CCA-177 541.65:547.478.6:547.279.4

Optically Active Trisulphides and Tetrasulphides Related to L-Cystine*

*B. Gašpert, Z. Štefanac, R. Marušić, and K. Balenović***

Chemical Laboratory, Faculty of Science, University of Zagreb, Strossmayerov trg 14, Zagreb, Croatia, Yugoslavia

Received May 13, 1960

A description is given of the preparation and properties of optically active bis(2-carboxy-2-phthalimidoethyl) disulphide (Ia), $[\alpha]_D$—275°, bis(2-carboxy-2-phthalimidoethyl) trisulphide (Ib), $[\alpha]_D$ —472° and bis(2-carboxy-2-phthalimidoethyl) tetrasulphide (Ic), $[\alpha]_D$—388°. The starting material for these compounds was *N*--phthaloyl-L-cysteine (II), $[\alpha]_D$—64° prepared from L-cysteine hydrochloride and sodium *o*-carbethoxythiobenzoate.

The peculiar optical properties of sulphur-containing amino acids were pointed out by van't Hoff[1]. Despite many subsequent experimental and theoretical investigations, there still are a number of unexplained phenomena connected with the abnormally high rotations of cystine and other optically active disulphides.

The great difference between the relatively low rotations of optically active mercaptans and the high rotations of some corresponding disulphides was explained by Kauzmann and Eyring[2] as the result of the restriction of freedom, or of orientation, of the substituents on the centre of asymmetry.

According to Fieser[3], the formation of a system of three ten-membered rings with stable hydrogen bonds could explain the high rotatory power of cystine. Thus, in L-homocystine ($[\alpha]_D^{21}$ —16°, c, 0.06 in water)[4] as in L-lanthionine ($[\alpha]_D$ + 6°, c, 1 in *N* NaOH)[5] the low rotatory power should result from the inequality in size of the three possible rings, preventing the formation of stable hydrogen bonds. If these propositions were correct, we can expect a comparatively low rotatory power in the case of L-β-homocystine, as that of homocystine. The optical rotation, however, of L-β-homocystine ($[\alpha]_D$—264°) fits excellently[6] into the experimentally founded suggestions of Fredga[7], namely, that the vicinity of the -S-S- bond to the centre of asymmetry is responsible for the high rotatory power of cystine and other disulphides.

The great influence of disulphide bonds on optical rotation is evident in some other instances as well[8].

It is known that the value of the specific rotation observed in the visible is largely determined by electronic transitions associated with absorption bands in the near ultraviolet[9]. In this respect weak absorption bands in this region might be especially important.

* Communication No. 83 from this Laboratory.

** To whom correspondence concerning this article should be addressed.

In fact cyclic or acyclic -S-S- compounds have weak absorption bands in their ultraviolet spectra, with maxima in the region between 250 and 350 mμ[10,11], and a rotatory dispersion study of some optically active disulphides has shown that their absorption bands were indeed optically active. The rotatory dispersion curve of cystine itself has the extremum at 268 mμ, which is associated with a Cotton effect curve centered about the disulphide band of cystine at 255 mμ[12].

It is known that the disulphide bridge has the dihedral angle of 90° and that the rotation about the bond is strongly hindered with a potential barrier between 10 and 20 Kcals.[13] A consequence of this geometry, and of such a large potential barrier, is the essential asymmetry of the disulphide bridge. These facts have recently been applied to speculations about possible explanations of the abnormally high rotations of cystine[14].

In connection with this problem it was desirable, *inter alia*, to prepare optically active trisulphides and tetrasulphides of the type I (n = 3,4) analogues of L-cystine, a description of which is now given[15].

