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Chemical Studies on Lichens

7.* Acaranoic and Acarenoic Acid, Two New Aliphatic Lichen Acids

JOHAN SANTESSON

The Institute of Chemistry, University of Uppsala, Uppsala, Sweden

Acarospora chlorophana (Wg.) Mass. contains two new aliphatic lichen acids, named acaranoic and acarenoic acid. On the basis of biogenetical considerations, spectral data, and comparison with synthetically prepared material, acaranoic acid is assigned the structure II (4-hydroxypentadecane-1,2-dicarboxylic acid, γ-lactone) and acarenoic acid the structure IV(4-hydroxy-2-pentadecene-1,2-dicarboxylic acid, γ-lactone).

In 1895 Zopf isolated an acid from the yellow, crustaceous lichen *Acarospora chlorophana* (Wg.) Mass. (= *Pleopsidium chlorophanum* (Wg.) Rabenh.) and named it "Pleopsidsäure".[1] He suggested a formula of $C_{17}H_{28}O_4$ and concluded that the acid was unsaturated. Zopf's "Pleopsidsäure" has since been shown [2] to be a mixture of two aliphatic acids, for which the names acaranoic and acarenoic acid were suggested (in order to avoid confusion, the name "Pleopsidsäure" was dropped).

The acid mixture is readily obtained from the lichen. If the mixture is subjected to ozonolysis, acaranoic acid can be recovered unchanged, whereas acaraneoic acid is destroyed. By preparative thin layer chromatography according to Bendz *et al.*[2] it is possible to obtain both acids in a pure state.

Acaranoic acid melts at 154—155°, is optically active ($[\alpha]_D^{25} = -30°$), and titrates as a dibasic acid, m.w. 302. The analytical values, together with mass spectral data suggest a molecular formula of $C_{17}H_{30}O_4$ (m.w. 298). The infrared spectrum shows carbonyl bands at 1695 and 1755 cm^{-1}, in fair agreement with the frequencies ascribed to aliphatic carboxylic acids and saturated γ-lactones.[3,4]

Acarenoic acid is very similar to acaranoic acid, but melts at 144—146.5° and has $[\alpha]_D^{25} = -39°$. From the analytical and mass spectral data, a formula of $C_{17}H_{28}O_4$ can be derived. It discolours cold, alkaline permanganate solu-

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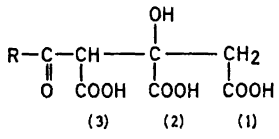
tion. The infrared spectrum shows one band (at 1630 cm^{-1}) corresponding to C=C unsaturation and two carbonyl bands (at 1692 and 1740 cm^{-1}) which can be due to a carboxylic and an α:β-unsaturated γ-lactonic group.[3,4]

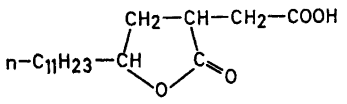
Upon hydrogenation, acarenoic acid is converted to a saturated acid, m.p. 148—150°, with an IR spectrum virtually identical with that of acaranoic acid. Thus it can be safely concluded that acaranoic acid is a dihydro-acarenoic acid. The very slight difference in the IR spectra and the somewhat different melting points could be attributed to the steric nonspecificity of the hydrogenation. While the biologically synthetized acaranoic acid ought to be a pure antipode, the acid prepared by hydrogenation would be partly racemic.

The biosynthesis of the known aliphatic lichen acids can readily be explained by assuming a common hypothetical precursor of the type I.[5] Retention of all carboxyl groups will give rise to the "tribasic" aliphatic lichen acids: caperatic, rangiformic, and norrangiformic acid. Removal of the carboxyl group 1 will account for the formation of the other known aliphatic

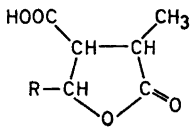


I

R = n−$C_{11}H_{23}$ or n−$C_{13}H_{27}$



II



IIIa: R = n−$C_{11}H_{23}$

IIIb: R = n−$C_{13}H_{27}$

lichen acids (*e.g.* roccellic, protolichesterinic, and nephrosteranic acid). No example is known of compounds formed by the removal of the carboxyl group 3. However, such a decarboxylation (of a β-keto acid) could readily be visualised, and would, after biosynthetical transformations of the same type as encountered in the formation of, *e.g.*, nephrosteranic acid (IIIa), give rise to a compound of the type II.

