The Structure of Spiroveitchionolide, an Unusual Lanostane-type Triterpene Lactone from *Abies veitchii*

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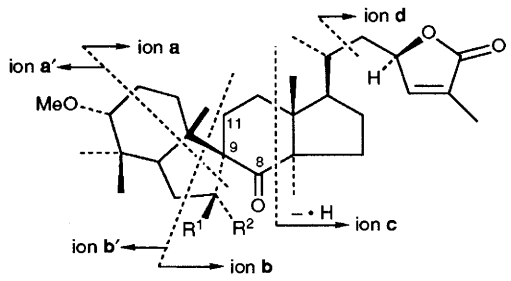
The structure of spiroveitchionolide, a novel triterpene lactone having an unusual skeletal system, isolated from *Abies veitchii*, is established as (3*R*, 7*S*, 9*R*, 23*R*)-7-hydroxy-3-methoxy-8-oxo-7(8→9) *abeo*-lanost-24-eno-26,23-lactone on the basis of chemical, spectral and single crystal X-ray crystallographic evidence.

The isolation of several (23*R*)-9β-lanosta-7,24-dieno-26,23-lactones showing significant *in vitro* antineoplastic activity against mouse leukaemia L_{1210} and human epidermoid KB cells† from the stem bark of *Abies mariesii*,[1] *A. firma*[2,3] and *A. veitchii*[4,5] (Pinaceae) and the discovery of several strong anti-tumour-promoting agents from the derivatives of the above triterpene lactones[6] prompted us to search for constituents having more effective biological activities from the same plant sources. Successive extraction of the stem bark of *A. veitchii* with diethyl ether and methanol and careful column chromatography of the ether-soluble fraction separated from the methanol extract led to the isolation of a highly oxygenated tetracyclic triterpene lactone bearing a novel skeletal system, named spiroveitchionolide **1**, in a yield of 0.017% from the fraction. We now report on the structure of this compound.

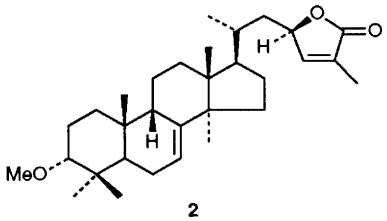
† *In vitro* antineoplastic activity was assayed by Dr T. Sasaki, Cancer Institute, Faculty of Medicine, Kanazawa University, according to the protocol described in ref. 11. Detailed evaluation of **1** as a selective antineoplastic agent is in progress.

Spiroveitchionolide **1** had the molecular formula $C_{31}H_{48}O_5$ (*m/z* 500.3501, M^+). The IR, ^1H and ^{13}C NMR spectra‡ exhibited the presence of five quaternary methyl groups, a

‡ Colourless prisms, m.p. 239.5–242 °C ($CHCl_3$–MeOH), $[\alpha]_D^{23}$ − 14.2 (*c* 0.27 in $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3474, 2820, 1753sh and 1732s, 1693, 1662, 849 and 797; δ_H (400 MHz, $CDCl_3$) 0.70 (3H, s, Me-18), 0.88 (3H, s, Me-28), 0.95 (3H, s, Me-29), 1.03 (3H, d, *J* 6.5 Hz, Me-21), 1.19 (3H, s, Me-30), 1.42 (3H, s, Me-19), 1.92 (3H, t, *J* 1.7 Hz, Me-27), 2.82 (1H, t, *J* 2.7 Hz, H–C–OMe), 3.30 (3H, s, OMe), 4.22 (1H, ddd, *J* 7.5, 6.7, 3.5 Hz, H-7), 4.97 (1H, ddd, *J* 9.5, 4.2, 1.8 Hz, H-23) and 6.99 (1H, quintet, *J* 1.6 Hz); δ_C (100 MHz, $CDCl_3$) 10.63 (C-27), 16.45 (C-19), 17.15 (C-18), 18.66 (C-21), 19.68 (C-30), 20.99 (C-2), 22.64 (C-29), 26.58 (C-1), 27.01 (C-16), 28.42 (C-28), 29.63 (C-15), 30.00 (C-11), 30.83 (C-12), 32.91 (C-20), 34.44 (C-6), 37.94 (C-4), 40.33 (C-22), 44.36 (C-5), 47.49 (C-13), 48.94 (C-10), 50.85 (C-17), 57.41 (OMe), 61.18 (C-14), 64.53 (C-9), 78.67 (C-23), 80.26 (C-7), 84.67 (C-3), 129.63 (C-25), 149.37 (C-24), 174.21 (C-26) and 215.67 (C-8); *m/z* (%) 500 (M^+, 16), 482 (10), 468 (14), 453 (6), 450 (6), 357 (**a**, 15), 333 (**b**, 70), 304 (**b** − CHO, 27), 235 (10), 233 (**c**, 12), 175 (13), 168 (**b'**, 38), 161 (42), 147 (28), 143 (**a'**, 19), 136 (**b'** − MeOH, 100), 121 (56) and 111 (d, 21); CD (dioxane) $[\theta]_{209}$ − 25 000° $[\theta]_{211}$ − 42 500° (trough), $[\theta]_{220}$ − 26 000°, $[\theta]_{235}$ 0° and $[\theta]_{310}$ − 4600°.



