ACTA CHEMICA SCANDINAVICA 20 (1966) 2781–2794

Anomalous Optical Rotation and Circular Dichroism of *N*-Thioacylated α-Amino Acids and Derivatives in Various Solvents*

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The preparation of *N*-thiobenzoyl and *N*-phenylthioacetyl derivatives of L-valine, L-tyrosine, L-proline, and, partly, L-leucine, as well as the corresponding salts, esters, and amides, is described.

Ultraviolet spectra, as well as optical rotatory dispersion and circular dichroism curves are reported for the above compounds in water, methanol, dioxane, and isooctane for the wave length region above 275 mμ.

In a number of cases unexpected and striking shifts in the sign of the observed Cotton effects are noticed on going from one solvent to another. The experimental results indicate that differently solvated species coexist in solution in a number of cases. The observed anomalies are discussed in terms of solvent-influenced changes in rotamer composition, possibly to the extent of the existence of solvent-dependent equilibria involving differently solvated species.

Important conclusions of the present study are: (i) considerable caution must be observed in assigning configuration to chiral molecules

* Partly abstracted from a dissertation submitted by Erik Bach to the Royal Veterinary and Agricultural College in partial fulfillment of the requirements for the degree of lic.agro.

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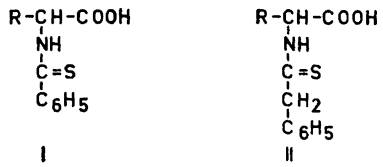
*** Part CVIII of the Stanford series 'Optical Rotatory Dispersion Studies'. Part CVII: Van Horn, A. R. and Djerassi, C. *J. Am. Chem. Soc. In press.*

solely on basis of the sign of the Cotton effect in systems where the solute-solvent interaction is unknown, and (ii) even in a given solvent cases are recorded of homologous compounds, of identical chirality, exhibiting Cotton effects of opposite sign.

Among the many types of optically active molecules which have been subjected to optical rotatory dispersion studies various derivatives of α-amino acids constitute an important group. Since the longest wave-length transition of the carboxylic acid group ($n \rightarrow \pi^*$) in simple α-amino acids appears at about 210 mμ, *i.e.* outside the region accessible to standard spectropolarimeters at the time of the present investigation (1963) * much interest has been associated with producing 'chromophoric derivatives'** possessing Cotton effects (c.e.) in the accessible wave-length region. Two such types of derivatives are the *N*-thioacylated α-amino acids (I, and II), the rotatory



I II

dispersion curves of which were first reported by Sjöberg *et al.*[6] Unaware of these studies, the same type of compounds, prepared several years ago by another of the authors, (A.K.), were subjected to a similar investigation in the Stanford laboratories with results partly deviating from those reported. It was soon realized that unexpected solvent effects were operating, and it was decided to undertake a joint investigation in order to clarify the apparent discrepancies and possibly gain further insight into the important problem of solvent-dependent anomalous rotatory dispersion and circular dichroism. The present paper presents the results of these studies. After their completion, two papers have appeared dealing with closely related problems.*** [14,16]

COMPOUNDS AND METHODS

L-Valine, L-leucine, L-tyrosine, and L-proline were selected as representative amino acids, the last of these chosen with a view to studying the influence of restricted rotation. The *N*-thiobenzoyl- and *N*-phenylthioacetyl-derivatives of the four amino acids, as well as the corresponding cyclohexylammonium salts, methyl esters,† and amides (III—VI), were subjected to rotatory dispersion (RD) and circular dichroism (CD) studies in solvents of varying polarity, *viz.* water (or aqueous alkali for the acids), methanol, dioxane, and isooctane, to the extent solubility permitted. For the sake of brevity the symbols presented in Table 1 will be used for the individual compounds in the following discussion.

* Only recently has improved instrumentation permitted Cotton effects to be measured on solutions of free amino acids in water and acid.[1-4]

** For a survey of such functional groups, *cf.* Ref. 5.

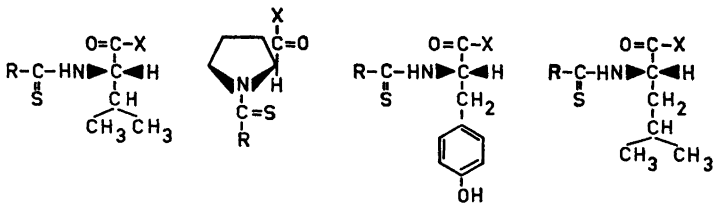
*** *Note added in proof.* After the submission of the present paper, three more publications dealing with the same problem have appeared.[23-25]

† In the leucine series the ethyl esters were employed.

Table 1. *N*-Thioacylated amino acids and derivatives, with abbreviations used throughout the present work.

