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Anomalous Rotatory Dispersion Curves of N-Thiobenzoyl and N-Phenylthioacetyl α-Amino Acids

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Submitted in honour of the sixtieth birthday of Professor *Arne Fredga*

N-Thiobenzoyl and N-phenylthioacetyl derivatives of α-amino acids have been shown to possess desirable spectroscopic properties for spectropolarimetric studies; they exhibit absorption bands of low extinction at about 365 and 330 mμ, respectively. Both series of derivatives exhibit Cotton effects related to these absorption bands. N-Phenylthioacetyl derivatives of α-amino acids have strong Cotton effects which are suitable for establishing the absolute configurations.

Recent investigations[1,2] have demonstrated the utility of anomalous rotatory dispersion measurements for stereochemical assignments and conformational problems. Unfortunately, most α-amino acids[3a] and peptides[3b] absorb far down in the ultraviolet region and thus exhibit only plain dispersion curves[4] over the currently accessible spectral range. However, it was found[5] that N-dithiocarbalkoxy (dithiocarbamate) derivatives of α-amino acids absorb at about 330 mμ and yield Cotton effects which can be related to the absolute configuration of the α-asymmetric center — a positive Cotton effect corresponding to the L-configuration, and a negative one to the D-configuration. In an later study[6] it was shown that also N-thionocarbethoxy-α-amino acids and 3-phenyl-2-thiohydantoins have Cotton effect curves which may be used for configurational assignments. In the present work, we have extended the study to other derivatives of amino acids for the same purpose.

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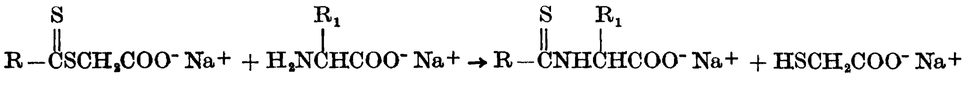
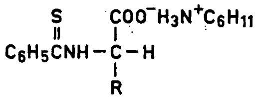
The C=S chromophore has proved to be useful [7] in this context for several other classes of substances, hence we looked for a suitable thiono derivative of α-amino acids. We were especially interested in investigating the N-thiobenzoyl and N-phenylthioacetyl derivatives of amino acids, since these radicals can easily be introduced into the parent compound. Thus Holmberg [8] prepared in 1944 some N-thiobenzoyl amino acids by the facile introduction of the thiobenzoyl group by means of the very reactive carboxymethyl ester of dithiobenzoic acid, and some years later Kjær [9] prepared a number of N-phenylthioacetyl amino acids by introducing the phenylthioacetyl group with the carboxymethyl ester of phenyldithioacetic acid.



$$R-\overset{S}{\overset{\|}{C}}SCH_2COO^-Na^+ + H_2N\overset{R_1}{\overset{|}{C}}HCOO^-Na^+ \rightarrow R-\overset{S}{\overset{\|}{C}}NH\overset{R_1}{\overset{|}{C}}HCOO^-Na^+ + HSCH_2COO^-Na^+$$

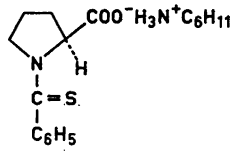
$R = C_6H_5$ or $C_6H_5CH_2$

In order to investigate the suitability of N-thiobenzoyl and N-phenylthioacetyl derivatives for rotatory dispersion work, it was first necessary to determine whether the C=S chromophore in these groups possessed suitable ultraviolet absorption bands. Preliminary ultraviolet measurements showed that N-thiobenzoyl α-amino acids have a low extinction band at about 365 mμ (log ε ~ 2.4) and two strong bands at 285 and 240 mμ (log ε ~ 3.9 and 4.1), while N-phenylthioacetyl-α-amino acids exhibit bands at about 330 mμ (log ε ~ 1.8) and 270 mμ (log ε ~ 4.1). The observation of the excellent spectroscopic properties of these derivatives prompted us to synthesize a number of optically active N-thiobenzoyl and N-phenylthioacetyl α-amino acids (I—V

