

## Triterpenoids in Lichens

### I. The Occurrence of Friedelin and *epi*Friedelinol\*

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Whilst acidic substances found in lichens have been extensively investigated, little is known about neutral constituents of lichens. In particular, triterpenoids seem to be scarce, the only ones known so far being zeorin (for a summary see<sup>1</sup>, compare also<sup>2</sup>), ursolic acid<sup>3</sup> and leucotylin<sup>2</sup>. The classifying of zeorin as a triterpenoid substance is due to Asahina and Akagi<sup>2</sup>, who deduced this from the fact that on dehydrogenation with selenium naphthalene hydrocarbons characteristic as dehydrogenation products of triterpenoids were isolated. Its relationship to the known triterpenoid compounds is obscure, and the basic hydrocarbon, zeorinane, is not identical with any of the known triterpenoid hydrocarbons<sup>4</sup>.

In an attempt to establish such a relationship it was decided to investigate lichens other than those known to contain zeorin, to see whether there might occur triterpenoids which on the one hand could be related to zeorin and on the other to a triterpenoid of known constitution. As a first object *Cetraria nivalis* (L.) Ach. was chosen, because it could be collected relatively easily in large quantities. During purification of the collected material some other, easily recognisable lichens were taken care of: *C. cucullata* (Bell.) Ach., *Cladonia alpestris* (L.) Rabh., *Alectoria ochroleuca* (Ehrh.) Nyl. and *Stereocaulon paschale* (L.) Fr. The remaining waste lichens, consisting mainly of various *Cladonias*, were also extracted, to take advantage of the fact that some of the isolated substances could thus be obtained in larger quantities. Furthermore were investigated *Cetraria islandica* (L.) Ach., since this lichen can be easily obtained commercially as a drug, and *C. Delisei* (Bory) Th. Fr. [= *hiascens* (Fr.)] and *C. crispa* (Ach.) Nyl., both readily collected in a pure state.

As it happened, the extraction was carried out without the use of cork. After sparingly soluble substances had been removed by filtration and acidic substances had been completely extracted by alkali, the neutral fraction was subjected to a chromatographic purification. *C. nivalis* was first investigated,

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With petroleum-benzene (1 : 1) and with benzene there was eluted a colourless substance, m.p. 262—263° (vac.),  $[\alpha]_D - 21^\circ$ , which analysed as  $C_{30}H_{50}O$ , gave a 2 : 4-dinitrophenylhydrazone, m.p. 300° (dec.), light abs. max. at 3 700 Å, and showed an absorption band at 1 708  $cm^{-1}$  in chloroform solution in the infra-red. Indications, therefore, were that the isolated substance might be a triterpenoid ketone with the carbonyl function in a six-membered ring. Wolff-Kishner reduction of the ketone afforded the basic hydrocarbon, m.p. 246—247°,  $[\alpha]_D + 21^\circ$ . Inspection of the literature revealed that friedelin had m.p. 255—261°,  $[\alpha]_D - 29^\circ$ <sup>5</sup>, m.p. 264—265° (vac., corr.),  $[\alpha]_D - 28^\circ$ <sup>6</sup>, 2 : 4-dinitrophenylhydrazone, m.p. 297—299°<sup>7</sup>, whilst the basic hydrocarbon, friedelane, prepared by various methods, is reported to melt at 245—249°<sup>5</sup>, m.p. 243—244° (corr.),  $[\alpha]_D + 42^\circ$ <sup>6</sup>, m.p. 244°,  $[\alpha]_D + 42^\circ$ <sup>8</sup>. For comparison friedelin, together with cerin, was extracted from cork<sup>5,6</sup>. The obtained specimen showed m.p. 262—263° (vac.),  $[\alpha]_D - 21^\circ$ , 2 : 4-dinitrophenylhydrazone, m.p. 297° (dec.), light abs. max. at 3 710 Å, and there was no depression of m.p. on admixture of the respective substances. Such was also the case with friedelane, prepared by the Wolff-Kishner method, m.p. 245—246°,  $[\alpha]_D + 18^\circ$ <sup>\*</sup>.

In a similar way friedelin was obtained from the other lichens mentioned above. The identity was secured by comparison of the ketone and its 2 : 4-dinitrophenylhydrazone with authentic specimens. The yield of friedelin was quite small, 250 mg having been obtained from nearly 4 kg of *Cetraria nivalis*, and relatively less from the other pure lichens. Quite astonishing, in view of this, is the amount isolated from the waste lichens, 5 g from 16 kg. This fact strongly indicates that some lichen(s) must contain friedelin in large amounts, but there are no hints as to which this might be.

