Two New Taxoids from *Taxus chinensis*

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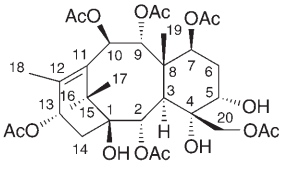
Two new taxoids, 2,20-*O*-diacetyltaxumairol N (**1**) and 14β-hydroxy-10-deacetyl-2-*O*-debenzoylbacatin III (**2**), were isolated from the needles and stems of *Taxus chinensis*. Their structures were determined on the basis of extensive 1D- and 2D-NMR-spectral analysis. Compound **1** showed weak cytotoxicity activity against T-24 ($IC_{50} = 34$ μg/ml) and QGY-7701 ($IC_{50} = 22$ μg/ml) cancer lines. Compound **2** showed no obvious cytotoxicity activity against T-24 ($IC_{50} > 100$ μg/ml) and QGY-7701 ($IC_{50} > 100$ μg/ml) cancer lines.

1. Introduction. – *Taxus chinensis* (Taxaceae), widely distributed in the southwest part of China, has attracted much attention in the last 20 years due to the presence of paclitaxel and structurally related taxanes. Although paclitaxel is an interesting and effective anticancer agent, the multidrug resistance (MDR) limited its application during the therapy. For this reason, recent research was aimed at the discovery of more effective and less toxic analogues of paclitaxel [1]. To obtain the semi-synthesis predecessor, we investigated the chemical constituents of a large-scale extract of needles and stems of *T. chinensis*. Except for the large-scale taxanes for semi-synthesis, some new compounds were also isolated as by-products [2]. In the course of our continuing phytochemical studies, two new taxanes, 2,20-*O*-diacetyltaxumairol N (**1**) and 14β-hydroxy-10-deacetyl-2-de(benzoyloxy)bacatin III (**2**), were isolated from the needles and stems of this plant. Compound **1** showed weak cytotoxicity activity against T-24 ($IC_{50} = 34$ μg/ml) and QGY-7701 ($IC_{50} = 22$ μg/ml) cancer lines. Compound **2** showed no obvious cytotoxicity activity against T-24 ($IC_{50} > 100$ μg/ml) and QGY-7701 ($IC_{50} > 100$ μg/ml) cancer lines. In this paper, the structure elucidation of these two new compounds was reported.



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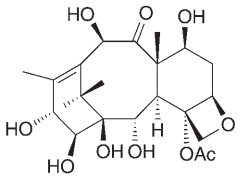

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2. Results and Discussion. – The extract of needles and stems of *T. chinesis* afforded, after extensive chromatographic purification and finally purification by HPLC, two new taxoids, **1** and **2**.