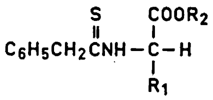


$$C_6H_5\overset{S}{\overset{\|}{C}}NH-\overset{COO^-H_3N^+C_6H_{11}}{\overset{|}{\underset{\underset{R}{|}}{C}}}-H$$

I $R = CH_2CH(CH_3)_2$
II $R = CH_2CONH_2$
III $R = CH_2CH_2COO^-H_3N^+C_6H_{11}$
IV $R = C_6H_5$

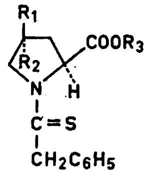


V



$$C_6H_5CH_2\overset{S}{\overset{\|}{C}}NH-\overset{COOR_2}{\overset{|}{\underset{\underset{R_1}{|}}{C}}}-H$$

VI $R_1 = CH_3$; $R_2 = H_3NC_6H_{11}$
VII $R_1 = CH(CH_3)_2$; $R_2 = H_3NC_6H_{11}$
VIII $R_1 = CH(CH_3)C_2H_5$; $R_2 = H_3NC_6H_{11}$
IX $R_1 = CH_2CONH_2$; $R_2 = H$
X $R_1 = C_6H_5$; $R_2 = H_3NC_6H_{11}$
XI $R_1 = CH_2C_6H_5$; $R_2 = H_3NC_6H_{11}$



XII $R_1 = R_2 = R_3 = H$
XIII $R_1 = H$; $R_2 = OH$;
$R_3 = H_3NC_6H_{11}$

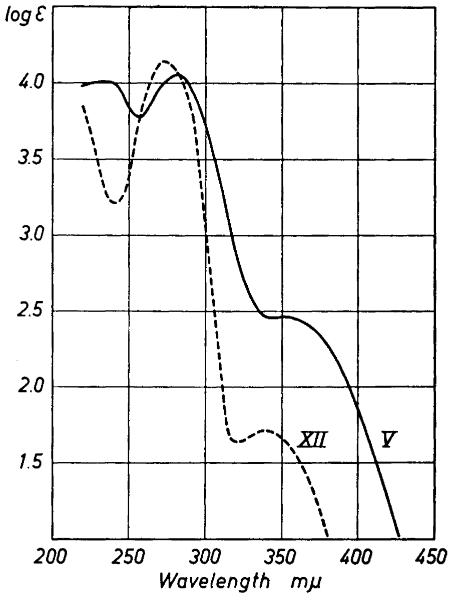


Fig. 1. Ultraviolet absorption in methanol of the cyclohexylammonium salt of N-thiobenzoyl-L-proline (V) and N-phenylthioacetyl-L-proline (XII).

and VI—XIII, respectively), following the experimental procedures described by Holmberg [8] and Kjær [9,10]. The reaction products were usually obtained as non crystallising oils. However, the cyclohexylammonium salts of the thioacylated amino acids crystallised excellently, hence these salts were used for purification and physical measurements. The ultraviolet spectra of one N-thiobenzoyl and one N-phenylthioacetyl amino acid are shown in Fig. 1.

The rotatory dispersion results are summarised in Figs. 2—5. Cotton effects are found in the region of the long wave length absorption bands indicating that these bands are anisotropic ("optically active"). The cyclohexylammonium salts of N-thiobenzoyl-L-leucine (I) as well as of N-thiobenzoyl-L-asparagine (II) exhibit positive Cotton effects (Fig. 2) of very low amplitudes while the corresponding derivative of L-proline (V) apparently has a negative Cotton effect. A positive effect is found for the cyclohexylammonium salt of N-thiobenzoyl-D-α-aminophenylacetic acid (IV). This irregularity is somewhat unexpected in view of the very consistent relationship between configuration and sign of the Cotton effects in the series of dithiocarbamates [5,6], where all the investigated compounds of L-configuration have positive Cotton effects. A similar anomaly was, however, found [6] for the N-thionocarbethoxy derivatives of hydroxyproline and acetoxyproline.

