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Short Communications

Lower Homologues of Thioctic Acid

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A few years ago I prepared 1,2-dithiolane-3-carboxylic acid[1], the lowest homologue of 6,8-thioctic acid [2-4]. It was my intention to prepare other homologues with side chains more like the biologically important acid. In 1956, however, Thomas and Reed prepared the nearest lower and higher homologues of 6,8-thioctic acid, 1,2-dithiolane-3-butyric acid and 1,2-dithiolane-3-caproic acid [5]. These homologues exhibited much lower biological activity than DL-6,8-thioctic acid in the acetate-replacing factor assay [6,7].

Preparation of the homologues between 1,2-dithiolane-3-carboxylic acid and 6,8-thioctic acid has thereby lost part of its biological interest. The acids with a carboxyl group in a position α to sulphur in the 1,2-dithiolane or 1,2-dithiane ring, however, show some interesting properties, which are not observed in the homologues where the carboxyl is screened by a side chain. This is a preliminary report of the syntheses and UV spectra of some carboxyl substituted cyclic disulphides.

The UV spectrum of 1,2-dithiane contains a characteristic absorption maximum at 290 mμ and that of 1,2-dithiolane a maximum at 330 mμ[8]. When a carboxylic group is substituted in a position α to the disulphide group, it causes a withdrawal of electrons from the disulphide group, resulting in a shift of the absorption band towards shorter wavelengths. This is observed for 1,2-dithiolane-3-carboxylic acid, which has an absorption peak at 280 mμ (Fig. 1) that is, a hypsochromic shift of 50 mμ. A still greater hypsochromic shift was observed by Schotte[9] for 1,2-dithiolane-3,5-dicarboxylic

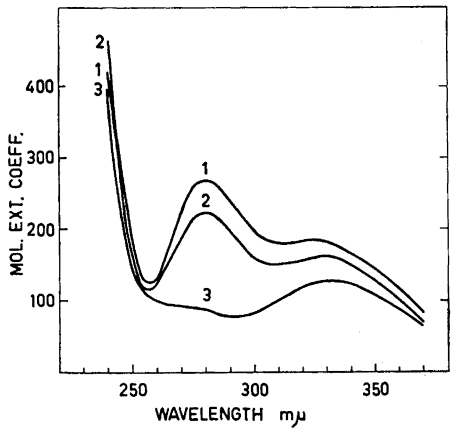

Fig. 1. UV absorption spectra of 1,2-dithiolane-3-carboxylic acid: (1) in 0.6 M HCl (solvent: alcohol); (2) in alcohol; (3) in 0.01 M NaOH (solvent: alcohol).

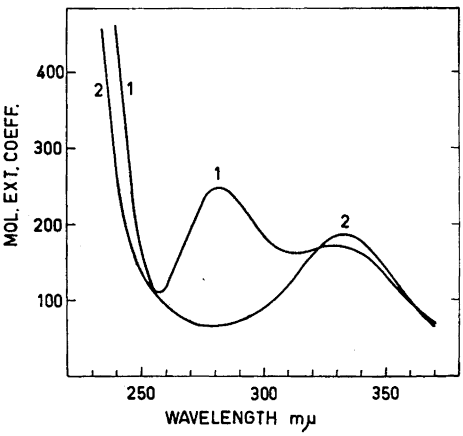

Fig. 2. UV absorption spectra of: (1) 5-methyl-1,2-dithiolane-3-carboxylic acid; (2) 5-methyl-1,2-dithiolane-3-acetic acid. Solvent: alcohol.

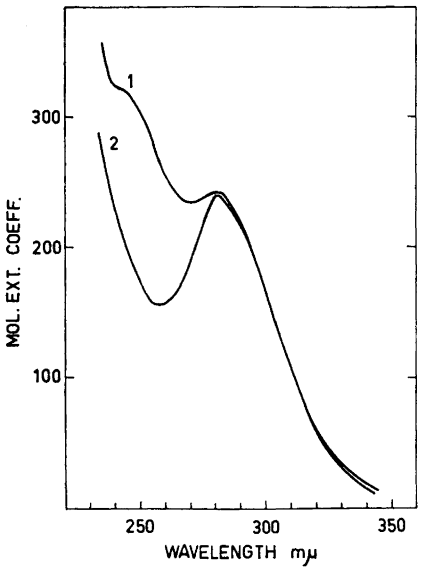

Fig. 3. UV absorption spectra of 1,2-dithiane-3-carboxylic acid: (1) in alcohol; (2) in 0.025 M KOH (solvent: alcohol).

acid (λ_{max} = 250 mμ). The spectrum of 5-methyl-1,2-dithiolane-3-carboxylic acid shows an absorption peak at 283 mμ (Fig. 2). The small bathochromic shift caused by methyl group in the latter acid will be discussed.[10]

