

Triterpenoids in *Cetraria nivalis* (L.) Ach.

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The presence in *Cetraria nivalis* of α -amyrin, lupeol, ursolic acid, cerin, and a new compound, $C_{30}H_{50}O_2$, is reported.

During a previous investigation of *Cetraria nivalis* the triterpenoids friedelin (friedelan-3-one) and epifriedelinol (friedelan-3 β -ol) were isolated.¹ In addition there was isolated a substance $C_{30}H_{50}O_2$, for details see Experimental, which contained one hydroxyl and one ketone function. The originally isolated amount from two collections was insufficient for a structure determination, and since later collections of material from the same narrow region did not contain this substance, but different triterpenoids, an account is given of the present state.

The unknown substance from *C. nivalis* has been characterised as a mono-alcohol by the formation of a mono-acetate and a mono-benzoate, and as a ketone by the formation of a mono-acetate 2,4-dinitrophenylhydrazone, the UV absorption maximum of which, at 3720 Å, indicates a ketone derivative.² This is supported by absence of a signal in the NMR spectrum which could be attributed to aldehyde proton. The substance could be oxidised to

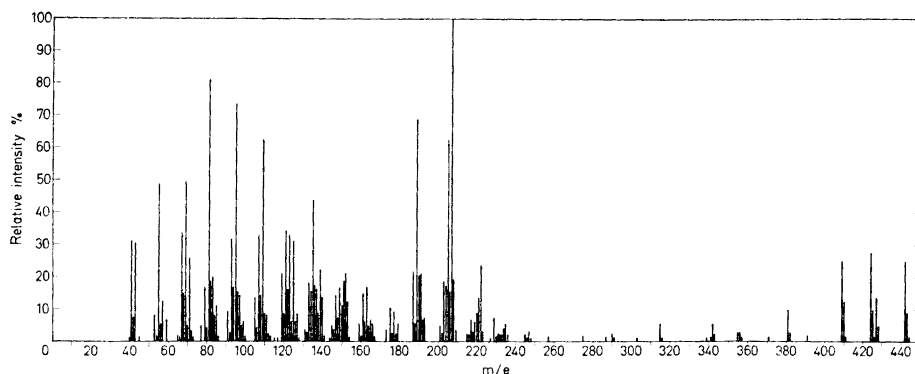


Fig. 1. Mass spectrum of ketol $C_{30}H_{50}O_2$.

a dicarbonyl compound, which appeared to furnish a mono-2,4-dinitrophenylhydrazone. Wolff-Kishner reduction of the ketol afforded a substance which gave a monoacetate and a monobenzoate, and on oxidation a carbonyl compound, which formed a 2,4-dinitrophenylhydrazone, the UV absorption maximum of which, at 3710 Å, again indicated a ketone.² The mass spectra of the ketol and the monol are reproduced in Figs. 1 and 2, respectively.

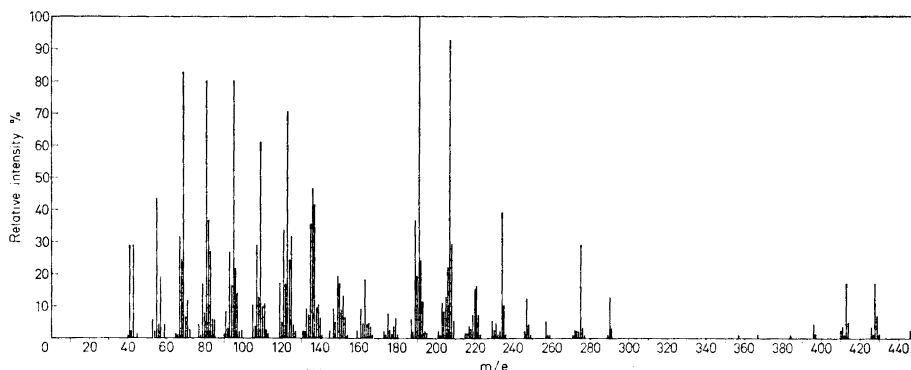


Fig. 2. Mass spectrum of alcohol $C_{30}H_{52}O$.

The constants thought to be reliable of the ketol and derived substances are summarised in Table 1.

The identification of triterpenoids found in later samples of *C. nivalis* was greatly facilitated when it was found that two of them occurred also in the

Table 1. Some constants of the ketol $C_{30}H_{50}O_2$ and derived substances.