The first absorption band of the N-thiobenzoyl amino acids is situated in a very convenient wave length range for spectropolarimetric studies and the extinction value (log $\varepsilon \sim 2.4$) is generally fairly reasonable. However, bearing in mind the low amplitudes of the Cotton effects this extinction is too high to allow convenient and reliable rotatory dispersion measurements through the absorption region. The corresponding absorption band of the N-phenylthio-

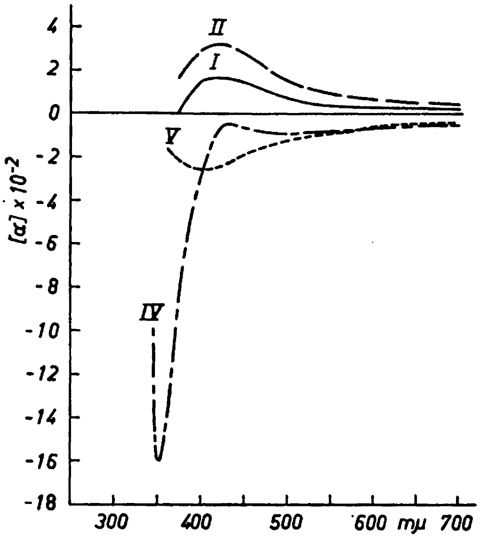


Fig. 2. Optical rotatory dispersion curves of the cyclohexylammonium salts of N-thiobenzoyl-L-leucine (I), N-thiobenzoyl-L-asparagine (II), N-thiobenzoyl-D-α-aminophenylacetic acid (IV) and N-thiobenzoyl-L-proline (V).

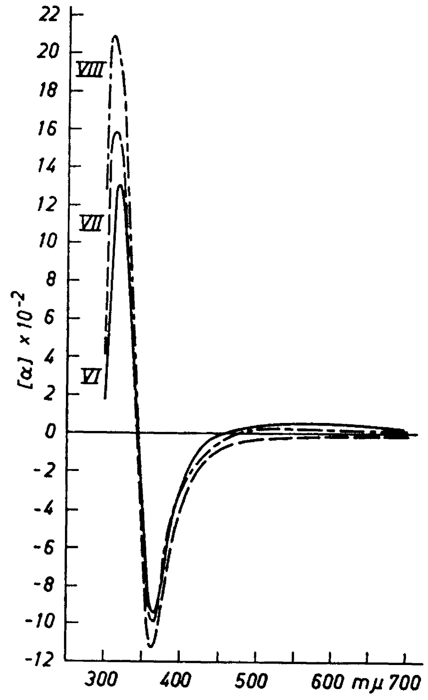


Fig. 3. Optical rotatory dispersion curves of the cyclohexylammonium salts of N-phenylthioacetyl-L-alanine (VI), N-phenylthioacetyl-L-valine (VII) and N-phenylthioacetyl-L-isoleucine (VIII).

acetyl amino acids falls still in a suitable wave length range ($\lambda_{max} \sim$ 330 mμ), and in conjunction with the lower extinction value (log $\varepsilon \sim$ 1.8) these derivatives appear to be more suitable for rotatory dispersion studies.

The rotatory dispersion curves of the cyclohexylammonium salts of N-phenylthioacetyl-L-alanine (VI), N-phenylthioacetyl-L-valine (VII) and N-phenylthioacetyl-L-isoleucine (VIII) are shown in Fig. 3. These three compounds have very similar dispersion curves, with negative Cotton effects of large amplitudes. N-Phenylthioacetyl derivatives of asparagine, α-aminophenylacetic acid and β-phenylalanine of L-configuration also exhibit negative Cotton effects (Fig. 4 and Experimental) while those of L-proline (XII) and L-hydroxyproline (XIII) have positive Cotton effects (Fig. 5).

