HETEROCYCLES, Vol. 65, No. 6, 2005, pp. 1403 - 1408
Received, 13th December, 2004, Accepted, 20th April, 2005, Published online, 22nd April, 2005

TWO NEW TAXOIDS FROM THE NEEDLES AND STEMS OF *TAXUS CHINENSIS*

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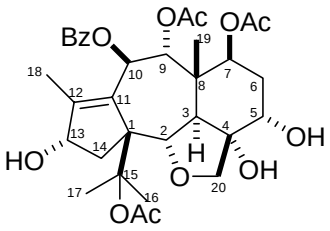
Abstract –Two new taxoids, named 10-*O*-benzoyl-15-*O*-acetyltaxumairol X (**1**) and 5-*O*-acetyl-20-*O*-deacetyl-4,20-*p*-hydroxylbenzylidenedioxytaxuyunnanine L (**2**), were isolated from the needles and stems of *Taxus chinensis.* Their structures were determined on the basis of combined 1D and 2D spectral techniques.

INTRODUCTION

Taxus species are a rich source of biologically active taxoids with unique structural features. Although more than four hundred taxoids have been isolated from various yew trees, there are still new ones being isolated continually, some of which may either be directly active, or serve as precursors for the semi-synthesis of other active analogs.[1] In our previous studies, some new taxoids from the needles and stems of *T. chinensis* were reported.[2] Further investigation on the extract of the same part of this plant (collected in June, 2000) resulted in the isolation of two new taxoids, 10-*O*-benzoyl-15-*O*-acetyl taxumairol X (**1**) and 5-*O*-acetyl-20-*O*-deacetyl-4,20-*p*-hydroxylbenzylidenedioxytaxyunnanine L (**2**). In general, taxoids without side chain and oxetane have no cytotoxity activity. Two compounds were assessed using MTT assay. However, neither of them showed obvious cytotoxicity activity against T-24, QGY-7701 and MCF-7 cancer line. The IC_{50} of two new compounds against three cancer lines were more than 100 μg/mL. In this paper, we describe the isolation and the structural elucidation of the two new compounds (**1**) and (**2**).



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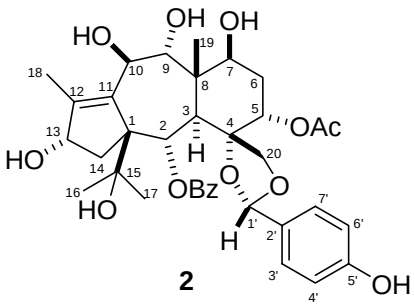

2

RESULTS AND DISCUSSION

Compound (**1**), colorless needles, mp 130-131°C, $[\alpha]_D^{25}$: +64.1° (*c* 0.27, MeOH), showed a positive HR-ESIMS $[M + Na]^+$ ion peak at *m/z* 653.2583 (calcd for $C_{33}H_{42}O_{12}Na$ 653.2574), corresponding to the formula of $C_{33}H_{42}O_{12}$. Its IR absorptions at 1637, 1724 and 3443 cm^{-1} indicated the presence of double bonds, ester and hydroxy groups, respectively. The UV spectrum showed absorption at 203 (4.48), 230 (4.10) nm. The characteristic ^1H NMR signals of **1** at δ_H 1.51 (3H), 1.53 (3H), 1.67 (3H) and 1.86 (3H) implied that it possesses taxane skeleton. On the basis of H-H COSY, HMQC and HMBC, full assignments of the ^1H and ^{13}C NMR signals of **1** were made (see experimental section). The downfield chemical shifts for C-1 (δ_C 67.1) suggested that **1** possesses the rearranged 11(15→1) abeo-taxane skeleton with reference to the spectral data of known compounds.[3] In comparison of the NMR spectral data of **1** with those of taxumairol X,[4] the structure of **1** was similar to taxumairol X except for C-15 and C-10. The presence of three acetoxy groups and one benzoyloxy group was indicated by ^1H and ^{13}C NMR spectra. The positional assignments of the esters were elucidated by HMBC spectrum (Figure 1). The correlations of δ_H 5.50 (H-7) with δ_C 170.3 (s), δ_H 4.91 (H-9) with δ_C 170.5 (s), and δ_H 6.23 (H-10) with δ_C 166.6(s) suggested that two acetoxy groups and one benzoyloxy group were attached at C-7, C-9 and C-10, respectively. The remain acetoxy group was assigned to C-15 according to the unusual downshift at 89.0 (C-15), which was confirmed by HMBC correlations between the signals at δ_H 4.91 (H-2) and δ_C 89.0 (C-15), and between the signals at δ_H 1.53 (Me-16), 1.51 (Me-17) and δ_C 67.1 (C-1). The presence of a tetrahydrofuran ring fused to C-2, C-3, C-4 and C-20 was revealed by HMBC correlations between the signal at δ_H 3.49 (H-20b) and signals at δ_C 76.8 (C-2) and 81.7 (C-4).

