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Note

A diterpene alkaloid from the needles of *Taxus baccata*†

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A diterpene alkaloid **2** has been isolated from the methanol extract of *T. baccata*. Its structure has been established as 2′-desacetoxyaustrospicatine on the basis of spectral analysis and comparison with the reported data.

Taxol, the highly functionalized diterpene natural product isolated in 1971[1], has recently received an enormous publicity both in scientific[2] and local media[3]. Taxol is presently isolated from the bark of *Taxus brevifolia*, the pacific yew tree or *Taxus baccata*, its European relative. Removal of the bark destroys the trees and endangers the survival of the species. However as a result of their high content of 10-deacetylbaccatin III, a compound that can be transformed into taxol, the needles of the European yew (*T. baccata* L.) represent a valuable and renewable source of taxol. A project was initiated in NCL during 1992 to isolate taxol and 10-deacetylbaccatin III from the needles of *T. baccata*. During our chemical investigation of the above plant we have isolated 10-deacetylbaccatin III (**1**) (0.07%) as a major compound along with a diterpene alkaloid **2** (0.01%). The compound 2′-desacetoxyaustrospicatine **2**, which we have isolated for the first time from *T. baccata*, has been reported from *Austrotaxus spicata*[4] and a Japanese Yew *Taxus cuspidata*[5]. Kobayashi *et al.* have mentioned that compound **2** increases cellular accumulation of vincristine in multidrug-resistant tumor cells, while taxol does not show such activity, rather and shows weak toxicity. Keeping in view the importance of compound **2**, we have elucidated its structure by detailed spectral analysis.

Compound **2**, a gummy liquid, $[\alpha]_D^{30}$ + 64° ($CHCl_3$), Mol. formula $C_{39}H_{53}O_{10}N$, showed in its IR spectrum peaks at 1745, 1740, 1725 for carbonyls and at 1630, 1430 cm^{-1} revealing the presence of unsaturation and an aromatic ring. The ^1H NMR spectrum of **2** showed the presence of three tertiary methyls and a methyl on a double bond, and exhibited four singlets in the region δ 2.08 to 2.20 assignable to methyl protons of acetyl groups. Two broad signals appearing at δ 4.95 and 5.25 showing the connectivities with each other clearly indicated the presence of an exocyclic methylene group at C-20 position. Furthermore, the proton signals at δ 5.58 (d, J = 10 Hz) and 6.30 (d, J = 10 Hz) showed the connectivities on its ^1H-^1H homodecoupling spectrum, indicating that both the positions C-9 and C-10 are substituted by acetyl groups which are *trans* to each other.

The triplet of a quartet at δ 5.95 assignable to the C-13 proton showed the connectivities with C-14 proton signals. The presence of a proton at C-1 was evident by the fact that C-2 protons appeared as a multiplet and both the protons showed the coupling with each other (Figure 1). Further, the C-2 proton also showed the connectivities with the proton at δ 2.80 assignable at C-3. It clearly suggested that C-1 and C-2 positions are not substituted with any oxygenated function. It was further supported by the ^{13}C NMR spectrum; the signal appeared at δ 40.77 (d) for C-1 and 27.51 (t) for C-2. It was also confirmed by ^1H-^{13}C heterocoupling experiment (Figure 2).

The assignment of the signal at δ 5.40 (br, t) to the C-5 proton and the signal appearing at 5.45 (dd) to the C-7 proton was made on the basis of its ^1H-^1H homonuclear decoupling experiment (Figure 1). The upfield chemical shift of the aromatic protons and the appearance of signals at δ 3.95 (t, 1H, J = 7 Hz), 2.20 (s, 6H), 3.0 (dd, 1H, J = 16, 7 Hz), and 2.80 (dd, 1H, J = 16, 7 Hz) clearly indicated the presence of unusual ester side chain as $-OCOCH_2(NMe_2)Ph$. Assignments of remaining protons were achieved by ^1H-^1H homonuclear decoupling experiment. Moreover,