Compound	Formula			Symbol
	No.	R	X	
N-Thiobenzoyl-L-valine [a]	III	C_6H_5	OH	V(T/H)
N-Thiobenzoyl-L-valine, cyclohexylammonium salt [a]	III	C_6H_5	$O^-C_6H_{11}NH_3^+$	V(T/C)
N-Thiobenzoyl-L-valineamide	III	C_6H_5	NH_2	V(T/N)
N-Thiobenzoyl-L-valine methyl ester	III	C_6H_5	OCH_3	V(T/E)
N-Phenylthioacetyl-L-valine [b]	III	$C_6H_5CH_2$	OH	V(P/H)
N-Phenylthioacetyl-L-valine, cyclohexylammonium salt [c]	III	$C_6H_5CH_2$	$O^-C_6H_{11}NH_3^+$	V(P/C)
N-Phenylthioacetyl-L-valineamide	III	$C_6H_5CH_2$	NH_2	V(P/N)
N-Phenylthioacetyl-L-valine methyl ester	III	$C_6H_5CH_2$	OCH_3	V(P/E)
N-Thiobenzoyl-L-proline [a]	IV	C_6H_5	OH	P(T/H)
N-Thiobenzoyl-L-proline, cyclohexylammonium salt [a c]	IV	C_6H_5	$O^-C_6H_{11}NH_3^+$	P(T/C)
N-Thiobenzoyl-L-prolineamide	IV	C_6H_5	NH_2	P(T/N)
N-Thiobenzoyl-L-proline methyl ester	IV	C_6H_5	OCH_3	P(T/E)
N-Phenylthioacetyl-L-proline [c]	IV	$C_6H_5CH_2$	OH	P(P/H)
N-Phenylthioacetyl-L-proline, cyclohexylammonium salt	IV	$C_6H_5CH_2$	$O^-C_6H_{11}NH_3^+$	P(P/C)
N-Phenylthioacetyl-L-prolineamide	IV	$C_6H_5CH_2$	NH_2	P(P/N)
N-Phenylthioacetyl-L-proline methyl ester	IV	$C_6H_5CH_2$	OCH_3	P(P/E)
N-Thiobenzoyl-L-tyrosine [a]	V	C_6H_5	OH	T(T/H)
N-Thiobenzoyl-L-tyrosine cyclohexylammonium salt [a]	V	C_6H_5	$O^-C_6H_{11}NH_3^+$	T(T/C)
N-Thiobenzoyl-L-tyrosineamide [d]	V	C_6H_5	NH_2	T(T/N)
N-Thiobenzoyl-L-tyrosine methyl ester	V	C_6H_5	OCH_3	T(T/E)
N-Phenylthioacetyl-L-tyrosine	V	$C_6H_5CH_2$	OH	T(P/H)
N-Phenylthioacetyl-L-tyrosine, cyclohexylammonium salt	V	$C_6H_5CH_2$	$O^-C_6H_{11}NH_3^+$	T(P/C)
N-Phenylthioacetyl-L-tyrosine-amide [b]	V	$C_6H_5CH_2$	NH_2	T(P/N)
N-Phenylthioacetyl-L-tyrosine methyl ester	V	$C_6H_5CH_2$	OCH_3	T(P/E)
N-Thiobenzoyl-L-leucineamide	VI	C_6H_5	NH_2	L(T/N)
N-Thiobenzoyl-L-leucine ethyl ester	VI	C_6H_5	OC_2H_5	L(T/E)
N-Phenylthioacetyl-L-leucineamide	VI	$C_6H_5CH_2$	NH_2	L(P/N)
N-Phenylthioacetyl-L-leucine ethyl ester	VI	$C_6H_5CH_2$	OC_2H_5	L(P/E)

[a] Previously described, Ref. 14. [b] Previously described, Ref. 7. [c] Previously described, Ref. 6. [d] Previously described, Ref. 8.



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The thioacylated amino acids, salts, esters, and amides (Table 1) were generally prepared by means of the carboxymethyl esters of dithiobenzoic [8] and dithiophenylacetic acid [7] as the thioacylating agents.* All free acids and cyclohexylammonium salts, as well as some esters and amides, were prepared in aqueous solution, whereas a few esters were thioacylated in pyridine solution.[10] In a few cases, the thioacylated amides were produced by ammonolysis of the corresponding esters. Thiobenzoyl-L-valine methyl ester was prepared from the corresponding acid and diazomethane. Several of the thioacyl-derivatives appeared as oils or amorphous solids. The method of preparation, physical constants, and analytical data for the individual compounds are presented in Table 2.

RESULTS

The *N*-thiobenzoyl-amino acids and derivatives all exhibit two major absorption bands above 250 mμ,** one within the wave-length range 340—400 mμ (log ε 2.4—2.6) and another of higher intensity (log ε 3.7—4.3) at 270—290 mμ (Table 3). The former is bathochromically displaced by decreasing the polarity of the solvent and thus can be attributed to an $n \rightarrow \pi^*$ transition of the thioamide grouping, whereas the high-intensity band, far less susceptible to solvent change, most likely arises from a $\pi \rightarrow \pi^*$ transition.[11] Similarly, two absorption bands of the same relative intensities are present in the phenylthioacetyl series at 320—360 mμ (log ε 1.6—2.5) and 265—280 mμ (log ε 3.9—4.3), respectively (Table 3). In both series these transitions are 'optically active' and give rise to anomalous rotatory dispersion and, implicitly, circular dichroism curves. The Cotton effect associated with the highest wave-length transition will be denoted 'the first', that arising from the $\pi \rightarrow \pi^*$ transition 'the second' c.e. In all cases it has been possible to determine the sign of the first c.e., and in many instances also that of the second.

In Figs. 1—4 are presented the RD- and CD-curves of all compounds studied in the present investigation in the wave-length region above 275 mμ and in the various solvents specified above.*** In a number of cases (Figs. 2 and 4), CD-curves were not recorded due to the unambiguous information provided by the first determined RD-curves.

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* For a review of this method, *cf.* Ref. 9.

** In all cases an additional absorption maximum at shorter wavelengths (230—245 mμ) was recorded (Table 3), but no attempts were made to account for its rotational contributions.

*** Due to the bulk of the experimental observations it is not possible here to present the tabulated data (numerical values for the RD- and CD-measurements) *in extenso*. For those who may be interested in these, copies are available upon request from the Copenhagen laboratory.

Table 2. Synthetic procedures, physical constants, and analytical data for the *N*-thioacylated amino acids and derivatives studied in the present work.