Dithiocarbamates of all the L-α-amino acids which have so far been examined [5,6] show a positive Cotton effect, as well as all the N-thionocarbethoxy derivatives except those of L-hydroxyproline and L-acetoxyproline [6]. In the N-phenylthioacetyl α-amino acids series even proline is exceptional, and it is

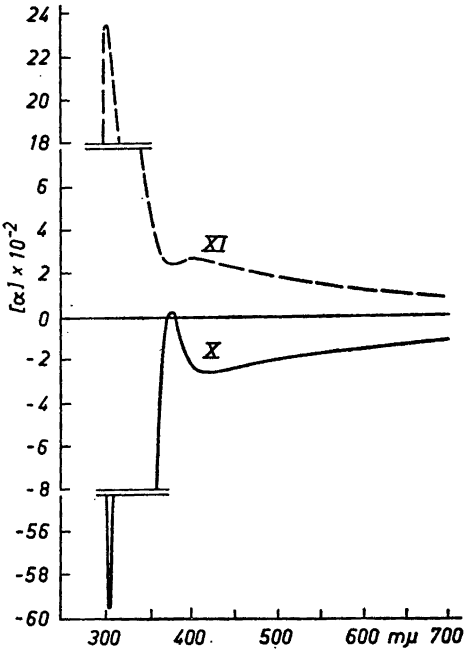


Fig. 4. Optical rotatory dispersion curves of the cyclohexylammonium salts of N-phenylthioacetyl-D-α-aminophenylacetic acid (X) and N-phenylthioacetyl-L-β-phenylalanine (XI).

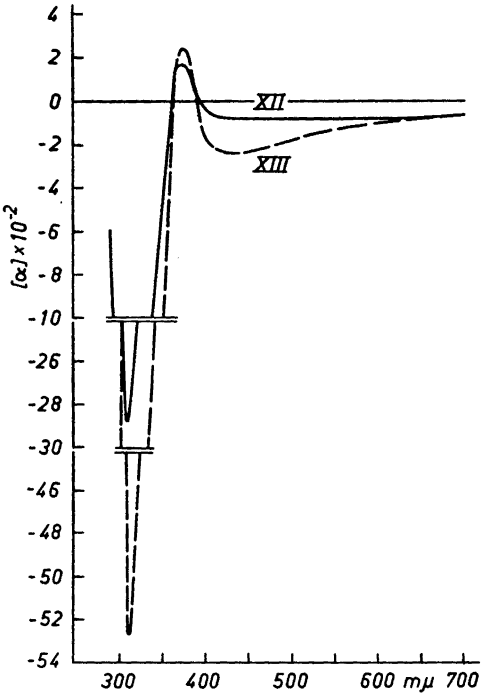


Fig. 5. Optical rotatory dispersion curves of N-phenylthioacetyl-L-proline (XII) and the cyclohexylammonium salt of N-phenylthioacetyl-L-hydroxyproline (XIII).

difficult to rationalise these exceptions. In order to see if the observed anomaly in the proline series will be found for N-phenylthioacetyl derivatives of other α-amino acids and peptides we have undertaken further studies of this subject *, and any possible reason for this anomaly will be discussed when further experimental data will be available.

It is interesting that the sign of the Cotton effects is generally negative for N-phenylthioacetyl L-α-amino acids whereas it is positive for dithiocarbamates and N-thionocarbethoxy derivatives. This reversal of sign is, however, systematic.

N-Phenylthioacetyl α-amino acids are easily prepared [8,9], and in view of the large amplitudes of their rotatory dispersion curves and particularly since their relevant absorption bands have low extinction and occur in a convenient region, these derivatives seem to be very suitable for stereochemical assign-

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* When the present paper was prepared we learnt that Professor A. Kjær had started similar studies, and the planned continuation will be a joint work between Professor Kjær and us.

ments of α-amino acids and constitute a valuable complement to the dithiocarbamates and N-thionocarbalkoxy derivatives.