Compound **1**, colorless crystals, $[\alpha]_D^{25} = +46.1$ ($c = 0.35$, MeOH), was proved to have a molecular formula $C_{32}H_{46}O_{15}$ from combined analysis of its HR-ESI-MS at *m/z* 693.2729 ($C_{32}H_{46}NaO_{15}^+$; calc. 693.2734) and ^{13}C-NMR spectra. Intense absorptions at 1632, 1742, and 3451 cm^{-1} in its IR spectrum implied that **1** possesses C=C bonds, esters, and OH groups, respectively. The ^{1}H- and ^{13}C-NMR (including DEPT) spectra of **1** indicated the presence of four Me, two CH_2, one methyleneoxy, one methine, and six methinoxy moieties, two quaternary, two oxyquaternary, and two olefinic quaternary C-atoms, and six AcO groups (see *Exper. Part*). Complete assignments of the ^{1}H- and ^{13}C-NMR signals were achieved by using various NMR techniques such as ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra. The ^{1}H-NMR signals at δ(H) 1.30, 2.05, 2.19, and 2.65 ppm due to four Me groups were characteristic of the taxane skeleton. Furthermore, the signals at δ(C) 75.9 (*s*, C(1)), 48.2 (*s*, C(8)), and 43.4 (*s*, C(15)) in the ^{13}C-NMR spectrum indicated that **1** possesses the basic 6/8/6 taxane skeleton [3]. Comparing the spectral data of **1** with those of taxumairol N indicated that it was very similar to taxumairol N, except for the two AcO groups located at C(2) and C(20) [4]. Interpretation of HMBC correlations permitted the 6/8/6 skeleton confirmation and the positional assignment of the six AcO groups (*Fig. 1*). The HMBC correlations for H–C(2) (δ(H) 6.39)/C(1) (δ(C) 75.9) and C(8) (δ(C) 48.2), Me(16) (δ(H) 2.19) and Me(17) (δ(H)1.30)/C(1) (δ(C) 75.9), Me(19) (δ(H) 2.05)/C(9) (δ(C) 76.6), C(8) (δ(C) 48.2), C(7) (δ(C) 70.3), and C(3) (δ(C) 45.0) confirmed the 6/8/6 skeleton system of **1**. The positional assignments of the six AcO groups of **1** were achieved by HMBC correlations of H–C(2) (δ(H) 6.39)/C(MeCO) (δ(C) 169.8), H–C(7) (δ(H) 6.38)/C(MeCO) (δ(C) 170.2), H–C(9) (δ(H) 6.69)/C(MeCO) (δ(C) 170.6), H–C(10) (δ(H) 6.81)/C(MeCO) (δ(C) 169.4), H–C(13) (δ(H) 6.26)/C(MeCO) (δ(C) 170.6), and H_a–C(20) (δ(H) 5.76) and H_b–C(20) (δ(H) 5.28)/C(MeCO) (δ(C) 171.5). The ROESY experiment established the relative configuration of **1** (*Fig. 2*). The β-orientation of H–C(2), H–C(9), H–C(5), and H–C(13) was confirmed by ROESY cross-peaks of H–C(2)/Me(19), H–C(2)/H–C(9), H–C(5)/H_a–C(20) and H_b–C(20), and H–C(13)/Me(16), while the α-orientation of H–C(3), H–C(7), and H–C(10) was deduced from the correlations of H–C(3)/H–C(7), H–C(7)/H–C(10), and H–C(10)/H–C(7). So, comparing these spectral data with those of taxumairol N, we established the structure of **1** to be *2,20*-O-*diacetyltaxumairol N.*

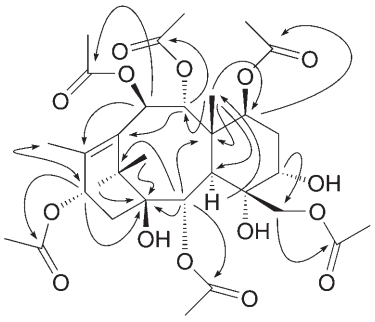

Fig. 1. *Key HMBC correlations of* **1**

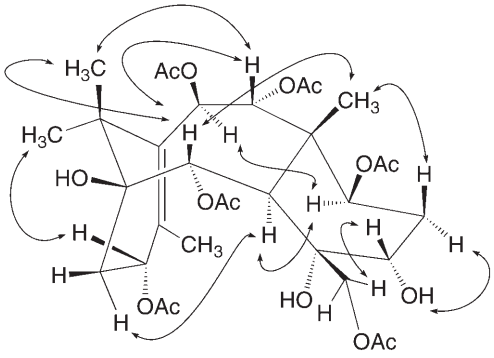

Fig. 2. *Selected ROESY correlations of* **1**

Compound **2** was isolated as colorless needles. The positive FAB-MS afforded a molecular-ion peak at *m/z* 457 ($[M+1]^+$), which was confirmed by positive HR-FAB-MS ($C_{22}H_{33}O_{10}^+$; found 457.1893, calc. 457.1893), corresponding to the molecular formula $C_{22}H_{32}O_{10.}$ Its IR spectrum showed a C=O (1702 cm^{-1}) and an OH group (3400 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of 14β-hydroxy-10-deacetylbacatin III, except for the absence of one PhCOO group at C(2) [5]. Relative upfield shift of H−C(2) (δ(H) 3.93) suggested that one OH group was located at C(2). The relative configuration of **2** was proved to be the same as that of baccatin III according to the ROESY spectrum. The β-orientation of H−C(2) and H−C(13) was confirmed by ROESY cross-peaks of H−C(2)/Me(19), H−C(2)/Me(17), and H−C(13)/Me(16), while the α-orientation of H−C(3), H−C(7), H−C(10), and H−C(14) was deduced from the correlations of H−C(3)/H−C(7), H−C(7)/H−C(10), H−C(10)/H−C(7), and H−C(14)/H−C(3). The α-orientation of H−C(5) was determined by the ROESY correlation between H−C(5) and H_α-C(20). Therefore, compound **2** was characterized as *14β-hydroxy-10-deacetyl-2*-O-*debenzoylbacatin III*.