Since acaranoic acid in many respects (*e.g.* the thin layer chromatographical behaviour,[2] the IR spectrum) differs strikingly from nephrosteranic (III a) and nephromopsinic acid (III b), it was assumed that it might be a compound of the type II.

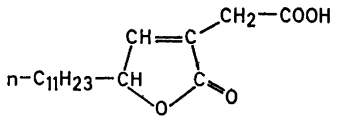
By condensation of methyl β-ketotetradecanoate with dimethyl bromosuccinate, hydrolysis and simultaneous decarboxylation of the reaction product, and subsequent reduction with sodium borohydride, II was formed in fair yield upon acidification.

Naturally, the synthetic product is a mixture of two diastereomeric racemates. A comparison with (optically active) acaranoic acid shows that:

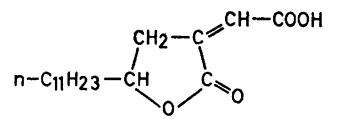
1. The two acids are chromatographically indistinguishable.
2. The IR spectra are very similar, but show some differences in the fingerprint region.
3. The mass spectra are almost identical (probably acaranoic acid is partly racemized at the elevated temperature in the mass spectrometer).

From the above data, it can be concluded that acaranoic acid probably has the structure II.

From biogenetical considerations (*cf.* Ref. 5) the unsaturated acid should have either structure IV or V. Only the former structure is consistent with the IR spectrum. In structure V, a significant lowering of the frequency of the



IV



V

band due to the carboxyl group would be expected.[3,4] Upon ozonolysis, acarenoic acid produces no oxalic acid, (maleic acid, ozonolyzed under the same conditions, does). This finding is in harmony with IV but not with V. Hence, it seems reasonable to attribute structure IV to acarenoic acid.

EXPERIMENTAL

All melting points are uncorrected. The thin layer chromatography was carried out according to Bendz *et al.*[2] The IR spectra were recorded on a Perkin-Elmer 237 (KBr discs). The mass spectra were recorded by the Mass Spectrometric Laboratory, Karolinska Institutet, Stockholm. The microanalyses were carried out by the Analytical Department at the Institute of Chemistry, Uppsala.

Extraction. Dry and ground *Acarospora chlorophana* (collected in the vicinity of Abisko, Sweden, and Hjerkinn, Norway, and on the Varanger Peninsula, Norway) (5.5 g) was extracted continuously with ether (300 ml) for 48 h. The ether extract was shaken with aqueous sodium hydrogen carbonate (5 %, 3 × 100 ml). Evaporation of the ether extract thus treated yielded a solid mass, which was recrystallized from aqueous ethanol to give rhizocarpic acid as yellow needles (90 mg, 1.6 %), m.p. 175–177°.

Acidification (1 N hydrochloric acid) of the combined sodium hydrogen carbonate extracts gave a precipitate, which was recrystallized once from ether to give "Pleopsidsäure" (130 mg, 2.4 %), m.p. 135–137°.

Ozonolysis. "Pleopsidsäure" (50 mg) was dissolved in a glacial acetic acid-ethyl acetate mixture (1:2, 75 ml) and ozonolyzed at room temperature. The reaction mixture was treated with sulphuric acid (0.5 N, 50 ml) and hydrogen peroxide (3 %, 25 ml) in order to decompose the ozonide, and then extracted with ether (2 × 25 ml).

To the aqueous layer was added manganese dioxide (8 × 2 mg) at short intervals to decompose the excess hydrogen peroxide. It was then concentrated (10 ml) and the pH adjusted to 1.5. To a part of this solution (1.0 ml) was added a mixture of 2 N sulfosalicylic acid and 2 N ferric chloride (0.1 ml). No discolouration of the added reagent was observed. (Maleic acid, ozonolyzed under the above conditions, caused a complete discolouration of the reagent.) Hence oxalic acid was absent in the aqueous layer. This method for detecting oxalic acid is described in detail in Ref. 6.