By elution with benzene/ether (1 : 1) and with ether a mixture of substances was obtained from *C. nivalis*. Nearly all of it was very soluble in chloroform, but when treated with this solvent on the filter a small amount of a colourless crystalline substance was left. It was crystallised from chloroform, m.p. 280—281° (vac.),  $[\alpha]_D + 23^\circ$ . This substance also occurred, in somewhat larger quantities, in the waste lichen extract, m.p. 281—282° (vac.),  $[\alpha]_D + 15^\circ$ . It analysed as  $C_{30}H_{52}O$  and in the usual way gave an acetate, m.p. 275—276° (vac.),  $[\alpha]_D + 12^\circ$  and a benzoate, m.p. 232—233° (vac.),  $[\alpha]_D + 26^\circ$ . Chromic acid oxidation furnished friedelin; this substance, therefore, must be a friedelinol. In literature the preparation of a friedelinol, m.p. 301—304°, by sodium-alcohol reduction of friedelin has been described<sup>9</sup>, and it is reported that a lower melting friedelinol, m.p. 278—282° has been prepared, compare<sup>10</sup>, foot-note 3. This low melting friedelinol must contain the hydroxyl group in the polar position, since the other isomer has been prepared by a method, which gives an alcohol with the hydroxyl in the thermodynamically more stable equatorial position, compare<sup>11</sup>. This isolated friedelinol presumably is identical with the low-melting friedelinol of the literature. The view that it contains the hydroxyl function in the polar position was confirmed by dehydration with phosphorus oxychloride in pyridine, which afforded a hydrocarbon mixture. Assuming that the hydroxyl group of this alcohol is situated at a

\* It will be noticed that there is a large discrepancy between reported and found values for  $[\alpha]_D$ . This will be dealt with in the following paper.

position corresponding to the position 2 of the usual triterpenoids, this friedelinol should be designated *epifriedelinol*, in analogy with the commonly accepted nomenclature.

#### EXPERIMENTAL

M.p.'s are uncorrected. All rotations were measured in chloroform solution in a 1 dm tube at room temperature (20–25°) unless specified to the contrary. The values recorded have been approximated to the nearest degree. Petroleum refers to the fraction of b.r. 40–70°. Ether solutions were dried, when necessary, with anhydrous sodium sulphate, before removal of the solvent. 2 : 4-Dinitrophenylhydrazones were purified by filtration through alumina and crystallisation from a suitable solvent (mixture). Their light absorption was measured in chloroform solution. Infra-red absorption spectra were recorded in chloroform solution on a Perkin-Elmer double beam instrument. The term "in the usual way" refers to pouring into water, extraction of organic material with ether and washing of the ether solution successively with alkali and dilute sulphuric acid solutions, when necessary. Saponifications were carried out by refluxing for 1–2 hours with several equivalents of potassium hydroxide in methanol. Substances were acetylated and benzoylated with acetic anhydride and benzoyl chloride, respectively, in pyridine solution at room temperature.

The lichens used in these investigations were collected in the Oppdal region, some 75 miles south of Trondheim in early September. The samples were purified, dried at 50° and ground coarsely before extraction with ether in an all-glass, ground-joint Soxhlet extractor. The waste lichens were extracted in a large metal Soxhlet extractor. The general procedure by working up the extracts was as follows: Substances which had precipitated during extraction were removed by filtration, and then the ether solution was concentrated to a small volume, cooled and again filtered. Finally the filtered ether extract was extracted with potassium hydroxide solution to remove all acidic substances. The ether solution was evaporated to dryness and the residue redissolved in petroleum and chromatographed on alumina.

*Cetraria nivalis* (L.) Ach. 3 950 g of dried lichen furnished 17.5 g of neutral material. Careful chromatography afforded several fractions eluted with benzene, and with benzene/ether (99 : 1) and (49 : 1). All of them gave the same colourless substance from acetone with m.p. ranging from 259–260° to 262–263° (vac.). They were combined and crystallised to constant m.p. 262–263° (vac.) and constant rotation,  $[\alpha]_D - 21^\circ$  (c, 2.34). (Found C 84.0, 84.4; H 11.8, 11.95.  $C_{30}H_{50}O$  (426.70) requires C 84.45; H 11.8). The total yield of this substance in the slightly impure form was 250 mg. It formed a 2 : 4-dinitrophenylhydrazone, m.p. 300° (dec.), abs.max. at 3 700 Å,  $\epsilon$  28 000. There was no depression of m.p. on admixture of the ketone and its 2 : 4-dinitrophenylhydrazone with specimens of friedelin and its 2 : 4-dinitrophenylhydrazone obtained from cork (see below).