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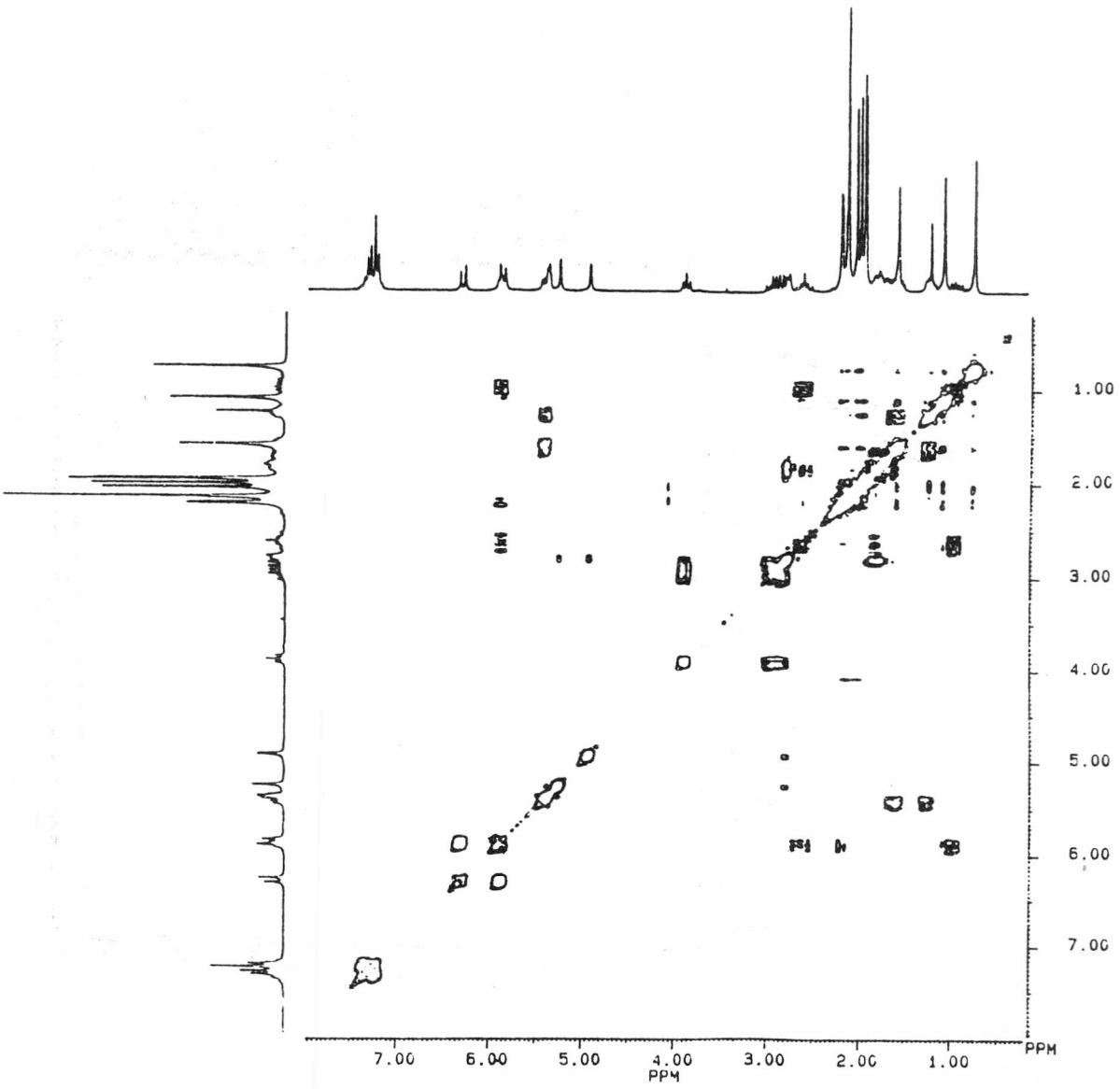

Figure 1

DEPT and ^{13}C NMR spectra also confirmed the proposed structure for compound **2**.