The relative stereochemistry of **1** was elucidated by the ROESY experiment (Figure 2). In its ROESY spectrum, the correlations of H-2/Me-19, H-5/H-20β, H-9/ Me-19, and H-13/ H-14β and Me-16 suggested that H-2, H-5, H-9 and H-13 of **1** were β-oriented. H-3, H-7 and H-10 were α-oriented according to the ROESY correlations of H-3/H-7 and H-10/H-18. Thus, the structure of **1** was assigned to be 10-*O*-benzoyl-15-*O*-acetyltaxumairol X.

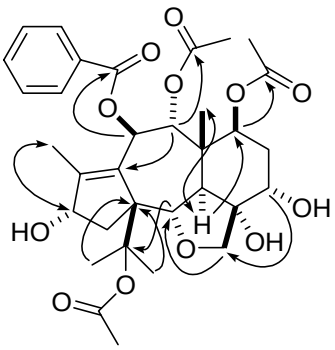

Figure 1. The key HMBC correlations of **1**

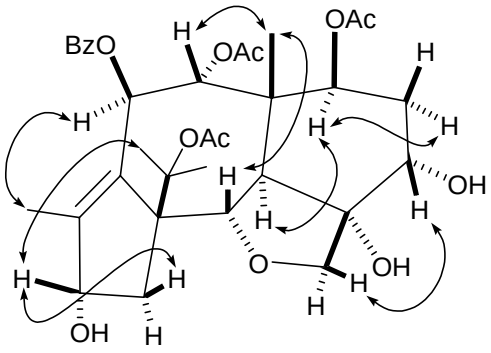

Figure 2. The selected ROESY correlations of **1**

Compound (**2**), colorless needles, mp 239-240°C, showed a quasi-ion $[M + Na]^+$ at *m/z* 691.2736 (calcd for $C_{36}H_{44}O_{12}Na$ 691.2731) in its positive HR-ESIMS spectrum, consistent with the molecular formula of $C_{36}H_{44}O_{12}$. The IR spectrum indicated the presence of double bonds (1616 cm^{-1}), ester (1712 cm^{-1}) and hydroxyl (3419 cm^{-1}) groups. The UV spectrum showed absorption at 203 (4.48), 230 (4.10), 254 (4.37) nm. Comparing the NMR spectral data of **2** with those of **1**, we could make a conclusion that the two compounds possessed the same taxane skeleton. The ^1H and ^{13}C NMR spectral data of **2** showed the presence of one acetoxy group and one benzoyloxy group. The proton signals at δ_H 5.29 (1H, s), 6.65 (2H, dd, J = 6.7, 2.0 Hz) and 6.83 (2H, dd, J = 6.7, 2.0 Hz) and carbon signals at δ_C 104.2 (d), 129.6 (s), 129.5 (d), 115.4 (d), and 159.0 (s) indicated the existence of a *p*-hydroxylbenzylidenedioxy. Prominent fragment ion peaks at *m/z* 547[M - $C_7H_6O_2^+$ + H^+], 515 [M - $C_7H_6O_2^+$ - $2H_2O$ + H^+], 139 [$C_7H_6O_3^+$ + H^+] and 107 [$C_7H_6O_2^+$ – H_2O + H^+] in its positive ESIMS spectrum proved the above deduce. The *p*-hydroxylbenzylidenedioxy was attached to C-4 and C-20, which was supported by the HMBC correlations of H-1'/C-4 and C-2', and H-20β/C-1'. The acetoxy group was assigned to C-5 based on the observation of HMBC correlation between H-5 (δ_H 4.99, br t, J = 2.2 Hz) and the carbonyl of acetoxy group (δ_C 170.4, s), while the benzoyloxy group was located at C-2 because of the HMBC correlation between H-2 (δ_H 6.05, d, J = 6.9 Hz) and the signal located at δ_c 168.3 (s, O<u>C</u>OC_6H_5).