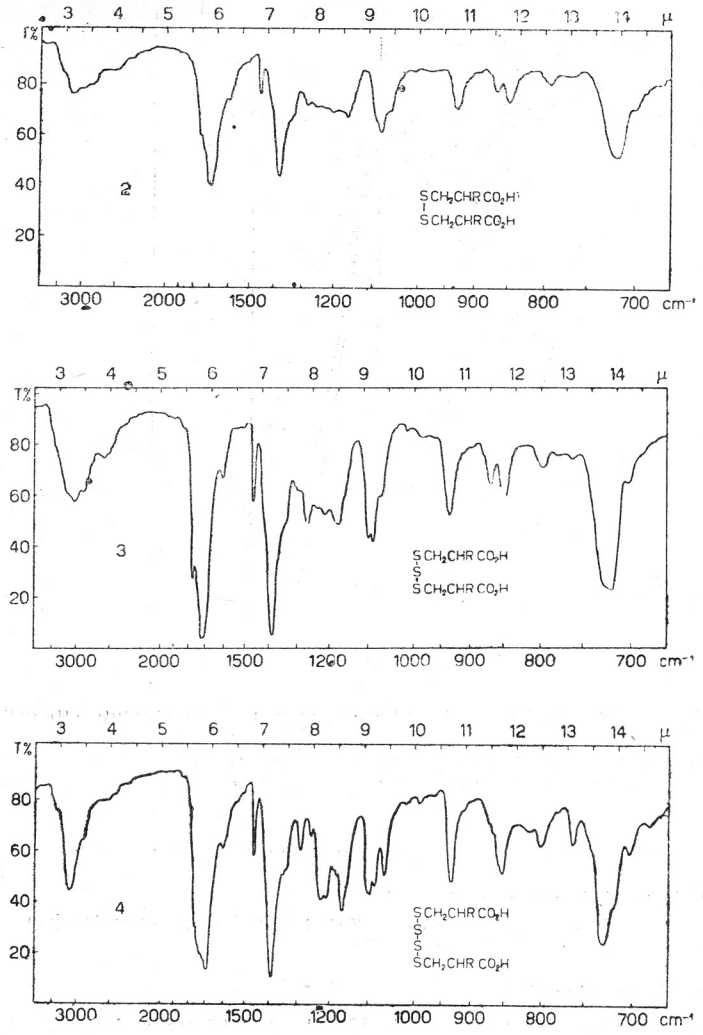
		$[\alpha]_D$	$[\phi]_D$
$CH_2CH(R)CO_2H$			
|	(a) n = 2	— 275°	— 1380°
$(S)_n$			
|	(b) n = 3	— 472°	— 2510°
$CH_2CH(R)CO_2H$			
I	(c) n = 4	— 388°	— 2190°
$HSCH(R)CO_2H$			
II		— 64°	— 160°

$R = N(CO)_2C_6H_4$

The starting material for these compounds was *N*-phthaloyl-L-cysteine (II), $[\alpha]_D$—64°, prepared from L-cysteine hydrochloride and sodium *o*-carbethoxythiobenzoate in *N,N*-dimethylformamide at 80° according to the earlier described method[16,17]. As expected the compound II gave a strongly positive nitroprusside test characteristic of thiols. Bis(2-carboxy-2-phthalimidoethyl) disulphide (Ia) was obtained by the aerial oxidation of a solution of compound II in aqueous ammonia at *p*H 8 in the presence of ferric ion. The disulphide Ia showed the m. p. 166—168° and $[\alpha]_D$—275°. Bis(2-carboxy-2-phthalimidoethyl) trisulphide (Ib) was obtained by the reaction of compound II with freshly distilled sulphur dichloride in chloroform, and had the m. p. 95—97° (decomp.) and $[\alpha]_D$—472°. Bis(2-carboxy-2-phthalimidoethyl) tetrasulphide (Ic) was prepared from II and disulphur dichloride in chloroform, and showed the m. p. 110—112° and $[\alpha]_D$—388°.

To the best of our knowledge the compounds Ib and Ic are the first optically active polysulphides described hitherto. The chemistry of polysulphides has not been as extensively studied as that of some other types of sulphur compounds[18], and we provisionally write, therefore, the formulae of Ib and Ic with linear arrangement of sulphur atoms. It is evident that they exhibit much higher rotations than analogous disulphides. Following recent propositions[19] the symbol $[\phi]$ is used for molecular rotation throughout this paper.

The infrared spectra of compounds Ia—c are shown in Figs. 1—3, and the absorption spectra in the ultraviolet region in Fig. 4. The -S-S-linkage absorption



Figs. 1—3. Infrared absoption spectra of compounds Ia—c in potassium bromide plates.

in the infrared occurs as a weak band in the 500—400 cm.$^{-1}$ region[20]. This is probably the cause of the essential identity of the main bands of the infrared spectra in the region between 3000 and 700 cm.$^{-1}$ measured for these three compounds.