The ketone (60 mg) was heated for 18 hours at 180° with hydrazine (2 ml) and sodium (0.25 g) in ethanol (6 ml). The product, isolated in the usual way, was filtered through alumina in petroleum, and the eluted substance crystallised from chloroform, m.p. 246–247°,  $[\alpha]_D + 21^\circ$  (c, 0.93, 2 dm tube);  $+ 22^\circ$  (c, 0.36, 2 dm tube). The hydrocarbon gave no depression on admixture with authentic friedelane (see below).

*epiFriedelinol*. The material eluted with benzene/ether (1 : 1) and with ether (0.8 g) was dissolved in petroleum, when crystals separated. Treatment on the filter with cold chloroform removed most of the material. The substance sparingly soluble in chloroform was crystallised and recrystallised from chloroform, m.p. 280–281° (vac.),  $[\alpha]_D + 23^\circ$  (c, 0.52). There was no depression on admixture with a substance of the same m.p. obtained from the waste lichens (see below). Oxidation with chromic acid in acetic acid at room temperature afforded friedelin, m.p. 260–262°, converted to the 2:4-dinitrophenylhydrazone, m.p. 298–299° (dec.), no depression on admixture with authentic specimens.

*The waste lichens*. 16.2 kg of dried material furnished 179 g of neutral fraction, which, when subjected to a treatment similar to that reported for *Cetraria nivalis* (see above), afforded altogether 5 g of a slightly impure friedelin. It was crystallised (very wastefully) from chloroform to give a sample of m.p. 263–264° (vac.),  $[\alpha]_D - 21^\circ$  (c, 1.99, 2 dm tube), no depression on admixture with friedelin obtained from *C. nivalis* (see above).

*epiFriedelinol*. This compound precipitated in admixture with other substances from a benzene solution of material which was sparingly soluble in ether, and from a benzene

solution of the unsaponifiable matter resulting after saponification of the neutral fraction. It was crystallised from chloroform to constant m.p. 281–282° (vac.) and constant rotation,  $[\alpha]_D + 15^\circ$  (c, 1.00, 2 dm tube), (67 mg). (Found C 83.9; H 12.25.  $C_{30}H_{52}O$  (428.72) requires C 84.0; H 12.2). It gave an *acetate*, m.p. 275–276° (vac.), from chloroform/methanol, depressed to 255–260° on admixture with *epifriedelinol*,  $[\alpha]_D + 12^\circ$  (c, 0.81). (Found \*C 80.95; H 11.5.  $C_{32}H_{54}O_2$  (470.75) requires C 81.5; H 11.55) and a *benzoate*, m.p. 232–233° (vac.), from chloroform/methanol,  $[\alpha]_D + 25^\circ$  (c, 1.29), +27° (c, 1.35). (Found \*C 83.3; H 11.25.  $C_{37}H_{56}O_2$  (532.82) requires C 83.4; H 10.6). Oxidation of *epifriedelinol* with chromic acid in acetic acid at room temperature furnished *friedelin*, m.p. 263–264° (vac.), no depression on admixture with an authentic specimen,  $[\alpha]_D - 15^\circ$  (c, 0.98), converted to the 2:4-dinitrophenylhydrazone, m.p. 300–301° (dec.), no depression on admixture with authentic material.

*epiFriedelinol* was dehydrated with phosphorus oxychloride (1 ml) in pyridine (12 ml) with standing overnight at room temperature. The dehydration product was isolated with petroleum and filtered through alumina. After crystallisation from chloroform the eluted material melted at 250–258°.

*Cetraria islandica* (L.) Ach. 10 kg of dried lichen, purchased through the local branch of Tollef Bredal, and said to originate from Poland, afforded 19 g of neutral matter which was chromatographed on a column 34 × 4.8 cm of alumina. Benzene/ether (99 : 1) and (49 : 1) eluted 0.3 g and 0.1 g, respectively, which on treatment with petroleum furnished altogether 30 mg of *friedelin* of m.p. 259–260°,  $[\alpha]_D - 21^\circ$  (c, 2.21), 2:4-dinitrophenylhydrazone, m.p. 296–297° (dec.), no depression on admixture with authentic specimens.

*Cetraria cucullata* (Bell.) Ach. 945 g of dried material afforded 4.5 g of neutral matter. After chromatography on alumina *friedelin* (23 mg) was obtained, m.p. 250–255° (vac.), from chloroform/methanol,  $[\alpha]_D - 18^\circ$  (c, 2.11), converted to the 2:4-dinitrophenylhydrazone, m.p. 297–298° (dec.), no depression on admixture with authentic specimens.

*Cetraria crispa* (Ach.) Nyl. 896 g of dried lichen furnished 4.9 g of neutral matter. *Friedelin* (22 mg) was obtained as above, m.p. 250–255° (vac.) from acetone,  $[\alpha]_D - 17^\circ$  (c, 2.00), converted to the 2:4-dinitrophenylhydrazone, m.p. 295–296° (dec.), from chloroform-ethyl acetate, no depression on admixture with authentic specimens.