The relative stereochemistry of **2** was determined on the basis of ROESY experiment (Figure 4). The ROESY correlations of H-2/Me-19, H-5/H-20β, H-9/Me-19, and H-13/H-14β and Me-16 suggested that H-2, H-5, H-9 and H-13 of **1** were β-oriented. The configurations of H-3, H-7 and H-10 were α-oriented judged by the ROESY correlations of H-3/H-7 and H-10/H-18. The configuration of H-1' was proved to be β-oriented according to the ROESY correlation between H-1'and H-20β.

Except for the *p*-hydroxylbenzylidenedioxy moiety, compound (**2**) was very similar to taxuyunnanine L,[5] in which one α-oriented acetoxy group and one benzoyloxy group was located at C-20 and C-2, respectively. So, compound (**2**) was determined as 5-*O*-acetyl-20-*O*-deacetyl-4, 20-*p*-hydroxylbenzyliden edioxytaxuyunnanine L.

Cytotoxicities of two compounds were assessed using MTT assay against T-24, QGY-7701 and MCF-7 cancer line. However, neither of them showed obvious activity (IC_{50}>100 μg/mL).

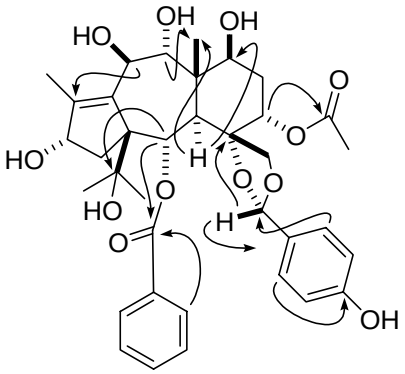

Figure 3. The key HMBC correlations of **2**

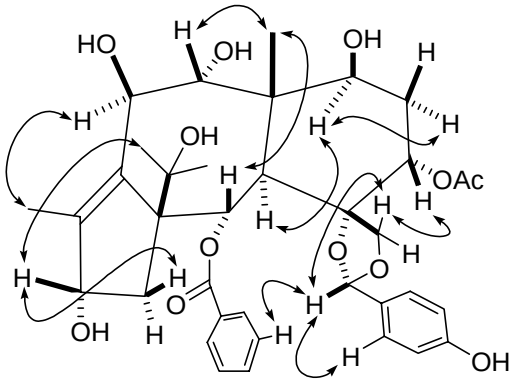

Figure 4. The selected ROESY correlations of **2**

EXPERIMENTAL

General Experimental Procedures. Melting points were obtained on an XRC-1 apparatus, and are uncorrected. Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were taken on a Shimadzu double-beam 210A Spectrophotometer. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer as KBr pellets. 1D and 2D NMR spectra were run on Bruker AM-400 and DRX-500 instruments. MS spectra were recorded on a VG Auto Spec-3000 spectrometer. Column chromatography was performed on silica gel (200-300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China) and silica gel H (10-40 μm, Qingdao Marine Chemical Inc.). half-preparative HPLC was performed on a Hewlett Packard instrument (column: Zorbax SB-C18, 250×9.4 mm; UV DAD detector). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The needles and stems of *T. chinensis* (*Taxaceae*) were collected in Sichuan Province of People's Republic of China in June 2000, and identified by Prof. Zhong-Wen Lin. A voucher specimen (No. 20013) has been deposited at the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The needles and stems of *T. chinensis* (100 Kg) were air-dried, milled, and extracted three times with EtOH (3×500 L) at rt for 24 h. The extract was fractionated