EXPERIMENTAL

The ultraviolet spectra were measured in methanol solution with a Beckman DK-2 spectrophotometer. The rotatory dispersion measurements were determined in methanol solution (25°) by means of an automatically recording Rudolph spectropolarimeter by Mr. Rolf Bäckström, whose assistance is gratefully acknowledged. The data are recorded below as outlined in Ref.[4]

Melting points were determined in an electrically heated metal block, using calibrated Anschütz thermometers.

The microanalyses were carried out in the laboratory of Dr. A. Bernhardt, Mülheim Germany. Before analysis, the compounds were dried at 50° and 0.05 mm.

N-Thioacylamino acids

The L-amino acids were all commercially available.

D-α-Aminophenylacetic acid was prepared according to Marvel and Noyes[11]. Rotation: $[\alpha]_D^{23} = -156.7°$ ($c = 7.0$ %; in 1.36 equiv. HCl).

Carboxymethyl dithiophenylacetate and *carboxymethyl dithiobenzoate* were prepared as described by Kjær[9,10] by reacting the Grignard reagent obtained from benzyl chloride and bromobenzene, respectively, with carbon disulphide, followed by treatment of the crude reaction mixture with chloroacetic acid.

The amino acids were phenylthioacetylated and thiobenzoylated according to the procedures described by Holmberg[8] and Kjær[9,10]. The amino acid (0.002–0.01 mole) was dissolved in two equivalents (glutamic acid in three equivalents) of N sodium hydroxide followed by the addition of one equivalent of carboxymethyl dithiophenylacetate or carboxymethyl dithiobenzoate. The solution was kept at room temperature overnight and then acidified with 5 N hydrochloric acid. The reaction product separated as an oil, which crystallised only in two cases (compounds IX and XII). The oil was therefore extracted with ether, and the extract washed thoroughly with water to remove the mercaptoacetic acid formed. The thioacylated amino acid was then converted to the well crystallising *cyclohexylammonium salt* by the addition of an ethereal solution of cyclohexylamine. The salt was collected and purified by recrystallisation. By this procedure the following compounds were prepared in yields ranging from 60–80 %.

Cyclohexylammonium salt of N-thiobenzoyl-L-leucine (I). M.p. 159–161° (decomp.), from ethanol-ether. (Found: C 65.2; H 8.48; N 7.87; S 9.33. Calc for $C_{19}H_{30}N_2O_2S$: C 65.1; H 8.63; N 7.99; S 9.15).

Ultraviolet absorptions: $\lambda_{\text{shoulder}}$ 365 mμ (log ε 2.43), $\lambda_{\max}$ 285 and 239 mμ (log ε 3.85 and 4.06).

R.D. (Fig. 2), c, 0.109: $[\alpha]_{700}$ + 20°, $[\alpha]_{589}$ + 35°, $[\alpha]_{415}$ + 165°, $[\alpha]_{375}$ 0°.

Cyclohexylammonium salt of N-thiobenzoyl-L-asparagine (II). M.p. 183–184° (decomp.), from ethanol-ether. (Found: C 58.5; H 6.93; N 11.9; S 8.84. Calc. for $C_{17}H_{25}N_3O_3S$: C 58.1; H 7.17; N 12.0; S 9.13).

Ultraviolet absorptions: $\lambda_{\text{shoulder}}$ 370 mμ (log ε 2.43), $\lambda_{\max}$ 286 and 241 mμ (log ε 3.87 and 4.08).

R.D. (Fig. 2), c, 0.089: $[\alpha]_{700}$ + 45°, $[\alpha]_{589}$ + 70°, $[\alpha]_{420}$ + 320°, $[\alpha]_{375}$ + 180°.

Cyclohexylammonium salt of N-thiobenzoyl-L-glutamic acid (III). M.p. 207–210° (decomp.), from ethanol-water. (Found: C 61.9; H 8.40; N 8.80; S 6.62. Calc. for $C_{24}H_{39}N_3O_4S$: C 61.9; H 8.44; N 9.02; S 6.89).