Experimental Section

IR spectrum was measured in $CHCl_3$, ^1H and ^{13}C NMR spectra in $CDCl_3$ with TMS as internal standard. The ^1H-^1H homonuclear decoupling and ^1H-^{13}C heterodecoupling experiments were carried out in $CDCl_3$ on an AC 200 MHz FT NMR (BRUKER) spectrometer. Optical rotation was determined on a JASCO DIP-181 digital polarimeter.

Plant material. Needles of *T. baccata* L. were collected from Darjeeling, West Bengal in November, 1993. A voucher specimen is depositedd in our laboratory.

Extraction and isolation. *T. baccata* needles were dried (2 kg) and powdered. The powdered plant material was extracted with methanol for three days at room temperature, filtered, and the solvent was removed under reduced pressure to yield a dark greenish extract (65 g). This extract was chromatographed over silica gel (450 g, 60-120 mesh) column using acetone-pet. ether as elution gradient with increasing polarity of acetone to collect seven broad fractions, A (15.5 g), B (7.0 g), C (6.2 g), D (8.7 g), E (5.0 g), F (12.3 g) and G (8.6 g).

Fraction D (8.7 g) on repeated column chromatography coupled with prep. TLC using $CHCl_3$ – MeOH (10 : 1) as irrigant yielded 1.40 g crystalline compound **1**, m.p. 223-225 (Lit[6]., m.p.