The new compounds were assessed using MTT assay [6] against against T-24 and QGY-7701 cancer lines. Compound **1** showed weak cytotoxicity activity against T-24 (IC_{50} = 34 μg/ml) and QGY-7701 (IC_{50} = 22 μg/ml) cancer lines, whereas **2** showed no obvious cytotoxicity activity against T-24 (IC_{50} > 100 μg/ml) and QGY-7701 (IC_{50} > 100 μg/ml) cancer lines.

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Experimental Part

General. Column chromatography (CC): silica gel (100–200 mesh, *Qingdao Marine Chemical Inc.*, Qingdao, P. R. China), and silica gel *H* (10–40 μm, *Qingdao Marine Chemical Inc.*). Fractions were monitored by TLC, and spots were visualized by heating silica-gel plates sprayed with 10% H_2SO_4 in EtOH. M.p.: *XRC-1* apparatus; uncorrected. Optical rotations: *Horiba SEAP-300* spectropolarimeter. UV Spectra: *Shimadzu* double-beam *210A* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS-135* IR spectrophotometer, with KBr discs; in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker AM-400* and *DRX-500* instruments with TMS as

internal standards; chemical shifts δ in ppm with reference to the solvent signals, *J* in Hz. MS: *VG Auto Spec-3000* spectrometer; in *m/z* (rel. %).

Plant Material. The leaves and stems of *T. chinensis* were collected in Sichuan Province, P. R. China, in June 2000, and were authenticated by Prof. *Zhong-Wen Lin*. A voucher specimen (No. 20013) has been deposited at the Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The needles and stems of *T. chinensis* (100 kg) were air-dried, milled, extracted (EtOH), and fractionated on silica-gel CC (2.0 kg, 100–200 mesh) by gradient solvent systems $CHCl_3/Me_2CO$ (9:1, 8:2, 7:3, 6:4, 5:5, resp.) to afford seven fractions. *Fr. 1* was subjected to *RP-18*, *Sephadex*, and silica gel repeatedly to give a residue (276 mg), which was purified by HPLC (*SB-C18*, $MeOH/H_2O$ 40:60; t_R 22.736 min) to give compound **1** (35 mg). *Fr. 7* was subjected to dry-CC on silica gel, with i-PrOH/$CHCl_3$ (1:19), and six fractions were collected. Then, *Fr. 2* was subjected to *RP-18*, *Sephadex*, and silica gel repeatedly to give a residue (194 mg), which was purified by HPLC (*SB-C18*, H_2O/MeOH 20:80, t_R 8.084 min) to afford compound **2** (22 mg).

*2,20-*O*-Diacetyltaxumairol N* (**1**): colorless crystals (acetone). M.p. 121–123° (acetone). $[\alpha]_D^{25} = +46.1°$ ($c = 0.35$; MeOH). UV (MeOH): 206 (3.87). IR (KBr): 3451, 2992, 2940, 1742, 1632, 1441, 1375, 1238, 1034, 984. ^{1}H-NMR ((D$_5$)pyridine, 400 MHz): 6.81 (*d*, $J = 11.2$, H_α–C(10)); 6.69 (*d*, $J = 11.2$, H_β–C(9)); 6.39 (*d*, $J = 3.5$, H_β–C(2)); 6.38 (overlapped, H_α–C(7)); 6.26 (br. *d*, $J = 8.3$, H_β–C(13)); 5.76 (*d*, $J = 9.5$, H_a–C(20)); 5.28 (*d*, $J = 9.5$, H_b–C(20)); 4.48 (br. *s*, H_β–C(5)); 3.37 (*d*, $J = 3.5$, H_α–C(3)); 3.00 (*m*, H_β–C(14)); 2.80 (*m*, H_α–C(14)); 2.71 (*m*, H_β–C(6)); 2.65 (*s*, Me(18)); 2.19 (*s*, Me(16)); 2.18 (*s*, MeCOO–C(9)); 2.18 (*s*, MeCOO–C(2)); 2.15 (*s*, MeCOO–C(13)); 2.12 (*m*, H_α–C(6)); 2.07 (*s*, MeCOO–C(7)); 2.05 (*s*, Me(19)); 2.03 (*s*, MeCOO–C(10)); 1.84 (*s*, MeCOO–C(20)); 1.30 (*s*, Me(17)). ^{13}C-NMR ((D$_5$)pyridine, 100 MHz): 171.5 (*s*, Me*C*OO–C(20)); 170.6 (*s*, Me*C*OO–C(9)); 170.6 (*s*, Me*C*OO–C (13)); 170.2 (*s*, Me*C*OO–C(7)); 169.8 (*s*, Me*C*OO–C(2)); 169.4 (*s*, Me*C*OO–C (10)); 140.5 (*s*, C(12)); 136.5 (*s*, C(11)); 78.1 (*s*, C(4)); 76.6 (*d*, C(9)); 75.9 (*s*, C(1)); 73.4 (*d*, C(2)); 72.2 (*d*, C(10)); 70.9 (*d*, C(13)); 70.3 (*d*, C(7)); 69.8 (*t*, C(20)); 69.6 (*d*, C(5)); 48.2 (*s*, C(8)); 45.0 (*d*, C(3)); 43.4 (*s*, C(15)); 38.7 (*t*, C(14)); 33.5 (*t*, C(6)); 29.3 (*q*, C(17)); 22.5 (*q*, C(16)); 21.8 (*q*, *Me*COO–C(20)); 21.7 (*q*, *Me*COO–C(13)); 21.0 (*q*, *Me*COO–C(9)); 21.0 (*q*, *Me*COO–C(7)); 20.9 (*q*, *Me*COO–C(2)); 20.9 (*q*, *Me*COO–C(10)); 16.0 (*q*, C(18)); 14.9 (*q*, C(19)). ESI-MS (pos.): 693 (100, $[M + Na]^+$). HR-ESI-MS (pos.): 693.2729 ($C_{32}H_{46}NaO_{15}^+$; calc. 693.2734).