1 R^1 = OH, R^2 = H
1a R^1 = OAc, R^2 = H
1b R^1R^2 = O



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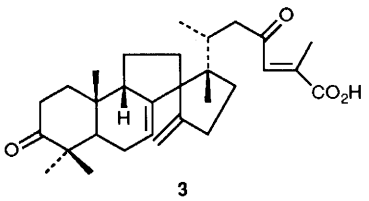

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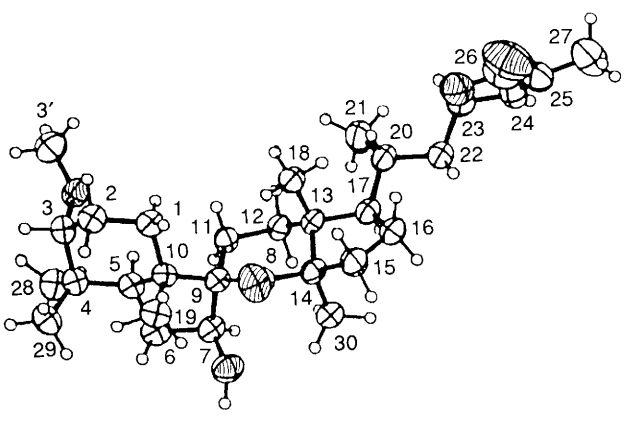

Fig. 1 X-Ray structure of **1**

secondary methyl group, a secondary methoxy group which can be located at the usual C-3 axial position,[4] a secondary hydroxy group, a six-membered ring ketone and a 4-substituted 2-methylbut-2-enolide moiety showing the same negative Cotton effect curve as that of abieslactone **2**;[1,4] it showed no IR and ^{1}H NMR signals due to CH_2 or CH vicinal to the oxo-group. This indicates that the oxo-group is located between two quaternary sp^3 carbons. Its DEPT spectrum showed that the carbon composition had one more methine and quaternary sp^3 carbons and lacked two methylene groups, in comparison with that of the corresponding lanostane. This fact, along with the appearance of typical fragment peaks corresponding to ions **a**, **a'**, **b**, **b'**, **c** and **d** in the mass spectrum,‡ suggested **1** to have an unusually migrated lanostane skeleton involving a spiro-ring system.