Ultraviolet absorptions: $\lambda_{\text{shoulder}}$ 365 mμ (log ε 2.42), $\lambda_{\max}$ 286 and 240 mμ (log ε 3.87 and 4.06).

R.D., c, 0.059: $[\alpha]_{700}$ + 30°, $[\alpha]_{589}$ + 45°, $[\alpha]_{420}$ + 145°, $[\alpha]_{380}$ + 55°.

Cyclohexylammonium salt of N-thiobenzoyl-D-α-aminophenylacetic acid (IV). M.p. 188–189.5° (decomp.), from ethanol-ether. (Found: C 68.6; H 7.47; N 8.05; S 8.91. Calc. for $C_{21}H_{26}N_2O_2S$: C 68.1; H 7.07; N 7.56; S 8.66).

Ultraviolet absorptions: λ_{max} 371, 286 and 238 mμ (log ε 2.40, 3.86 and 4.13).

R.D. (Fig. 2), *c*, 0.065: $[\alpha]_{700}$ −65°, $[\alpha]_{589}$ −65°, $[\alpha]_{500}$ −95°, $[\alpha]_{430}$ −45°, $[\alpha]_{353}$ −1600°.

*Cyclohexylammonium salt of N-thiobenzoyl-*L*-proline (V).* M.p. 215−216° (decomp.), from ethanol-ether. (Found: C 64.6; H 7.81; N 8.15; S 9.23. Calc. for $C_{18}H_{26}N_2O_2S$: C 64.6; H 7.84; N 8.38; S 9.59).

Ultraviolet absorptions: λ_{max} 352, 281 and 231 mμ (log ε 2.47, 4.06 and 4.02).

R.D. (Fig. 2), *c*, 0.109: $[\alpha]_{700}$ −35°, $[\alpha]_{589}$ −70°, $[\alpha]_{400}$ −250°, $[\alpha]_{360}$ −165°.

*Cyclohexylammonium salt of N-phenylthioacetyl-*L*-alanine (VI).* M.p. 129.5−131° (decomp.), from ethanol-ether. (Found: C 62.8; H 8.57; N 8.49. Calc. for $C_{17}H_{26}N_2O_2S$: C 63.3; H 8.12; N 8.69).

Ultraviolet absorptions: λ_{max} 319 and 267 mμ (log ε 1.98 and 4.01).

R.D. (fig. 3), *c*, 0.0624: $[\alpha]_{700}$ + 10°, $[\alpha]_{589}$ + 40°, $[\alpha]_{550}$ + 60°, $[\alpha]_{365}$ −960°, $[\alpha]_{317}$ + 1300°, $[\alpha]_{300}$ + 160°.

*Cyclohexylammonium salt of N-phenylthioacetyl-*L*-valine (VII).* M.p. 166.5−167.5° (decomp.), from ethanol-ether. (Found: C 65.7; H 8.93; N 8.14. Calc. for $C_{19}H_{30}N_2O_2S$: C 65.1; H 8.63; N 7.99).

Ultraviolet absorptions: λ_{max} 333 and 271 mμ (log ε 1.83 and 4.08).

R.D. (Fig. 3), *c*, 0.0622, $[\alpha]_{700}$ −5°, $[\alpha]_{589}$ −10°, $[\alpha]_{363}$ −1130°, $[\alpha]_{312}$ + 1590°, $[\alpha]_{300}$ + 430°.

*Cyclohexylammonium salt of N-phenylthioacetyl-*L*-isoleucine (VIII).* M.p. 170.5−171.5° (decomp.), from ethanol-ether. (Found: C 66.3; H 8.96; N 7.82. Calc. for $C_{20}H_{32}N_2O_2S$: C 65.9; H 8.85; N 7.69).

Ultraviolet absorptions: λ_{max} 332 and 270 mμ (log ε 1.83 and 4.08).

