

Studies on the Chemistry of Lichens

II*. Chemical Components of *Haematomma ventosum* (L.) Mass.
var. *lapponicum* (Räs.)

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Thamnolic acid and a new lichen substance, called *ventosic acid*, $C_{22}H_{44}O_6$, have been isolated from *Haematomma ventosum* (L.) Mass. var. *lapponicum* (Räs.). The new substance is an aliphatic compound. It contains four hydroxyl and one carboxyl group. Reaction with hydriodic acid and reduction with zinc and glacial acetic acid affords *n-docosanoic acid* (behenic acid). This result is in harmony with the new substance being tetrahydroxy behenic acid.

Haematomma ventosum is a crustose lichen and is rather common on boulders and rocks on mountain-wilds. Sometimes it covers large areas. Our material was collected in Trabelia, the middle part of Gudbrandsdal, Norway, and the greatest possible care was taken to avoid contamination by other species.

The lichen *Haematomma ventosum* (L.) Mass. is known in two varieties. The one is *lapponicum* giving a bright red colour with *p*-phenylenediamine, the other does not give any colour with this reagent.

Knop, Hesse and Zopf¹⁻⁵ were the first to examine samples of this lichen, but they did not indicate which of the varieties they dealt with. The obsolete methods employed could not yield complete information regarding the composition of the lichen.

A thorough examination of *Haematomma ventosum* (L.) Mass. var. *lapponicum* (Räs.) has now been made using a series of solvents in succession for extraction. During the years 1955 and 1956 in all about four kilogrammes were extracted. As a result of this investigation it has been found that the important components of this lichen are usnic, divaricatic and thamnolic acid and a new lichen substance, for which the name *ventosic acid* was adopted. Previous investigators did not obtain thamnolic acid from this lichen. The structure of thamnolic acid has been established by Asahina and coworkers, both by degradation and by synthesis⁶⁻¹⁰.

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The crude thamnolic acid obtained from ether and acetone extracts was collected and thoroughly washed with chloroform and ethanol. Recrystallisation was effected several times conveniently from dioxan giving thamnolic acid with one molecule dioxan as light yellow prisms¹¹, m.p. 210—211° (decomp.). After drying *in vacuo* thamnolic acid gave analytical values agreeing with the formula $C_{19}H_{16}O_{11} \cdot C_4H_8O_2$. The crystalline product gave the same colour reactions and has the same properties as mentioned for thamnolic acid in the literature^{12,13}. Thamnolic acid is very sparingly soluble in most organic solvents but may be crystallised from acetone or dioxan.

With barium hydroxide the substance gave the same characteristic yellow crystals as reported by Zopf for hirtellic acid¹² (identical with thamnolic acid).

A proof of the identity was possible by mixed melting point determination and X-ray diffraction patterns. The comparison of X-ray diffraction patterns of authentic thamnolic acid and the substance isolated here, both crystallised from dioxan, gave the spacing values in Å, recorded in Table 1.

Table 1. Spacing values in Å. (w = weak, m = medium, s = strong, vs = very strong).

| Substance from <i>Haematomma ventosum</i> var. <i>lapponicum</i> | Authentic thamnolic acid. |
|--|---------------------------|
| 15.77 m | 15.77 m |
| 9.02 » | 9.02 » |
| 7.64 » | 7.65 » |
| 7.16 s | 7.17 » |
| 6.56 m | 6.58 » |
| 5.34 » | 5.25 s |
| 4.57 » | 4.59 w |
| 4.35 » | 4.33 » |
| 3.60 vs | 3.60 vs |
| 3.17 m | 3.17 w |
| 2.92 w | 2.91 » |
| 2.76 » | 2.77 » |

By working up the mother liquors from the acetone extractions another aldehydic substance was obtained which was found to be identical with decarboxy-thamnolic acid. This substance has so far not been detected in lichens and it is most likely produced during the extraction with acetone. Asahina⁸ made the same observation.

Zopf³ and Hesse⁵ isolated small quantities of an aldehydic substance from *Haematomma ventosum*, for which the name *ventosaric acid* was adopted by Zopf. Zopf gave the melting point at 205—207°, Hesse at 190° which is not far from that of thamnolic acid. Ventosaric acid further resembles thamnolic acid in having a low solubility in ethanol and acetone and in its colour reactions with ferric chloride, sodium hydroxide and concentrated sulphuric acid. These facts would suggest that ventosaric acid is identical with thamnolic acid.