	M.p.	$[\alpha]_D$
$C_{30}H_{50}O_2$	221–222°	+ 105°
$C_{30}H_{48}O_2-OC-CH_3$	284–285° (vac.)	+ 59°
$C_{30}H_{48}O_2$	240–242° (vac.)	+ 112°
$C_{30}H_{52}O$	219–220°	+ 23°
$C_{30}H_{51}O-OC-CH_3$	245° (vac.) with sintering at 226–227°	– 18°
$C_{30}H_{51}O-OC-C_6H_5$	225°, sintering at 185°	– 8°

“waste lichens”.¹ By the aid of this material they were identified as α -amyryn and lupeol, for details see Experimental, and by comparison of their 3,5-dinitrobenzoates their presence in *C. nivalis* was demonstrated. It seems to be worth-while to mention that these two substances could not be separated on ordinary silica gel thin layer chromatograms as alcohols, acetates, benzoates, or 3,5-dinitrobenzoates, but they were readily separated on silver nitrate coated silica gel. However, it was not possible to separate impurities of un-

known nature by the two adsorbents, although silver nitrate coated silica gel was much the better. Crystallisations afforded better separation, but was very wasteful. The m.p. of the 3,5-dinitrobenzoates seemed to be least sensitive to impurities.

The presence of ursolic acid and of cerin in *C. nivalis* has been demonstrated. For details, see Experimental.

EXPERIMENTAL

M.p.'s are uncorrected. Mixed m.p. determinations were done by observing the m.p.'s of the two substances and their admixture at the same time. Rotations were measured in chloroform solutions in a 1 dm tube. Petroleum refers to a fraction of b.r. 60–70°. Infrared spectra were recorded on a Perkin-Elmer Model 21 spectrometer in (a) 10 % solution in chloroform in an 0.05 mm cell or in (b) suspension in potassium bromide discs. NMR spectra were recorded in deuteriochloroform solutions on a Varian A–60 A instrument. Mass spectra were obtained with an AEI MS9 spectrometer. Esterifications were done in pyridine solutions with acetic anhydride or the appropriate acid chloride. Light absorptions of 2,4-dinitrophenylhydrazones were measured in chloroform solutions on a Perkin-Elmer Model 13 instrument.

The "waste lichens"

For origin of the material, see Ref.1. The neutral material remaining after saponification and from which epifriedelinol had been isolated (60 g) was chromatographed crudely on alumina, and then carefully on silica gel. Materials eluted from silica gel with benzene-ether 99:1 and 49:1 in several fractions contained nicely crystalline materials. The various fractions on thin layer showed one spot, which in mixed chromatograms appeared identical. However, the crystalline materials melted over a wide range. The materials were converted to the 3,5-dinitrobenzoates, which were crystallised from acetone or ethyl acetate to furnish a mixture of crystals: light yellow plates which by decantation could be separated from denser and darker yellowish-red prisms.

Lupeol. The light yellow plates, above, after several crystallisations from ethyl acetate melted at 274–275° (80 mg), $[\alpha]_D + 55^\circ$ (c, 2.28). The NMR spectrum contained a somewhat broadened peak at 102 Hz, indicating a $-C=C-CH_3$ grouping. There was no depression of the m.p. on admixture with authentic 3,5-dinitrobenzoate of lupeol (below).

α -Amyrin. The denser prisms likewise were crystallised repeatedly from ethyl acetate, m.p. 252–253° (2.4 g), $[\alpha]_D + 81^\circ$ (c, 2.15).

The 3,5-dinitrobenzoate was saponified, and the alcohol was converted to the acetate. Part of the acetate crystallised from the pyridine solution and was filtered off. The crude crystals melted at 218–220°, whilst the material remaining in solution melted over a wide range and below 220°. The former crystals were repeatedly crystallised from chloroform-methanol to give a top fraction of m.p. 229–230°, $[\alpha]_D + 79^\circ$ (c, 2.02) (510 mg).

Saponification furnished the alcohol, which again was crystallised repeatedly from chloroform-methanol, m.p. 191–191.5°, $[\alpha]_D + 81^\circ$ (c, 2.05) (32 mg). From this alcohol were prepared the acetate, m.p. 227.5–228°, $[\alpha]_D + 76^\circ$ (c, 2.02), the benzoate, m.p. 197–198°, $[\alpha]_D + 86^\circ$ (c, 0.95), and the 3,5-dinitrobenzoate, m.p. 255–256°, $[\alpha]_D + 79^\circ$ (c, 0.99). The 3,5-dinitrobenzoate did not depress the m.p. of an authentic sample of α -amyrin 3,5-dinitrobenzoate (see below), and their IR (a) and NMR spectra were identical, as was found also for their benzoates.

