The Circular Dichroism of *N*-Thiobenzoyl-L-α-amino-acids. Part VI.[1] † Assessment of the Resolution of Amino-acids as their *N*-Thiobenzoyl Derivatives

By **Graham C. Barrett** * and **Peter R. Cousins**, Oxford Polytechnic, Headington, Oxford OX3 0BP

C.d. spectra of crops and mother liquors obtained during fractional crystallisation of diastereoisomeric alkaloidal salts of *N*-thiobenzoyl-DL-α-amino-acids indicate directly the degree of resolution and, in principle, the absolute configuration of the predominant *N*-substituted amino-acid component in each fraction. Improvements resulting from c.d. monitoring of the resolution of a DL-amino-acid as its *N*-thiobenzoyl derivative include the ability to operate on a scale smaller than that possible by polarimetric methods, as illustrated for the resolution of 2-amino-3,3-dimethylbutyric acid (for which a new synthesis from ethyl acetoacetate is described).

RESOLUTION of a DL-amino-acid commonly involves its conversion into an *N*-acyl derivative, followed by fractional crystallisation of the mixture of diastereoisomeric salts formed with a chiral amine [*e.g.* (*R*)- or (*S*)-phenethylamine, -brucine, or -morphine.[2] When conditions have been established under which partial crystallisation takes place from a solution of the diastereoisomeric salts, the crop is collected and an attempt is made to establish whether preferential crystallisation of one of the diastereoisomeric salts has occurred, by determining the specific rotation of the crop. There follows a routine sequence of collection of successive crops, and their recrystallisation to constant specific rotation.

The monitoring of the resolution would be simplified if the contribution of the acid component to the optical rotatory power of a partly resolved diastereoisomeric salt mixture could be determined directly. The specific rotation of the crop will include contributions from both the partly resolved acid and the chiral base; o.r.d. and c.d. spectra of such salts would also be composed of contributions from acid and base components, since the absorption wavelength region of an aromatic amine, or of an alkaloid [from *ca.* 330 nm (ref. 3) to shorter wavelengths], overlays that of the *N*-acylamino-acid. However, since an *N*-thioacyl-D- or -L-amino-acid ($R^1CS{\cdot}NH{\cdot}CHR^2{\cdot}CO_2H$) and its amine salts show weak absorption features near the visible wavelength region, associated with the $n \longrightarrow \pi^*$ transition of the thioamide chromophore, and show an associated Cotton effect within the 330—400 nm wavelength range,[4,5] then o.r.d. or c.d. spectra of crops obtained by fractional crystallisation of alkaloidal salts of *N*-thioacyl-DL-amino-acids should include a Cotton effect at wavelengths longer than *ca.* 330 nm when some degree of resolution has been effected.

The potential of c.d. spectroscopy in this area has now been established for monitoring the resolution of *N*-thiobenzoyl-DL-α-amino-acids ($PhCS{\cdot}NH{\cdot}CHR{\cdot}CO_2H$). These thioacyl derivatives, and their amine salts, are stable to the operations involved in their resolution; isolated examples {resolution of *N*-[ethylthio(thiocarbonyl)]aspartic acid,[6] *N*-ethoxythiocarbonyl-leucine,[7] and *O*-[ethylthio(thiocarbonyl)]lactic acid [8]} show that other thioacyl derivatives are adequately stable for the same purpose. However, *N*-thiobenzoyl-amino-acids are particularly suitable since their long-wavelength Cotton effect is centred at a wavelength [4] longer than that of other thioacyl derivatives; [5] also, the sign of the long-wavelength Cotton effect shown by crops from a resolution of an *N*-thiobenzoyl-DL-amino-acid will indicate the absolute configuration of the predominant enantiomer of the *N*-substituted amino-acid in each crop, by comparison of c.d. data with those reported [4,9] for a large number of *N*-thiobenzoyl-L-α-amino-acids.

