cahydrate. A solution of sodium disulphide in excess (0.11 moles), prepared about 48 hours before use from $Na_2S \cdot 9\ H_2O$ and sulphur in water as solvent, was added cautiously with cooling. The total volume was 200 ml, and the solution was allowed to stand for 8 hours with occasional shaking. Some sulphur, having separated, was filtered off and the still alkaline solution acidified with dilute sulphuric acid. After the removal of precipitated amounts of non-crystalline material, mainly sulphur, the aqueous part was extracted 10 times with 50 ml portions of ether. The ether was allowed to evaporate spontaneously, leaving a semi-solid, colourless mass. By treatment with a small volume of hot water, this product mostly dissolved, and after rapid filtration the solution was placed in a refrigerator over night. The dicarboxylic acid crystallized as beautifully formed prisms. Repeated recrystallizations yielded 1.9 g (11 %) of a pure substance with the melting point 197—199°. The melting point depends on the rate of heating; the value given was found by plunging the capillary tube into the preheated (about 190°) apparatus. (Found: Equiv.wt. 97.8; S 33.33; Mol.wt. 191, 186, 188. Calc. for $C_5H_6O_4S_2$ (194.2)· Equiv. wt. 97.1; S 33.02; Mol. wt. 194.2).

*Preliminary experiments on resolution. (+)-1,2-Dithiolane-3,5-dicarboxylic acid.* 0.39 g (0.002 moles) of the *racemic* acid and 0.94 g (0.002 moles) of brucine were dissolved in a hot mixture of water (10 ml) and ethanol (6 ml). Crystallization over night at room temperature yielded 0.60 g (45 %) of salt. The acid was set free from its salt by acidifying with dilute sulphuric acid and extraction with ether in the usual manner. The rotatory power was measured in alcohol. M.p. 176—180°.

$$[\alpha]_D^{25} = + 414°$$

*(—)-1,2-Dithiolane-3,5-dicarboxylic acid.* 0.47 g (0.001 mole) of brucine was added to the mother liquor from above together with 5 ml of ethanol. Crystallization as before yielded 0.60 g of salt. The measurement of the rotatory power was performed as described above. M.p. 177—181°: $[\alpha]_D^{25} = - 564°$, Equiv. wt. Calc. 97.1. Found 97.3.

The author is indebted to Professor Arne Fredga for valuable discussions and stimulating help. A grant from the *Swedish Natural Science Research Council* is gratefully acknowledged.

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Received December 10, 1953.

## Some Sulphur Compounds Related to Pimelic Acid

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In the preceding paper [1] attention is drawn to the study of α,α'-dimercapto dicarboxylic acids and the important results, that might be obtained by investigations already shortly outlined. No such derivatives related to pimelic acid have until now been published, and only those of adipic acid have been more thoroughly investigated [2,3].

The corresponding dibromo acids are usually employed as starting materials, but for α,α'-dibromo pimelic acid the isolation of one of the stereoisomeric forms still remains. Fehnel and Oppenlander [4] report and discuss the preparation of the high melting form in connection with an examination of some symmetrical 2,6-disubstituted tetrahydrothiapyrans. In a reaction with sodium sulphide they obtained *cis-(meso)*-2,6-tetrahydrothiapyrandicarboxylic acid, and they thus concluded that the high melting isomer of α,α'-dibromo pimelic acid possesses the *meso* configuration. Already before these results were published, the same compounds had been prepared in a slightly different way by the present author. As his observations are in good agreement with those of Fehnel and Oppenlander, it seems unnecessary to publish the experimental details.

The present publication deals with some further sulphur derivatives obtained from the high melting dibromo acid, while attempts to isolate the other stereoisomer are in progress.

For the preparation of mercapto-substituted carboxylic acids two methods are

mainly taken into consideration, namely the reduction of the corresponding disulphides and the treatment of the xanthogen-substituted acids with ammonia [5,6].