Compound[a]	Formula	Method[b]	M.p.[c]	Specific Rotation[d]		Analyses Calculated				Found			
				[α]°	Conc. g/100 ml	C	H	N	S	C	H	N	S
V(T/H)	$C_{12}H_{15}NO_3S$[n]	A	oil[e]			60.7	6.37	5.90		60.4	6.65	5.85	
V(T/C)	$C_{18}H_{28}N_2O_2S$[o]	A	176–177(d.)	+ 41	1.0	64.2	8.39	8.33		64.2	8.40	8.33	
V(T/E)	$C_{13}H_{17}NO_2S$	—[f]	oil	+ 19	1.0	62.1	6.82	5.57	12.8	62.3	6.59	5.39	12.5
V(T/N)	$C_{12}H_{16}N_2OS$	C	174–175	+ 20	0.8	61.0	6.83	11.8	13.6	61.0	6.84	11.8	13.4
V(P/H)	$C_{13}H_{17}NO_2S$[g]	A	98.5–99.5	− 18	1.0	62.1	6.82	5.57		61.9	6.60	5.63	
V(P/C)	$C_{19}H_{30}N_2O_2S$[h]	A	175(d.)	− 20	1.0	65.1	8.63	7.99		65.4	8.68	7.83	
V(P/E)	$C_{14}H_{19}NO_2S$	C	oil	− 58	0.7	63.4	7.22	5.28		62.6	7.26	5.14	
V(P/N)	$C_{13}H_{18}N_2OS$	C	151–152	− 74	0.9	62.4	7.25	11.2	12.8	61.8	7.30	11.0	13.0
P(T/H)	$C_{12}H_{13}NO_3S$[n]	A	solid[i]	− 96	1.0	61.2	5.57	5.95		60.9	5.72	5.78	
P(T/C)	$C_{18}H_{26}N_2O_2S$[h,o]	A	219(d.)	− 48	1.0	64.6	7.84	8.38	9.59	64.3	7.62	8.55	9.45
P(T/E)	$C_{13}H_{15}NO_2S$	B	oil	−215	0.5	62.6	6.06	5.62		62.4	6.13	5.39	
P(T/N)	$C_{12}H_{14}N_2OS$	C	solid[i]	−136	0.9	61.5	6.02	12.0	13.7	61.1	6.24	11.6	13.1
P(P/H)	$C_{13}H_{15}NO_3S$[h]	A	126	− 69	1.0	62.6	6.06	5.62		62.4	5.94	5.80	
P(P/C)	$C_{19}H_{28}N_2O_2S$	A	195(d.)	− 35	0.7	65.5	8.10	8.04		65.4	8.12	8.04	
P(P/E)	$C_{14}H_{17}NO_2S$	B	66.5–69	−106	1.1	63.9	6.51	5.32	12.2	63.6	6.53	5.26	12.0
P(P/N)	$C_{13}H_{16}N_2OS$	C	105	− 93	0.8	62.9	6.49	11.2	12.9	62.8	6.52	11.4	13.1
T(T/H)	$C_{16}H_{15}NO_3S$[n]	A	solid[i]	+151	1.0	63.8	5.02	4.65		—[j]	5.31	4.42	
T(T/C)	$C_{22}H_{28}N_2O_3S$[o]	A	165(d.)	+213	1.0	66.0	7.05	7.00		65.9	7.06	6.96	
T(T/E)	$C_{17}H_{17}NO_3S$	C	oil	+ 85	0.2	64.7	5.43	4.44		64.6	5.74	4.50	
T(T/N)	$C_{16}H_{16}N_2O_2S$[h]	D	184–185	+167[l]	2.8								
T(P/H)	$C_{17}H_{17}NO_3S$	A	92–93	+117	1.0	64.7	5.43	4.44		—[m]	5.67	4.30	
T(P/C)	$C_{23}H_{30}N_2O_3S$	A	158–160	+119	0.5	66.6	7.29	6.76		66.7	7.42	6.82	
T(P/E)	$C_{18}H_{19}NO_3S$	C	118	+106	0.8	65.6	5.81	4.25		65.7	5.88	4.28	
T(P/N)	$C_{17}H_{18}N_2O_2S$[g]	D	87.5–89	+ 82	2.5								
L(T/E)	$C_{15}H_{21}NO_2S$	B	oil	+ 38	0.8	64.5	7.58	5.01	11.5	64.6	7.60	4.97	11.3
L(T/N)	$C_{13}H_{18}N_2OS$	D	162–163.5	+ 59	0.8	62.4	7.25	11.2	12.8	62.5	7.49	11.4	12.9
L(P/E)	$C_{16}H_{23}NO_2S$	B	oil	− 58	2.9	65.5	7.90	4.77	10.9	65.0	8.13	5.23	11.1
L(P/N)	$C_{14}H_{20}N_2OS$	C	107	− 72	0.9	63.6	7.63	10.6	12.1	63.7	7.72	10.6	12.1

[a] For symbols, see Table 1. [b] See: Experimental. [c] Melting points of ester and amide derivatives are uncorrected and determined in capillary tubes in a bath; other m.p. are determined in an electrically heated block. [d] When not otherwise indicated, rotations are measured in methanol at room temperature (20–27°). [e] In the course of 6 months the oil changed into an amorphous solid, devoid of optical activity. [f] Prepared from the corresponding acid and diazomethane. [g] Previously described (Ref. 7). [h] Previously described (Ref. 6). [i] Amorphous; liquefies at about 60°. [j] Satisfactory C-analysis could not be obtained, equivalent weight (microtitration): 301 (Calc. 301). [k] Previously described (Ref. 8). [l] Determined in 96 % ethanol. [m] Satisfactory C-analysis could not be obtained, equivalent weight (microtitration): 315 (Calc. 315). [n] Previously described as a non-analyzed oil (Ref. 14). [o] Previously described (Ref. 14).