In 6,8-thioctic acid the carboxylic group is too far away to have any influence on the -S-S-group, and the side chain therefore acts upon the disulphide group as an alkyl group, λ_{max} = 333 mμ[8]. Even if a carboxylic group is substituted in the β-position, it does not affect the disulphide group seriously. The spectrum of 5-methyl-1,2-dithiolane-3-acetic acid with an absorption maximum at 334 mμ, makes this clear (Fig. 2). The recently prepared 1,2-dithiolane-3-acetic acid also has an absorption maximum at the same wavelengths as 6,8-thioctic acid[11]. Schotte has shown that a carboxylic group in the 4-position, as well as other groups situated there, has no effect on the characteristic absorption peak of the five-membered ring[12,13]; *cf.* also Schotte.[14]

Fredga has prepared 1,2-dithiane-3,6-dicarboxylic acid[15,16], and Schotte has recorded its UV spectrum[9]. The carboxylic group shifts the absorption maximum to shorter wavelengths, but because the disulphide peak is so close to the absorption band at about 200 mμ the spectrum becomes nearly continuous above 200 mμ where the readings were made. 1,2-Dithiane-3-carboxylic acid has now been prepared. As could be expected, the hypsochromic shift of λ_{max} was here smaller. The spectrum is shown in Fig. 3. The inflexion at about 245 mμ corresponds to the 280 mμ maximum of 1,2-dithiolane-3-carboxylic acid, and the peak at 282 mμ corresponds to the absorption at 333 mμ in the dithiolane analogue. On the other hand, 1,2-dithiane-4-carboxylic acid gives nearly the same absorption band as the unsubstituted ring and 1,2-dithiane-3-butyric acid (5,8-thioctic acid)[17].

Figs. 1 and 2 show that the alkali salts of the two acids have absorption curves which are quite different in character from those of the free acids. The peaks attributed to the usual disulphide band have disappeared, but the lower intensity bands at longer wavelengths remain.

Thus it is clear from the spectra that 1,2-dithiolane-3-carboxylic acid and 1,2-dithiane-3-carboxylic acid show two maxima in the actual region for the disulphide absorption, and one of these maxima disappears when the acid is converted to its ionized form. Schotte has found that the absorption curves for 1,2-dithiolane-3,5-dicarboxylic acid and some other acids changed character in alkaline solution[9], but he did not obtain the pronounced maxima seen in Figs. 1 and 3. Such a split of the disulphide absorption band has not been previously reported in the literature. These double absorption bands may be explained by the assumption that the two sulphur atoms in the disulphide group need not be regarded as

Acid	M.p.	*Found* S	*Found* Eq.wt.	*Calc.* S	*Calc.* Eq.wt.
1,2-Dithiolane-3-carboxylic	81—82° *	42.61	150.6	42.69	150.2
5-Methyl-1,2-dithiolane-3-carboxylic	77—79°	39.14	164.7	39.05	164.3
5-Methyl-1,2-dithiolane-3-acetic	83—86.5°	35.67	177.8	35.97	178.3
1,2-Dithiane-3-carboxylic	75—76°	39.09	165.0	39.05	164.3

* This acid polymerizes easily and the polymer melts at about 147°.

a unit. In ultraviolet absorption the exited electron may originate from either of the two sulphur atoms, and in an electrically asymmetrical disulphide there must be two separate exitation energies for electrons from the two different sulphur atoms, resulting in two UV absorption bands.

Experimental. A Beckman model DU photoelectric quartz spectrophotometer equipped with a hydrogen discharge lamp was used for the ultraviolet absorption measurements.

The synthetic methods for the acids (*cf.* p. 1710) will be published later.

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On the Effect of Ionizing Radiations on Cyanocobalamin

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The destruction of cyanocobalamin (vitamin B_{12}) by ionizing radiations has generally been estimated spectrophotometrically or microbiologically [1-4]. Smith [4] mentions in his recent article on the instability of radioactive vitamin B_{12} that absorption spectroscopy gives a moderately accurate measure of the residual vitamin B_{12}. He also states that a microbiological assay can be used as there is no evidence of a significant breakdown to other microbiologically active compounds. In our preliminary studies of the effect of γ-rays on cyanocobalamin we have been unable to correlate the changes in the absorption spectra with the microbiological activity of irradiated solutions of vitamin B_{12}. Moreover, evidence has been obtained for the formation of microbiologically active breakdown products.

Solutions of cyanocobalamin containing 5 mg per ml in a phosphate buffer of pH 5 were irradiated under aerobic conditions with γ-rays from a ^{60}Co source. The doses given were 2, 5 and 10 Mrad. The spectra of the irradiated and nonirradiated solutions of vitamin B_{12} were measured in a Beckman Model DU spectrophotometer. A tube assay with *Escherichia coli* 113—3 [5] was used for the microbiological tests. The assay media were those of Diding [6] and of Burkholder [7], to which 1.5 mg of KCN per l had been added. Growth was measured turbidimetrically after incubation of the tubes for 20 h at 37°C.

The spectra of the non-irradiated and of the irradiated solutions of cyanocobalamin are shown in Fig. 1. Concomitant with the decrease in the absorption maxima following irradiation, there was also a tendency for the peaks in the 360 and 550 mμ regions to shift towards lower wavelengths. Calculation of the degree of destruction by comparing the absorption at 361 mμ of irradiated and non-irradiated solutions indicated that 60 % of the vitamin originally present had been destroyed by a dose of 2 Mrad. According to the microbiological assay approximately 85 % was inactivated by this dose.