The monomeric disulphide related to α,α'-dimercapto pimelic acid has a seven-membered ring structure and can be obtained from the dibromo acid and sodium disulphide, although in a rather low yield. As could be expected considerable amounts of polymeric linear disulphides are formed. It is further interesting to note that as a by-product the above mentioned *cis*-2,6-tetrahydrothiapyrandicarboxylic acid has been isolated. When α,α'-dibromo *adipic* acid is treated with potassium disulphide, both the cyclic sulphide and disulphide are formed, the former with separation of sulphur [7]. The difference between the homologues thus only implies that the dibromo pimelic acid gives also polymeric products, obviously due to the unfavourable conditions for cyclization to a ring of the size in question. — The structure of the monomeric disulphide is definitely settled by reduction with zinc powder in ammoniacal solution, which yielded dimercapto pimelic acid, identified by titration with sodium hydroxide and with iodine. Owing to its solubility properties the mercapto acid has, however, not yet been obtained quite pure. — The formation of a dithiol from the cyclic disulphide shows that the structure $>S=S$ for the disulphide group is excluded. On the other side, a substance of this type might possibly act as an intermediate in the formation of the cyclic monosulphide dicarboxylic acid, which is obtained with separation of sulphur. — The relative difficulties in obtaining the seven-membered ring system in question, dithiacycloheptane, of which only the unsubstituted heterocycle [8] is known previously, are further elucidated by experiments on the oxidation of the dimercapto acid with hydrogen peroxide. Preliminary results show that only polymeric products are formed as has also been found for α,α'-dimercapto suberic acid [9]. The lower homologue, dimercapto adipic acid, yields the six-membered cyclic disulphide without complications [2,3]. The influence of the dilution on the course of such oxidation reactions is being investigated.

From the high melting form of α,α'-dibromo pimelic acid the corresponding *bis*-ethylxanthogen acid has been prepared in the usual way. By treatment with ammonia the dimercapto derivative is obtained in a good yield, the report of which, however, will be given later, when the purification operations are quite finished. The definite evidence of the structure of these compounds from stereochemical point of view will be discussed later.

*EXPERIMENTAL. 1,2-Dithiacycloheptane-3,7-dicarboxylic acid.* A solution of sodium disulphide (0.058 moles) in water, prepared in the usual manner [1] 48 hours before use, was added to an aqueous solution of the neutral sodium salt of α,α'-dibromo pimelic acid, obtained by rapid neutralization of 15.9 g (0.05 moles) of the acid (m.p. 150—151°) with sodium carbonate decahydrate. The total volume was 120 ml. After 27 hours, separated sulphur (0.4 g) was filtered off and the solution was acidified with dilute sulphuric acid. Polymeric products immediately precipitated as a viscous oil, and the aqueous solution was extracted repeatedly with 35 ml portions of ether. On spontaneous evaporation the ether solution left a semi-solid, colourless mass. Recrystallizations (twice) from water gave 1.2 g (11 % yield) of the dicarboxylic acid as hard prisms. The melting point is 193—194° but depends somewhat on the rate of heating; the value given was found by plunging the capillary tube into the preheated (about 188°) apparatus. The melting seems to occur without any decomposition. (Found: Eq.wt. 112.3; S 29.12; Mol.wt. 223, 222, 224, 224; Calc. for $C_7H_{10}O_4S_2$ (222.3): Eq. wt. 111.1; S 28.85; Mol. wt. 222.3).

It is possible that the yield above of monomeric substance can be somewhat improved, if the reaction is performed in more dilute solution.

The mother liquor from the first recrystallization was extracted with ether, and the crude product obtained by evaporation of the solvent was purified by fractionated crystallization from small amounts of water. 0.4 g of a pure substance, m.p. 208—210°, (more soluble in water than the disulphide) was isolated and identified as *cis*-2,6-tetrahydrothiapyrandicarboxylic acid (mixed m.p. 208—209°).

