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Studies on Optically Active Amino Acids. XIX.[1] Solvent Effects on Optical Rotatory Dispersion and Circular Dichroism of N-Dithiocarbethoxy-L-α-amino Acids[2]

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Optical rotatory dispersion and circular dichroism curves of twenty N-dithiocarbethoxy-L-α-amino acids were measured in water, methanol, dioxane, chloroform, and benzene. Striking solvent effects on Cotton effect curves, due to $n\rightarrow\pi^*$ transition of the >N-CSS chromophore, were found. The Cotton effect was inverted by changing solvents. These solvent effects were classified in five groups based on the chemical structure of their amino acids.

Cyclohexylammonium salts of these dithiocarbamates produced different solvent effects on Cotton effect curves due to the same transition of the >N-CSS chromophore.

The most reliable conditions for correlating the absolute configuration of α-amino acids to the sign of Cotton effect curves using dithiocarbamate derivatives were discussed.

Solvent effects on optical rotatory dispersion (ORD) and on circular dichroism (CD) have been reported for a variety of compounds, attracting attention to the solvent used in ORD and CD measurements and arousing interest in the investigation of phenomena related to conformational and/or solvational equilibria. Striking inversions of Cotton effects with ketonic chromophoric compounds, *i.e.* α-halo-[4a,b,d] and α-hydroxy-[4c] cyclohexanones were found by Djerassi, *et al.*[4] when solvents were changed. These phenomena are best rationalized by suggesting that the conformational equilibria in these compounds depend on solvent polarity.[4–6a]

However, different kinds of Cotton effects, changed by solute and solvent interaction, were observed for rigid molecules such as isofenchone,[7a] epiisofenchone,[7a] α-acetoxycamphor[7b] and camphor.[6a] This is considered due to equilibria associated with solvation.[6a] Relationships between the ORD and CD curves of ketonic chromophores and the solvents used for measurements, have been described elsewhere.[5f,6]

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[1] Part XVIII: K. Achiwa, S. Terashima, H. Mizuno, N. Takamura, T. Kitagawa, K. Ishikawa, and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **18**, 61 (1970).

[2] Preliminary communication concerning part of this work has been published by Shu-ichi Yamada, Kiyoyasu Ishikawa, and Kazuo Achiwa, *Chem. Pharm. Bull.* (Tokyo), 13, 892 (1965), (Ref. 8*a*).

[3] Location: *Hongo, Bunkyo-ku, Tokyo.*

[4] *a*) C. Djerassi and L. E. Geller, *Tetrahedron*, 3, 319 (1958); *b*) C. Djerassi, L. E. Geller, and E. J. Eisenbraun, *J. Org. Chem.*, **25**, 1 (1969); *c*) C. Djerassi, R. Records, and B. Bach, *Chem. & Ind.*, **1961**, 258; *d*) K.M.Wellman, E. Bunnenberg, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 1870 (1963).

[5] *a*) N.L. Allinger, J. Allinger, L.E. Geller, and C. Djerassi, *J. Org. Chem.*, **25**, 6 (1960); *b*) C. Djerassi, E.J. Warawa, R.E. Wolff, and E.J. Eisenbraun, *ibid.*, **25**, 917 (1960); *c*) C. Djerassi and J. Staunton, *J. Am. Chem. Soc.*, **83**, 736 (1961); *d*) J. Allinger, N. L. Allinger, L.E. Geller, and C. Djerassi, *J. Org. Chem.*, **26**, 3521 (1961); *e*) A. Moscowitz, K.M. Wellman, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3515 (1963); *f*) K.M. Wellman, P.H.A. Laur, W.S. Briggs, A. Moscowitz, and C. Djerassi, *ibid.*, **87**, 66 (1965); *g*) K.M. Wellman, W.S. Briggs, and C. Djerassi, *ibid.*, **87**, 73 (1965).

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[7] *a*) H.P. Gervais and A. Rassat, *Bull. Soc. Chim. France*, **1961**, 743; *b*) C. Coulombeau and A. Rassat, *ibid.*, **1963**, 2673.

In 1965, striking solvent effects in ORD and CD measurements for non-ketonic chromophores, *i.e.*, dithiocarbamates[2,8a)] and N-thioacyl derivatives[8b)] were first reported from our laboratory, then independently by Barrett.[9a)] Successive papers on this subject have been published by these authors.[8,9)] Changes in Cotton effect curves by changing solvents for thionamides, N-thiobenzoyl and N-phenylthioacetyl derivatives of amino acids have been reported, respectively, by Djerassi, *et al.*[10a)] and Kjaer, *et al.*[10b)] This type of solvent effect was connected with conformational mobility of molecules using the technique of low temperature CD measurements.[10a, 11)]

N-Dithiocarbamates of α-amino acids were first reported as the most suitable compounds for correlating the absolute configuration of the α-asymmetric centers of α-amino acids with their ORD curved.[12)] A positive Cotton effect corresponded to the L-configuration, and a negative one to the D-configuration. This optical method, including CD measurement,[13)] has subsequently been well studied stereochemically,[11)] and is often employed to establish the absolute configuration of various α-amino acids.[14)] However, no attention had been paid to the solvents used for these ORD and CD measurements until we published a communication,[8a)] in which the sign of the Cotton effect was remarkably inverted depending upon the solvents used. This should be noted in assigning the absolute configuration of α-amino acids by this optical method.

This paper deals with further detailed results of our studies, in which several types of solvent effects exist for measurements of ORD and CD curves of N-dithiocarbethoxy-α-amino acids and their cyclohexylammonium salts. We report the most reliable solvent for establishing the absolute configuration of α-amino acids.

The solvents selected for our ORD and CD measurements are as follows; water, methanol, dioxane, chloroform, and benzene. Even when selection is made empirically, these solvents are typical in a sense; water and methanol are protic and polar, chloroform is non-polar, and dioxane is non-polar, aprotic and capable hydrogen bonding with a -NH_2 or -OH group. With chloroform as solvent, care must be taken since commercially available G.R. (Guaranteed Reagnet) chloroform contains about 1% (v/v) of ethanol as a stabilizer. This amount of ethanol is not necessarily negligible, since the concentration of a sample for ORD and CD measurements is usually 0.1—0.5% (w/v). This should be considered particularly when the Cotton effect is inverted by changing the solvent from ethanol to chloroform and vice versa. Accordingly, CD measurements of N-dithiocarbethoxy-L-alanine (I), which invert a positive Cotton effect to a negative one when the solvent is changed from methanol to chloroform, were performed in chloroform containing various amounts of ethanol. Results are shown in Table I. No severe change in the CD maximum was observed in changing the solvent from pure chloroform[15)] (no ethanol content) to 10% ethanol–containing chloroform. However, the molecular ellipiticity $[\theta]$ at a negative CD maximum in pure chloroform is slightly smaller than

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8) *a*) S. Yamada, K. Ishikawa, and K. Achiwa, *Chem. Pharm. Bull.* (Tokyo), **13**, 892 (1965); *b*) *Idem*, *ibid.*, **13**, 1266 (1965); *c*) *Idem*, *ibid.*, **14**, 921 (1966).

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10) *a*) J.V. Burakevich and C. Djerassi, *J. Am. Chem. Soc.*, **87**, 51 (1965); *b*) E. Bach, A. Kjaer, R. Dahlbom, T. Walle, B. Sjöberg, E. Bunnenberg, C. Djerassi, and R. Records, *Acta Chem. Scand.*, **20**, 2781 (1966).

11) W.S. Briggs and C. Djessassi, *Tetrahedron*, **21**, 3455 (1965).

12) B. Sjöberg, A. Fredga, and C. Djerassi, *J. Am. Chem. Soc.*, **81**, 5002 (1959).

13) C. Djerassi, H. Wolf, and E. Bunnenberg, *J. Am. Chem. Soc.*, **84**, 4552 (1962).

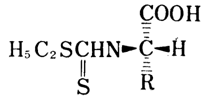
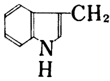
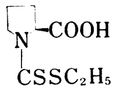
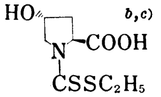
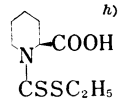
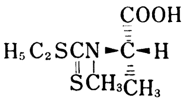
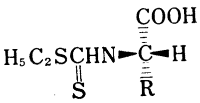
14) *a*) C. Djerassi, K. Undheim, R. C. Sheppard, W.G. Terry, and B. Sjöberg, *Acta Chem. Scand.*, **15**, 903 (1961); *b*) J.S. Dalby, G.W. Kenner, and R.C. Sheppard, *J. Chem. Soc.*, **1962**, 4387; *c*) H. Pracejus and S. Winter, *Chem. Ber.*, **97**, 3173 (1964); *d*) H. Ripperger and K. Schreiber, *Tetrahedron*, **21**, 407 (1965); *e*) S. Nakamura, T. Chikaike, H. Yonehara, and H. Umezawa, *Chem. Pharm. Bull.* (Tokyo), **13**, 599 (1965); *f*) S. Gronowitz, I. Sjögren, I. Wernstedt, and B. Sjöberg, *Arkiv Kemi*, **23**, 129 (1965).

15) Ethanol free chloroform was obtained by elution of G.R. chloroform through an alumina column.