Acaranoic acid. The ether extract of the above reaction mixture was concentrated (2 ml) and the precipitate that appeared was filtered off and recrystallized from ether

to give acaranoic acid (18 mg), m.p. 154—155°, $[\alpha]_D^{25} = -30°$ ($CHCl_3$, *c* 0.29). The molecular weight was determined by mass spectroscopy and the equivalent weight by titration (0.05 N sodium hydroxide, phenolphthalein). (Found: C 68.2; H 9.88; m.w. 298; equiv. w. 151. $C_{17}H_{30}O_4$ requires: C 68.4; H 10.13; m.w. 298; equiv. w. (as a dibasic acid) 149). IR: ν_{max}^{CO} 1695 and 1755 cm^{-1}.

Acarenoic acid. Preparative thin-layer chromatography of "Pleopsidsäure" (30 ml) (silica gel HF, ether-butyric acid 20:1) yielded acaranoic acid (12 mg) and acarenoic acid (recrystallized from benzene) (11 mg), m.p. 144—146.5°, $[\alpha]_D^{25} = -39°$ ($CHCl_3$, *c* 0.25). The molecular weight was determined by mass spectroscopy. (Found: C 68.4; H 9.30; m.w. 296. $C_{17}H_{28}O_4$ requires: C 68.8; H 9.45; m.w. 296). IR: $\nu_{max}^{C=C}$ 1630 cm^{-1}, ν_{max}^{CO} 1692 and 1740 cm^{-1}. A solution of acarenoic acid in sodium carbonate discoloured an alkaline potassium permanganate solution in the cold.

Hydrogenation of acarenoic acid. Acarenoic acid (4 mg) and Adams' catalyst (0.5 mg) in ethanol (10 ml) was hydrogenated at atmospheric pressure and room temperature for 1 h. After removal of the catalyst, the ethanol was evaporated. The residue was recrystallized from ether to give a compound (2 mg), m.p. 148—150°, practically identical with acaranoic acid (IR, thin layer chromatography).

4-Hydroxypentadecane-1,2-dicarboxylic acid, γ-lactone. To a solution of sodium (0.7 g) in superdry ethanol (100 ml) was added methyl β-ketotetradecanoate [7] (7.7 g) and dimethylbromosuccinate [8] (6.9 g). The mixture was refluxed (4 h) and poured onto a mixture of ice (300 g) and hydrochloric acid (0.5 N, 50 ml). The precipitate was removed by filtration and refluxed for 30 min in ethanolic potassium hydroxide (5 % in 85 % EtOH, 100 ml). The solution was poured onto ice (300 g) and filtered as soon as the ice had melted. The filtrate was extracted with ether (2 × 200 ml) and the ether extract discarded, The filtrate was then acidified (2 N hydrochloric acid) and extracted with ether (2 × 200 ml). The combined extracts were washed with 5 % ethanol (2 × 100 ml; the presence of ethanol was found essential to obtain a good separation of the layers), the ether was evaporated, and the residue dissolved in sodium hydroxide (0.5 N, a slight excess). To the solution was added dropwise under stirring sodium borohydride (1 g) in sodium hydroxide (0.1 N, 10 ml). After 15 min, the reaction mixture was acidified (N sulphuric acid in 10 % ethanol) and poured onto ice (100 g). The precipitate was collected by filtration, dried, washed with light petrol (30—40°, 10 ml) and repeatedly recrystallized from an ether-light petrol (30—40°) mixture to yield 4-hydroxy-pentadecane-1,2-dicarboxylic acid, γ-lactone (2.1 g, 24 %), m.p. 112—116°. The molecular weight was determined by mass spectroscopy and the equivalent weight by titration (0.05 N sodium hydroxide, phenolphthalein). (Found: C 68.7; H 9.95; m.w. 298; eq.w. 152. $C_{17}H_{30}O_4$ requires: C 68.4; H 10.13; m.w. 298; eq.w. (as a dibasic acid) 149).

The comparison of the compound with acaranoic acid is described in the general part (*vide supra*).

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