Table 3. UV-Maxima in various solvents for the *N*-thioacylated amino acids and derivatives investigated. The measurements were performed in 1 cm cells at room temperature with concentrations ranging from *ca.* 0.01 to 0.7 mg/ml in the short, and from 0.02 to 4.2 mg/ml in the long wavelength region.

Compound [a]	Water		Methanol		Dioxane		Isooctane		Water		Methanol		Dioxane		Isooctane	
	mμ	log ε	mμ	log ε	mμ	log ε	mμ	log ε	mμ	log ε	mμ	log ε	mμ	log ε	mμ	log ε
V(T/C)	279[f]	3.92	286[e]	3.92	286	3.85			356	2.54*i*	375	2.41*i*	385	2.47		
V(T/H)	278[d]	3.80*i*[b]	286[c]	3.59	289[e]	3.78			364	2.57*i*	371	2.36	389	2.41		
V(T/N)			288[e]	3.89	290[e]	3.81					388	2.39*i*	388	2.46		
V(T/E)			289[d]	3.84	287[d]	3.82	289[e]	3.71			374	2.38	387	2.46	401	2.33
V(P/C)	269	4.02	271	4.10	271	4.03			324	1.90	336	1.82	360	1.73*i*		
V(P/H)	267	4.26	270	4.02	269	4.05			323	1.84	340	1.74	356	1.67		
V(P/N)			271	4.04	274	3.93					344	1.73	356	1.61		
V(P/E)			267	4.10	267	4.06	268	4.08			338	1.82*i*	380	1.51*i*	364	1.62
T(T/C)	278[f]	4.00	279[e]	3.94	278[e]	3.95			356	2.57*i*	373	2.37*i*	384	2.42		
T(T/H)	282[d]	4.03	275[e]	3.95	280[e]	3.93			360	2.53*i*	372	2.38	388	2.42		
T(T/N)			280[e]	3.95	287[g]	3.95					380	2.25	387	2.49		
T(T/E)			280	3.86	287[h]	3.58					384	2.29	380	1.76		
T(P/C)	268	4.07	270	4.15	268	4.09			325	1.99*i*	330	1.92*i*	320	2.37*i*		
T(P/H)	267[d]	4.07	269	4.14	269	4.07			300	3.32*i*	337	1.75	353	1.70		
T(P/N)			272	4.08	274	4.03					341	1.81	355	1.72		
T(P/E)			272	4.09	272	4.09					343	1.72	358	1.73		
P(T/C)	279[d]	4.04	281[c]	4.04	285[d]	3.99			345	2.60*i*	360	2.46	380	2.50		
P(T/H)	278[d]	3.96	280[c]	3.72	282[c]	3.97			340	2.58	367	2.44	385	2.46		
P(T/N)			283[d]	4.01	288[c]	4.27					368	2.37	383	2.48		
P(T/E)			280[c]	4.02	286[c]	4.07	287[c]	3.94			363	2.10	385	1.71	395	2.39
P(P/C)	272	4.09	278	4.10	277	4.12			325	2.10*i*	340	1.89*i*	375	1.83*i*		
P(P/H)	272	4.22	275	4.13	276	4.14			327	1.99*i*	340	1.85*i*	359	1.69*i*		
P(P/N)			276	4.15	279	4.12					344	1.69	358	1.69		
P(P/E)			275	4.12	278	4.17	276	4.12			319	2.71*i*	330	2.71	325	2.64
L(T/N)			284[d]	3.86	289[d]	3.87					374	2.42	384	2.53		
L(T/E)			286[e]	3.83	289[e]	3.80	289	3.70			377	2.27	387	2.44	400	2.26
L(P/N)			265	4.02	265	4.05					341	1.76	352	1.74		
L(P/E)			268	4.30	270	4.28	268	4.09			335	2.20	362	2.02*i*	365	1.75*i*

[a] The abbreviations are those defined in Table 1. [b] *i* indicates inflection.
[c] Additional maximum observed at 230–235 mμ. [d] Additional maximum at 236–240 mμ.
[e] Additional maximum at 241–245 mμ. [f] Additional maximum at 247 mμ.
[g] Additional maxima at 280 and 227 mμ. [h] Additional maxima at 280 and 242 mμ.

Both the *N*-thiobenzoyl- (Figs. 1 and 2) and the *N*-phenylthioacetyl-series (Figs. 3 and 4) exhibit several examples of change in sign of c.e. with change in solvent. To illustrate the remarkable changes, the observed signs of the first and second c.e. of four *N*-thiobenzoyl-L-valine derivatives in various solvents are presented in Table 4. Here, as in all observed cases of inversion

Table 4. The signs of the first and second c.e. of *N*-thiobenzoyl-L-valine derivatives in various solvents.

Solvent	Compound	V (T/C)[a]	V (T/H)	V (T/N)	V (T/E)
Water		+(−)[b]	+(−)		
Methanol		−(+)	−(+)	+(−)	+
Dioxane		−(+)	−(+)	−(−)	+
Isooctane					−(+)

[a] Abbreviations are those defined in Table 1. [b] The sign in parentheses indicates that of the second c.e.

of sign of the first c.e., the positive c.e. is associated with the more polar solvent. Generally, though exceptions are noted, solvent induced changes in sign of the first c.e. are accompanied by inversions in sign of the second c.e.; if so, the positive c.e. of the latter is always observed in the less polar solvent. In the thiobenzoyl derivatives solvent-induced changes of the sign of the first c.e. are limited to the valine-derivatives (Table 4) and the leucine ester (L(T/E), (Fig. 1); none of the tyrosine or proline derivatives exhibit observable changes. In the phenylthioacetyl series (Figs. 3 and 4), however, changes have been observed for virtually all compounds, with the proline derivatives as a notable exception.