R.D. (Fig. 3), *c*, 0.0578: $[\alpha]_{700}$ 0°, $[\alpha]_{589}$ + 15°, $[\alpha]_{500}$ + 20°, $[\alpha]_{364}$ −985°, $[\alpha]_{309}$ + 2100°, $[\alpha]_{300}$ + 1350°.

*N-Phenylthioacetyl-*L*-asparagine (IX)* was prepared as described by Kjær[9], m.p. 137−138° (decomp.) (lit. 139−140°).

Ultraviolet absorptions: λ_{max} 340 and 267 mμ (log ε 1.75 and 4.05).

R.D., *c*, 0.056: $[\alpha]_{700}$ + 35°, $[\alpha]_{589}$ + 70°, $[\alpha]_{450}$ + 215°, $[\alpha]_{365}$ −35°, $[\alpha]_{305}$ + 1470°, $[\alpha]_{290}$ + 860°.

*Cyclohexylammonium salt of N-phenylthioacetyl-*D*-α-aminophenylacetic acid (X).* M.p. 198−200° (decomp.), from ethanol. (Found: C 68.8; H 7.17; N 7.28; S 8.58. Calc. for $C_{22}H_{28}N_2O_2S$: C 68.7; H 7.34; N 7.29; S 8.34).

Ultraviolet absorptions: λ_{max} 334 and 269 mμ (log ε 1.77 and 4.07).

R.D. (Fig. 4), *c*, 0.0447: $[\alpha]_{700}$ −115°, $[\alpha]_{589}$ −150°, $[\alpha]_{425}$ − 270°, $[\alpha]_{380}$ + 20°, $[\alpha]_{305}$ −5950°, $[\alpha]_{300}$ −2420°.

*Cyclohexylammonium salt of N-phenylthioacetyl-*L*-β-phenylalanine (XI).* M.p.186.5−187.5° (decomp.), from ethanol-ether. (Found: C 69.7; H 7.81; N 7.09. Calc. for $C_{23}H_{30}N_2O_2S$: C 69.3; H 7.59; N 7.03).

Ultraviolet absorptions: $\lambda_{shoulder}$ 325 mμ (log ε 1.89), λ_{max} 268 mμ (log ε 4.10).

R.D. (Fig. 4), *c*, 0.0887: $[\alpha]_{700}$ + 90°, $[\alpha]_{589}$ +120°, $[\alpha]_{395}$ + 265°, $[\alpha]_{375}$ + 240°, $[\alpha]_{302}$ +2340°, $[\alpha]_{297}$ + 1660°.

*N-Phenylthioacetyl-*L*-proline (XII).* M.p. 122−124° (decomp.), from light petroleum-ether. (Found: C 62.5; H 5.81; N 5.91. Calc. for $C_{13}H_{15}NO_2S$: C 62.6; H 6.06; N 5.62).

Ultraviolet absorptions: λ_{max} 339 and 274 mμ (log ε 1.72 and 4.14).

R.D. (Fig. 5), *c*, 0.061: $[\alpha]_{700}$ −65°, $[\alpha]_{589}$ −80°, $[\alpha]_{375}$ + 170°, $[\alpha]_{308}$ −2890°, $[\alpha]_{290}$ −590°.

*Cyclohexylammonium salt of N-phenylthioacetyl-*L*-hydroxyproline (XIII).* M.p. 181−181.5° (decomp.), from ethanol-ether. (Found: C 63.0; H 7.59; N 7.51. Calc. for $C_{19}H_{28}N_2O_3S$: C 62.6; H 7.74; N 7.69).

Ultraviolet absorptions: $\lambda_{shoulder}$ 330 mμ (log ε 1.82), λ_{max} 276 mμ (log ε 4.14).

R.D. (Fig. 5), *c*, 0.0672: $[\alpha]_{700}$ −60°, $[\alpha]_{589}$ −90°, $[\alpha]_{435}$ −240°, $[\alpha]_{374}$ + 240°, $[\alpha]_{314}$ −5270°, $[\alpha]_{305}$ −1170°.

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