*14β-Hydroxy-10-deacetyl-2-*O*-debenzoylbacatin III* (**2**): colorless needles (acetone). M.p. 201–203° (acetone). $[\alpha]_D^{25} = -8.4$ ($c = 0.062$; MeOH). UV (MeOH): 203 (4.16). IR (KBr): 3433, 3019, 2939, 1702, 1633, 1439, 1390, 1373, 1242, 1169, 1135, 1093, 1059, 1019, 992. ^{1}H-NMR ((D$_6$)acetone, 500 MHz): 5.16 (*s*, H_α–C(10)); 4.90 (*dd*, $J = 9.6, 2.0$, H_β–C(5)); 4.65 (br. *d*, $J = 6.7$, H_β–C(13)); 4.59 (*d*, $J = 8.9$, H_β–C(20)); 4.42 (*d*, $J = 8.9$, H_α–C(20)); 4.28 (*dd*, $J = 10.7, 6.4$, H_α–C(7)); 3.93 (*d*, $J = 6.8$, H_β–C(2)); 3.82 (*d*, $J = 6.3$, H_α–C(14)); 3.60 (*d*, $J = 6.8$, H_α–C(3)); 3.38 (*m*, H_α–C(6)); 2.10 (*s*, MeCO); 1.98 (*s*, Me(18)); 1.80 (*m*, H_β–C(6)); 1.64 (*s*, Me(19)); 1.06 (*s*, Me(16)); 0.95 (*s*, Me(17)). ^{13}C-NMR ((D$_6$)acetone, 100 MHz): 212.7 (*s*, C(9)); 170.9 (*s*, Me*C*O); 140.6 (*s*, C(12)); 136.8 (*s*, C(11)); 84.8 (*d*, C(5)); 82.3 (*s*, C(4)); 78.5 (*t*, C(20)); 76.9 (*s*, C(1)); 76.4 (*d*, C(13)); 75.7 (*d*, C(10)); 73.7 (*d*, C(2)); 73.5 (*d*, C(14)); 72.4 (*d*, C(7)); 58.7 (*s*, C(8)); 47.0 (*d*, C(3)); 43.4 (*s*, C(15)); 37.8 (*t*, C(6)); 26.8 (*q*, C(16)); 22.5 (*q*, *Me*CO); 21.7 (*q*, C(17)); 15.1 (*q*, C(18)); 10.4 (*q*, C(19)). FAB-MS (pos.): 457 (100, $[M + 1]^+$), 421 (6), 360 (10), 328 (51), 239 (2), 175 (37). HR-FAB-MS (pos.): 457.1893 ($C_{22}H_{33}O_{10}^+$; calc. 457.1893).

Cytotoxic Activity. The compounds **1** and **2** were examined for their cytotoxic activity against human cell lines (T-24 and QGY). Cancer cells were incubated for 48 h at 37° in the presence of various concentrations of compounds from DMSO-diluted stock soln. The growth inhibitory property was determined by *in-vitro* treatment of respective cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

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