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3-*O*-MALONYLBETULAFOLIENTRIOL OXIDE I FROM *BETULA NANA* SUBSP. *EXILIS*

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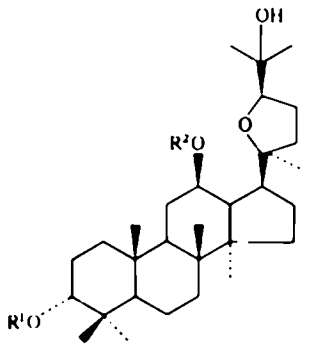
Abstract The major ether-soluble metabolite of *Betula nana* subsp. *exilis* was determined by chemical and spectral methods to be 3-*O*-malonylbetulafolientriol oxide I.

Following our discovery that papyriferic acid (**1**) plays an important role in defending Alaskan paper birch (*Betula papyrifera* subsp. *humilis* = *resinifera*) from browsing by vertebrate herbivores [1, 2], we have initiated a phytochemical investigation of other birches found in boreal regions. In particular, our attention has been drawn to Alaskan populations of dwarf birch (*Betula nana* subsp. *exilis*) which, like juvenile *B. resinifera*, has resinous deposits on the exterior of its bark of its current year twigs. These twigs are rather unpalatable to snowshoe hares (Bryant, J. P., personal communication).

Preliminary chemical investigation of *B. nana* subsp. *exilis* by thin-layer chromatography (TLC) revealed that its major ether-soluble metabolite was a compound slightly more polar than papyriferic acid but possessing similar visualization characteristics with a variety of reagents. TLC examination of a number of Eurasian birches indicated that this substance is also produced by *B. nana* subsp. *nana* from Finland and *B. middendorffi* from Siberia. In this paper we assign the structure of this new secondary metabolite as 3-*O*-malonyl-betulafolientriol oxide I (**2**).

Following extensive chromatography of an ether extract of *B. nana* subsp. *exilis*, compound **2** was isolated as an amorphous white powder (mp 168–172°) which resisted recrystallization efforts. The 60 MHz ^{1}H NMR spectrum of **2** appeared very similar to that of **1**, the only significant observable differences being the lack of an acetyl methyl signal (*ca* δ2) and the apparent shift of a one-hydrogen multiplet from δ4.8 to 3.5. Thus it appeared that **2** was desacetyl papyriferic acid. Verification of this tentative assignment was obtained from ^{13}C NMR and high-resolution ^{1}H NMR spectroscopy. The fully decoupled ^{13}C NMR spectrum of **2** exhibited peaks including only two carbonyl signals (δ169.5 and 167.0) compared to three (δ170.1, 169.1 and 167.3) observed for papyriferic acid. Examination of the 250 MHz ^{1}H NMR spectrum of **2** revealed that the signal (δ4.83, *dt*, J = 5.2, 10.4 Hz) assigned to the proton on C-12 of papyriferic acid had shifted to δ3.47 (*dt*, J = 3.7, 11.0 Hz) in **2**, that the acetyl methyl signal (δ2.02) of papyriferic acid was absent in **2**, and that **2** contained three rather than two exchangeable hydrogens. These observations taken together with the molecular weight of **2** (MH^+ = 563.3965 as determined by FAB mass spectrometry) and the saponification of **2** to betulafolientriol oxide I (**3**) [3, 4] allowed assignment of the structure of **2** as 3-*O*-malonylbetulafolientriol oxide I (**2**).



	R¹	R²
1	HOOCCH$_2$CO	Ac
2	HOOCCH$_2$CO	H
3	H	H

Two unusual features of the NMR spectra of compound **2** merit comment here. The ^{13}C NMR signals assigned to the two carbonyl carbons of the malonyl residue (δ167.0 and 169.5) are both extremely weak and broad (line width *ca* 10 Hz in each case) at 25°. These two signals sharpen and increase intensity at −30°. In the 250 MHz ^{1}H NMR spectrum of **2** taken at 25° the signal assigned to the malonyl protons (δ3.35) integrates for 1.6 protons (rather than the expected 2.0 protons) and the integral value increases slowly with decreasing temperature until its value reaches 1.9 protons at −40°. Apparently the malonyl residue of **2** undergoes a dynamic process which results in the exchange of the two carbonyl carbons. Experimental investigation of this phenomenon is presently being undertaken.

Although compound **2** has a structure very similar to papyriferic acid, a substance which deters feeding by hares [2], and is present in dwarf birch at rather high concentrations (*ca* 1% of wet weight as determined by the

^{1}H NMR method of ref. [2]), we presently have no evidence for the contribution of **2** towards the unpalatability of this plant to vertebrate herbivores. Indeed, Sinclair and Smith [5] have argued that the snowshoe hare's use of dwarf birch in British Columbia is governed by nitrogen concentrations. Investigation of the role of **2** in diet selection by the snowshoe hare is presently underway in our laboratory.