A DL-amino-acid is easily converted into its *N*-thiobenzoyl derivative by reaction [10] with carboxymethyl dithiobenzoate [11] at room temperature in aqueous solution at pH 7—8 during 4—12 h; we have recently shown [12] that thiobenzoylcholine iodide [13] brings about the same reaction under similarly mild conditions but within 20—30 min. The first stage in the resolution of *N*-thiobenzoyl-DL-2-aminobutyric acid was considerably simplified with the help of c.d. measurements. Whereas

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the crops from fractional crystallisation of the brucine salt of the *N*-substituted amino-acid showed no c.d. near 370 nm, the corresponding strychnine salt gave a first crop from aqueous acetone which was recognised as enriched in the L-enantiomer since it showed negative c.d. near 370 nm in methanol. Assignment of absolute configuration followed from the analogous c.d. behaviour of salts of L-homologues,[4,9] and was confirmed by data for a sample of authentic *N*-thiobenzoyl-L-2-amino-butyric acid.

The resolution of DL-proline as its *N*-thiobenzoyl derivative was accomplished within a short time, and without practical difficulties, through the same procedure, by using morphine as the chiral base.

A further example, the resolution of *N*-thiobenzoyl-DL-2-amino-3,3-dimethylbutyric acid (PhCS·NH·CHBu^t·CO_2H) demonstrated the small scale on which the resolution of a DL-amino-acid can be conducted with the aid of c.d. Fractional crystallisation to constant c.d. can be monitored easily for crops of 3—5 mg, and the same information can be obtained for smaller quantities when the acid is liberated from the salt, by making use of the much more intense c.d. exhibited by *N*-thiobenzoyl-D- or -L-amino-acids near 290 nm.[14] DL-2-Amino-3,3-dimethylbutyric acid was synthesised from ethyl 2-t-butylacetoacetate[15] by Schmidt reaction followed by hydrolysis; treatment of the crude DL-amino-acid (contaminated with glycine) with carboxymethyl dithiobenzoate gave an aqueous solution of DL-2-amino-3,3-dimethylbutyric acid free from glycine as a result of the more rapid thiobenzoylation of the less-hindered amino-acid. Prolonged reaction with carboxymethyl dithiobenzoate gave the *N*-thiobenzoyl derivative (0.25 g), which was resolved by using brucine. The first crop, which showed negative c.d. near 390 nm, was concluded to be the salt of the D-acid, since the acid liberated from the salt gave more positive $\varepsilon_L - \varepsilon_R$ values in polar solvents near 390 nm, in comparison with c.d. data for solutions in non-polar solvents (*cf.* refs. 4 and 9).

Hydrolysis of *N*-thiobenzoyl-D- or -L-amino-acids can be brought about without racemisation in 6N-hydrochloric acid,[16] as for benzoyl analogues; conversion of the *N*-thiobenzoyl-amino-acid into the benzoyl analogue by treatment with aqueous silver nitrate[4] followed by electrolysis[17] provides a mild alternative procedure for liberating the resolved amino-acid.

EXPERIMENTAL

C.d. spectra were determined for *ca.* 0.001M-solutions by using either a Roussel-Jouan Dichrographe (North-East London Polytechnic) or a Roussel-Jouan Dichrographe CD-185 (Department of Biochemistry, University of Oxford).

N-*Thiobenzoyl*-DL-2-*aminobutyric Acid*.—Carboxymethyl dithiobenzoate[11] (10.6 g, 0.05 mol) and DL-2-aminobutyric acid (5.2 g, 0.05 mol) were dissolved in N-sodium hydroxide (100 ml). After 12 h at room temperature the solution was acidified to give N-*thiobenzoyl*-DL-2-*aminobutyric acid* (9.0 g, 80%), m.p. 140° (from benzene) (Found: C, 58.65; H, 5.7; N, 6.25; S, 13.95. $C_{11}H_{13}NO_2S$ requires C, 59.15; H, 5.85; N, 6.25; S, 14.35%).

