Studies on the Chemistry of Lichens, XVIII * Chemical Investigation of the Species *Letharia vulpina* (L.) Hue and New Derivatives of Vulpinic Acid

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Lichen *Letharia vulpina*, Vulpinic Acid Derivatives

The crystalline and amorphous constituents of *Letharia vulpina* collected at Flensjøen, Sør-Trøndelag, Norway, have been identified as atranorin, vulpinic-, pulvinic-, usnic-, benzoic- and hydroxyfatty acids. Vulpinic acid thallate (I) and vulpinic acid isopropylether have been prepared as new derivatives. The identity of the compounds was established by consideration of elemental analyses, infrared, mass and nuclear magnetic resonance spectra, and thin layer chromatography.

Introduction

The study of the lichen species *Letharia vulpina* (L.) Hue has been carried out by several groups of investigators, and vulpinic acid was reported to be the principal chemical constituent. Atranorin is for the first time isolated and described from this lichen by Hesse as early as 1898 [1]. He established that vulpinic acid was always accompanied by atranorin in *Letharia vulpina*. In a work by Stahl and Schorn [2] on TLC of, among other items, lichen substances, usnic acid is mentioned as a component of lichen species. Bachelor and Cheriyan [3] investigated the species and reported the occurrence of free benzoic acid. Pulvinic acid or other derivatives of this compound have not as yet been reported from *Letharia vulpina*.

The purpose of the present work is to report the results of a chemical re-examination of the lichen *Letharia vulpina* subjected at our institute. In agreement with existing literature atranorin, vulpinic-, usnic- and benzoic acid were found as the major constituents. It is of interest that pulvinic acid, too was found to occur in our Norwegian sample of *Letharia vulpina*. An amorphous mass was isolated and established as a mixture of high molecular hydroxyfatty acids. The 2-aminoethanesulfonic acid *taurine* was detected in a water extract by means of an amino acid analyzer. No attention has been given to the occurrence of alditols or sugar compounds in this report.

Vulpinic acid thallate (I) and vulpinic acid isopropyl ether were prepared as new derivatives of vulpinic acid.

Experimental

Thin layer chromatography of the isolated aromatic compounds were carried out on plates coated with silica gel and cellulose as absorbents. Developments were performed with the following solvent combinations: S1] *n*-butyl alcohol-*n*-propyl alcohol-ammonia-water (17 + 4 + 6 + 3), S2] acetone-chloroform-ethyl alcohol (4 + 4 + 1), S3] chloroform-acetone (4 + 1), S4] chloroform-acetone (1 + 1), S5] chloroform-pyridine (24 + 1), S6] toluene-acetic acid (85 + 15), S7] 2-butyl alcohol-ammonia-water (21 + 6 + 3).

Usnic- and benzoic acid were located on the chromatograms by spraying with anisaldehyde-sulfuric acid [2] and hydrogen peroxide-ferric chloride [4] respectively. The identifications of the spots were performed by comparison to appropriate standard substances.

Material and isolation of the lichen substances. The lichen *Letharia vulpina* (L.) Hue occurs in Norway as relatively small tufts on wooden roofs, fences and the like. The material used in the present investigation was collected on dead *Pinus silvestris* at Flensjøen, Sør-Trøndelag. The fresh lichen obtained was air-dried (221 g), ground into a small size and extracted for 20 h with ethyl ether and chloroform in succession. On concentration of the yellow extracts in vacuo at 40 °C to a small volume, a crystalline mixture of vulpinic acid and atranorin (16.1 g), small amounts of benzoic acid, and a colourless, amorphous substance were deposited. The last mentioned product was recrystallized from hot acetic acid, yield 154 mg, *Fraction 1*. The filtrates were combined, evaporated, and the dark residue treated with small quantity of hot acetone. By the aid of preparative thin layer chromatography of the acetone solution on pre-washed silica gel coated plates with solvent system S1, the *Fraction 2* was obtained, in addition to a yellow coloured band that is due to a still unidentified substance.

Vulpinic acid thallate (I) was readily prepared by addition of thallium (I) ethoxide to a solution of

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vulpinic acid in chloroform. The crude salt (yield 94%) was conveniently recrystallized from ethyl alcohol giving beautiful, yellow crystals, which decompose at 195 °C. UV (methyl alcohol): λ_{max} 372 and 287 nm, λ_{min} 337 and 243 nm. ^{1}H NMR (60 MHz, DMSO-d_6): δ 3.80 (singlet, methoxy group), 6.93 – 7.73 (multiplet, aromatic protons, 8H), 8.22 – 8.38 ppm (multiplet, aromatic protons, 2H). Found: C 43.32, H 2.51, O 15.08, Tl 38.68. $C_{19}H_{13}O_5Tl$ requires: C 43.41, H 2.49, O 15.22, Tl 38.88.

Vulpinic acid isopropyl ether. Vulpinic acid thallate was suspended in isopropyl iodide and refluxed for 18 h, followed by filtration of thallium(I) iodide. Concentration of the filtrate to a smaller volume produced a crystalline mass. Recrystallisation from ethyl alcohol-water (2 + 1) yielded a colourless product, m.p. 148.6 °C. UV (methyl alcohol): λ_{max} 322 and 224 nm, λ_{min} 256 and 213 nm. ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.17 (doublet, 6H, C-methyl groups), 3.89 (singlet, ester-methyl), 4.65 (distorted septet, methine-group), 7.23 – 7.78 ppm (multiplet, 10H, aromatic protons). Found: C 72.81, H 5.57, O 21.47. $C_{22}H_{20}O_5$ requires: C 72.51, H 5.53, O 21.95.