When the extraction was carried out with acetone and ethanol a white amorphous, sparingly soluble precipitate was formed in the extraction flask. This precipitate gave after purification, as described in the experimental part, ventosic acid as a white amorphous powder, m.p. 183—185°. The substance was soluble in hot ethanol, glacial acetic acid, dioxan, aniline and pyridine. In other organic solvents it had a very low solubility. After drying the acid gave analytical values agreeing with the formula $C_{22}H_{44}O_6$. Several attempts were made by means of the method of Pirsch to obtain a molecular weight, but without result. Ventosic acid did not contain methoxyl groups and exhibited no carbonyl function. It was recovered unchanged after boiling with sodium hydroxide solution as well as on diluting its solution in cold concentrated sulphuric acid.

Ventosic acid on treatment with acetic anhydride and a trace of concentrated sulphuric acid gave a colourless glassy compound, m.p. about 54°, the composition of which corresponded to a tetra-acetate. The acetate has so far not crystallised. On boiling with ethanolic potassium hydroxide it was deacetylated to the original substance.

With *p*-phenylazobenzoyl chloride¹⁴ the substance yielded an orange-red tetrabenzoate, $C_{37}H_{38}O_5N_4$, m.p. 153—157°.

Treated with hydriodic acid ($d = 1.7$) ventosic acid yielded a dark brown oil which could not be purified or crystallised. As the crude oily product contained iodine it was reduced with zinc dust in glacial acetic acid. The resulting product crystallised in colourless needles, m.p. 75°. Repeated recrystallisations from different solvents did not raise the melting point discernibly and no evidence was obtained for the presence of more than one component. It contained no hydroxyl groups and was identified as behenic acid, $C_{22}H_{44}O_2$, by preparation of the methyl ester, m.p. 52—53°, and by X-ray analysis. All these values correspond well with those given in the literature. (Similar reduction has been made with 13,14-dihydroxy-behenic acid by Urwanzopp¹⁵. The behenic acid obtained in this manner had the m.p. 74—76°)

The higher fatty acids exhibit at least three polymorphic forms and X-ray measurements show that these crystal modifications possess different spacings. The most important of these modifications are designated as α - and β -, the former showing a C crystal spacing and the latter a somewhat longer B spacing, (Ref.¹⁶). The X-ray investigations of Müller and Shearer¹⁷⁻¹⁹ showed that the fatty acids exhibit three characteristic lines d_1 , d_2 and d_3 . The last two

Table 2.

| | Behenic acid obtained by reduction of ventosic acid | Authentic sample of behenic acid | Behenic acid, Refs. ¹⁶⁻¹⁸ |
|-------------------------|---|----------------------------------|--------------------------------------|
| Melting point | 75° | 78—79° | 80.0° |
| d_1 \left\{ B spacing | 54.2 Å | 53.2 Å | 53.6 Å |
| C spacing | 49.6 » | 49.0 » | 48.5 » |
| d_2 | 4.14 » | — | 4.1 » |
| d_3 | 3.73 » | — | 3.7 » |

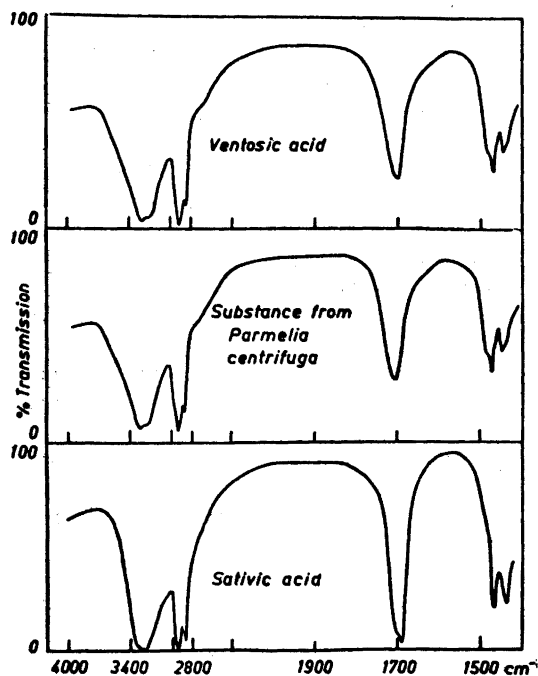


Fig. 1. Infrared absorption spectra from 4 000—1 450 cm^{-1} . All substances as pressed KBr—disks.

lines are essentially constant for a specific series and are the diffractions from planes parallel to the long axes of the molecules. The long spacing d_1 is a function of the number of carbon atoms in the chain.