Resolution. (i) A solution of the *N*-thiobenzoyl-amino-acid (7.0 g, 0.03 mol) and brucine (14.0 g, 0.03 mol) in water (500 ml) gave crops which on recrystallisation from aqueous ethanol afforded material showing negligible c.d. at wavelengths beyond 340 nm. (ii) A solution of the *N*-thiobenzoyl-amino-acid (4.4 g, 0.02 mol) and strychnine (6.6 g, 0.02 mol) in acetone (*ca.* 10 ml) was diluted with water to 400 ml. A crop which separated overnight showed negative c.d. centred at 390 nm in methanol; after two recrystallisations from 30% aqueous acetone (50 ml per g of salt) material showing $\varepsilon_L - \varepsilon_R = -0.18$ at 380 nm, in methanol, was obtained. The salt was dissolved in an excess of 2N-hydrochloric acid and ether, and the *N*-thiobenzoyl-L-amino-acid (84%) was isolated from the dried ($MgSO_4$) ether solution as its cyclohexylammonium salt, m.p. 139° (from ethyl acetate–ether). The mother liquors from the resolution gave *N*-thiobenzoyl-D-2-aminobutyric acid, m.p. 76° (from benzene), $\varepsilon_L - \varepsilon_R = +1.23$ in ether for the c.d. maximum at 393 nm.

N-*Thiobenzoyl*-L-2-*aminobutyric Acid*.—L-2-Aminobutyric acid was converted into its *N*-thiobenzoyl derivative through the method used for the synthesis of its DL-analogue; it gave a *cyclohexylammonium salt*, m.p. 139° (Found: C, 63.15; H, 8.25; N, 8.7; S, 9.75. $C_{17}H_{26}N_2O_2S$ requires C, 63.3; H, 8.15; N, 8.7; S, 9.95%). The free acid showed positive c.d. in MeOH centred at 388 nm, $\varepsilon_L - \varepsilon_R = +0.24$, *ca.* 10% larger than that of the corresponding salt obtained by resolution of the DL-analogue.

Resolution of N-*Thiobenzoyl*-DL-*proline*.—The oil formed by addition of morphine (1.43 g, 0.005 mol) to a solution of *N*-thiobenzoyl-DL-proline, m.p. 156°[18] (1.185 g, 0.005 mol) in acetone (7 ml) was taken into solution by dropwise addition of water (3 ml) to the stirred suspension. Crystals (0.993 g, 38%) which separated overnight showed $\varepsilon_L - \varepsilon_R = +0.28$ at 389 nm, and $\varepsilon_L - \varepsilon_R = -0.15$ at 343 nm in methanol, indicating the salt to be substantially enriched in *N*-thiobenzoyl-D-proline. The acid liberated from the salt by suspension in 2N-hydrochloric acid and extraction into ether showed $\varepsilon_L - \varepsilon_R = +0.47$ at 402 nm and $\varepsilon_L - \varepsilon_R = -0.05$ at 352 nm, confirming the assignment of the D-configuration and showing that better than 95% configurational purity had been achieved, since a sample of *N*-thiobenzoyl-L-proline[4] prepared from L-proline showed $\varepsilon_L - \varepsilon_R = -0.48$ at 402 nm in methanol. A second crop (1.314 g), which separated from the mother liquors during a further 24 h, showed antipodal c.d. curves in the 330—420 nm range, and was 72% configurationally pure (based on c.d. data). Liberation of the *N*-thiobenzoyl-amino-acid from this salt gave an oil from which crystals (0.082 g) of *N*-thiobenzoyl-DL-proline, m.p. 155°, separated on trituration with petrol containing traces of ether. A third crop 0.172 g, 6%) from the mother liquors was shown to contain the DL-acid with a few percent of the D-isomer by virtue of its weak c.d. ($\varepsilon_L - \varepsilon_R = -0.01$ near 400 nm). Acidification of the mother liquors and extraction into ether gave *N*-thiobenzoylproline (0.041 g), estimated by c.d. data to be a 1 : 3 mixture of D- and L-enantiomers.