on silica gel column chromatography (2.0 kg, 100-200 mesh) by gradient elution with $CHCl_3$ by increasing concentration of Me_2CO [$CHCl_3$-Me_2CO/9:1, 8:2, 7:3, 6:4, 5:5, 0:100 respectively] to afford 6 fractions. The $CHCl_3$-Me_2CO/5:5 fractions was subjected to silica gel (100-200 mesh) eluted by $CHCl_3$-Me_2CO/19:1 to give a residue (285 mg), which was purified by HPLC (HPLC conditions for **1**:

MeOH:H_2O/55:45, 254 nm; for **2**: MeOH:H_2O/55:45, 254 nm) in conjunction with crystallization to obtain two new compounds (**1**) (33 mg) and (**2**) (19 mg).

10-*O*-Benzoyl-15-*O*-acetyltaxumairol X (**1**)**:** colorless needles, mp 130-131°C (MeOH), $[\alpha]_D^{25}$: + 64.1° (*c* 0.27, MeOH), UV $_{MeOH}$ λ_{max} (log ε): 203 (4.48), 230 (4.10) nm, IR (KBr) ν_{max} cm^{-1}: 3443, 2952, 1724, 1637, 1451, 1372, 1270, 1149, 1070, 1028, 933. ^{13}C and ^1H NMR (acetone-d_6): ^{13}C NMR: 67.1 (C-1, s), 76.8 (C-2, d), 43.6 (C-3, d), 81.7 (C-4, s), 68.6 (C-5, d), 31.4 (C-6, t), 71.6 (C-7, d), 43.6 (C-8, s), 76.5 (C-9, d), 71.6 (C-10, d), 134.1 (C-11, s), 152.6 (C-12, s), 77.4 (C-13, d), 41.2 (C-14, t), 89.0 (C-15, s), 23.4 (C-16, q), 24.2 (C-17, q), 13.3 (C-18, q), 16.0 (C-19, q), 75.1 (C-20, t), 22.9 (q, 15-OCO$\underline{C}H_3$), 21.1 (q, 7-OCO$\underline{C}H_3$), 21.1 (q, 9-OCO$\underline{C}H_3$), 170.3 (s, 7-O$\underline{C}OCH_3$), 170.5 (s, 9-O$\underline{C}OCH_3$), 170.8 (s, 15-O$\underline{C}OCH_3$), 166.6 (s, O$\underline{C}OC_6H_5$), 131.2 (s, *i*-OCO$\underline{C}_6H_5$), 130.5 (d, *o*-OCO$\underline{C}_6H_5$), 129.5 (d, *m*-OCO$\underline{C}_6H_5$), 134.1 (d, *p*-OCO$\underline{C}_6H_5$). ^1H NMR: 4.91 (1H, d, *J* = 11.3 Hz, H-2β), 2.49 (1H, d, *J* = 11.3 Hz, H-3α), 3.99 (1H, br s, H-5β), 2.30 (1H, m, H-6α), 1.76 (1H, m, H-6β), 5.50 (1H, dd, *J* = 12.1, 4.5 Hz, H-7α), 4.91 (1H, d, *J* = 5.2 Hz, H-9β), 6.23 (1H, d, *J* = 5.2 Hz, H-10β), 4.64 (1H, br t, *J* = 5.5 Hz, H-13β), 2.47 (1H, m, H-14α), 1.69 (1H, m, H-14β), 1.53 (3H, s, Me-16), 1.51 (3H, s, Me-17), 1.86 (3H, s, Me-18), 1.67 (3H, s, Me-19), 3.89 (1H, d, *J* = 8.2 Hz, H-20α), 3.49 (1H, d, *J* = 8.2 Hz, H-20β), 2.08 (3H, s, 9-OCOCH_3), 1.90 (3H, s, 7-OCOCH_3), 1.51 (3H, s, 15-OCOCH_3), 8.00 (2H, m, *o*-OCOC_6H_5), 7.53 (2H, m, *m*-OCOC_6H_5), 7.65 (1H, m, *p*-OCOC_6H_5). Positive ESIMS *m/z* (%): 653 $[M + Na]^+$ (100). Positive HR-ESIMS: 653.2583 (Calcd for $C_{33}H_{42}O_{12}Na$ 653.2573).