In several cases the existence of more than one 'species' of a given compound in a given solvent can be directly inferred from the experimental data. Thus, the CD-curves of V(P/C), V(P/H), and T(P/C) in water, and T(P/H) in methanol, (Fig. 4), are composed of segments of opposite sign, separated by about 30 mμ, and hence diagnostic of the existence of solvational and conformational equilibria.[17] Even more striking are the CD-curves for the thiobenzoyl-proline derivatives in various solvents (Fig. 2), where in every case the $n \rightarrow \pi^*$ transition gives rise to two oppositely signed CD extrema, separated by 30—40 mμ and of widely varying amplitude ratios. Significantly, the amplitude of the negative extremum, at longest wavelength, decreases on increasing the polarity of the solvent whereas that of the positive extremum increases. The observed UV-maxima always fall within the two CD-extrema, closest to the one of greatest amplitude.

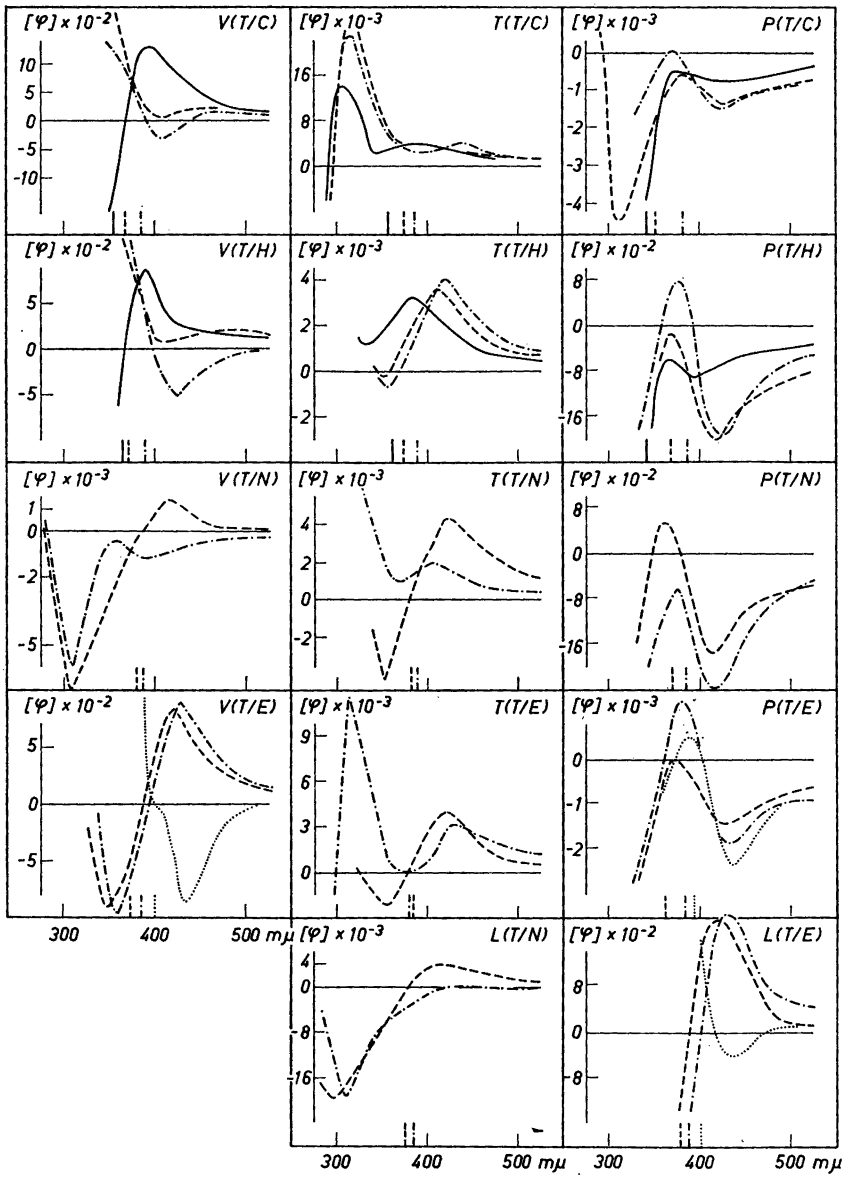

Fig. 1. RD-Curves of *N*-thiobenzoyl-L-amino acids and derivatives. The symbols are those defined in Table 1. [φ] indicates molecular rotation (*cf.* Ref. 12). —: Water (or 1 N NaOH for the acids); – – –: methanol; —·—: dioxane; ···: isooctane. The positions of the UV-maxima in the various solvents are indicated on the abscissa.

