzylmercapto-DL-butyric Acid. A. Threo Isomer (DL-8a). A solution of acetic anhydride (7 ml) in 20 ml of acetone was added dropwise in 1 hr to a stirred suspension of 11.25 g (50 mmol) of 2-amino-3-benzylmercapto-DL-butyric acid (**9a**) in 50 ml of 1 *N* NaOH and 40 ml of acetone. During the addition the pH was kept at 9.7 with 2 *N* NaOH. After 30 min of reaction 120 ml of EtOAc was added and the pH was adjusted to 1.8 with 2 *N* HCl. The EtOAc layer was separated and the aqueous layer was extracted with EtOAc. The combined layers were dried (Na_2SO_4) and concentrated to an oil, which was crystallized from EtOAc, yielding 11 g (82%) of DL-**8a**, mp 104.5–107°, ir (KBr) ν_{max} 1730, 1410 (COOH), 3350, 1640, 1545 (amide), 715, 695 cm^{-1} (C_6H_5).

B. Erythro Isomer (DL-8b). This compound, mp 140–141.5°, ir (KBr) ν_{max} 1715, 1430 (COOH), 3350, 1625, 1545 (amide), 712, 692 cm^{-1} (C_6H_5), was prepared in an identical way.

Resolution of DL-8a with Acylase. Acylase (200 mg) and $Co(NO_3)_2 \cdot 8H_2O$ (83 mg) were added to a solution of DL-**8a** (3.3 g, 11.8 mmol) in 250 ml of sterile water. The mixture was adjusted to pH 7.5 with NH_4OH and incubated at 37°. During incubation the pH was maintained at this value. After 400 hr. the solution was filtered, acidified to pH 1.8 with HCl, and extracted with EtOAc. The aqueous layer was concentrated to a volume of 15 ml and desalted over Dowex 2-X8 ion-exchange resin (OH^- form) as described by Dreze, *et al.*,[15] yielding 100 mg of L-**9a** (7.4% of the theoretical amount), $[\alpha]^{25}D$ +70° (*c* 1, 1 *N* HCl). The material recovered from the EtOAc layer was hydrolyzed with concentrated HCl, yielding 1.3 g of **9a**, $[\alpha]^{25}D$ −10° (*c* 1, 1 *N* HCl).

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Registry No. DL-**1a**, 43083-47-2; DL-**1b**, 43083-48-3; D-**1a**, 43083-49-4; D-**1a** HCl, 43083-50-7; L-**1a**, 43083-51-8; L-**1a** HCl, 43083-52-9; D-**1b**, 43083-53-0; D-**1b** HCl, 43083-54-1; L-**1b**, 43083-55-2; L-**1b** HCl, 43083-56-3; *cis*-**2**, 43083-57-4; *trans*-**2**, 43083-58-5; DL-**4a**, 43083-59-6; DL-**4b**, 43083-60-9; D-**4a**, 43083-61-0; D-**4a** brucine salt, 49613-55-0; L-**4a**, 43083-62-1; L-**4a** brucine salt, 43083-63-2; D-**4b**, 43083-64-3; D-**4B** dehydroabietylamine salt, 43083-65-4; L-**4b**, 43083-66-5; L-**4B** dehydroabietylamine salt, 43083-67-6; DL-**8a**, 43083-68-7; DL-**8b**, 43083-69-8; DL-**9a**, 43083-70-1; DL-**9b**, 43083-71-2; L-**9a**, 43083-72-3; D-**9a**, 43083-73-4.

References and Notes

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