Table I. CD Maxima of I in Ethanol–Chloroform Mixture

Amount of EtOH (v/v)	0%	1%	10%	50%
$[\theta]^{20}$ (mμ)	−3420 (343)	−3850 (343)	−3860 (343)	−669 (342)

Table II. Signs of Cotton Effects for N-Dithiocarbethoxy-L-α-amino Acids in Various Solvents

	Group	R	Solvt.				
			H_2O	CH_3OH	Dioxane	$CHCl_3$	C_6H_6
	1	CH_3 I		+	+	−	+,−
		$(CH_3)_2CH$ II		+	+	−	+,−
		(*S*) $C_2H_5CH(CH_3)$[a] III		+	+	−	+,−
		$HOOC(CH_2)_2$[b] IV		+	+	−	+,−
		$C_2H_5SSCHN\ (CH_2)_4$ V		+	+	−	+,−
	2	$C_6H_5CH_2$ VI		+	+,−	−	−
		p-$HOC_6H_4CH_2$[b] VII		+	+,−	−	−
	3	$HOOC\ CH_2$[c,d] VIII	+	(+)	−	−	
		$H_2NOC\ CH_2$[c] IX	+	(+)	−	−	
		$HO\ CH_2$[c,e] X	+	(+)	−	−	
		CH_3COOCH_2[f] XI	+	(+)	−	−	
		 [g] XII	+	(+)	−	−	
		(*S*) $CH_3CH(OH)$[a] XIII	+	(+)	−	−	
XIV 	4			+	+	+	+
XV  [b,c]				+	+	+	+
XVI  [h]				+	+	+	+
XVII 				+	+	+	+
	5	(*R*) $C_2H_5CH(CH_3)$[a] XVIII		+	+	+,−	+
		$(CH_3)_3C$ XIX		+	+	+,−	+
		(*R*) $CH_3CH(OH)$[a] XX	+	+	+	+,−	

+: positive Cotton effect −: negative Cotton effect +,−: double humped CD maxima in which positive maximum locates in longer wave length (+): small positive Cotton effect

a) (*R*) and (*S*) denote the absolute configuration of β-asymmetric center; (*S*) $C_2H_5CH(CH_3)$=isoleucine, (*R*) $C_2H_5CH(CH_3)$=alloisoleucine, (*S*) $CH_3CH(OH)$=allothreonine, (*R*) $CH_3CH(OH)$=threonine

b) insoluble in C_6H_6

c) CH_3OH(10—20%) was added to $CHCl_3$ to dissolve the sample.

d) +,− CD maxima were observed in dioxane but the positive maximum was very small.

e) No CD maximum was observed in CH_3OH.

f) +,− CD maxima were observed in CH_3OH.

g) insoluble in H_2O

h) D-Isomer was used for measurements.

that in chloroform containing 1—10% ethanol. This may be due to solvation of ethanol or dimerization of the carboxyl group in pure chloroform. In these experiments, G.R. chloroform was used for measurements without the removal of ethanol. When a sample was difficult to dissolve in chloroform, an appropriate amount of methanol (10—20%) was added to the chloroform.

With cyclohexylammonium salts of N-dithiocarbethoxy-α-amino acids, water and methanol were chosen as solvents, due to the insolubility of these salts in other solvents.

Result and Discussion

N-Dithiocarbethoxyl-L-α-amino Acids

Detailed ORD and CD investigations covering twenty N-dithiocarbethoxy-L-α-amino acids in several solvents, showed various kinds of changes in signs of the Cotton effect, depending upon the solvents used. Results are classified into five groups according to the close relationships between the type of solvent effect and the structure of the N-dithiocarbethoxy-L-α-amino acid, regardless of small variations and exceptions shown in Table II.

Group 1——Solvent effects represented by N-dithiocarbethoxy-L-alanine (I).

Alanine is the simplest α-amino acid with an asymmetric center at α-position and no functional group at β-position. Compound I shows a positive CD maximum at 340 mμ and 349 mμ in methanol and dioxane, respectively. However, it shows a negative one at 343 mμ in chloroform, and double humped CD maxima at 368 mμ and 340 mμ in benzene. The former is positive and the latter negative (Fig. 1). These CD maxima (except in benzene) are approximately identical in location with the wavelength maximum for ultraviolet absorption associated with the $n \rightarrow \pi^*$ transition of the -NHCSS- group in these solvents (Table III). Though two CD maxima are shown in benzene, only a single ultraviolet absorption maximum was observed in the region of 347 mμ.[16] The ultraviolet (UV) absorption maximum is bathochromically displaced by decreasing the polarity of solvents. This is attributable to the $n \rightarrow \pi^*$ transition of the -NHCSS- group (Table III).

As shown in Table II, N-dithiocarbethoxy-L-valine (II), -L-isoleucine (III), and -L-glutamic acid (IV) and N$^{\alpha}$, N$^{\omega}$-di(dithiocarbethoxy)-L-lysine (V) exhibit the same solvent effects on ORD and CD curves as I, *i.e.* Cotton effect signs are positive in methanol and dioxane, and negative in chloroform. There are double humped CD maxima in benzene, even though the Cotton effects of II, and III in ORD measurements are apparently negative in benzene (Table IV).

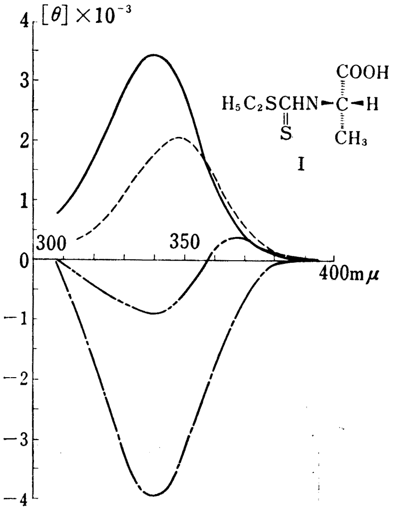


Fig. 1. CD Curves of N-Dithiocarbethoxy-L-alanine (I)

———: in CH_3OH
- - - - -: in dioxane
———: in $CHCl_3$
———: in C_6H_6

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[16] In all double humped CD maxima, a single UV absorption band was observed for the corresponding $n \rightarrow \pi^*$ transition associated with the >N-CSS- group. The UV absorption band usually falls within the two CD maxima, closest to the one of greatest amplitude.

[17] Solvent effects of N-dithiocarbethoxy-L-phenylglycine on ORD and CD measurements were the same as for group 1. However, this compound, which has a phenyl group at an asymmetric center, is different, from other amino acids in this group.[8c] Thus, it was excluded from the group.

Table III. $n \rightarrow \pi^*$ Transition of I and Is associated with the N-Dithiocarbethoxy Group

		Solvt.				
		H_2O	CH_3OH	Dioxane	$CHCl_3$	C_6H_6
I	λ_{max}(mμ)	320—330	335	343	343	347
	ε	shoulder	75	75	76	59
Is	λ_{max}(mμ)	320—330	330—340			
	ε	shoulder	shoulder			

concentration, 0.05%(w/v) temperature, at 21°

No amino acid in this group has a functional group at β-position.[17] Signs of Cotton effect curves characteristic of this group show positive in methanol and dioxane, clearly negative in chloroform. They are also abnormal in ORD measurements and double humped CD maxima as the shape of I in benzene. Values for molecular rotations [*M*] and molecular ellipticities [θ] of these derivatives are given in Table IV.

The solvent effect of N-dithiocarbethoxy-L-alanine ethyl ester (XXI) was next studied to examine whether a carboxyl hydrogen of I contributes to its solvent effect. Since XXI gave almost similar solvent effects as I, *i.e.* positive Cotton effect in methanol and dioxane, negative one in chloroform and double humped CD maxima in benzene on CD measurement (Table IV), we concluded that dimerization or intra- or inter-molecular hydrogen bonding of the carboxyl group has little influence on this solvent effect.

Group 2——Solvent effects represented by N-dithiocarbethoxy-L-phenylalanine (VI).

Solvent effects of N-dithiocarbethoxy-L-phenylalanine (VI) differed somewhat from those of I. A positive Cotton effect curve occured only in methanol, and a negative one in chloroform and benzene. In dioxane an almost plain curve appeared from ORD measurement and double humped CD maxima (Fig. 2) which were quite similar to those for I in benzene. As expected from its structure, N-dithiocarbethoxy-L-tyrosine (VII) exhibited the same solvent effects as VI (Tables II and IV).

In conclusion, the dithiocarbamates of α-amino acids in this group, with a phenyl group at β-position, show a positive Cotton effect in methanol, a double humped one in dioxane but not in benzene, and a negative one in benzene and chloroform.

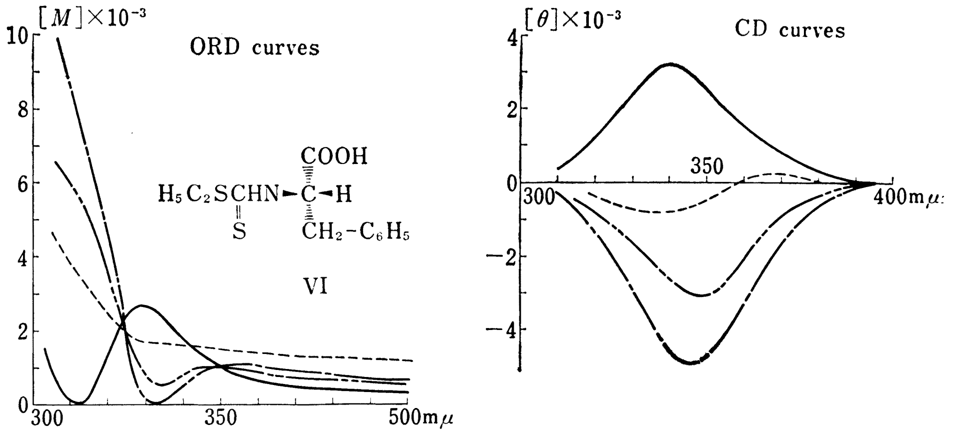


Fig. 2. ORD and CD Curves of N-Dithiocarbethoxy-L-phenylalanine (VI)

———: in CH_3OH, - - - -: in dioxane, —·—: in $CHCl_3$, — — —: in C_6H_6

TABLE IV. Values of Cotton Effects for N-Dithiocarbethoxy-L-α-amino Acids on ORD and CD Measurements in Various Solvents

