isoThiocyanates XXXVII *. Rotatory Dispersion Studies of Optically Active Sulphoxides Derivable from isoThiocyanates of Natural Origin
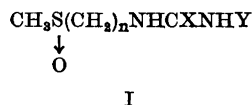
W. KLYNE and JANET DAY

Postgraduate Medical School, University of London, England

ANDERS KJÆR

Organic Chemical Laboratory, Royal Veterinary and Agricultural College, Copenhagen, Denmark

Within the group of naturally occurring sulphoxides, in which optical activity can be partly or wholly attributed to the presence of an asymmetric sulphur atom (*cf. e.g.* Refs. [1,4]), a homologous series of *iso*thiocyanates presents an interesting stereochemical problem. In 1948, Schmid and Karrer [2] assigned the structure $CH_3SOCH = CHCH_2CH_2NCS$ to sulphoraphen, a strongly *laevo*rotatory mustard oil, isolated from radish seed. This finding was soon followed by the isolation by Schultz and Gmelin [3] of the glucoside, glucoiberin, enzymically hydrolyzed to another sulphoxide-mustard oil, for which the structure $CH_3SO(CH_2)_3NCS$ was suggested [3] and later proved [4]. More recently, Kjær *et al.* [4,6-8] reported the isolation of additional sulphoxide-mustard oils, subsequent to enzymic hydrolysis of glucosides present in various crucifers. Thus, alyssin [4,5] ($CH_3SO(CH_2)_5NCS$), hirsutin [6] ($CH_3SO(CH_2)_8NCS$), arabin [7] ($CH_3SO(CH_2)_9NCS$) and camelinin [8] ($CH_3SO(CH_2)_{10}NCS$) were encountered as species of natural provenance. Lately, Procházka [9] added sulphoraphan, $(-)-CH_3SO(CH_2)_4NCS$, the saturated compound corresponding to sulphoraphen [2], to the above series (*cf.* also Ref. [6]).

Identical configuration around the asymmetric sulphur atom in the homologous series of naturally derived sulphoxide-mustard oils was suggested by the fact that the optical rotations at 589 mμ [4] of the

isothiocyanates measured, as well as of various thiourea derivatives*, were all negative and of comparable magnitude.

In order to substantiate this conclusion, a rather unusual and extensive series of homologous, naturally derived sulphoxides of the general structural type (I) was subjected to rotatory dispersion studies in methanol solution.

$$CH_3S(CH_2)_nNHCXNHY$$
$$\downarrow$$
$$O$$

I

X = O, or S
Y = H, C_6H_5, or $C_6H_5CH_2$
n = 3, 4, 5, 8, 9, or 10

In all cases the rotation becomes *more negative* as one moves to shorter wave lengths (negative plain curves; for nomenclature, see Djerassi and Klyne [10]). Except

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* No rotation was reported for the aromatic thiourea-derivatives employed in the characterisation of sulphoraphan [9]. A small specimen of the phenylthiourea, kindly placed at our disposal by Dr. Procházka, Prague, was measured in the laboratory of one of the authors (A.K.) and gave: $[a]_D^{25}$ −54° (*c* 1.8, 96 % EtOH, microtube).

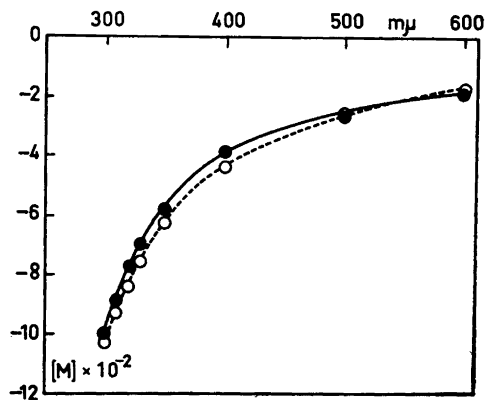*Fig. 1.* Rotatory dispersion curve of straight-chain N-(ω-methylsulphinylalkyl)-thioureas, $CH_3SO(CH_2)_nNHCSNH_2$, in methanol. - - - - : n = 5; ———: n = 10.