Treatment of **1** in a boiling mixture of acetic anhydride and pyridine gave an amorphous acetate **1a**, δ_H 1.96 (3H, s, OAc) and 5.10 (1H, ddd, H-7*S*), while it resisted the usual acetylation at room temperature. Chromium trioxide oxidation of **1** afforded the dioxolactone **1b**, m.p. 257–259 °C, $[\alpha]_D^{23}$ – 87.9 (*c* 0.13 in $CHCl_3$), which exhibited IR bands for a five-membered ring ketone bearing a vicinal methylene group, besides bands for both the lactone carbonyl and six-membered ring ketone [$\nu_{max.}$ (KBr)/cm^{-1} 1746, 1732, 1694 and 1410]. These results, together with biogenetic consideration, indicated that **1** must have 7(8→9) *abeo*-9ξ-lanostane skeleton involving a $-H_2C-CH(OH)-C-CO-C-$ grouping in the *B*–*C*-ring system in which the hydroxy group is sterically a little hindered. As no definitive proof supporting the above assumption could be obtained from either 2D long-range ^1H–^{13}C COSY and NOESY experiments, the single crystal X-ray structure of **1** was determined.§ Fig. 1 shows a perspective view of **1**, proving unambiguously its structure. The carbon skeleton involves a characteristic spiro-*B*/*C* ring system which can be considered to have been reconstructed from 9β-lanostane through the 1,2-migration of the C(7)–C(8) bond to the C(9) position.

This is the first report on the isolation of **1**, although abiesonic acid **3** and five of its analogues have previously been isolated from the leaves of *A. sibirica*[7–9] and the seeds of *A. mariesii*.[10]

Compound **1** seems to be derived from abieslactone **2** *via* oxidative fission of the Δ^7-double bond caused by a powerful and unusual oxidase enzyme, and subsequent retro-aldol condensation of the resulting keto-aldehyde in the plant. Investigation of the biological activity of **1** is now in progress.

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References

1 S. Uyeo, J. Okada, S. Matsunaga and J. W. Rowe *Tetrahedron*, 1968, **24**, 2859.
2 R. Tanaka, A. Inoshiri, M. Yoneda, T. Ishida, A. Numada and S. Matsunaga, *Phytochemistry*, 1990, **29**, 3263.
3 R. Tanaka and S. Matsunaga, *Phytochemistry*, 1991, **30**, 1983.
4 R. Tanaka and S. Matsunaga, *Phytochemistry*, 1990, **29**, 3267.
5 R. Tanaka and S. Matsunaga, *J. Nat. Prod.*, 1991, **54**, 1337.
6 J. Takayasu, R. Tanaka, S. Matsunaga, H. Ueyama, H. Tokuda, T. Hasegawa, A. Nishino, H. Nishino and A. Iwashima, *Cancer Lett.* 1990, **53**, 141.
7 V. A. Raldugin, Yu. V. Gatilov, I. Yu. Bagryanskaya and N. I. Jaroshenko, *Khim. Prir. Soedin.*, 1986, 584.
8 V. A. Raldugin, S. A. Shichevtsov, Yu. V. Gatilov, I. Yu. Bagryanskaya, L. I. Demenkova and V. A. Pentegova, *Khim. Prir. Soedin.*, 1987, 842.
9 V. A. Raldugin, M. M. Shakirov, T. V. Leibyuk and S. A. Shevtsob, *Khim. Prir. Soedin.*, 1991, 511.
10 S. Hasegawa, T. Miura, N. Kaneko, Y. Hirose and Y. Iitaka, *Tetrahedron*, 1987, **43**, 1775.
11 Y. Sonoda, K. Ichinose, T. Yoshimura, Y. Sato and T. Sasaki, *Chem. Pharm. Bull.*, 1991, **39**, 100.

§ *Crystal data*: $C_{31}H_{48}O_5$, *M* = 500.720, triclinic, space group *P*1, *a* = 11.763(2), *b* = 17.021(14), *c* = 7.104(1) Å; α = 97.15(3), β = 90.71(3), γ = 93.08(4)°; *U* = 1408.9(12) Å^3, D_c = 1.1803 g cm^{-3}, *Z* = 2. A total of 4806 independent reflection intensities up to 2θ = 130° were measured on a Rigaku automatic four-circle diffractometer with graphite-monochromated Cu-*K*α radiation. 4601 reflections with $F_o > 0.0$ were used for the structure analysis by direct methods. The non-hydrogen atoms were refined anisotropically by block-diagonal least-squares. Hydrogen atoms were located from a difference Fourier synthesis. The structure was finally refined to $R = 0.056$ ($R_w = 0.081$). Atomic coordinates, thermal parameters, and bond lengths and angles, have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.