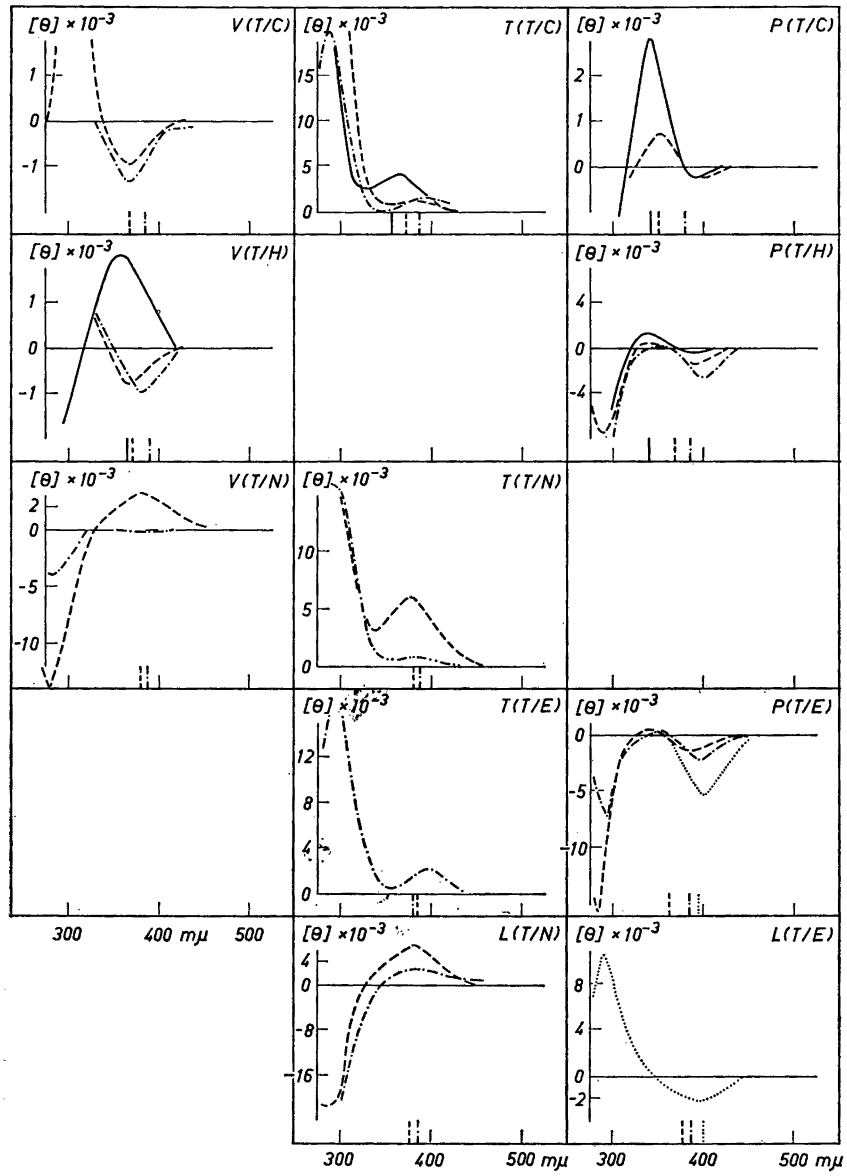

Fig. 2. CD-Curves of *N*-thiobenzoyl-L-amino acids and derivatives. [θ] indicates molecular ellipticity (*cf.* Ref. 13). The symbols are those used in Fig. 1.

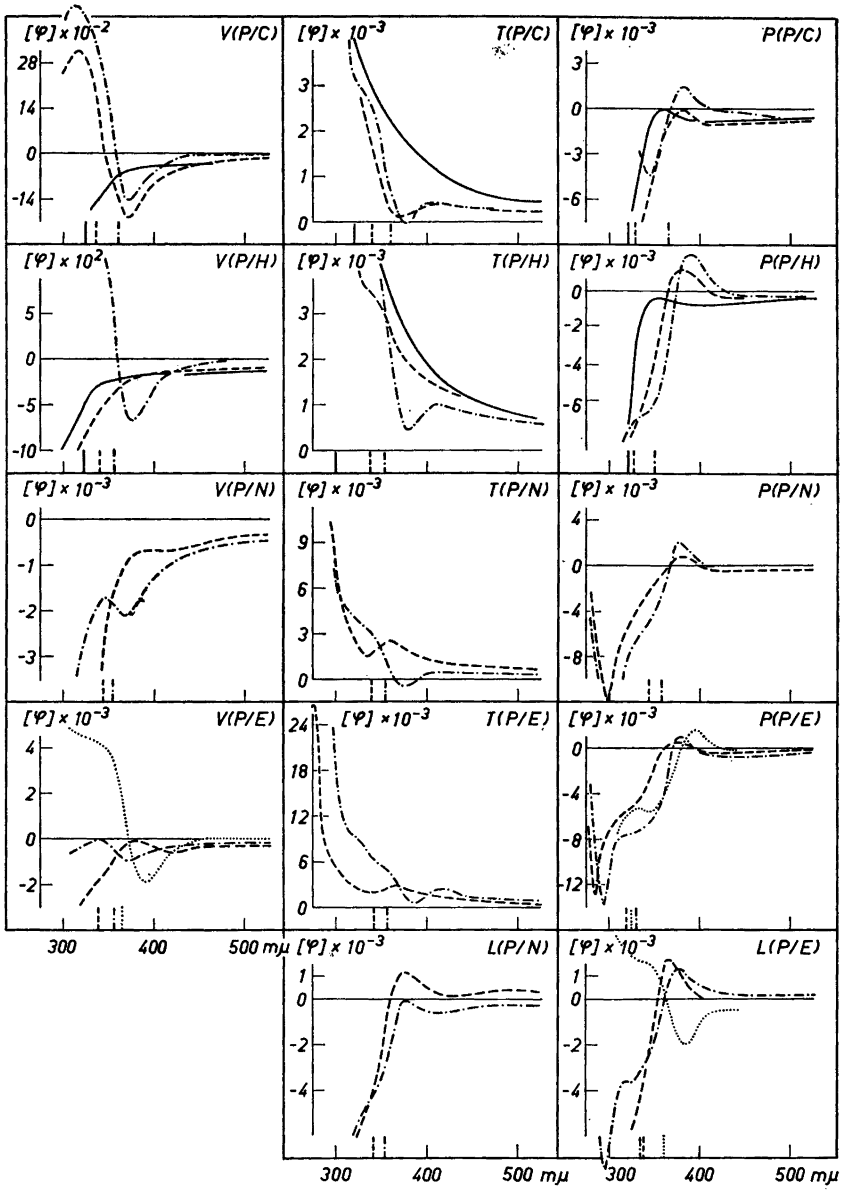

Fig. 3. RD-Curves of *N*-phenylthioacetyl-L-amino acids and derivatives. The symbols are those used in Fig. 1.