*α,α'-Bis-ethylxanthogen pimelic acid.* 15.9 g (0.05 moles) of α,α'-dibromo pimelic acid (m.p. 150—151°) was neutralized in water solution with 14.3 g (0.05 moles) of $Na_2CO_3\,10H_2O$, the total volume being 100 ml. 18.4 g (15 % excess) of solid potassium ethyl xanthate was added immediately and the mixture shaken until homogeneous. After standing at room temperature for three days, the solution was acidified with dilute sulphuric acid. The *bis*-xanthogen acid separated as an oil, which soon crystallized (if the reaction time is shorter

a lower yield is obtained, and the product crystallizes less readily). The crude product was filtered off and treated with water in order to remove any dibromo acid left. The yield of acid thus obtained was 18 g (calc. 20.2 g), and the melting point was about 130°. After five recrystallizations from formic acid the product was pure and showed the m.p. 135—136°. (Found: Eq.wt. 199.6; S 31.93; Calc. for $C_{13}H_{20}O_6S_4$ (400.4): Equiv. wt. 200.2; S 32.02).

The author wants to express his gratitude to Professor Arne Fredga for valuable discussions. A grant from the *Swedish Natural Science Research Council* is gratefully acknowledged.

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Received December 22, 1953.

## On the Growth-Inhibition of *Lactobacillus bifidus* by Certain Fatty Acids

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Recent investigations [1,2] have shown that free long-chain (C>6) saturated fatty acids as well as unsaturated oleic and linoleic acids inhibit the growth of *Lactobacillus bifidus in vitro*. According to Barbero *et al.*[3] the same is true *in vivo*; in their experiments the number of *L. bifidus* in the intestinal tract of infants increased when the inhibitory saturated acids were removed from the cow's milk used in the feeding. A comparison of the composition of human milk and cow's milk shows that their content of the most strongly inhibiting saturated acids (lauric and myristic acids) is approximately equal [4]. Since, however, it is known that in practice human milk promotes the growth of *L. bifidus* in the intestinal tract of infants, it has been concluded that human milk must contain substances which can reverse the effect of the inhibitory acids. Some evidence has been cited [1,5] to show that proteins, and lactalbumin in particular, act as the detoxifying agents. The stronger detoxifying effect of human milk compared with cow's milk could, accordingly, be explained by the relatively greater content of whey proteins in human milk.

The *L. bifidus* strains used in our investigation were isolated from the faeces of breast-fed infants. The basal medium was that proposed by Hassinen *et al.*[6], supplemented with tryptic digest of casein as a source of strepogenin, found by us [7,8] to be essential for *L. bifidus*. The growth was measured titrimetrically after incubation at 37° C for 72 hours. The fatty acids investigated were added to the media as potassium salts, obtained by saponification of the corresponding acids with KOH in ethanol.

Our results on the inhibition of *L. bifidus* by different fatty acids agree in general with the data given by Tomarelli *et al.*[1] and Hassinen *et al.*[2] Propionic and butyric acids were found to have no effect, caproic acid was slightly inhibitory, and with increasing length of the carbon chain the inhibitory effect increased until lauric acid, which had the strongest effect. With the chain-length increasing further the toxicity decreased, the effect of myristic acid being nearly identical with that of capric acid. According to Hassinen *et al.*[2] palmitic, stearic and oleic acids do not inhibit *L. bifidus;* in our experiments, however, these acids also showed inhibiting, although distinctly weaker, effects than those of linoleic, lauric, capric and myristic acids. It is interesting to note in this connection that ricinoleic acid had an effect that was about 5 times as strong as that of oleic acid.

In order to overcome the inhibitory effect of fatty acids we added different proteins and yeast extract to the media containing lauric or linoleic acids. The results are given in Table 1. It will be seen that the additions of egg albumin, casein and blood albumin have permitted a fairly good growth of the bacteria, even in the presence of the inhibiting acids. Gelatin had a somewhat weaker effect whereas