As a result of hydrazinolysis of the *N*-phthaloyl group in compounds Ia-c, L-cystine and phthaloyl hydrazine were obtained in each case. In the case of the polysulphides Ib and Ic elemental sulphur was also detected. The addition and loss of sulphur are the most characteristic reactions of polysulphides[18].

Further work concerning the preparation of free amino acids corresponding to compound Ib and Ic and the measurement of their optical rotatory dispersion is in progress.

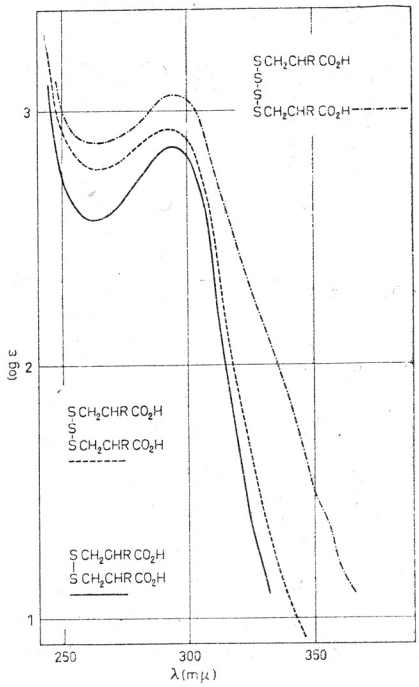


Fig. 4. Ultraviolet absorption spectra of compounds Ia—c in absolute ethanolic solution.
———— Ia, c: 1.06×10^{-2}, λ_{max}: 293 mμ, ε_{max}: 720
— — — — Ib, c: 1.05×10^{-2}, λ_{max}: 293 mμ, ε_{max}: 665
—.—.—. Ic, c: 1.128×10^{-2}, λ_{max}: 294 mμ, ε_{max}: 1170

EXPERIMENTAL

All melting points are uncorrected.

Spectrophotometric measurements were made by using a Beckman DU Model and a Perkin-Elmer Infracord Model 137.

N-*Phthaloyl*-L-*cysteine* (II)

A solution of L-cysteine hydrochloride (4.7 g., 0.03 mole, $[\alpha]_D + 7.7^0$) in *N,N*-dimethylformamide (40 ml.) was heated to 80—85° with stirring, and a solution of sodium *o*-carbethoxythiobenzoate (6.95 g., 0.03 mole) in *N,N*-dimethylformamide (15 ml.) added. Stirring at the same temperature was continued for 4 hours under nitrogen. The solvent was removed under reduced pressure at 80° (under nitrogen), and the residue poured into water (100 ml.), adjusted to pH 3 with *N*-hydrochloric acid and extracted with peroxide-free ether (3×30 ml.). The combined ethereal extracts were washed with water (2×20 ml.) and dried ($MgSO_4$). After evaporating the solvent under reduced pressure a nearly colourless oil remained (7 g., 92%) consisting of *N*-phthaloyl-L-cysteine showing $[\alpha]_D^{20}$ —64° (c, 1.3 in ethanol), $[\phi]_D^{23}$ —160°. The analytical sample was a viscous colourless oil distilling at 150°/0.04 mm.

Anal. 10.396 mg. subst.: 19.809 mg. CO_2, 3.772 mg. H_2O
$C_{11}H_9O_4NS$ (251.25) calc'd.: C 52.58; H 3.61%
found: C 52.00; H 4.06%

Sodium nitroprusside test was strongly positive.[21]

(—)-Bis(2-carboxy-2-phthalimidoethyl) disulphide (Ia)

N-Phthaloyl-L-cysteine (II, 0.5 g., $[\alpha]_D$ —64°) was dissolved in methanol (5 ml.) and adjusted with 10% ammonium hydroxide to *p*H 8. A trace of $Fe_2(SO_4)_3$ was added, and a stream of air passed through the solution overnight. The solution was filtered, and most of the solvent evaporated under reduced pressure. The residue was adjusted with *N* hydrochloric acid to *p*H 5, extracted with ether (3 × 20 ml.) and the ethereal extracts washed with water and dried ($MgSO_4$). After removing the solvent a viscous oil remained, consisting of (—)-bis (2-carboxy-2--phthalimidoethyl) disulphide, which on drying under reduced pressure became an amorphous powder (0.3 g., 60%). On addition of dichloromethane the oily disulphide crystallised, and after drying at 50°/0.004 mm. for 15 hours colourless neeedles were obtained, m. p. 166—168° (decomp.), showing $[\alpha]_D^{20}$ —275° ± 1° (c, 1 in ethanol), $[\phi]_D^{20}$ — 1380°.