Cetraria nivalis

For origin of the material, see Ref.1. When the neutral material was chromatographed crudely on alumina, a transition zone was obtained with the last 100 ml of benzene and the first 200 ml of ether. The material of this transition zone weighed 1.9 g and furnished

150 mg of a crystalline *substance*, m.p. 221–222° from benzene-petroleum, $[\alpha]_D + 105^\circ$ (c, 2.69); band in the infrared (a) at 1703 cm^{-1} . (Found: C 81.7; H 11.3, $\text{C}_{30}\text{H}_{50}\text{O}_2$ (442.50) requires: C 81.4; H 11.4). Mass spectrum, see Fig. 1.

The *substance* formed an acetate, m.p. 284–285° (vac.), $[\alpha]_D + 59^\circ$ (c, 2.02). (Found: C 78.6; H 13.1.* $\text{C}_{32}\text{H}_{52}\text{O}_3$ (484.4) requires: C 79.3; H 10.8). The *acetate 2,4-dinitrophenylhydrazone* melted at 298° (decomp.), λ_{max} 3720 Å, ϵ 41 000. (Found: N 8.35.* $\text{C}_{38}\text{H}_{56}\text{N}_4\text{O}_6$ (664.46) requires: N 8.45). Attempts at preparing a benzoate gave a product of constant m.p. 262–263° (vac.), but varying rotation. The ketol was oxidised to a dicarbonyl compound, m.p. 240–242° (vac.), $[\alpha]_D + 112^\circ$. (Found: C 81.0; H 8.3. $\text{C}_{30}\text{H}_{48}\text{O}_2$ (440.72) requires: C 81.8; H 10.95). M^+ at m/e 440 (76 %); B.P. at m/e 205. It formed a *mono-2,4-dinitrophenylhydrazone*, m.p. 278° (decomp.), λ_{max} 3710 Å, ϵ 28 000. (Found: N 9.1.* $\text{C}_{36}\text{H}_{52}\text{N}_4\text{O}_5$ (620.84) requires: N 9.02).

Wolff-Kishner reduction of $\text{C}_{30}\text{H}_{50}\text{O}_2$. A portion (50 mg) of the ketol was heated for 16 h at 180° with hydrazine (0.5 ml), sodium (0.25 g) and ethanol (6 ml). The *product* crystallised from chloroform-methanol, m.p. 219–220°, $[\alpha]_D + 23^\circ$ (c, 2.47). (Found: C 83.1; H 12.05. $\text{C}_{30}\text{H}_{52}\text{O}$ (428.4) requires: C 84.1; H 12.25). Mass spectrum, see Fig. 2.

The *acetate* melted at 245° with sintering at 226–227° (vac.), $[\alpha]_D - 18^\circ$ (c, 1.72). (Found: C 79.0; H 11.0. $\text{C}_{32}\text{H}_{54}\text{O}_2$ (470.4) requires: C 81.6; H 11.55). Mass spectrum: 470 (7 %), 191 (89 %), 189 (100 %).

The *benzoate* melted at 225° with sintering at 185°, $[\alpha]_D - 8^\circ$ (c, 1.06). (Found: C 82.4; H 10.3. $\text{C}_{37}\text{H}_{56}\text{O}_2$ (532.4) requires: C 83.4; H 10.6). Mass spectrum: 532 (4 %), 191 (59 %), 189 (50 %), 95 (100 %).

Oxidation with chromium trioxide in acetic acid afforded a carbonyl compound, m.p. 205–206°, $[\alpha]_D$ about $+30^\circ$ but varying. Mass spectrum: 426 (75 %), 191 (100 %). It formed a *2,4-dinitrophenylhydrazone*, m.p. 267–268° (decomp.), λ_{max} 3710 Å, ϵ 30 500. (Found: N 9.0. $\text{C}_{36}\text{H}_{54}\text{N}_4\text{O}_4$ (606.86) requires: N 9.25).

All derivatives were crystallised from chloroform-methanol.