Compd. No.	Solvt.	ORD				CD maximum	
		1st extremum		2nd extremum[a]			
		mμ	[M]	mμ	[M]	mμ	[θ]
I[b]	CH_3OH	359	1460	311[c]	−3790	340	3420
	dioxane	364	1060	319	−4710	349	2020
	$CHCl_3$	351	−1750	309	4490	343	−3850
	C_6H_6	353	−813	320[c]	171	(368 340	348 −900
II	CH_3OH	362	810	317	−1740	341	3600
	dioxane	371	386	333	−488	355	1275
	$CHCl_3$	364	−877	311[c]	4020	346	−5730
	C_6H_6	363	−487	314[c]	2080	(377 347	231 −2420
III	CH_3OH	359	1410	317	−2200	341	3000
	dioxane	367	745	329	−503	351	1120
	$CHCl_3$	361	−1000	311[c]	5500	344	−3990
	C_6H_6	360	−186	310[c]	2260	(373 345	182 −1230
IV	CH_3OH	360	1650	318	−2470		
	dioxane	364	1470	324	−1820		
	$CHCl_3$	364	−349	316[c]	4050		
V	CH_3OH	360	1280	320	−1340	340	2300
	dioxane	363	1040	330	−506	347	1360
	$CHCl_3$	362	−324	310[c]	3250	343	−1680
	C_6H_6	372	516	349	144	(361 326	369 −238
VI[d,e]	CH_3OH	357	2730	325	24	339	3330
	dioxane	360	1670[f]	——		(368 341	238 −765
	$CHCl_3$	356	20	310	11400	346	−4920
	C_6H_6	364	503	316[c]	6330	348	−3120
VII	CH_3OH	357	2370	327	105	338	2220
	dioxane	360	1550[f]	——		(371 341	41 −669
	$CHCl_3$	366	310	316[c]	10800	346	−4980
VIII[g]	H_2O	360	868	310[c]	−1700	346	1980
	CH_3OH	365	608	335	−78	354	710
	dioxane	354	−734	310[c]	3360	(370 340	56 −2820
	$CHCl_3$	358	−742	314[c]	5340	340	−4250[h]
IX	H_2O	359	1170	310[c]	−3010	336	3670
	CH_3OH	362	1370	329	−162	349	1890
	dioxane	357	−85	308[c]	5500	341	−4480
	$CHCl_3$	359	−477[h]	309[c]	4960	334	−2070[i]
X	H_2O	359	437	317	−702	336	1010
	CH_3OH	——[j]		310[c]	1470	400 −330	0
	dioxane	362	−79	312[c]	4050	344	−2390
	$CHCl_3$	364	−803	315[c]	6430	343	−3550[h]
XI	H_2O	358	791	327	232	322	780
	CH_3OH	361	831[f]	——		(360 341	140 −290
	dioxane	361	423	310[c]	3620	343	−1500
	$CHCl_3$	363	−1650	310[c]	7580	346	−6630
XII	CH_3OH	350	1870	330	1730	335	1050
	dioxane	364	1720[f]	330	4920	348	−950
	$CHCl_3$	363	−637	310[c]	14600	346	−5990
XIII	H_2O	359	446	322	−261	339	762
	CH_3OH	360	600	335	399	347	465
	dioxane	360	81	306[c]	2340	343	−920
	$CHCl_3$	364	−30	310[c]	4650	342	−2170
XIV	CH_3OH	357	1840	308	−9750	336	6410
	dioxane	358	1870	316[c]	−6150	340	6130
	$CHCl_3$	362	74	312	−8750	342	4120
	C_6H_6	360	750	314	−7880	346	4770
XV[k]	CH_3OH	355	1960	309[c]	−5840	335	4790
	dioxane	357	2430	313[c]	−5920	346	6050
	$CHCl_3$	356	2220	312[c]	−5610	337	4860
XVI[d,l]	CH_3OH	366	192	320[c]	−781		
	dioxane	367	307	320[c]	−900		
	$CHCl_3$	368	172	320[c]	−1100		
	C_6H_6	370	287	320[c]	−1050		
XVII[d]	CH_3OH	365	57	320[c]	−237	349	201
	dioxane	366	28	320[c]	−226		
	$CHCl_3$	372	−25	326[c]	−305	353	163
	C_6H_6	368	146	320[c]	−747		
XVIII	CH_3OH	361	1940	321	−2050	344	3900
	dioxane	364	1400	327	−1080	349	2230
	$CHCl_3$	359	−270	314[c]	3140	(373 341	63 −1940
	C_6H_6	374	905	342	−120	360	1080
XIX[d,m]	CH_3OH	364	508	320	−926	345	1030
	dioxane	370	144	324	−381	352	297
	$CHCl_3$	383	119	352	−232	(364 337	191 −245
	C_6H_6	376	390	333	−523	358	652
XX	H_2O	357	884	309[c]	−1650	335	2010
	CH_3OH	360	1650	305	−403	342	1980
	dioxane	361	1540	330	437	347	1480
	$CHCl_3$	350	16	315[c]	1280	(361 329	141 −716
XXI	CH_3OH	361	835	310	−5350	343	3540
	dioxane	367	550	310	−2730	347	1530
	$CHCl_3$	362	−1720	310[c]	4300	344	−7550
	C_6H_6	362	−1250	310[c]	2390	(379 344	65 −2320
XXII	CH_3OH	367	592	310	−1240	349	1440
	dioxane	357	−798	310	3670	(371 341	155 −2580
	$CHCl_3$	361	−921	310	7280	341	−5100

a) 2nd extrema were often observed as shoulders.
b) On ORD in C_6H_6 there was a small peak at the longer wave length ($[M]_{388}$ −11°). lit.[13] CD (c=0.738, CH_3OH) [θ] (mμ): 2580 (340) (positive maximum)
c) The limited value to be measured at the shortest wave length.
d) The optical purity of compound used is not high.
e) lit.[14f] ORD (c=0.143, CH_3OH) [M] (mμ): −4170° (352) (trough), −1900° (324) (peak) for D-isomer
f) shoulder
g) lit.[13] (Also see footnote (Ref. 32) of lit.[11]) ORD (c=0.119, dioxane) [M] (mμ): −760° (356) (through), 5980° (310) (peak) CD (c=1.15, dioxane) [θ] (mμ): −2500 (340) (negative maximum)
h) 10% CH_3OH (v/v) was added to $CHCl_3$ to dissolve the sample.
i) 20% CH_3OH (v/v) was added to $CHCl_3$ to dissolve the sample.
j) possitive plain curve
k) lit.[14a] ORD (c=0.18, CH_3OH) [α] (mμ): 690° (352.5) (peak), −1780° (310) (through)
l) D-isomer was used for measurements.
m) lit.[14c] ORD (CH_3OH) [M] (mμ): −613° (356) (through), 1865° (peak) CD (CH_3OH) [θ] (mμ): −1605 (negative maximum) for D-isomer

Group 3——Solvent effects represented by N-dithiocarbethoxy-L-serine (X).

Solvent effects of N-dithiocarbethoxy-L-aspartic acid (VIII), -L-asparagine (IX), -L-serine (X), -O-acetyl-L-serine (XI), -L-tryptophan (XII) and allo-L-threonine (XIII) are interesting because these compounds contain various functional groups at β-position. All showed a positive Cotton effect in water and a negative one in chloroform (Fig. 3 and 4, and Table IV),

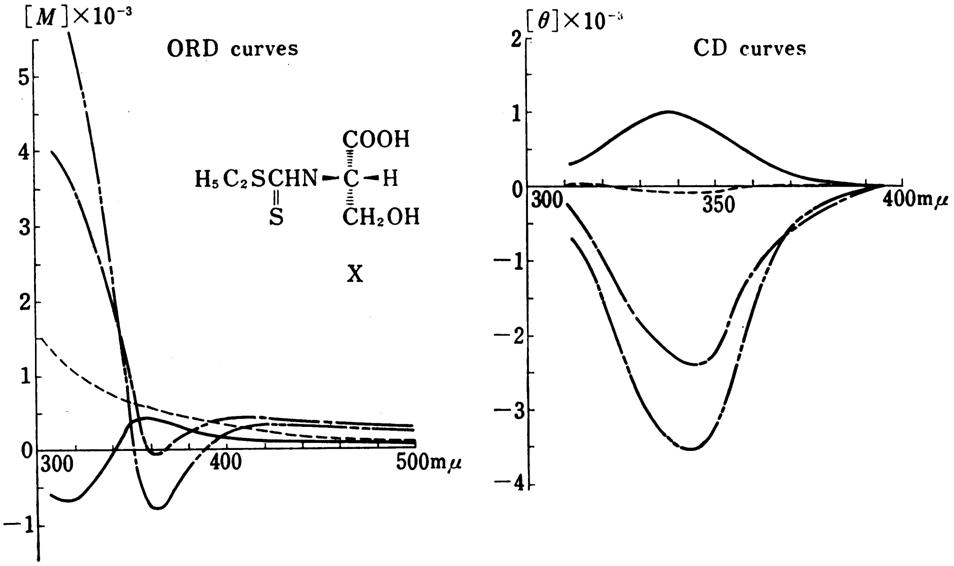


Fig. 3. ORD and CD Curves of N-Dithiocarbethoxy-L-serine (X)