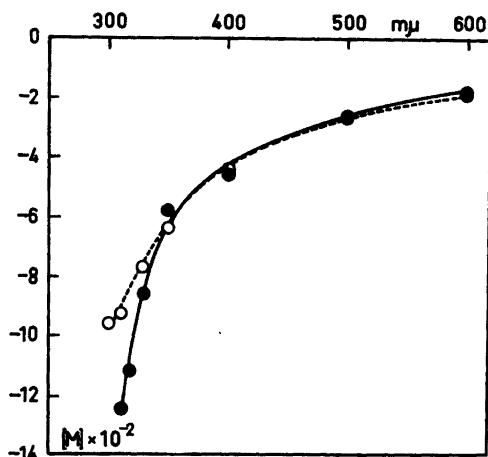* Part XXXVI of this series: *Acta Chem. Scand.* 13 (1959) 1575.

Fig. 2. Rotatory dispersion curve of straight-chain, N-substituted N′-(ω-methylsulphinyl-alkyl)-thioureas, $CH_3SO(CH_2)_nNHCSNHR$, in methanol. ————: n = 5, R=C_6H_5
----: n = 10, R=$C_6H_5CH_2$.

are almost superimposable. Representative examples are reproduced in Figs. 1 and 2. In Table 1, the values determined at 600 mμ, 500 mμ, 400 mμ and 300 mμ, are listed for all the compounds investigated.

Clearly, the rotatory dispersion curves provide a much better means of allotting configuration at the asymmetric sulphur atom of the compounds (I), than do "monochromatic" rotation analogies. From the present results it seems safe to conclude that all sulphoxide-*iso*thiocyanates encountered in Nature thus far, possess identical configurations. Their spatial relationship to (+)-*S*-methyl-L-cysteine sulphoxide, the sole sulphoxide of established absolute configuration [11], remains to be determined.

From the present rotatory dispersion curves it is evident that the presence of an aromatic nucleus at a considerable distance from the asymmetric sulphur atom in eleven of the compounds apparently has a negligible effect on the dispersion curve down to 300 mμ. In the phenylthiourea-derivative of sulphoraphen, with a double bond located αβ to the sulphur atom, a slight deviation in the rotatory dispersion curve is noticed at about 300 mμ, compared with those of the saturated series.

for a few points in the region of 300 mμ, the curves, when plotted as molecular rotations, are very closely similar, and some

Table 1. Molecular rotations in methanol at various wave lengths of compounds represented by formula (I).

Formula	Mol. weight	Concn. (mg/ml)	300 mμ	400 mμ	500 mμ	600 mμ
				Molecular rotations at λ		
			(ALL VALUES NEGATIVE)			
$CH_3SO(CH_2)_3NHCSNHCH_2C_6H_5$ [4,c]	270	0.1	670	360	280	160
$CH_3SO(CH_2)_4NHCSNHC_6H_5$ [9]	270	0.1	630 (320) [d]	330	250	160
$CH_3SOCH=CH(CH_2)_2NHCSNHC_6H_5$ [b]	268	0.1	1 040	460	300	200
$CH_3SO(CH_2)_5NHCSNH_2$ [4]	208	1	1 030	440	250	180
$CH_3SO(CH_2)_5NHCSNHC_6H_5$ [4]	284	0.1	1 250 (310)	450	260	180
$CH_3SO(CH_2)_8NHCSNH_2$ [6]	250	1	1 000	400	210	80
$CH_3SO(CH_2)_8NHCSNHC_6H_5$ [6]	327	1 and 0.1	820 (310)	420	270	170
$CH_3SO(CH_2)_9NHCSNH_2$ [7]	264	1	980	410	250	160
$CH_3SO(CH_2)_9NHCSNHC_6H_5$ [7]	341	1 and 0.1	610 (330) ?	400	210	150
$CH_3SO(CH_2)_9NHCSNHCH_2C_6H_5$ [7]	355	0.1	1 080	570	370	150
$CH_3SO(CH_2)_9NHCONHCH_2C_6H_5$ [7]	339	0.5	940	430	250	190
$CH_3SO(CH_2)_{10}NHCSNH_2$ [8]	279	1	1 000	380	270	190
$CH_3SO(CH_2)_{10}NHCSNHC_6H_5$ [8]	355	0.2	990 (320)	420	230	150
$CH_3SO(CH_2)_{10}NHCSNHCH_2C_6H_5$ [c]	369	1	950	450	250	170
$CH_3SO(CH_2)_{10}NHCONHCH_2C_6H_5$ [8]	352	0.5	1 040	440	250	150

[a] Determined at 25°. [b] A specimen prepared from sulphoraphen as described [2]. [c] A non-analyzed specimen [8]. [d] Lowest wave-length recorded, when not 300 mμ.