EXPERIMENTAL

Plant collection and extraction. Terminal branches (< 1 cm diameter) of dwarf birch [*Betula nana* subsp. *exilis* (Sukatsch) Hult.] were collected near Fairbanks, Alaska just after leaf abscission in September 1984. The plant was identified by Dr. John Bryant of the Institute of Arctic Biology, University of Alaska, Fairbanks. The sample (1.2 kg) was ground on a Wiley mill (2 mm screen) and the ground material stored at −20° in a sealed plastic bag. A portion (200 g) of the ground material was extracted (25°) with 1.9 l. of Et_2O for 27 hr. Filtration, drying ($MgSO_4$) and concn under vacuum provided 15.2 g of a dark-green resinous extract.

Isolation of 3-O-*malonylbetulafolientriol 1* (**2**). A portion (7.64 g) of the crude extract was subjected to CC on silica gel. Following elution of less polar substances with Me_2CO-petrol (1:1), fractions rich in **2** (TLC; silica gel; $CHCl_3$-MeOH HOAc, 92.4:7.0:0.6; R_f 0.34) were eluted with Me_2CO-petrol (3:1). A portion (1.12 g) of crude **2** obtained from the gravity column was further purified by flash chromatography [6] on silica gel with an eluant of $CHCl_3$-MeOH-HOAc (93:7:0.6). Fractions rich in **2** (TLC) were combined and evaporated. This sample (409 mg) was again chromatographed in this system to give 119 mg of a pale-green oil which exhibited a single spot in TLC. Purification of a portion (82 mg) of this sample by reverse-phase (C18) flash chromatography [6] using 85% aq. MeOH as eluant gave, after lyophilization, 57 mg **2** as a white amorphous solid: mp 168–172° (dec.); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3410, 1720; FABMS (found: MH$^+$ 563.3965; $C_{33}H_{55}O_9$ requires: M + H, 563.3948) *m/z* (rel. int.): 563 (20), 545 (18), 527 (10), 441 (10), 423 (18), 143 (100); ^{1}H NMR (250 MHz, $CDCl_3$): δ0.76 (3H, *s*, Me), 0.80 (3H, *s*, Me), 0.82 (3H, *s*, Me), 0.86 (3H, *s*, Me), 0.91 (3H, *s*, Me), 1.02 (3H, *s*, Me), 1.20 (6H, *s*, C(OH)Me_2), 3.35 (2H, *br s*, $W_{1/2}$ = 11 Hz, $COCH_2CO$), 3.47 (1H, *dt*, $J_{11\alpha,12} = J_{12,13}$ = 11.0, $J_{11\beta,12}$ = 3.7 Hz, H-12), 3.78 (1H, *t*, *J* = 7.4 Hz, H-21), 4.63 (1H, *s*, $W_{1/2}$ = 7 Hz, H-3), 5.02 (3H, *br s*, $W_{1/2}$ = 80 Hz, exchange with D_2O, OH); ^{13}C NMR (62.1 MHz, $CDCl_3$): δ169.7, 166.8, 86.5, 85.2, 80.1, 71.0, 70.3, 52.1, 50.7, 50.1, 49.1, 47.8, 39.8 (× 2), 37.0, 36.7, 34.5, 34.0, 32.5, 31.1, 31.0, 28.5, 27.8, 27.7, 27.5, 25.9, 24.9, 22.7, 21.5, 18.3, 18.0, 16.0, 15.3; $[\alpha]_D$ −1.0° ($CHCl_3$; *c* 3.0).

Saponification of compound **2**. Saponification (KOH 95% EtOH) of **2** (19 mg) afforded a white crystalline material (16 mg) which exhibited a single TLC spot (R_f 0.48) identical to that obtained from an authentic sample of betulafolientriol oxide I obtained from saponification of **1** [1]. Recrystallization (Me_2CO) gave pure **3**: mp 235 237° (lit. [3] 237.9°); mmp 235–238°.

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REFERENCES

1. Reichardt, P. B. (1981) *J. Org. Chem.* **46**, 4576.
2. Reichardt, P. B., Bryant, J. P., Clausen, T. P. and Wieland, G. D. (1984) *Oecologia (Berlin)* **65**, 58.
3. Nagai, M., Tanaka, N., Tanaka, O. and Ichokawa, S. (1973) *Chem. Pharm. Bull.* **21**, 2061.
4. Ohmoto, T., Nikaido, T. and Ikuse, M. (1978) *Chem. Pharm. Bull.* **26**, 1437.
5. Sinclair, A. R. E. and Smith, J. N. M. (1984) *Oecologia (Berlin)* **61**, 403.
6. Still, W. C. (1978) *J. Org. Chem.* **43**, 2933.