——: in H_2O, – – –: in CH_3OH, ——: in dioxane, — · —: in $CHCl_3$

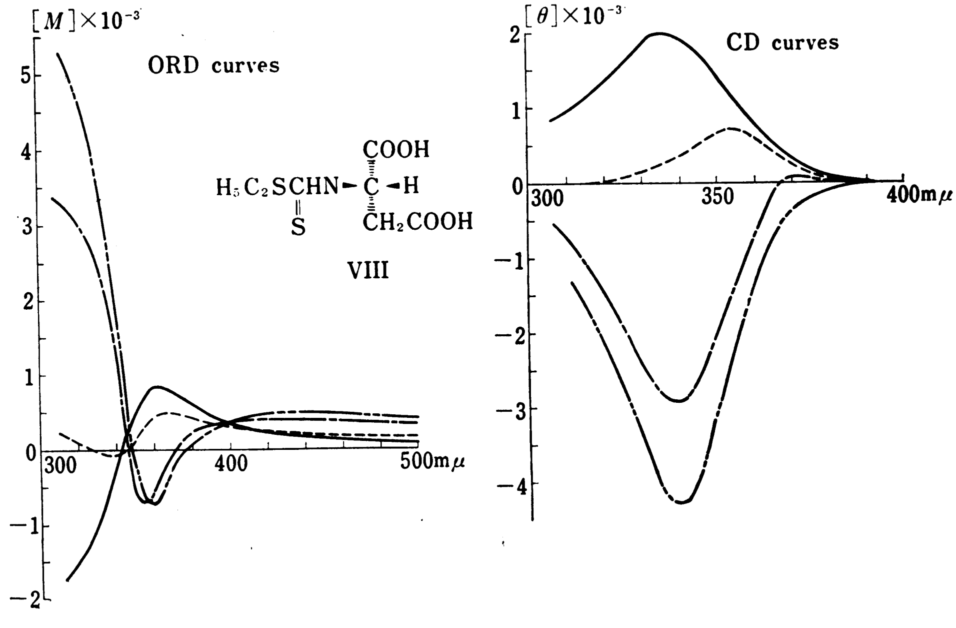


Fig. 4. ORD and CD Curves of N-Dithiocarbethoxy-L-aspartic Acid (VIII)

——: in H_2O, – – –: in CH_3OH, ——: in dioxane, — · —: in $CHCl_3$

as did Groups 1 and 2. In dioxane, except for VIII, they showed a single negative Cotton effect. VIII gave a simple negative Cotton effect in ORD measurement, but in the double humped CD maxima the positive maximum at the longer wavelength is very small compared to the negative one ($[\theta]_{370}$ +56, $[\theta]_{340}$ −2820) (Fig. 4). Hence, VIII is somewhat exceptional for this group. Generally, in methanol, the Cotton effect is positive but small. This is one characteristics of this group (Fig. 3 and 4, and Table IV). An extreme example of this characteristic is X. ORD measurement of X in methanol showed no Cotton effect curve. It exhibited only a plain curve, with very weak double humped CD maxima, barely discernible in CD measurement (Fig. 3). This is the first reported case, in which the dithiocarbamate derivative shows no Cotton effect in ORD and CD measurements in the N-CSS chromophore range. When there are two CD extreme of the same amplitude but of opposite signs, at the same wave length, it is evident that the CD curves cancel each other. When the positions of CD extrema are slightly separated, the apparent CD curve is double humped and fairly well separated.[5f)] Clearly whenever one observes a double–humped CD curve with separated maxima for a substance containing only a single N-CSS chromophore, either conformational and/or solvational equilibria are implied. Hence, dithiocarbamate derivatives of this group exhibit some exceptions in methanol and dioxane, however, they always show a positive Cotton effect in water (Table IV).

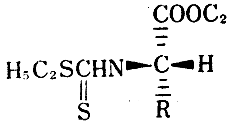
Comparison of VIII with glutamic acid derivative IV, suggests a relationship between the type of solvent effect and the position of the functional group in amino acids. The solvent effect of VIII, with a functional group at β-position, belongs to group III. However, IV, which has a functional group at γ-position, but not at β-position, belongs to Group 1, the alanine series.

Solvent effects of XI and N-dithiocarbethoxy-L-aspartic acid diethyl ester XXII were examined to assess the effect of hydrogen bonding from the β-functional groups. XI and XXII exhibited almost the same solvent effects as X and VIII, respectively, suggesting that differences in β-substituents do not have much affect on solvent effects.

Consequently, we concluded that N-dithiocarbethoxy-L-amino acids with functional groups, *i.e.* $-COOH$, $-CONH_2$, $-COOC_2H_5$, $-OH$ and $-OCOCH_3$ at β-position, generally exhibit Group 3 type solvent effects, as represented by X. XII showed solvent effects similar to X although, from its chemical structure, XII should belong to the same category as VI and VII. This contradiction has not yet been resolved.

Group 4——Solvent effects represented by N-dithiocarbethoxy-L-proline (XIV).

In contrast to the preceding three types of solvent effects, compounds in this group show no remarkable effect for inverting the Cotton effect sign and show only limited change in amplitude, depending upon the solvents used. amino acids belonging to this group are N-monosubstituted α-amino acids including cyclic amino acids, such as proline, hydroxyproline and pipecolic acid. N-Dithiocarbethoxy-L-proline (XIV) always exhibited a positive Cotton effect with only slight differences in amplitude in all solvents; methanol, dioxane chloroform and benzene (Fig. 5). Similar results were obtained for N-dithiocarbethoxy-L-hydroxyproline (XV), -L-pipecolic acid (XVI) and N-dithiocarbethoxy-N-methyl-L-alanine (XVII) (Tables II and IV). Other proline derivatives, N-thionocarbomethoxy-(XXIII),[18)] and N-phenyl-thioacetyl (XXIV)[8b)] derivatives also showed solvent effects similar to dithiocarbamate derivatives. As previously suggested[8b)] a relationship between this kind of solvent effect and the restriction of bond rotation around asymmetric carbon and nitrogen atoms apparently exists.



$$H_5C_2S\underset{\underset{S}{\|}}{C}HN\blacktriangleright\overset{COOC_2H_5}{\underset{R}{C}}\blacktriangleleft H$$

XXI : $R=CH_3$
XXII : $R=CH_2COOC_2H_5$

[18)] Unpublished data.

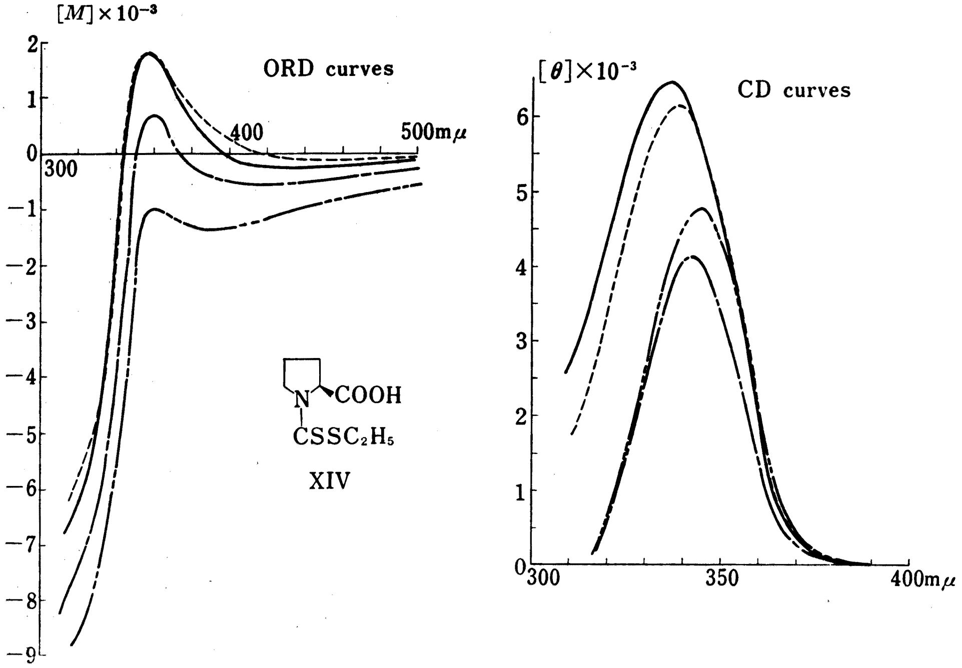
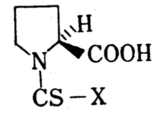
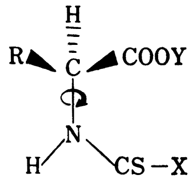


Fig. 5. ORD and CD Curves of N-Dithiocarbethoxy-L-proline (XIV)

———: in CH_3OH, - - - -: in dioxane, ———: in $CHCl_3$, ———: in C_6H_6



X=SC_2H_5, OCH_3, $CH_2C_6H_5$



X=SC_2H_5, OCH_3, $CH_2C_6H_5$
Y=H, C_2H_5

This proposal has been supported by Djerassi, *et al.*[11] who studied low-temperature CD measurement of the dithiocarbamate of L-proline,[19] which is comparable to measurements for IV and VIII.

For the dithiocarbamates of this group no remarkable solvent effects were observed, which differs from Groups 1—3 and derivatives of the L-series constantly show a positive Cotton effect with a limited change of amplitude depending upon the solvents used.

Group 5——Solvent effects induced by β-asymmetry.

We have classified various types of solvent effects into four groups based on empirical data, but no theoretical explanation of these classifications is yet possible due to the complexities of these phenomena.

We found that the β-asymmetry of amino acids contributes to the solvent effects. Djerassi, *et al.*[12] reported that for the ORD of N-dithiocarbalkoxy-α-amino acids in methanol[11] the Cotton effect signs of these derivatives are dependent upon asymmetry of the α-carbon

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[19] Actually they used an N-dithiocarbomethoxy derivative. There is probably little difference between the thioethoxy(-S-C_2H_5) and thiomethoxy(-S-CH_3) groups.

atom and are independent of the β-asymmetric center. They recommended use of these derivatives to determine the absolute configuration of the α-carbon atom.