The results of X-ray examinations of the behenic acid obtained by reduction of ventosic acid and of authentic sample of behenic acid are shown in Table 2. The acids gave extremely good reflections.

Infrared analysis of ventosic acid ascertained the presence of an aliphatic carbonyl-hydroxy-compound. The infrared absorption spectrum in the region 4 000 cm^{-1} —700 cm^{-1} showed strong bands at 3 280 cm^{-1} and at 1 710 cm^{-1} . The observed values indicate the presence of hydroxyl and carbonyl groups, respectively. The frequency at 1 710 cm^{-1} would suggest the presence of a dimer aliphatic acid^{20,21}. Further bands at 2 920 cm^{-1} and at 1 470 cm^{-1} —1 445 cm^{-1} corresponded to C-H and C-H₂ stretching frequencies in saturated aliphatic compounds. The 1 382 cm^{-1} band shows that a C-CH₃ group is present. There were only few bands below 1 450 cm^{-1} .

An infrared spectrum of sativic acid (tetrahydroxystearic acid), m.p. 168—171°, was very similar to that of ventosic acid in the region 4 000 cm^{-1} —1 450 cm^{-1} . The same characteristic bands were observed (Fig. 1). This adds strength to the conclusion that ventosic acid must be an aliphatic hydroxy acid.

X-Ray diffraction patterns of sativic acid showed a certain similarity with that of ventosic acid. Characteristic are the two strong peaks in the region 4.0—4.9 Å, (Fig. 2).

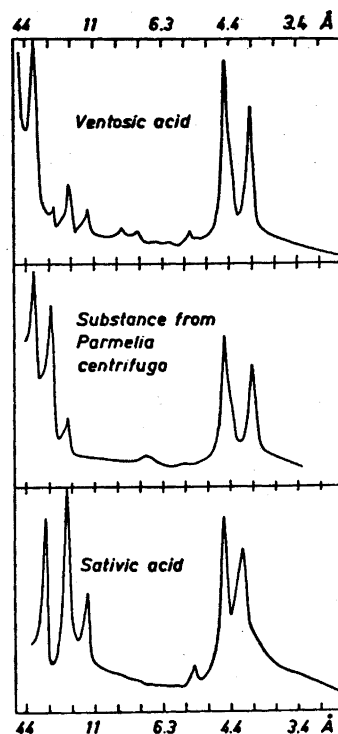


Fig. 2. X-Ray powder diagrams taken with Cu- $K\alpha_1$ radiation.

It was quite impossible to obtain an ester of ventosic acid. This is remarkable because the isolated substance most probably is an aliphatic tetrahydroxy carboxylic acid. On the other hand saticvic acid was easily converted into the methyl ester by reaction with methanol and concentrated sulphuric acid.

Both ventosic and saticvic acid were practically insoluble in sodium hydroxide solution.

Metabolic products of *Parmelia physodes* (L.) Ach. and *Parmelia furfuraceae* (L.) Ach. have been studied in detail by Zellner^{22,23}. He obtained two white, amorphous substances which he called *hypogymnole*. The physical and chemical properties of these substances are in agreement with those of ventosic acid from *Haematomma ventosum*. An allied substance has been isolated by Klima²⁴ from *Alectoria ochroleuca* (Ehrh.) Nyl. Zellner and Klima did not, however, establish the chemical composition of these substances.

A preliminary examination of these three lichens showed that the substances detected by Zellner and Klima most likely are identical with ventosic acid from *Haematomma ventosum*. Also the lichens *Parmelia centrifuga* (L.) Ach., *Cetraria nivalis* (L.) Ach. and *Cladonia alpestris* (L.) Rabh. were investigated. Hereby substances either identical or closely related to ventosic acid have been isolated. X-Ray and infrared analysis have been used.

As an example X-ray diffraction pattern and infrared absorption spectrum of the substance isolated from *Parmelia centrifuga* are shown in Figs. 1 and 2.

Lack of material has so far not permitted degradative experiments on ventosic acid on a larger scale. However, it is of interest to note that ventosic rapidly acid is oxidised by periodic acid. It is hoped to establish the structure of ventosic acid by investigation of the oxidation products.