Ursolic acid. The ethereal extract of *C. nivalis* was extracted successively with solutions of sodium hydrogen carbonate, disodium carbonate, and sodium hydroxide. The material extracted with sodium hydroxide was liberated with dilute sulphuric acid and extracted with ether. On standing the ether solution deposited colourless crystals, very sparingly soluble in the usual organic solvents. The methyl ester, obtained with diazomethane, crystallised from petroleum with a trace of chloroform as colourless needles (200 mg), m.p. 106–107°, $[\alpha]_D + 67^\circ$ (c, 2.24). The methyl ester afforded a methyl ester acetate, m.p. 237–239°, $[\alpha]_D + 64^\circ$ (c, 2.43), and a methyl ester carbonyl compound, m.p. 192–194°, $[\alpha]_D + 80^\circ$ (c, 0.54). These values, apart from the m.p. of the methyl ester, are very close to those reported for ursolic acid:³ methyl ester, m.p. 171°, $[\alpha]_D + 69^\circ$, methyl ester acetate, m.p. 246°, $[\alpha]_D + 62^\circ$, methyl ester ketone, m.p. 191–193°, $[\alpha]_D + 84^\circ$ (pyridine). On the basis of this Professor D.H.R. Barton suggested that the original acid might still be ursolic acid. When authentic ursolic acid was esterified with diazomethane and crystallised from chloroform-petroleum, as above, it melted at 107–108°, immediately solidifying and remelting at 170–171°. A mixed m.p. determination showed that the mixture behaved as described for the authentic ester, whilst the methyl ester of the isolated acid remained clear, but stuck to the walls of the m.p. tube until 170°, when it ran down.

C. nivalis (5 kg), collected in the same place as the former samples,¹ was extracted with ether as before. After acidic material had been removed by alkali, an ether solution of the neutral part deposited a mixture of crystals (1.4 g). The remainder (31.1 g) was saponified to give 15 g of neutral material.

Part (0.5 g) of the material deposited from the ether solution was chromatographed on a column of silver nitrate coated silica gel. The transition zone from benzene to ether (50 ml) contained material (46 mg) which on thin layer chromatograms of silver nitrate coated silica gel gave two spots. The faster running was very sparingly soluble in chloroform, and when crystallised from this solvent, melted at 258–260°, no depression on admixture with cerin from cork. Their infrared (b) and mass spectra were identical. The neutral material remaining after the saponification, (15 g) above, first was chromatographed carefully on alumina, but none of the compound $\text{C}_{30}\text{H}_{50}\text{O}_2$, above, was obtained. Material eluted with benzene-ether 9:1 (1.8 g) and 3:1 (1.0 g) on thin layer chromatograms showed the same spot as had been observed in material from the waste lichens, and which corresponded to the α -amyrin-lupeol mixture (above). The fractions were con-

verted to the 3,5-dinitrobenzoates and chromatographed carefully on silver nitrate coated silica gel. Benzene-petroleum 2:1 eluted material which, crystallised from ethyl acetate, furnished a 3,5-dinitrobenzoate (0.2 g) m.p. 254–255°, $[\alpha]_D + 80^\circ$ (c, 2.74), undepressed on admixture with the 3,5-dinitrobenzoate of α -amyrin from the waste lichens (above). Their IR (b) and NMR spectra were identical.

Similarly, material eluted with benzene afforded crystals (80 mg), m.p. 277–278° from ethyl acetate, $[\alpha]_D + 54^\circ$ (c, 2.43), undepressed on admixture with 3,5-dinitrobenzoate of lupeol from the waste lichens (above). Their IR (b) and NMR spectra were identical.

α -Amyrin 3,5-dinitrobenzoate. α -Amyrin was esterified with 3,5-dinitrobenzoyl chloride, and the ester was crystallised from chloroform-methanol, m.p. 254–255°, $[\alpha]_D + 80^\circ$ (c, 2.08), literature,⁴ m.p. 220–221°. (Found: C 72.2; H 8.4; N 4.5. ** $C_{37}H_{52}N_2O_6$ (620.84) requires: C 71.6; H 8.45; N 4.5).

Lupeol 3,5-dinitrobenzoate. Lupeol was converted to the ester and crystallised, as described for α -amyrin (above), m.p. 279–280°, $[\alpha]_D + 54^\circ$ (c, 2.15). (Found: C 72.2; H 8.4; N 4.55. ** $C_{37}H_{52}N_2O_6$ (620.84) requires: C 71.6; H 8.45; N 4.5).

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