As noted, N-dithiocarbethoxy-L-isoleucine (III) exhibited the same type of solvent effect as I, but the solvent effects of dithiocarbethoxy-allo-L-isoleucine (XVIII) differed somewhat from those of III, since XVIII showed double humped CD maxima in chloroform and a positive Cotton effect in benzene (Fig. 6), while III showed double humped CD maxima in benzene and a negative Cotton effect in chloroform. Solvent effects of N-dithiocarbethoxy-L-*tert*-leucine (XIX) were also examined. XIX has the same solvent effects as XVIII, not as III (Tables II and IV).

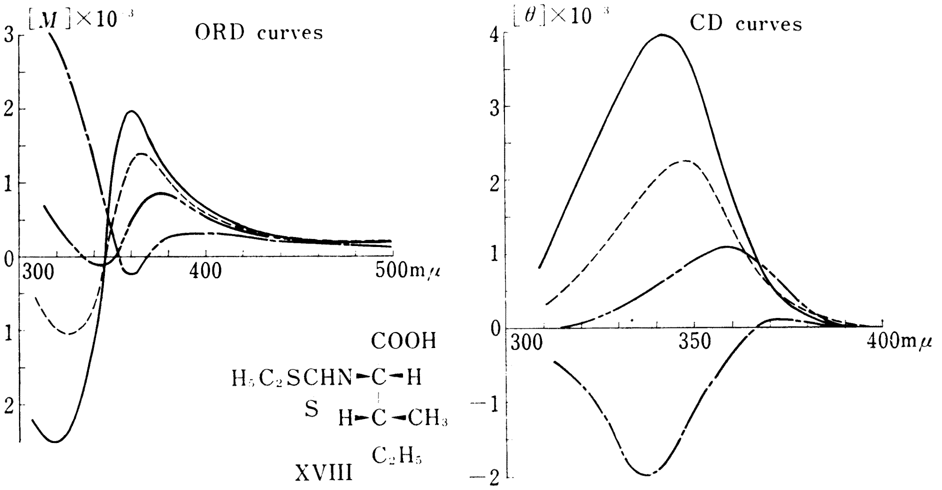


Fig. 6. ORD and CD Curves of N-Dithiocarbethoxy-allo-L-isoleucine (XVIII)

———: in CH_3OH, - - - - -: in dioxane, —·—·—: in $CHCl_3$, —··—··—: in C_6H_6

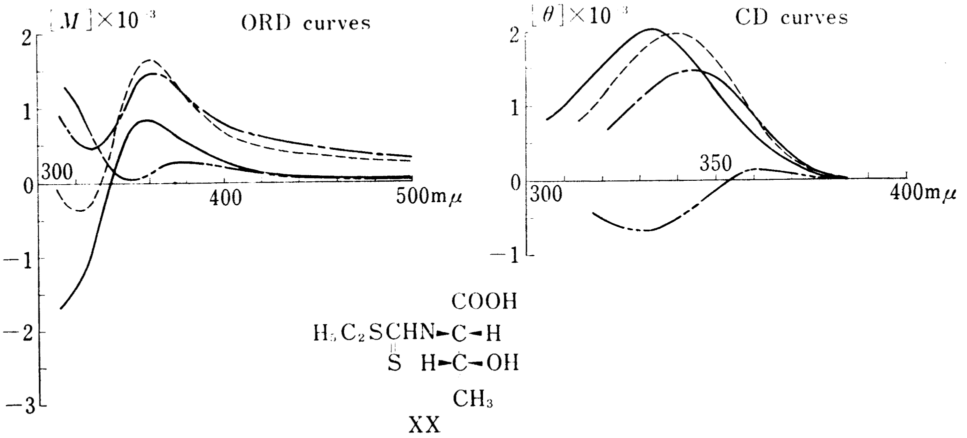


Fig. 7. ORD and CD Curves of N-Dithiocarbethoxy-L-threonine (XX)

———: in H_2O, - - - - -: in CH_3OH, —·—·—: in dioxane, —··—··—: in $CHCl_3$

N-Dithiocarbethoxy-L-threonine (XX) showed double humped CD maxima in chloroform and a positive Cotton effect in dioxane (Fig. 7) however, N-dithiocarbethoxy-allo-L-threonine (XIII) showed a negative Cotton effect in chloroform and dioxane, similar to the solvent effect of X (Tables II and IV).

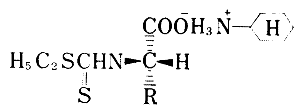
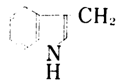
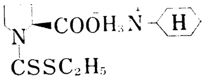
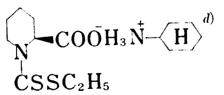
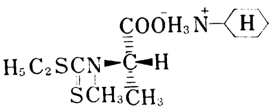
These two results show that the β-asymmetric center of the amino acid produces a change in solvent effect, even though the α-asymmetric center is the same.

It is very difficult to explain these phenomena from the observations, however, dithiocarbamates of this group may be characterized as showing double humped CD maxima in chloroform and positive ones in water, methanol, dioxane and benzene.

Cyclohexylammonium Salts of N-Dithiocarbethoxy-α-amino Acids

Cotton effect signs in water and methanol are summarized in Table V for thirteen kinds of cyclohexylammonium salts of N-dithiocarbethoxy-L-α-amino acids. Values of $[M]$ and $[\theta]$ are given in Table VI. ORD and CD measurements were performed only in water and methanol because of insolubility in other solvents. Regardless of variations, results for the three types of solvent effects should be classified differently than results for N-dithiocarbethoxy-α-amino acids.

Table V. Signs of Cotton Effects for the Cyclohexylammonium Salt of N-Dithiocarbethoxy-L-α-amino Acids in H_2O and CH_3OH

	Group	R		Solvt.	
				H_2O	CH_3OH
	1	CH_3 Is		+	−
		$C_6H_5CH_2$ VIs		+	−
		p-$HOC_6H_4CH_2$ VIIs		+	−
		H_2NOCCH_2 IXs[a]		+	−
		$HOCH_2$ Xs		+	−
		CH_3COOCH_2 XIs[b]		+	−
		 XIIs		+	−
		(R) $CH_3CH(OH)$ XXs		+	−
	2	$\langle H \rangle$-$\overset{+}{N}H_3\overset{-}{O}OC(CH_2)_2$	IVs[c]	+	+
		$\langle H \rangle$-$\overset{+}{N}H_3\overset{-}{O}OCCH_2$	VIIIs[c]	+	+
 XIVs	3			+	+
 XVIs[d]				+	+
 XVIIs				+	+

Notations are the same as in Table II.
a) +,− CD maxima were observed in CH_3OH.
b) +,− CD maxima were observed in H_2O.
c) CD maxima in CH_3OH were of abnormal shape.
d) D-Isomer was used for measurements.

Group 1——Solvent effects represented by cyclohexylammonium salt of N-dithiocarethoxy-L-alanine (Is)[20]

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[20] Subscript s denotes the cyclohexylammonium salt of the corresponding N-dithiocarbethoxy-L-amino acids.

Table VI. Values of Cotton Effects for Cyclohexylammonium Salts of N-Dithiocarbethoxy-L-α-amino Acids on ORD and CD Measurements in H_2O and CH_3OH

Compd. No.	Solvt.	ORD				CD maximum	
		1st extremum		2nd extremum[a]			
		mμ	[M]	mμ	[M]	mμ	[θ]
Is	H_2O	355	874	310[b]	−3780	322	3890
	CH_3OH	356	−1130	310[b]	2790	335	−3290
IVs[c,d]	H_2O	355	1530	310[b]	−4490	330	4480
	CH_3OH	341	858	319	704	360 328	110[e] 587
VIs	H_2O	355	1610	322	−35	338	1930
	CH_3OH	361	862	309[b]	10000	337	−4180
VIIs	H_2O	355	1840	329	811	340	1520
	CH_3OH	361	494	309	13300	337	−4890
VIIIs[c,f]	H_2O	355	1630	310[b]	5030	328	5530
	CH_3OH	362	1060	337	680	351	679
IXs	H_2O	360	1300	303[b]	−5030	336	1910
	CH_3OH	351	348	301[b]	2890	362 330	72 −483
Xs	H_2O	360	565	321	−943	340	1740
	CH_3OH	359	−1260	320[b]	6910	337	−4710
XIs	H_2O	361	796	331	196	345 315	472 −368
	CH_3OH	359	−948	306	7640	339	−2880
XIIs	H_2O	357	1630	310	5030	349	506
	CH_3OH	362	1060	337	680	336	−2520
XIVs	H_2O	349	489	305[b]	−6720	324	4080
	CH_3OH	358	679	311	−6250	336	3690
XVIs[g,h]	H_2O	360	−363	310[b]	−8990		
	CH_3OH	364	235	320[b]	−6100		
XVIIs[g]	H_2O	358	−213	310	−5700	330	2190
	CH_3OH	366	−97	310	−3590	339	893
XXs	H_2O	358	880	307[b]	−2770	333	2320
	CH_3OH	358	−460	310	4530	344	−3290

a) 2nd extrema were often observed as shoulders.
b) The limited value to be measured at the shortest wave length.
c) dicyclohexylammonium salt
d) CD maximum in CH_3OH was of abnormal shape.
e) shoulder
f) CD maximum in CH_3OH was located in a longer wave length than in other cases.
g) Optical purity of the compound used is not high.
h) D-Isomer was used for measurements.