EXPERIMENTAL

(Melting points are uncorrected.)

Extraction and isolation of the lichen substances

Usnic acid. The fresh lichen obtained was air-dried and ground into coarse powder (1 000 g), and exhaustively extracted with ether in a Soxhlet apparatus. During the extraction usnic acid separated as large yellow crystals in the flask. After 20 h when the extraction was found to be completed the crystals were removed by filtration and washed with ethanol. Usnic acid was recrystallised from chloroform-ethanol (1:3). Yield 24.5 g; m.p. 199–200°; $[\alpha]_D^{25} + 500^\circ$ (chloroform, $c = 2.355$).

Divaricatic acid. The ether extract was evaporated. The residue which consisted of crystals and an oily substance was treated with small quantities of ether. The ether-soluble portion was evaporated and the residue recrystallised from 60 % ethanol. The substance was identified as divaricatic acid. Repeated recrystallisations from ethanol and glacial acetic acid yielded 3.7 g; m.p. 135–137°. The acid dissolved readily in ether and ethanol at room temperature, and also in hot chloroform, benzene and glacial acetic acid. An alcoholic solution of the substance turned violet on the addition of a drop of ferric chloride solution.

The ether insoluble portion was washed with alcohol. Pale yellow crystalline product (Fraction A).

The residual lichen was subsequently extracted with acetone for 20 h. During the extraction the new compound (ventosic acid) separated as a colourless amorphous powder. It was removed by filtration and washed with acetone, ethanol and ether. Finally dried at 70° for 30 min (Fraction B). The dark red extract was concentrated by distilling off the solvent. More yellow crystalline product separated (Fraction D). The solid was filtered off and the mother liquid evaporated. The residue, a reddish brown resinous mass was shaken with 800 ml ethanol for one hour. The alcohol-soluble part was not further investigated. The insoluble part could be divided into two portions by treatment with hot chloroform. The chloroform-insoluble portion was collected by filtration and washed with alcohol (Fraction D).

Decarboxythamnolic acid. The chloroform extract yielded a pale yellow crystalline product on standing in a refrigerator. This was filtered off and washed with small quantities of ether, (1.6 g). Repeated recrystallisations from ethyl acetate yielded decarboxythamnolic acid in a pure state. Yield 23 mg; m.p. 213–215° (decomp). (Found: C 57.18; H 4.12; Calc. for $C_{18}H_{14}O_8$: C 57.46; H 4.29). As described by Asahina the decarboxythamnolic acid was soluble in hot acetone, ethanol, ethyl acetate and chloroform. On heating with potassium hydroxide and chloroform the substance gave a deep red solution. With barium hydroxide no characteristic crystals were obtained.

Thamnolic acid. Fraction A from the ether extraction and the fractions C and D from the acetone extraction of the lichen were found to be the same substance. The fractions were collected (17.7 g), and recrystallised repeatedly from dioxan. Pale yellow needles, m.p. 210–212° (decomp.). Mixed melting point with authentic sample of thamnolic acid showed no depression. (Found: C 54.43; H 4.70; Calc. for $C_{18}H_{14}O_{11} \cdot C_4H_8O_2$: C 54.33; H 4.76).

Ventosic acid. The residual lichen powder was finally extracted with 96 % ethanol for 48 h. On standing the extract gave some ventosic acid. Together with the fraction B from the acetone extract, the yield of crude ventosic acid was about 0.8 %. The sub-

stance was purified by recrystallisation from dioxan, ethanol and glacial acetic acid. White, amorphous powder, m.p. 183°. (Found: C 65.29; H 10.88; Calc. for $C_{22}H_{44}O_6$: C 65.30; H 10.96). It gave none of the colour reactions characteristic for lichen substances. The compound was without taste.

Derivatives of ventosic acid

Acetylation. Ventosic acid (1.0 g) was heated on a steam bath for 45 min with 30 ml of the following mixture: 30 g acetic anhydride + 220 ml pyridine. During the reaction the solution became brown. The reaction mixture was then poured into ice-water with good stirring. The brown oily product which separated was extracted with ether and the ether solution evaporated. The dark oily residue was dissolved in ethanol and treated with active carbon, filtered and the colourless solution evaporated to dryness. The acetyl compound was thus obtained as a colourless glassy product, m.p. 54°. It could not be crystallised. (Found: for material dried *in vacuo* over KOH: C 62.67; H 9.21; Calc. for $C_{30}H_{52}O_{10}$: C 62.92; H 9.15).