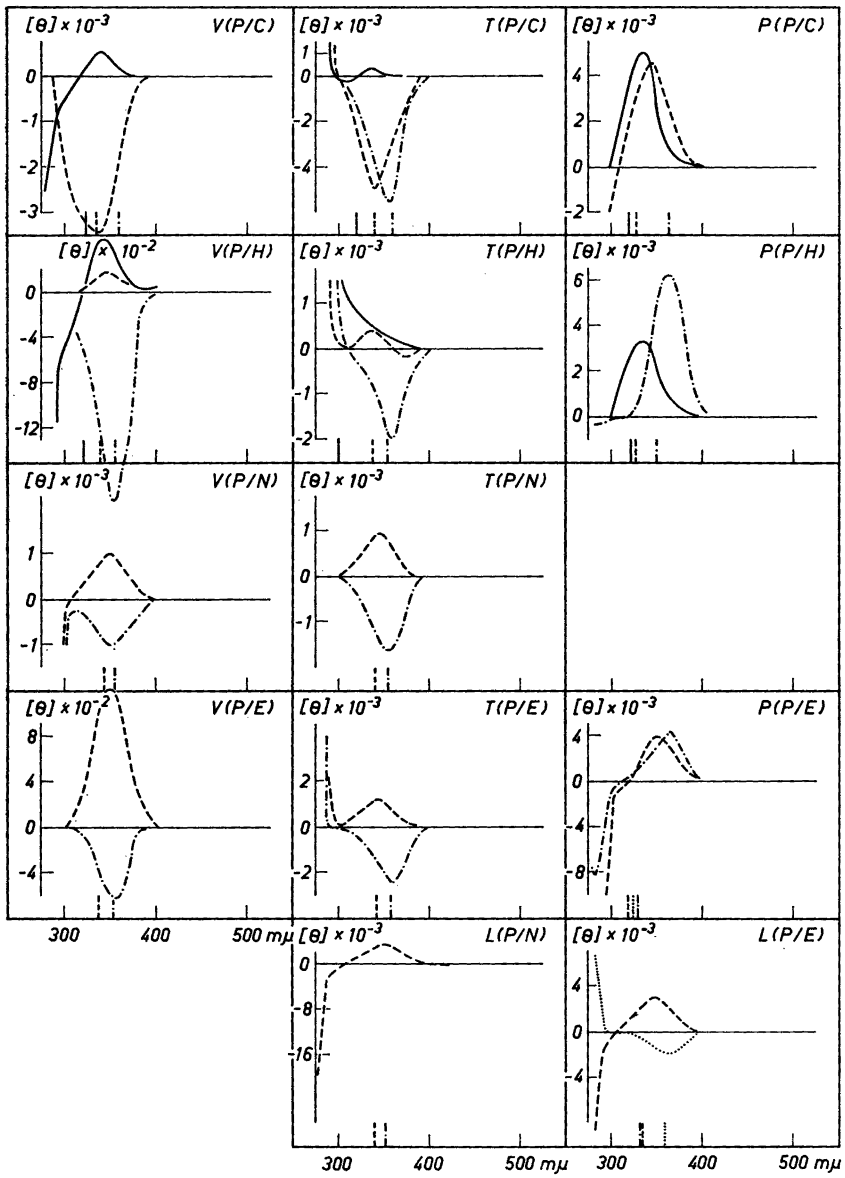

Fig. 4. CD-Curves of N-phenylthioacetyl-L-amino acids and derivatives. The symbols are those used in Fig. 1.

DISCUSSION

Since the thiobenzoyl- and phenylthioacetyl-groupings were first introduced as a means of producing 'chromophoric' α-amino acid derivatives [6] several limitations as to their general usage for configurational assignments have become apparent. Much as the original conclusion,[18] that a positive c.e. of *N*-dithiocarbalkoxy-amino acids is indicative of the L-configuration, has necessitated more precise specification as to the solvent employed,[19] it has now become apparent that similar precautions obtain in the case of the *N*-thiobenzoyl- and *N*-phenylthioacetyl derivatives. Apart from the present investigation, Yamada *et al.*[16] recently demonstrated the solvent influence on the anomalous rotation of certain *N*-phenylthioacetyl-α-amino acids, whilst Barrett [14] observed similar phenomena working with the corresponding *N*-thiobenzoyl derivatives. Hence, there can be no doubt that mono-*N*-substituted thioamides can interact drastically with the solvent either by producing one or more solvated species, or, alternatively or additionally, by adopting rotamer (or other isomer) compositions which are highly dependent on the solvent.

The general principles governing the interaction between solvent and chiral solutes, as reflected in CD measurements, have recently been reviewed, and the operation of conformational or solvation equilibria, separately or combined, has been discussed.[20] More specifically, examples of inversion in sign of the c.e. with increasing *N*-substitutions in thioamides have recently been presented.[21]

The present investigation does not provide sufficient data for a detailed discussion of the solute-solvent interaction, but it appears that the type of compounds here studied may be of considerable potential interest in this respect. The intrinsic difficulty in such studies is associated with the possible dual or combined operation of conformational changes and solvent interaction. Conceivably, low temperature CD- measurements, as conducted for several other compounds (*cf.* Ref. 22), might prove helpful also within the present group. Despite the lack of understanding regarding the actual solvent interaction, the present study provides an instructive illustration of the caution required when assigning configuration to chiral molecules on the basis of anomalous rotation data. Thus, the change from one solvent to even a very similar solvent may, in certain cases, result in a shift in sign of the c.e. as amply demonstrated in Figs. 1—4. However, also structurally analogous compounds, such as, *e.g.* L(P/E) and V(P/E) (Figs. 3—4), or L(T/N) and V(T/N) (Figs. 1—2), may exhibit c.e. of opposite signs in the same solvent, here dioxane, reflecting a markedly different solute-solvent interaction in the two sets of homologues. Although the —C(S)—NH-grouping appears to be particularly prone to enter such solvent interactions (*cf.* also Refs. 14, 16, and 21), our knowledge regarding other structural types possessing the same ability is still so limited that extreme caution is warranted in all cases where configuration is attributed to chiral molecules on the basis of anomalous rotation arising from 'chromophoric groups' other than those extensively studied and familiar to the observer.