A remarkable effect was observed for Is, which showed a clear positive Cotton effect in water and a negative one in methanol (Fig. 8). Almost the same solvent effect was observed for the sodium salt of I, suggesting that the species of cation does not affect the solvent effect (see Experimental). Seven amino acid salts, *i.e.* cyclohexylammonium salts of N-dithiocarbethoxy-L-phenylalanine (VIs), -L-tyrosine (VIIs), -L-asparagine (IXs), -L-serine (Xs), -O-acetyl-L-serine (XIs), -L-tryprophan (XIIs) and -L-threonine (XXs), all gave solvent effects similar to Is.[21] This group in salt form includes almost all the amino acids, which are classified in three groups (1, 2, and 3) in the free acid form. Characteristically this group always shows a negative Cotton effect in methanol for the most salt forms, even though IXs gives

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21) Solvent effects of the cyclohexylammonium salt of N-dithiocarbethoxy-L-phenylglycine on ORD and CD measurements were also examined and showed the same effect as Is. However, this compound was excluded from this group for the reasons given in footnote 17.

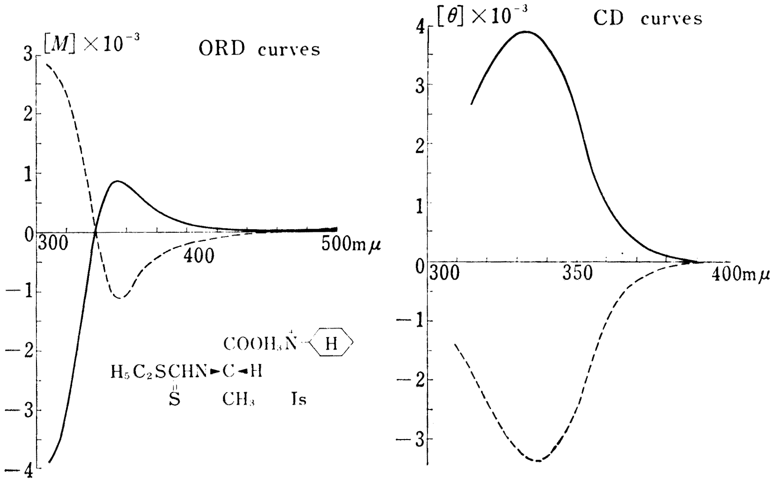

Fig. 8. ORD and CD Curves of Cylohexylammonium Salt (Is) of N-Dithiocarbethoxy-L-alanine

——: in H_2O ----: in CH_3OH

double humped CD maxima (Table VI), and N-dithiocarbamates of the salt and free acid forms often produce reversed Cotton effects in methanol. Therefore, it is necessary to distinguish between data for the salt form and for the free acid form, especially in determining the absolute configuration of amino acids even when the same solvent, methanol, is used. The same phenomena have also been reported for salts of N-phenylthioacetyl amino acids.[8b)]

Group 2——Solvent effects represented by dicyclohexylammonium salts of N-dithiocarbethoxy-L-glutamic acid (IVs) and -L-aspartic acid (VIIIs).

ORD and CD curves of the di-salt forms of the acidic amino acids in this series are interesting. Both the dicyclohexylammonium salts of N-dithiocarbethoxy-L-glutamic acid (IVs) and -L-aspartic acid (VIIIs), gave positive Cotton effects in water and methanol (Fig. 9 and 10). However, in methanol IVs and VIIIs had a very small amplitude in the positive Cotton effect. Cotton effect curves for IVs in the ORD and CD are particularly abnormal. The Cotton

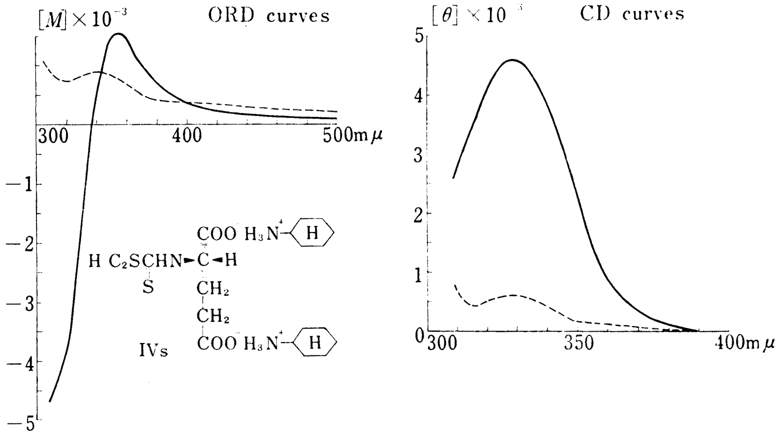

Fig. 9. ORD and CD Curves of Dicycohexylammonium Salt (IVs) of N-Dithiocarbethoxy-L-glutamic Acid

——: in H_2O, ----: in CH_3OH

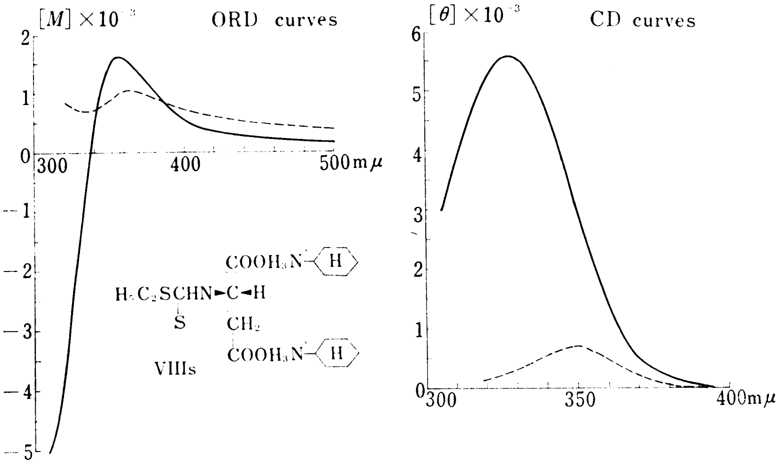

Fig. 10. ORD and CD Curves of Dicyclohexylammonium Salt (VIIIs) of N-Dithiocarbethoxy-L-aspartic Acid

——: in H_2O, ----: in CH_3OH

effects of VIIIs and IVs, which are positive in methanol, are quite different from the results for Group 1 of this salt series. This is ascribed to the γ-carboxylate anion in IVs and the β-carboxylate anion in VIIIs participation in this solvent effect, even though no definitive explanation can be given.

Group 3——Solvent effects represented by cyclohexylammonium salt of N-dithiocarbethoxy-L-proline (XIVs).

As in free acids, cyclohexylammonium salts of N-dithiocarbethoxy-L-proline (XIVs) (Fig. 11), -L-pipecolic acid (XVIs) and -N-methyl-L-alanine (XVIIs), all gave positive Cotton effects in water and in methanol. The CD maximum of XIVs is at 324 mμ in water and at 336 mμ in methanol, corresponding to each ultraviolet maximum. These results are the same as for the free acid forms of this group, which always show positive Cotton effects in all solvents besides water and methanol. This may be due to restricted rotation of the carbon and nitrogen bonding.

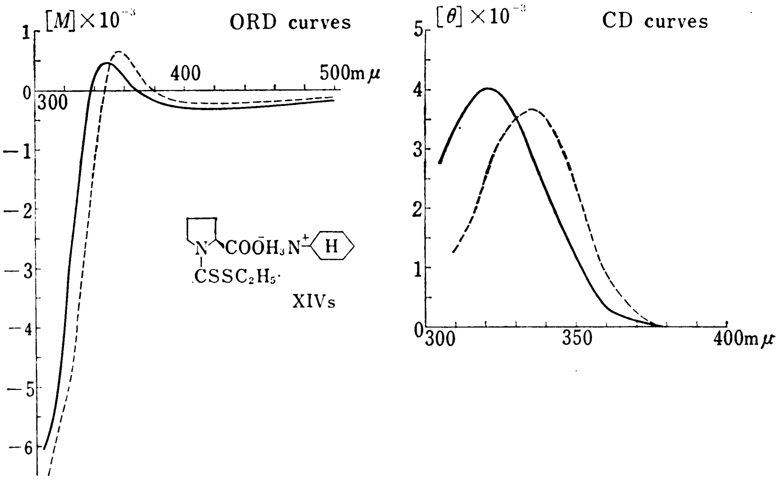

Fig. 11. ORD and CD Curves of Cyclohexylammonium Salt (XIVs) of N-Dithiocarbethoxy-L-proline

——: in H_2O, ----: in CH_3OH

Conclusion

Though ORD and CD measurements are thought to be the most convenient methods for establishing the absolute configuration of amino acids, various solvent effects were observed in ORD and CD measurements of the N-dithiocarbethoxy derivatives of twenty amino acids in free acid or in salt form. It is very dangerous to correlate absolute configuration with the sign of an apparent Cotton effect without considering the solvent used, and the form (free acid or salt) of the dithiocarbamate of the amino acids. The reason for this solvent effect is not clear, but from an empirical point of view it is the most reliable method for correlating the absolute configuration of amino acids to the sign of the Cotton effect measured with a salt form of N-dithiocarbethoxy-α-amino acid in water, because only one small exception, XIs, has been observed. If it shows a positive Cotton effect, the amino acid which forms dithiocarbamate belongs to the L-series. In assigning an absolute configuration the patterns of solvent effects in acid and salt forms should be examined.

Experimental[22)]

N-Dithiocarbethoxy-L-α-amino Acids and Their Cyclohexylammonium Salts (I-X, XII-XVIII and XX)——General Method: An amino acid (0.05—0.01 mole) and K_2CO_3 (two equivalents)[23)] were dissolved in a mixture of H_2O (50 ml) and EtOH (30—50 ml). CS_2 (3—4 equivalents) was added and stirring was continued for 1 hr. EtBr (3—4 equivalents) was added and the mixture was stirred for another 7—8 hr. The reaction mixture was concentrated under reduced pressure and washed with ether. It was then acidified with 10% HCl and extracted with ether, AcOEt or $CHCl_3$. The extract was washed with NaCl satd. solution in H_2O and dried over Na_2SO_4. After removal of the solvent, a crude product was obtained in a 50—90% yield. This crude product was purified by one of the following methods.