Results and Discussion

Detection of the constituents of *Letharia vulpina*

By reducing the ether extract of the lichen material, vulpinic acid (main component) and atranorin separated out. Vulpinic acid was purified by recrystallisation and later on used for preparation of vulpinic acid thallate and vulpinic acid isopropyl ether.

A compound with low solubility in common organic solvents, was obtained as a white powder from the chloroform extract of the lichen, *Fraction 1*. The higher part of the IR spectrum harmonized completely with the spectra of hydroxyfatty acids isolated from several other lichen species [5]. The lower part of the spectrum showed a lower intensity of the hydroxyl band (3300 cm^{-1}) as compared with the asymmetric carbon-hydrogen stretching band observed around 2900 cm^{-1}. This part of the spectrum showed a close similarity to those given in Part XI [6] (Fig. 3 a, b) in this series.

The low resolution mass spectrum failed to show the molecular ions. Outstanding peaks in the spectrum occur at *m/e* 283, 265, 253, 235, 213, 199, 195, 183, 181, 163, 121 and 111, all corresponding to fragments thoroughly discussed in Part XI [6] and XVI [7] of this series. Other important fragments were observed at *m/e* 481, 383, 365 and 347 corresponding to the fragments [$C_{30}H_{60}O_6 - H_2O - OH$], [$C_{30}H_{60}O_6 - H_2O - OH - C_7H_{14}$], [*m/e* 383 – H_2O] and [*m/e* 383 – 2 H_2O] respectively.

By comparing these results with previous accounts [5, 6], it must be concluded that *Fraction 1* isolated from the chloroform extract of the lichen contains a mixture of *tetrahydroxyfatty acid homologues* of the general formula $C_nH_{2n}O_6$, where n = 30 is the highest tentative value.

By reducing the volume of the combined filtrates, a colourless and silky crystalline mass was deposited on the inside of the rotation flask when allowed to stand at room temperature. This product was found to be identical with *benzoic acid.*

The mother liquid was at last concentrated to remove all the solvent and left a dark, viscous residue. Preliminary TLC analyses of the residue indicated the presence of vulpinic acid, atranorin, usnic acid, atranol, methyl β-orcinolcarboxylate and chlorophyll together with some unidentified constituents. Preparative thin layer chromatography of the residue readily furnished *Fraction 2.* This compound was visible as a yellow spot in daylight on the chromatograms. TLC of the fraction on silica gel as absorbent with use of the solvent systems S2 – S6, and on cellulose layer with solvent S7, unambiquously identified it as *pulvinic acid.*

The remaining lichen material was then extracted with 0.05 N hydrochloric acid in 10% isopropyl alcohol yielding a coloured and viscous residue. The evidence of the presence of 2-aminoethanesulfonic acid, *taurine,* was obtained by means of methods described in an earlier report [8]. The amount of taurine in this lichen was calculated to be about *287 mg per 1000 g material.* Previously taurine has been detected at our institute from the lichen species *Xanthoria parietina, Anaptychia fusca, Alectoria species, Lecanora achariana, Cladonia gonecha, Stereocaulon evolutum* and *Pertusaria corallina* with an average of 167 mg taurine per 1000 g material.

New derivatives of vulpinic acid

It is known that stable and crystalline thallium(I) salts are readily prepared in nearly quantitative yield by addition of thallium(I) ethoxide to a solution of acidic compounds in an inert solvent such

as benzene, petroleum ether or chloroform [9 – 11]. We have found that treatment of a solution of vulpinic acid in chloroform with equimolecular quantity of thallium(I) ethoxide for a short period at room temperature, affords *vulpinic acid thallate*. The medium OH band at about 3450 cm^{-1} and the strong OH stretching vibration band at 2510 cm^{-1} (chelate compounds) in the IR spectrum of vulpinic acid are completely absent in the spectrum of vulpinic acid thallate. It is interesting to note that the strong carbonyl bands at 1770 [$\alpha\beta$-unsaturated-γ-lactone] and 1675 cm^{-1} [intramolecular hydrogen-bonded ester carbonyl] of vulpinic acid are replaced by only one carbonyl band at 1690 cm^{-1} in the spectrum of the thallium salt.

Vulpinic acid thallate is sufficiently volatile for MS analysis.

Characteristic ions corresponding to the fragments $[M(Tl^{205}) - CO_2 - 1]$, $[M(Tl^{203}) - CO_2 - 1]$, $[M(Tl^{205}) - C_6H_6 - CO_2CH_3]$, $[M(Tl^{205}) - OCH_3]$, $[M(Tl^{203}) - OCH_3]$, $Tl^{205}O$, $Tl^{203}O$ and pulvinic acid lactone were observed in a low and a high resolution mass spectrum.

The thallium(I) salt of vulpinic acid upon treatment with an excess of isopropyl iodide gave a beautiful crystalline and sharp melting solid of *vulpinic acid isopropyl ether* in good yield. The compound exhibits a strong carbonyl absorption at 1770 cm^{-1} in IR responsible to the mono-$\alpha\beta$-unsaturated-γ-lactone ring. It is of interest to note the expected $\alpha\beta$-unsaturated ester band at 1723 cm^{-1} instead of the intramolecular hydrogen-bonded ester band in vulpinic acid at 1675 cm^{-1}. Three bands at 1397, 1385 and 1373 cm^{-1} support the presence of the methyl groups.

MS of vulpinic acid isopropyl ether shows the molecular ion at *m/e* 364. A metastable peak at 284.9 indicates that the ion *m/e* 322 is is formed by loss of the isopropylidene radical. Elimination of MeOH and C_3H_6 from the molecular ion affords the stable base peak of pulvinic acid lactone ion at *m/e* 290.

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