Anal. 7.550 mg. subst.: 14.602 mg. CO_2, 2.242 mg. H_2O
$C_{22}H_{16}O_8N_2S_2$ (500.51) calc'd.: C 52.79; H 3.22%
found: C 52.78; H 3.32%

The sodium nitroprusside test was negative.

(—)-Bis (2-carboxy-2-phthalimidoethyl) trisulphide (Ib)

To a solution of *N*-phthaloyl-L-cysteine (II, 4 g., 0.016 mole, $[\alpha]_D$—64°) in anhydrous chloroform a solution of freshly distilled sulphur dichloride (0.82 ml., 0.008 mole) in chloroform (15 ml.) was added. After the vigorous developing of hydrogen chloride ceased, the reaction mixture was refluxed for one hour, the solvent was then evaporated under reduced pressure, and the oily residue consisting of (—)-bis (2-carboxy-2-phthalimidoethyl) trisulphide dissolved in dichloromethane. On addition of petroleum ether (b. p. 40—60°) an oily layer separated, which crystallized on standing at 0° as colourless prismatic needles (2 g., 47%) with the m. p. 80—85° (decomp.). Further purification of the substance by dissolving in ether, removing the solvent, and adding dichloromethane yielded crystals which after drying at 50°/0.004 mm. had the m. p. 95—97° (decomp.) and $[\alpha]_D^{20}$ —472° ± 2° (c, 1.5 in ethanol), $[\phi]_D^{20}$ —2510°. Last traces of solvent were removed by drying at 100°/0.004 mm. for 12 hours. A melt remained which was analysed.

Anal. 6.538 mg. subst.: 11.844 mg. CO_2, 2.000 mg. H_2O
8.489 mg. subst.: 11.265 mg. $BaSO_4$
$C_{22}H_{16}O_8N_2S_3$ (532.57) calc'd.: C 49.61; H 3.02; S 18.06%
found: C 49.44; H 3.42; S 18.23%

(—)-Bis (2-carboxy-phthalimidoethyl) tetrasulphide (Ic)

To a solution of *N*-phthaloyl-L-cysteine (II, 7 g., 0.028 mole) in chloroform (15 ml.), a solution of disulphur dichloride (1.87 g., 0.014 mole) in chloroform (25 ml.) was added. After the vigorous evolution of hydrochloric acid had subsided, the mixture was refluxed for 1 hour. The solvent was removed under reduced pressure, and dichloromethane was added to the pale yellow oily residue. After standing at 0° the oil crystallized. The crystals were dissolved in ether, treated with a small amount of charcoal, and filtered. The filtrate was evaporated under reduced pressure, dichloromethane added, and (—)-bis(2-carboxy-2-phthalimidoethyl) tetrasulphide separated as colourless prisms in a yield of 4 g. (50%), showing the m. p. 108—110°. The analytical sample was recrystallized from dichloromethane and showed the m. p. 110—112° and $[\alpha]_D^{20}$ —388° ± 2° (c, 1.5 in ethanol). $[\phi]_D^{20}$ —2190°. The substance was melted in high vacuum before analysis.

Anal. 5.947 mg. subst.: 10.274 mg. CO_2, 1.627 mg. H_2O
6.984 mg. subst.: 11.508 mg. $BaSO_4$
$C_{22}H_{16}O_8N_2S_4$ (564.64) calc'd.: C 46.80; H 2.85; S 22.72%
found: C 47.14; H 3.06; S 22.63%

Hydrazinolysis of (—)-bis(2-carboxy-2-phthalimidoethyl) disulphide (Ia)

A mixture of (—)-bis(2-carboxy-2-phthalimidoethyl) disulphide (250 mg., 0.5 mmole), sodium carbonate (50 mg.), hydrazine hydrate (50 mg.) and water (2 ml.) was left at room temperature for 2 days[22]. The gradually formed precipitate of L-cystine (63 mg., 52%) was filtered off, m. p. 242—248°, $[\alpha]_D^{20}$ —205° (c, 1.4 in *N* hydrochloric acid).