Method A: Recrystallization from a suitable solvent (I—III, VI, VIII, IX, XIV, XV, XVII, and XVIII).

Method B: When the crude product did not crystallize, a cyclohexylammonium salt was made from it with cyclohexylamine in ether, ether–EtOH or ether–EtOH–hexane. When solidified, this salt was purified by recrystallization from a suitable solvent and the free acid was again obtained by acidification, as described in the general method. This free acid was used for ORD and CD measurements after drying it in a desiccator in a vacuum (IV, VII, X, XII, XVI, XX).

Method C: When the crude free acid and the cyclohexylammonium salt failed to crystallize, the crude product was purified by silica gel chromatography and the product was dried in a desiccator *in vacuo*, then it was used for ORD and CD measurements (V, XIII).

Melting points, specific rotations and data on elemental analyses of crystallized samples are written in Table VII. Noncrystallized samples were checked by their IR spectra, shown in Table VIII. V and XIII were also checked by their NMR spectra.

N-Dithiocarbethoxy-L-alanine (I)——Recrystallized from H_2O needles mp 106°, $[\alpha]_D^{10}$ $-32°$ ($c=0.292$, H_2O) (lit.[24)] mp 106—107°, $[\alpha]_D^{25}$ $-27.2°$ ($c=1.97$, H_2O)). IR ν_{max}^{KBr} cm^{-1}: 3345 (NH), 1723 (COOH), 1523 (N-C=S); $\nu_{max}^{CHCl_3}$ cm^{-1}: 3384 (NH), 1723 (COOH), 1498 (N-C=S).

NMR Spectra of N-Dithiocarbethoxy-L-lysine (V) and -allo-L-threonine (XIII)——V (*ca.* 10% solution in $CDCl_3$) τ: 7.85—8.95 (12H, multiplet, 2($C\underline{H}_3$-CH_2-S) and 3 (C-$C\underline{H}_2$-C)), 6.10—7.30 (6H, multiplet, 2 (CH_3-$C\underline{H}_2$-S) and N-$C\underline{H}_2$-C), 5.40 (1H, broad, C$\underline{H}$-COO). XIII (*ca.* 10% solution, in $CDCl_3$) τ: 8.25—8.95 (6H, multiplet, $C\underline{H}_3CH_2S$ and $C\underline{H}_3CH(OH)$), 6.73 (2H, quartet, -S$C\underline{H}_2$-), 5.10—5.85 (1H, multiplet, C$\underline{H}$-COO), 4.50—4.95 (1H, multiplet, $CH_3C\underline{H}(OH)$).

O-Acetyl-N-dithiocarbethoxy L-Serine (XI) and Its Cyclohexylammonium Salt (XIs)——Crude O-acetylserine hydrochloride[25)] (460 mg, 0.0025 mole) was dissolved in 90% EtOH (30 ml), then Et_3N (760 mg, 0.0075

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22) All melting points are uncorrected. ORD, CD and UV data were measured with a JASCO ORD/UV/CD-5 Model and its CD attachment in concentrations of $c=0.1$—0.5. $[\alpha]_D$ values were measured with a Yanagimoto OR-20 Model and IR data were measured with a JASCO DS-402 Model or a DS-301 Model. Elemental analyses were made for samples dried in a vacuum for more than two days at room temperature.

23) When an amino acid hydrochloride or an acidic amino acid was used as the starting material, 2.5 equivalents of K_2CO_3 were generally used and 3 equivalents of K_2CO_3 were used for L-lysine hydrochloride.

24) A. Fredga, *Svensk Kem. Tidskr.*, **54**, 26 (1942).

25) M. Wilcheck and A. Patchornik, *J. Org. Chem.*, **29**, 1629 (1964).

26) A. Fredga, *Svensk Kem. Tidskr.*, **53**, 221 (1941).

27) E. Fischer, *Chem. Ber.*, **48**, 350 (1915).

TABLE VII. Melting Points, Specific Rotations and Elemental Analysis Data of N-Dithiocarbethoxy-L-α-amino Acids and Their Cyclohexylammonium Salts

Compd.	Appearance	Recryst. solvt.	mp (°C)	Specific rotation				Formula	Elemental analysis					
									Calcd.			Found		
				[α]D	Temp. (°C)	Conc. (C)	Solvt.		C	H	N	C	H	N
Is	needles[a]	EtOH–(iso-Pr)$_2$O	139—140[b]	4.7°	12	0.102	EtOH	$C_{12}H_{24}O_2N_2S_2$	49.28	8.27	9.58	49.31	8.25	9.47
II	needles	hexane	88—90	5.4°	8	0.368	C_6H_6	$C_8H_{15}O_2NS_2$	43.41	6.83	6.33	43.79	6.88	5.72
III	prisms	hexane	58.5—59.5	27°	15	0.602	dioxane	$C_9H_{17}O_2NS_2$	45.92	7.32	5.95	46.32	7.41	5.92
IVs[c]	needles	H_2O–EtOH	182[b]	6.5°	25	0.550	H_2O	$C_{20}H_{39}O_4N_3S_2$	53.42	8.74	9.35	53.16	8.68	9.45
VI[d]	needles	ether–hexane	59—60	131°	18	0.280	dioxane	$C_{12}H_{15}O_2NS_2$	53.50	5.61	5.20	53.77	5.67	5.37
VIs	needles	EtOH–(iso-Pr)$_2$O–hexane	130—131[b]	134°	12	0.718	EtOH	$C_{18}H_{28}O_2N_2S_2$	58.66	7.66	7.60	58.37	7.42	7.56
VIIs	leaflets	EtOH–(iso-Pr)$_2$O–hexane	154—155[b]	162°	13	0.812	EtOH	$C_{18}H_{28}O_3N_2S_2$	56.22	7.34	7.55	56.52	7.04	7.62
VIII[e]	needles	ether–hexane	138—140	27°	13	0.202	H_2O	$C_7H_{11}O_4NS_2$	35.43	4.64	5.90	35.77	4.86	5.68
VIIIs[c]	needles	EtOH–(iso-Pr)$_2$O	160—161[b]	69°	22	0.232	MeOH	$C_{19}H_{37}O_4N_3S_2$	52.38	8.56	9.65	51.61	8.98	9.28
IX	leaflets	H_2O	148—149[b]	134°	34	0.302	dioxane	$C_7H_{12}O_3N_2S_2$	35.53	5.12	11.85	35.57	5.05	12.08
IXs	needles	MeOH–(iso-Pr)$_2$O	140[b]	61.1°	23	0.216	MeOH	$C_{13}H_{25}O_3N_3S_2$	46.53	7.51	12.50	46.05	7.33	12.63
Xs	needles	EtOH	152[b]	24.5°	26	0.482	H_2O	$C_{12}H_{24}O_3N_2S_2$	46.72	7.84	9.07	46.77	7.87	9.33
XIIs	needles	EtOH–(iso-Pr)$_2$O	176—177[b]	57.1°	29	0.904	MeOH	$C_{20}H_{29}O_2N_3S_2$	58.94	7.17	10.31	58.94	7.33	10.47
XIV	needles	hexane	72—74	−212°	12	0.202	MeOH	$C_8H_{13}O_2NS_2$	43.81	5.97	6.39	44.12	5.90	6.23
XIVs	needles	EtOH–(iso-Pr)$_2$O	204—205[b]	−48.3°	21	0.418	MeOH	$C_{14}H_{26}O_2N_2S_2$	52.79	8.23	8.80	52.99	8.31	8.86
XV[f]	pillars	AcOEt–hexane	148	−18.6°	10	0.430	dioxane	$C_8H_{13}O_3NS_2$	40.83	5.56	5.95	41.11	5.80	5.78
DXVIs[g,h]	needles	EtOH–(iso-Pr)$_2$O	188[b]	−38.1°	30	0.226	MeOH	$C_{15}H_{28}O_2N_2S_2$	54.18	8.49	8.43	54.36	8.60	7.97
XVII[h,i]	needles	ether–hexane	98—99	−2.9°	19	0.210	dioxane	$C_7H_{13}O_2NS_2$	40.55	6.32	6.76	40.36	6.18	7.00
XVIIs[h]	needles	EtOH–(iso-Pr)$_2$O	159—160[b]	−8.5°	21	0.216	MeOH	$C_{13}H_{26}O_2N_2S_2$	43.41	6.83	6.32	43.64	6.75	6.51
XVIII	needles	hexane	67—70	59°	17	0.202	dioxane	$C_9H_{17}O_2NS_2$	45.92	7.32	5.95	46.77	7.12	6.24
XXs	needles	H_2O–EtOH–(iso-Pr)$_2$O	163—164[b]	52°	29	0.394	MeOH	$C_{13}H_{26}O_3NS_2$	48.40	8.12	8.68	48.27	7.94	8.49

a) hygroscopic needles

b) melting points with decomposition

c) dicyclohexylammonium salt

d) lit.[14f] mp 92—94°

e) The reported melting point 141.5°[25] after solidification, it was melt again at 170°. The reported specific rotation $[\alpha]_D^{25}$ 34.0° (c=2.735, H_2O).[26]

f) lit,[14a] mp 89—91° (ether–light petroleum) Recrystallization of XV from ether–light petroleum did not change the the melting point.

g) subscript D represents D-isomer

h) Optical purity of the compound used is not high.

i) The starting material, N-methyl-L-alanine, was prepared according to the literature.[27]