Optical rotations were measured with a Rudolph photoelectric spectropolarimeter using a tube of 1 dm length. For the details, see Ref.[11]. The experimental error is probably $\pm 20°$ for molecular rotations (cf. Ref.[11]). In some cases it was impossible to make measurements down to 300 mμ because of intense light absorption by the compound under study. The course of a typical curve for $CH_3SO(CH_2)_5$ NHCSNH$_2$ between 350 and 300 mμ is indicated by the following values; [M], and λ in parentheses; -625 (350), -755 (330), -840 (320), -930 (310), $-1\,030$ (300 mμ).

1. Kjær, A. *Handbuch der Pflanzenphysiologie,* Springer-Verlag, Berlin-Göttingen-Heidelberg 1958, vol. **IX**, p. 70 f.f. —*Fortschr. Chem. org. Naturstoffe.* **18** (1960). *In press.*
2. Schmid, H. and Karrer, P. *Helv. Chim. Acta* **31** (1948) 1017.
3. Schultz, O.-E. and Gmelin, R. *Arch. Pharm.* **287**/59 (1954) 404.
4. Kjær, A. and Gmelin, R. *Acta Chem. Scand.* **10** (1956) 1100.
5. Schultz, O.-E. and Wagner, W. *Z. Naturforsch.* **11b** (1956) 417.
6. Kjær, A. and Christensen, B. *Acta Chem. Scand.* **12** (1958) 833.
7. Kjær, A. and Gmelin, R. *Acta Chem. Scand.* **10** (1956) 1358.
8. Kjær, A., Gmelin, R. and Boe Jensen, R. *Acta Chem. Scand.* **10** (1956) 1614.
9. Procházka, Ž. *Collection Czechoslov. Chem. Communs.* **24** (1959) 2429.
10. Djerassi, C. and Klyne, W. *Proc. Chem. Soc.* **1957**, 55.
11. Hine, R. and Rogers, D. *Chem. & Ind. London* **1956** 1428.
12. Jones, P. M. and Klyne, W. *J. Chem. Soc.* **1960** 871.

Purification of Human Plasminogen on DEAE-cellulose

PER WALLÉN and KURT BERGSTRÖM

Kemiska Institutionen II, Karolinska Institutet, Stockholm, Sweden

In a recent communication a method for the purification of human plasminogen was outlined [1]. The most important step of this method was an adsorption of a partially purified plasminogen preparation on columns of DEAE-cellulose equilibrated with an ammonium acetate buffer, pH 9.0, containing lysine to a concentration of 0.01 M. Under these conditions about 80 % of the protein was adsorbed, whereas 60 — 80 % of the plasminogen passed through the column in the frontal fraction together with about 20 % of the protein. We were prompted to use lysine in this connection by the observation of Alkjaersig, Fletcher and Sherry [2], who found that ε-aminocaproic acid and lysine increased the solubility of plasminogen and plasmin preparations at neutral reaction, probably due to a dissolution of a protein complex.

As starting material we used a plasminogen preparation obtained from Cohn's fraction III by dialysis against a phosphate buffer of pH 7.0 and low ionic strength. The specific activity ranged from 8 to 20 times that of fraction III. This variability was shown to be caused by the poor reproducibility of the first step. Therefore we now have excluded this step.

Plasminogen is easily adsorbed on DEAE-cellulose, previously equilibrated with ammonium acetate buffer, pH 9.0, but if lysine is added to the buffer it is not adsorbed. Thus it was near at hand first to adsorb plasminogen to DEAE-cellulose from a weakly alkaline buffer without lysine and then to try to elute the activity with the same buffer to which lysine had been added. In the following a method based on this principle is outlined.

A preparation obtained from Cohn's fraction II + III by precipitating the plasminogen at low ionic strength and pH 5.3 was used as starting material. Preparations of this type have almost the same specific activity as fraction III and the yields are higher. About 35 g DEAE-cellulose (Brown Comp. New Hampshire, U.S.A.) were suspended in 0.04 M ammonium acetate buffer pH 9.0. The slurry was poured in a column with a diameter of 4 cm and packed with