Hydrazinolysis of (—)-bis(2-carboxy-2-phthalimidoethyl) trisulphide (Ib) *and of tetrasulphide* (Ic)

The hydrazinolysis of Ib and Ic was carried out in the same manner as with Ia. The gradually formed precipitate consisting of amino acids and elemental sulphur was filtered off and washed with carbon disulphide. The remaining crystals consisted mainly of L-cystine and traces of three ninhydrine-active components, as shown by paper chromatography.

Acknowledgment. The authors wish to thank the Rockefeller Foundation for financial support of this work.

REFERENCES

1. J. H. van t'Hoff, *Die Lagerung der Atome im Raume*, Braunschweig, 1894, p 124.
2. W. Kauzmann and H. Eyring, *J. chem. Phys.* **9** (1941) 41.
3. L. Fieser, *Rec. trav. chim. Pays-Bas* **69** (1950) 410.
4. V. du Vigneaud and W. I. Patterson, *J. biol. Chem.* **109** (1935) 97.
5. G. B. Brown and V. du Vigneaud, *J. biol. Chem.* **140** (1941) 769.
6. K. Balenović, I. Jambrešić, B. Gašpert, and D. Cerar, *Rec. trav. chim. Pays-Bas* **75** (1956) 1252.
7. A. Fredga, *Acta chem. Scand.* **4** (1950) 1307.
8. *cf. e. g. lit. cit.* 5.
9. *cf.* W. Kuhn in *Stereochemie*, Editor K. Freudenberg, Leipzig, 1933, pp. 317—434.
10. J. E. Baer and M. Carmack, *J. Am. Chem. Soc.* **71** (1949) 1215.
11. M. Calvin and J. A. Barltrop, *J. Am. Chem. Soc.* **74** (1952) 6153.
12. J. A. Schellman in C. Djerassi, *Optical Rotatory Dispersion, Applications to Organic Chemistry*, New York 1960, Section 15-4.
13. M. Calvin, *U. S. Atomic Energy Comm.*, UCRL-2438, 1954, p. 3.
14. J. A. Schellman, *lit. cit.* 12, p. 224.
15. K. Balenović and B. Gašpert, *Chem. & Ind.* (1960) (in press).
16. *idem., ibid.* (1957) 115.
17. K. Balenović, B. Gašpert, and N. Štimac, *Croat. Chem. Acta* **29** (1957) 93.
18. *cf.* R. Connor, *Organic Sulphur Compounds* in »*Organic Chemistry*«, Vol. I, Editor H. Gilman, New York 1943, pp. 835—943.
19. *cf.* P. M. Jones and W. Klyne, *J. Chem. Soc.* **1960**, 871.
20. *cf. e. g.* L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, 2nd Edition, London 1959, p. 352.
21. *cf.* M. L. Anson in *The Chemistry of the Amino Acids and Proteins*, C. L. A. Schmidt, Editor, 2nd Edition, Springfield 1945, p. 410.
22. *cf.* F. E. King and D. A. A. Kidd, *J. Chem. Soc.* **1949**, 3315.

IZVOD

Optički aktivni trisulfidi i tetrasulfidi, analozi L-cistina

B. Gašpert, Z. Štefanac, R. Marušić i K. Balenović

Opisana je preparacija i svojstva optički aktivnog bis(2-karboksi-2-ftalimidoetil) disulfida (Ia), $[\alpha]_D$—275°, bis(2-karboksi-2-ftalimidoetil) trisulfida (Ib), $[\alpha]_D$—472° i bis (2-karboksi-2-ftalimidoetil) tetrasulfida (Ic), $[\alpha]_D$—388°. Ovi su spojevi priređeni iz *N*-ftaloil-L-cisteina (II), $[\alpha]_D$—64° dobivenog iz L-cistein hidroklorida i natrium *o*-karbetoksitiobenzoata.

KEMIJSKI ZAVOD
PRIRODOSLOVNO-MATEMATIČKI FAKULTET
ZAGREB

Primljeno 13. svibnja 1960.