Table VIII. IR Spectra of N-Dithiocarbethoxy-L-α-amino Acids

Compd.	Appearance	IR Spectrum	
		State	Characteristic absorptions (cm^{-1})
IV	pale yellow oil	$CHCl_3$ sol.	3350 (NH), 1717 (COOH), 1492 (N–C=S)
V	yellow oil	$CHCl_3$ sol.	3370 (NH), 1729 (COOH), 1492 (N–C=S)
VII	pale yellow oil	$CHCl_3$ sol.	3350 (OH, NH), 1725 (COOH), 1494 (N–C=S)
X	pale yellow oil	liq. film	3280 (OH, NH), 1737 (COOH), 1500 (N–C=S)
XII	yellow oil	$CHCl_3$ sol.	3475 (NH), 3370 (NH), 1726 (COOH), 1494 (N–C=S)
XIII	pale yellow oil	$CHCl_3$ sol.	3340 (OH, NH), 1734 (COOH), 1495 (N–C=S)
DXVI	yellow oil	$CHCl_3$ sol.	1717 (COOH), 1407 (N–C=S)[a]
XX	pale yellow oil	$CHCl_3$ sol.	3355 (OH, NH), 1720 (COOH), 1493 (N–C=S)

a) Compared with IR spectrum of XIV, $\nu_{max}^{CHCl_3}$ (cm^{-1}): 1724 (COOH), 1412 (N-C=S).

mole) and CS_2 (0.6 ml, 0.01 mole) were added. After 10 min of stirring, EtBr (0.8 ml, 0.01 mole) was added and stirring was continued for another 5 hr at room temperature. The reaction mixture was concentrated, washed with ether, then acidified with 10% HCl and extracted with ether. The extract was washed with satd. NaCl solution in H_2O, and dried over Na_2SO_4. Evaporation of ether *in vacuo* produced a yellow oil, which was converted to the cyclohexylammonium salt in $(iso\text{-}Pr)_2O$. The crude salt was recrystallized from EtOH–$(isoPr)_2O$ to give needles, XIs (450 mg, 0.0013 mole, 51% yield). mp 130—130.5° (decomp.), $[\alpha]_D^{13}+61.9°$ ($c=0.820$, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3235 (NH), 1731 (CH_3COO-), 1640, 1585, 1510 (N-C=S). *Anal.* Calcd. for $C_{14}H_{26}O_4N_2S_2$: C, 47.97; H, 7.48; N, 7.99. Found: C, 48.05; H, 7.05; N, 8.28.

The free acid, XI was obtained from XIs (described above in Method B) as a pale yellow oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3340 (NH), 1743 (CH_3COO-, COOH), 1494 (N-C=S). This oil became needles after standing for several days but further purification was difficult.

N-Dithiocarbethoxy L-*tert*-Leucine (XIX)——a) Trimethylpyruvic Acid Phenylhydrazone: Phenylhydrazine (25 g, 0.23 mole) was added to a solution of trimethylpyruvic acid[28] (26 g, 0.20 mole) in MeOH (200 ml). The reaction mixture was allowed to stand overnight at room temperature. A small amount of solvent was evaporated and precipitates were separated by filtration to give orange needles (31.0 g, 0.14 mole, 70% yield), mp 156—157° (decomp.). This product, without further purification, was used for the next reaction. A small portion of the crude product was recrystallized from EtOH–H_2O, to give an analytical sample. Yellow needles, mp 157—158° (decomp.). IR ν_{max}^{KBr} cm^{-1}: 3265 (NH), 1655 (COOH), 1538. *Anal.* Calcd. for $C_{12}H_{16}O_2N_2$: C, 65.43; H, 7.32; N, 12.72. Found: C, 65.67; H, 7.32; N, 12.89.

b) DL-*tert*-Leucine: Conc. HCl (100 ml) was added to a solution of trimethylpyruvic acid phenylhydrazone (24.2 g, 0.11 mole) in EtOH–ether (3:2, 500 ml). Zn powder (7.15 g, 1.1 atom) was added in small portions under vigorous stirring at a rate that maintained the temperature below 50°. When one-third of the Zn powder was added, conc. HCl (100 ml) was added again. An equal amount of conc. HCl was added when two-thirds of the Zn powder was added. Conc. HCl (20 ml) was added when all of the Zn powder had been added, and the reaction mixture was stirred for 4 hr at room temperature, then allowed to stand overnight. After concentration to *ca.* 300 ml, a solution of NaOH (120 g) in H_2O (200 ml) was added to the reaction mixture with stirring. The precipitate was filtered and washed with H_2O (100 ml × 2). The filtrate and washings were combined and washed with ether and saturated with H_2S. The precipitate (ZnS) was filtered and its filtrate was acidified with conc. HCl. It was then concentrated to dryness and extracted with refluxing EtOH (100 ml × 3). After evaporation of EtOH, the residue was extracted again with EtOH (130 ml) and neutralized with Et_3N (10.3 g, 0.10 mole). The precipitate was separated by filtration and recrystallized from H_2O–acetone to give colorless needles (9.8 g, 0.075 mole, 68%). A small portion of the crystals was recrystallized from H_2O–acetone to give an analytical sample. mp>245°. IR ν_{max}^{KBr} cm^{-1}: 1615, 1535. *Anal.* Calcd. for $C_6H_{13}O_2N$: C, 54.94; H, 9.99; N, 10.68. Found: C, 55.09; H, 9.89; N, 10.68.

c) L-*tert*-Leucine: DL-*tert*-Leucine was esterified by MeOH and HCl gas in the usual manner (86% yield). DL-*tert*-Leucine methyl ester was resolved by dibenzoyl-(+)-tartaric acid and hydrolyzed with conc. HCl, as in the literature.[14c)].

d) N-Dithiocarbethoxy-L-*tert*-leucine (XIX): Et_3N (400 mg, 0.004 mole), CS_2 (1 ml, 0.016 mole) and EtOH (50 ml) were added to a solution of L-*tert*-leucine ($[\alpha]_D^{37}=-5.41$, ($c=1.039$, H_2O), lit.[14c)] $[\alpha]_D^{21}$-9.4° ($c=1$, H_2O)) (260 mg, 0.002 mole) in H_2O (30 ml). This reaction mixture was stirred for 2 hr at room temperature. After adding EtBr (1.5 ml, 0.02 mole), stirring was continued for another 10 hr. The reaction mixture was concentrated, washed with ether, acidified with 10% HCl and extracted with ether. The ether

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28) A. Richard, *Ann. Chimie*, **21**, 323 (1910); A. Kjaer, *Acta Chem. Scand.*, **7**, 889 (1953).

extract was washed with NaCl satd. solution in H_2O, dried over Na_2SO_4, and concentrated to dryness under reduced pressure. The residue was chromatographed on silica gel and gave needles (260 mg, 55%), which were twice recrystallized from hexane to give colorless needles. mp 83—85°, $[\alpha]_D^{18}$ 11.3 ($c=1.080$, CH_3OH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380 (NH), 1718 (COOH), 1495 (N-C=S). *Anal.* Calcd. for $C_9H_{17}O_2NS_2$: C, 45.93; H, 7.32; N, 5.95. Found: C, 46.44; H, 7.27; N, 5.93. ORD ($c=0.200$, CH_3OH) $[M]^{31}$ (mμ): 21.2° (589), 98.7° (430). 508° (364) (peak), 0° (348), −926° (320) (trough). CD ($c=0.200$, CH_3OH) $[\theta]^{31}$ (mμ): 0 (385), 1030 (345) (positive maximum). This sample was not optically pure (lit.[14c]) mp 93—99° (CCl_4–petr. ether). ORD for D-isomer (CH_3OH) $[\phi]$ (mμ): 78° (589), 0° (430), −613° (356) (trough), 0° (345), 1865° (316). CD for D-isomer (CH_3OH) $[\theta]$ (mμ): 0 (385), −1605 (342) (negative maximum), 0 (300)).

N-Dithiocarbethoxy-L-alanine Ethyl Ester (XXI)——$SOCl_2$ (0.4 ml, 0.0055 mole) was added dropwise to a solution of I (390 mg, 0.002 mole) in dry EtOH (50 ml) with stirring and ice cooling. After standing at room temperature for 6 hr, the reaction mixture was concentrated under reduced pressure and extracted with $CHCl_3$. The extract was wahsed with dil. $NaHCO_3$ solution in H_2O and NaCl satd. solution in H_2O and dried over Na_2SO_4. Evaporation of $CHCl_3$ in vacuo gave crystals, which were recrystallized from hexane producing colorless needles (250 mg, 57% yield). mp 73—74°, $[\alpha]_D^{32}$ −21.8° ($c=0.101$, dioxane). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3345 (NH), 1735 ($-COOC_2H_5$), 1490 (N−C=S). *Anal.* Calcd. for $C_8H_{15}O_2NS_2$: C, 43.41; H, 6.83; N, 6.33. Found: C, 43.64; H, 6.75; N, 6.51.

N-Dithiocarbethoxy-L-aspartic Acid Diethyl Ester (XXII)——VIII (360 mg, 0.0015 mole) was treated with $SOCl_2$ (0.4 ml, 0.0055) in EtOH (30 ml) as described for XXI. Recrystallization of the crude product from hexane gave colorless pillars (250 mg, 57%) mp 46—48°, $[\alpha]_D^{19}+58.7°$ ($c=0.204$, dioxane). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3355 (NH), 1742 (−COO-), 1490 (N−C=S). *Anal.* Calcd. for $C_{11}H_{19}O_4NS_2$: C, 45.04; H, 6.52; N, 4.78. Found: C, 45.22; H, 6.48; N, 5.48.

ORD of the Sodium Salt of I——In H_2O: I was dissolved in H_2O containing an equivalent mole of NaOH, $c=0.193$ $[M]^{31}$(mμ): 875° (357) (peak), −3750° (310).

In CH_3OH: I was dissolved in CH_3OH containing an equivalent mole of NaOMe, $c=0.197$ $[M]^{32}$(mμ): −1380° (356) (trough), 3730° (312).

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