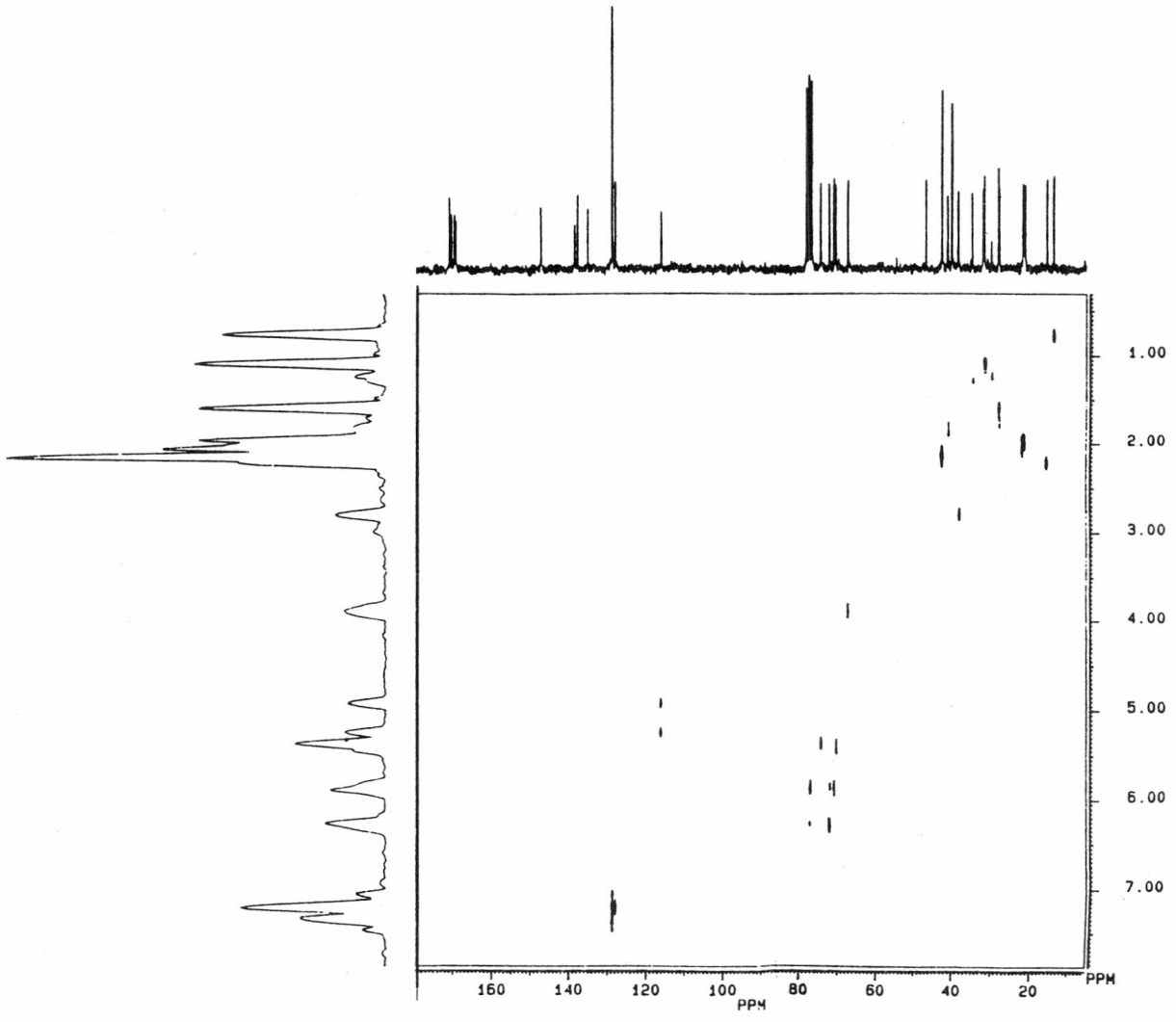

Figure 2

229-31°). It was identified by comparing its ^1H NMR and mass spectral data with those reported by Miller *et al.*[6] from *T. wallichiana* Zucc.

Fraction B (7.0 g) on repeated column chromatography and preparative TLC using $CHCl_3$ – MeOH (10 : 0.5) as irrigant afforded 200 mg compound **2** as a solid, m.p. 296°-98°; $[\alpha]_D^{30}$ + 64° ($CHCl_3$); IR ($CHCl_3$): 1745, 1740, 1725, 1715, 1630 and 1430 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 1.85 (m, 1H, H-1), 1.90 (m, 1H, H-2α), 2.60 (m, 1H, H-2β), 2.80 (m, 1H, H-3), 5.40 (brt, 1H, J = 1.5 Hz, H-5), 1.65 (m, 1H, H-6α), 1.30 (m, 1H, H-6β), 5.45 (dd, 1H, J = 4.0, 9.5 Hz, H-7), 5.85 (d, 1H, J = 10.0 Hz, H-9), 6.30 (d, 1H, J = 10.0 Hz, H-10), 5.93 (tq, 1H, J = 4.0, 1.0 Hz, H-13), 1.04 (m, 1H, H-14α), 2.62 (m, 1H, H-14β), 0.80 (s, 3H, H-19), 1.10 (s, 3H, H-17), 2.28 (d, 1H, J = 1.0 Hz, H-18), 1.65 (s, 3H, H-19), 5.25 (brs, 1H, H-20α); 4.95 (brs, 1H, H-20β), OCOR : 7.30m (5H, H-2″,3″,4″,5″,6″), 2.95 (1H, J = 16.0, 7.0 Hz, H-2′α), 2.80 (1H, J = 16.0, 7.0 Hz, H-2β), 3.95t (1H, J = 7.0 Hz, H-3′), $-N-(CH_3)_2$: 2.20 (s, 6H), OAc : 2.00 (s, 3H), 2.02 (s, 6H), 2.05 (s, 3H); ^{13}C NMR ($CDCl_3$, 50.13 MHz): δ 40.77 (d, C-1), 27.51 (t, C-2), 38.0 (d, C-3), 138.29 (s, C-4), 74.26 (d, C-5), 31.64 (t, C-6), 70.33 (d, C-7), 39.67 (s, C-8), 70.75 (d, C-9), 71.99 (d, C-10), 147.00 (s, C-11), 137.52 (s, C-12), 77.14 (d, C-13), 34.42 (t, C-14), 46.34 (s, C-15), 27.69 (q, C-16), 34.42 (q, C-17), 3.05 (q, C-18), 15.24 (q, C-19), 115.80 (t, C-20), OCOR : 128.50 (d, C-1″, C-5″), 127.67 (s, C-6″), 169.09 (s, C-1′), 39.67 (t, C-2′), 67.18 (d, C-3′), NMe_2: 42.25 (q, 6H), $OCOCH_3$: 21.42 (q), 21.30 (q), 20.99 (q), 20.85 (q), $OCOCH_3$: 170.75 (s), 170.54 (s), 170.21 (s) and 169.40 (s).

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