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Proposed Structures for Protogen-A and Protogen-B

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The structure of a dithioöctanoic acid is proposed for Protogen-A and that of a thiosulfinyloctanoic acid for Protogen-B on the basis of data obtained from infrared absorption, desulfurization, neutralization, saponification and iodine oxidation after saponification and after reduction of Protogen-B.

Following the isolation of Protogen-B[1] we made a preliminary announcement[2] of the partial structure of Protogen-A, and shortly thereafter presented synthetic evidence[3] showing that the most likely structure is that of 6-thioctic acid (6,8-dithioöctanoic acid). Subsequent investigations by other workers[4] led to the conclusion that α-lipoic acid is the cyclic disulfide of 4,8-, 5,8- or 6,8-dimercaptocaprylic acid. The biological similarity[5] of the protogens and the lipoic acids coupled with the close agreement in chemical properties suggests that the structure of α-lipoic acid is also that of 6-thioctic acid. For the sake of clarity, when synthetic material is being spoken of, the name 6-thioctic acid should be used, and the names protogen and lipoic acid should be reserved for naturally occurring materials possessing the appropriate biological activity.

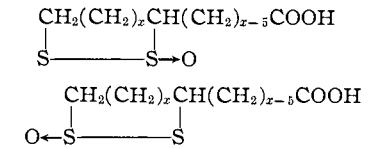
A highly purified sample of Protogen-B[6] was a light yellow oil having a neutralization equivalent of 224, and an apparent pK_a of 5.0 when titrated in aqueous alcohol with sodium hydroxide. The sodium salt could be converted to a water-insoluble S-benzylthiuronium salt whose analysis indicated $C_8H_{14}S_2O_3$ as the empirical formula for Protogen-B.

Desulfurization of Protogen-B with Raney nickel gave a volatile acid which was positively identified as octanoic acid by a comparison of the X-ray powder photograph of its S-benzylthiouronium salt with that of a known sample. The yield of octanoic acid was 82%, and no other organic products could be detected in more than trace amounts. Thus not only were the eight carbon atoms confirmed, but also the carbon skeleton was established as a straight chain.

The presence of the carboxyl group, indicated by titration, was confirmed by the infrared spectrum of Protogen-B which had the characteristic bands at *ca.* 3.1–3.3 μ (broad) and 5.78 μ. A detailed study in the CH stretching region, using a lithium fluoride prism, indicated the absence of any C-methyl groups (no band at 2960 cm.$^{-1}$), an observation confirmed by a blank Kuhn–Roth C-methyl determination. β-Mercaptobutyric acid, used as a model, gave 0.8 mole of acetic acid per mole. Thus it was established that the molecule is substituted with two sulfur atoms one of which is bonded to the terminal carbon atom. The third oxygen atom could not be bonded to carbon since the desulfurization would then have led to an oxygen-substituted octanoic acid.

The nature of the sulfur–oxygen functional group was indicated by the following observations. Fusion of Protogen-B with sodium hydroxide[7] gave rise to both sulfide and sulfite[8] indicating that the sulfur atoms existed in different states of oxidation. Furthermore, the infrared spectrum of Protogen-B had a band at 1040 cm.$^{-1}$ which could best be accounted for as absorption due to a sulfoxide or closely related group. Protogen-B did not give an immediate color with alkaline sodium nitroprusside, but if it were allowed to stand in alkali a short time before the addition of the nitroprusside, a bright wine-red color characteristic of a mercaptan was obtained. Although analytical material was not always available, by first determining the carboxylic acid content of a sample by titration it was then possible to demonstrate quantitatively that Protogen-B contained one saponifiable group per carboxyl, and that after saponification there was present a group which rapidly took up one equivalent of iodine per carboxyl group. Furthermore, if the saponified material was treated with sodium borohydride for a few hours and then titrated with iodine, it was shown that two equivalents of iodine were required per carboxyl group, whereas unsaponified Protogen-B did not take up iodine rapidly. Thus it appeared that Protogen-B was saponified to a monothiol compound which could be reduced to a dithiol. This dithiol on mild oxidation (iodine) gave a substance which could not be distinguished from Protogen-A by biological activity, countercurrent distribution, paper chromatography and infrared absorption. Previous work[6] has shown that Protogen-B was derived from Protogen-A by mild oxidation.

The functional group which best accounts for the above observations on Protogen-B is a cyclic thiolsulfinate represented by one of the structures



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Since degradative evidence for the position of the second sulfur atom and the position of the oxygen atom would be difficult to obtain from the small amount of material available to us, we decided to investigate synthetically the series of parent disulfides, the thioctic acids.[2] The results of these studies are reported in detail in the following article.[9]

Experimental

S-Benzylthiuronium Salt.—A 32-mg. sample of Protogen-B was treated by the usual procedures[10] to give 47 mg. of benzylthiuronium salt, m.p. 129–132°. Recrystallized from aqueous alcohol or absolute alcohol and acetone it had m.p. 132–134°. *Anal.* Calcd. for $C_{16}H_{24}N_2S_3O_3$: C, 49.45; H, 6.23; N, 7.21; S, 24.76. Found: C, 48.68; H, 6.33; N, 7.57; S, 23.84.