*Cetraria Delisei* (Bory) Th. Fr. [= *hiascens* (Fr.)]. 763 g of dried lichen gave 4.1 g of neutral material. Chromatography as above afforded *friedelin* (20 mg), m.p. 253–257° (vac.) from chloroform/acetone,  $[\alpha]_D - 22^\circ$  (c, 1.93), converted to the 2:4-dinitrophenylhydrazone, m.p. 297–298° (dec.), from chloroform/ethyl acetate, no depression on admixture with authentic specimens.

*Cladonia alpestris* (L.) Rabh. From 648 g of dried lichen 3.0 g of neutral material was obtained. When chromatographed, a very small amount of a very impure *friedelin* was obtained, m.r. 240–255°, admixture with authentic *friedelin*, m.r. 258–262°, all three taken at the same time and in evacuated tubes.

All neutral material was combined and saponified. The unsaponifiable matter (0.6 g) was isolated with ether and chromatographed on alumina. Benzene eluted 0.1 g, which, from chloroform/acetone, furnished a substance of m.p. 262–263° (vac.) undepressed on admixture with *friedelin*,  $[\alpha]_D - 13^\circ$  (c, 0.64), converted to the 2:4-dinitrophenylhydrazone, m.p. 299–300° (dec.), undepressed on admixture with *friedelin* 2:4-dinitrophenylhydrazone.

*Alectoria ochroleuca* (Ehrh.) Nyl. 612 g of dried lichen afforded 3.5 g of neutral material, which was chromatographed on alumina to give 0.5 g eluted with benzene. When this material was dissolved in petroleum and left standing, there gradually separated 17 mg of a mixture of substances, m.r. 225–250°. The mixture was heated twice with fresh amounts of petroleum and each time filtered whilst hot. The small amount of substance finally left on the filter melted at 262–263° (vac.), and did not depress a sample of authentic *friedelin*.

All material was combined and saponified, and the unsaponifiable matter (0.6 g) was isolated with ether and chromatographed on alumina. Benzene eluted material, which, from acetone furnished *friedelin*, m.p. 261–263° (vac.), undepressed on admixture with the authentic compound,  $[\alpha]_D - 21^\circ$  (c, 0.96).

*Stereocaulon paschale* (L.) Fr. 1.4 g of neutral matter was obtained from 360 g of dried lichen. When this was chromatographed, benzene eluted 0.1 g, which was treated with petroleum as above. Undissolved material was crystallised from chloroform/acetone, m.p. 261–263° (vac.), undepressed on admixture with *friedelin*,  $[\alpha]_D - 18^\circ$  (c, 1.11),

converted to the 2:4-dinitrophenylhydrazone, m.p. 298–299° (dec.), no depression on admixture with friedelin 2:4-dinitrophenylhydrazone.

*Isolation of friedelin and cerin from cork.* Cork dust (1 kg) was extracted with hot ethyl acetate (compare <sup>5,6</sup>). A small sample of the deposited material was chromatographed on alumina. The crystals eluted with benzene were crystallised from chloroform/acetone, and finally from benzene to give friedelin, m.p. 262–263° (vac.),  $[\alpha]_D -21^\circ$  (c, 2.40), 2:4-dinitrophenylhydrazone, m.p. 297° (dec.), light abs. max. at 3 710 Å,  $\epsilon$  28 000.

The main part of the material which separated from the ethyl acetate solution was crystallised from chloroform until a substance of constant m.p. 260–262° (vac.), and constant rotation,  $[\alpha]_D -49^\circ$  (c, 0.68);  $-50^\circ$  (c, 0.50, 4 dm tube) was obtained (cerin). A mixture of cerin and friedelin melted at 264–275° (vac.), all three taken at the same time.

*Friedelane.* Friedelin (0.2 g) was heated for 18 hours at 180° with sodium (0.25 g) and hydrazine (2 ml) in ethanol (6 ml). The reaction product, isolated in the usual way, was filtered through alumina in petroleum and the eluted substance crystallised from chloroform, m.p. 245–246°,  $[\alpha]_D +18^\circ$  (c, 0.57).

#### SUMMARY

Friedelin has been isolated from the following lichens: *Cetraria nivalis* (L.) Ach., *C. islandica* (L.) Ach., *C. cucullata* (Bell.) Ach., *C. crispa* (Ach.) Nyl., *C. Delisei* (Bory) Th. Fr. [= *hiascens* (Fr.)], *Cladonia alpestris* (L.), Rabh., *Alectoria ochroleuca* (Ehrh.) Nyl. and *Stereocaulon paschale* (L.) Fr.

The occurrence of epifriedelinol in *Cetraria nivalis* (L.) Ach. has been demonstrated.

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