Deacetylation. The acetyl compound (0.31 g) was boiled with alcoholic potassium hydroxide (25 ml 0.5 N) for 30 min. After a short time a white amorphous powder precipitated. After cooling the powder was removed by filtration and washed with water and alcohol. To remove ash-components the product was recrystallised from glacial acetic acid and washed with alcohol and ether; m.p. 182°. It was found to be identical with the original substance, ventosic acid, the mixed melting point being undepressed. X-Ray diffraction patterns were also identical. (Found: C 65.19; H 10.68; Calc. for $C_{22}H_{44}O_6$: C 65.30; H 10.96).

p-Phenylazobenzoate. A mixture of ventosic acid (1.0 g), *p*-phenylazobenzoylchloride (2.4 g) and dried pyridine (6 ml) was refluxed for one hour. The reaction mixture was filtered, cooled and poured into a solution of 10 % sodium hydrogen carbonate and ice. The orange-red precipitate obtained was filtered off and thoroughly washed with water. Recrystallisation from a benzene-ethanol mixture gave orange-red powder. Yield 0.68 g; m.p. 153–157°. (Found: C 71.59; H 6.63; N 8.15; Calc. for $C_{74}H_{76}O_{10}N_8$: C 71.89; H 6.19; N 9.06). The benzoate was easily soluble in benzene and ether with a dark red colour.

Reduction to behenic acid. Ventosic acid (4.35 g) was refluxed with 100 ml hydriodic acid (b.p. 126°) for 10 min. The solid rapidly turned to a dark brown oily product. The reaction mixture was diluted with water and extracted with ether. The ether extract was shaken with an aqueous solution of sodium thiosulphate and at last washed with water. The ether solution, dried and concentrated, yielded a light yellow viscous liquid, (3.1 g). As the liquid contained halogen it was boiled with zinc powder in glacial acetic acid (25 ml) for one hour. The reduction product was obtained in a crystalline state when the filtered solution was evaporated *in vacuo*. The residue was washed with much water and recrystallised several times from diluted acetic acid and petroleum ether (30–50°); m.p. 74–75°, undepressed on admixture with authentic behenic acid (m.p. 78–79°). Yield about 1 g. The substance was readily soluble in ether, acetone, ethanol and benzene. (Found: C 77.54; H 12.82; mol.wt. (Landsberger) 313, 314; Calc. for $C_{22}H_{44}O_2$: C 77.55; H 13.02; mol.wt. 341).

Behenic acid methyl ester. The methyl ester was prepared from behenic acid (110 mg) with diazomethane. The ester recrystallised from methanol (10 ml) gave a quite colourless and crystalline product; m.p. 52–53°. No depression of the melting point when mixed with an authentic sample of behenic acid methyl ester. (Found: C 78.10; H 13.01; CH_2O 9.89; $C_{23}H_{46}O_2$ requires C 77.89; H 13.07; CH_2O 8.75).

Tetrahydroxystearic acid (Sativic acid). The hydroxy acid was prepared from linolic acid tetrabromide (m.p. 115–116°) in the same manner as described by Rollett²⁵ and Hazura²⁶. The crude product of sativic acid was recrystallised from dilute hydrochloric acid, washed with water and dried. Then shaken with hot benzene and at last recrystallised from alcohol. Colourless, crystalline product; m.p. 168–171°. (Found: C 62.18; H 10.51; $C_{18}H_{36}O_6$ requires C 62.10; H 10.41).

Sativic acid methyl ester. Sativic acid (300 mg) was suspended in a solution of methanol (30 ml) and concentrated sulphuric acid (3.3 ml) and refluxed for 2 h. After cooling the solution was diluted with water. A white precipitate was formed. This was filtered off and recrystallised from methanol-water (1:1). The colourless, crystalline product was

removed by filtration and washed with methanol and ether. Yield 170 mg; m.p. 146–156°. Once more purified by recrystallisation from white spirit (160–180°) (50 ml). M.p. 147–151°. (Found: C 63.14; H 10.33. $C_{11}H_{18}O_6$ requires C 62.95; H 10.57).

By the X-ray diffraction analysis, a small amount of the substance was melted on a glass plate and spread out so as to form a very thin film. The instrument used was a Philips X-ray Geiger Counter Spectrometer with Cu- $K\alpha_1$ radiation.

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