Treatment with Raney Nickel.[11]—Twelve ml. of water, 0.2 ml. of 2 *M* sodium carbonate, 19.8 mg. of Protogen-B and 2 g. of Raney nickel catalyst (prepared without the use of ethanol) were shaken together 1.75 hr. at 78°. The catalyst was removed by centrifugation and washed with warm water. The combined washings and supernatant were distilled until 10 ml. of H_2O had been collected. By oxidation with acidic dichromate[12] at 100° it was found that only 12 μ equiv. of oxidizable material had distilled.

The alkaline boiler residue was acidified with sulfuric acid, and the oil which separated was extracted with ether. The extract was dried with sodium sulfate and the ether removed by evaporation with nitrogen at ice-bath temperature. The yield of crystalline residue, m.p. 13°, was 10.5 mg. The infrared spectrum of this material could not be distinguished from that of caprylic acid. The benzylthiuronium salt of this acid, m.p. 144.0–144.4° (elongated prisms from absolute ethanol), gave no depression in melting point with an authentic sample of the salt of caprylic acid, and the X-ray diffraction powder photographs of the unknown salt and the caprylate salt were indistinguishable although easily distinguished from those of the pelargonate and heptylate salts.

Titrations.—One to 3-mg. samples were obtained by evaporating aliquots of solutions of Protogen-B (purity greater than 80%) in chloroform. A syringe microburet[13] was used for delivering the titrating agents. Slow neutralization of an alcoholic solution with 0.2 *N* aqueous sodium hydroxide and with vigorous stirring (nitrogen stream) gave an equivalent weight of 224 (theory 222). Poor stirring or more concentrated alkali (1 *N*) gave low results indicative of the easily saponifiable group present. A potentiometric titration in 50% aqueous alcohol gave an apparent pK_a of 5.0.

Saponification was carried out under nitrogen in excess 0.2 *N* aqueous sodium hydroxide either for 16 hr. at room temperature or 1 hr. at 100–105°. Equivalent weights varied with the purity of the starting material, one of the better samples giving 119 (theory 111).

A 3.12-mg. sample of Protogen-B, found by neutralization and saponification to contain 12.8 μequiv. of carboxyl and 11.9 μequiv. of saponifiable group, was saponified under nitrogen with 200 μl of 0.2 *N* sodium hydroxide 1 hr. at 100°. One hundred μl. of 0.4 *N* acetic acid was added and standard 0.1 *N* iodine in potassium iodide solution added until an excess remained. After 5 minutes the excess was titrated with 0.1 *N* thiosulfate, showing that 11.7 μ eq. of iodine was reduced. Unsaponified Protogen-B did not take up any iodine under these conditions.

A second sample from the same batch, 3.07 mg. (12.6 μequiv. COOH), was saponified as above and then treated with 4 mg. of sodium borohydride at room temperature for 1.5 hr. One-hundred μl. of 2 *N* acetic acid was then added and after evolution of gas (H_2) ceased the sample was titrated with iodine and thiosulfate as above. A corresponding blank was run to take account of residual reducing action of the borohydride. The iodine required was 25.3 μequiv.

From a different batch of Protogen-B a 2.39-mg. sample required 10.3 μequiv. of sodium hydroxide for neutralization to phenolphthalein. The resulting solution was treated with 4.5 mg. of sodium borohydride for 2.5 hr. at room temperature. Acetic acid was then added and the iodine consumption found to be 20.3 μequiv. after correction for a corresponding blank.

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Syntheses in the Thioctic Acid Series

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Treatment of γ-(2-tetrahydrofuryl)-butyric acid with hydrogen bromide, acetyl bromide or a solution of potassium iodide in phosphoric acid produces primarily the 8-halogen substituted-δ-lactone of octanoic acid along with the expected rearrangement products. These lactones are converted to the corresponding dithioloctanoic acids by treatment with thiourea and hydrobromic or hydriodic acid. The dithiol acids are oxidized to the cyclic disulfides with iodine. The expected 5-thioctic acid (5,8-dithioöctanoic acid) was obtained in 22% over-all yield from γ-(2-tetrahydrofuryl)-butyric acid. Small amounts of the isomeric 4-thioctic acid (4,8-dithioöctanoic acid) and 6-thioctic acid (6,8-dithioöctanoic acid) were also isolated from the reaction mixture. The biologically important 6-thioctic acid has been prepared by another synthesis. Ethyl adipyl chloride was added to ethylene in the presence of aluminum chloride to yield on distillation ethyl 6-keto-Δ^7-octenoate. Addition of thioacetic acid to the vinyl ketone followed by a sodium borohydride reduction gave ethyl 8-acetylthio-6-hydroxyoctanoate. Saponification of this ester and treatment of the resulting 8-thiol-6-hydroxyoctanoic acid with hydriodic acid and thiourea gave, after hydrolysis of the intermediate thiuronium salt, dihydro-6-thioctic acid (6,8-dithioloctanoic acid). The dithiol acid was readily oxidized to the cyclic disulfide (6-thioctic acid) with gaseous oxygen using ferric iron as catalyst.

Preliminary communications[1,2] from this laboratory have described the preparation, physical constants and biological activity of 6-thioctic acid, 5-thioctic acid and 4-thioctic acid. The isolation of 6-thioctic acid (*dl*-α-lipoic acid) from a synthesis designed to yield 5-thioctic acid has been described.[1-4]

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