DL-2-*Amino*-3,3-*dimethylbutyric Acid*.—(i) The amino-

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acid was prepared by the method of Knoop and Landmann.[19] The poor yield reported by these workers could not be improved by modifying the solvent extraction step by prolonging the period of extraction after taking precipitated manganese dioxide into solution with sulphur dioxide. (ii) Of a number of established procedures for the t-butylation of active methylene compounds,[15] the reaction of ethyl acetoacetate with t-butyl chloride in the presence of silver perchlorate gives the best yield (40%) of ethyl 2-t-butylacetoacetate, but the use of boron trifluoride–diethyl ether complex in place of the silver salt is more convenient (yield 28%). Ethyl 2-t-butylacetoacetate (5.5 g, 0.03 mol), b.p. 95° at 14 mmHg (lit.,[15] 90—92° at 14 mmHg), gave DL-2-amino-3,3-dimethylbutyric acid contaminated with glycine (arising from residual ethyl acetoacetate) when subjected to the Schmidt rearrangement [20] at 5 °C with an excess of hydrazoic acid [from NaN_3 (4.0 g) and H_2SO_4 (3.0 g)] in chloroform, followed by dilution with water, evaporation of the dried ($MgSO_4$) chloroform solution, and hydrolysis of the crude *N*-acetyl-DL-amino-ester (3.2 g) thus obtained (m.p. 58—60°) in refluxing 6N-hydrochloric acid during 48 h.

N-*Thiobenzoyl*-DL-2-*amino*-3,3-*dimethylbutyric Acid*.—The crude amino-acid hydrochloride from (ii) above was dissolved in water and the solution was brought to pH 8; a solution of the sodium salt of carboxymethyl dithiobenzoate (estimated 0.25 equiv.) was added, and the mixture was set aside for 12 h at room temperature. Acidification and extraction into ether gave an aqueous solution shown by paper chromatography to be free from glycine; a solution of the sodium salt of carboxymethyl dithiobenzoate (*ca.* 1 equiv.) was added, and after 2 days the solution was acidified and extracted into ether. The dried ($MgSO_4$) extracts were evaporated, and the residue on trituration with petroleum (b.p. 40—60°) gave carboxymethyl dithiobenzoate, m.p. 126°, and *N*-thiobenzoyl-DL-2-amino-3,3-dimethylbutyric acid in small yield as an oil. Repetition of this procedure on several occasions during 4 weeks with the aqueous solution gave *N*-thiobenzoyl-DL-amino-acid cyclohexylammonium salt after treatment of the crude *N*-thiobenzoylamino-acid in ether solution with cyclohexylamine; the *salt* had m.p. 185° (from ethyl acetate) (Found: C, 64.45; H, 8.5; N, 8.2; S, 9.05. $C_{19}H_{30}N_2O_2S$ requires C, 65.1; H, 8.65; N, 8.0; S, 9.15%).

Resolution of N-*Thiobenzoyl*-DL-2-*amino*-3,3-*dimethylbutyric Acid*.—(i) A solution of the acid (from 0.35 g of cyclohexylammonium salt) and brucine (0.46 g) in acetone (10 ml) was diluted with water until cloudy. The mixture was warmed and filtered, and set aside for 3 months. The crop which had formed by this time was filtered off, and showed negative c.d. centred at 390 nm in methanol. One recrystallisation of the salt from aqueous methanol gave *N*-thiobenzoyl-D-amino-acid brucine salt (0.088 g) which showed $\varepsilon_L - \varepsilon_R$ values −0.12 (396 nm) and −0.22 (398 nm) in methanol and propan-2-ol, respectively, and −0.03 (425 nm) and +0.13 (368 nm) in dichloromethane, confirming the assignment of the D-configuration to this compound by analogy with the solvent-dependent c.d. behaviour of amine salts of other *N*-thiobenzoyl-D-amino-acids with alkyl side-chains.[9] (ii) A solution of *N*-thiobenzoyl-DL-amino-acid (from 0.218 g of cyclohexylammonium salt), and morphine (0.177 g) in 20% aqueous acetone (15 ml) gave crystals (0.083 g) after 3 days at room temperature; this crop, and a second crop (0.07 g) deposited after a further 2 days, showed negative c.d. in methanol centred at 390 nm. The *N*-thiobenzoyl-amino-acid liberated from these crops showed $\varepsilon_L - \varepsilon_R$ values −0.28 (382 nm) in methanol and +0.18 (390 nm) in ether, consistent with the D-configuration for the acid component in these crops. Third (0.10 g) and fourth crops (0.083 g) obtained from the mother liquors during a further 5 days showed $\varepsilon_L - \varepsilon_R = -0.08$ and −0.18, respectively, at 390 nm in ether, after liberation of their *N*-thiobenzoyl-amino-acid component; these crops were therefore enriched in the L-enantiomer.

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