EXPERIMENTAL

Ultraviolet spectra were recorded by means of a Bausch & Lomb Spectronic 505 or a Zeiss QII spectrophotometer. Rotatory dispersion curves were measured with a recording JASCO ORD-5 spectropolarimeter at Stanford, and with a recording Rudolph instrument by Mr. Rolf Bäckström at Uppsala. All circular dichroism measurements were performed with a Baird-Atomic/Jouan Dichrograph.

N-Thioacylamino acids and their cyclohexylammonium salts

Method A. The amino acids were phenylthioacetylated and thiobenzoylated according to the procedures described by Holmberg [15] and by Kjær.[7,8] The amino acid (0.01 mole) was dissolved in two equivalents (tyrosine in three equivalents) of 1 N sodium hydroxide, and one equivalent of carboxymethyl dithiophenylacetate or carboxymethyl dithiobenzoate was added. With L-valine and L-proline the solution was kept at room temperature overnight, whilst in the case of the less reactive L-tyrosine the reaction mixture was heated for 5—6 h at 60°. The solution was then acidified with 5 N hydrochloric acid, the oily precipitate extracted with ether, and the ether extract thoroughly washed with water to remove the mercaptoacetic acid formed as a by-product in the reaction. After drying over sodium sulphate, the ether was removed. In the case of the phenylthioacetyl derivatives, the residue could be brought to crystallization. These acids were recrystallized from a mixture of ether and light petroleum.

The cyclohexylammonium salts of the thioacylated amino acids vere prepared by mixing ethereal solutions of cyclohexylamine and the acid produced as described above. The crystalline salt was purified by recrystallization from a mixture of ethanol and ether.

In order to obtain the non-crystalline *N*-thiobenzoyl amino acids in a pure state, the crystalline cyclohexylammonium salts of the acids were decomposed with hydrochloric acid and the resulting oil was extracted with ether. The solvent was removed *in vacuo* and the oily residue maintained under vacuum for some time, affording an amorphous solid which could not be induced to crystallize.

Satisfactory analyses could not be obtained for the two thioacyl derivatives of tyrosine. However, equivalent weight determinations (microtitration) gave correct results, as did elementary analyses of the corresponding cyclohexylammonium salts.

N-Thioacylamino acid esters and amides

Method B. A solution of one equiv. of carboxymethyl dithiobenzoate or carboxymethyl dithiophenylacetate in pyridine (5—10 ml/g) was added in one portion to a solution of one equiv. of the amino acid ester hydrochloride in pyridine (5—10 ml/g), containing 2—2.5 moles of triethylamine. The preparations were normally performed on 0.5—1.5 g of amino acid ester hydrochloride. The mixtures were allowed to stand overnight at room temperature, except for those containing L (T/E) and L (P/E); the latter were kept at 60°. The reaction mixture was distributed between dilute sulphuric acid and ether, and the aqueous phase washed repeatedly with ether. The organic phase was washed with sodium bicarbonate, dried over sodium sulphate, and the ether removed through a small column.

In one case only (P (P/E)), the reaction product was crystalline and could be purified by recrystallization from an ethyl acetate:heptane mixture; colloidal impurities were removed by treatment with charcoal. In all other cases, however, the products were viscous oils, which were purified by repeated short-path distillation. In the case of L (T/E), the reaction product was purified by adsorption on an alumina column ('Al_2O_3, nach Woelm, akt.-stufe I, neutral') and elution with benzene prior to distillation.

Method C. A solution of one equiv. of carboxymethyl dithiobenzoate or carboxymethyl dithiophenylacetate in an equivalent amount of 1 N NaOH was added in one portion to a solution of one mole of the amino acid amide (as free base or hydrochloride) or amino acid ester hydrochloride in water, in quantities ranging from 0.2—2.5 g. When the hydrochlorides were used, the solutions were neutralized with the required quantity

of base before reaction. In most cases, the reactions started within a few minutes. The reaction mixtures were kept overnight at room temperature, except in three cases where longer reaction times were required. V (P/E) and T (T/E) were kept for 18 h at room temperature, and P (T/N) for 35 h at 55°. When crystalline products resulted, the reaction mixtures were filtered and the products recrystallized (T (P/E) from benzene:pentane, and the amides from aqueous ethanol); colloidal impurities were removed with charcoal. In cases where the reaction products were oils or amorphous solids, they were extracted from the reaction mixture with ether (in the case of P (T/N) with ethyl acetate), and the extracts were washed with sodium bicarbonate and dried. After removal of the solvent, the products were purified by repeated short-path distillation. In the case of P (T/N), the product was adsorbed on an alumina column (*cf.* above) and eluted with 1 % methanol in benzene prior to distillation.

Method D. The phenylthioacetyl- or thiobenzoyl-amino acid ester was dissolved in methanol, previously saturated with NH_3 at 0°, and allowed to stand in a sealed flask at room temperature for 2 days. After removal of the solvent and washing of the residue with ether, the product was purified by recrystallization from aqueous ethanol, if necessary with the application of charcoal.

Acknowledgement. Financial assistance (grant No. GP.-4304) from the *National Science Foundation* to the *Stanford group* and from the *Swedish Natural Science Research Council* to the *Swedish authors* is gratefully acknowledged.

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Received July 27, 1966.