5-*O*-Acetyl-20-*O*-deacetyl-4,20-*p*-hydroxylbenzylidenedioxytaxyunnanine L (**2**)**:** colorless needles, mp 239-240°C (MeOH), $[\alpha]_D^{25}$: +29.8° (*c* 0.51, MeOH). UV $_{MeOH}$ λ_{max} (log ε): 203 (4.48), 230 (4.10), 254 (4.37) nm. IR (KBr) ν_{max} cm^{-1}: 3419, 2975, 1712, 1616, 1519, 1451, 1378, 1262, 1073, 1045, 975, 836, 715. ^{13}C and ^1H NMR (acetone-d_6): 69.6 (C-1, s), 72.4 (C-2, d), 43.5 (C-3, d), 83.7 (C-4, s), 74.9 (C-5, d), 33.8 (C-6, t), 77.9 (C-7, d), 45.0 (C-8, s), 81.2 (C-9, d), 69.8 (C-10, d), 138.4 (C-11, s), 147.2 (C-12, s), 69.5 (C-13, d), 39.9 (C-14, t), 76.3 (C-15, s), 26.3 (C-16, q), 28.7 (C-17, q), 13.4 (C-18, q), 11.4 (C-19, q), 70.1 (C-20, t), 104.2 (C-1', d), 129.6 (C-2', s), 129.5 (C-3', d), 115.4 (C-4', d), 159.0 (C-5', s), 21.0 (q, 5-OCO$\underline{C}H_3$), 170.4 (s, 5-O$\underline{C}OCH_3$), 168.3 (s, O$\underline{C}OC_6H_5$), 132.1 (s, *i*-OCO$\underline{C}_6H_5$), 130.8 (d, *o*-OCO$\underline{C}_6H_5$), 129.8 (d, *m*-OCO$\underline{C}_6H_5$), 134.4 (d, *p*-OCO$\underline{C}_6H_5$). ^1H NMR: 6.05 (1H, d, *J* = 6.9 Hz, H-2), 3.47 (1H, d, *J* = 6.9 Hz, H-3), 4.99 (1H, br t, *J* = 2.2 Hz, H-5), 1.95 (1H, m, H-6α), 1.87 (1H, m, H-6β), 4.09 (1H, dd, *J* = 9.0, 4.2 Hz, H-7α), 4.38 (1H, dd, *J* = 7.9, 3.3 Hz, H-9β), 4.67 (1H, d, *J* = 7.9 Hz, H-10α), 4.67 (1H, overlap, H-13α), 2.63 (1H, m, H-14α), 2.34 (1H, m, H-14β), 1.09 (3H, s, Me-16), 2.08 (3H, s, Me-17), 1.95 (3H, s, Me-18), 1.67 (3H, s, Me-19), 4.10 (1H, d, *J* = 9.8 Hz, H-20α), 3.88 (1H, d, *J* = 9.8 Hz, H-20β), 5.29 (1H, s, H-1'), 6.83 (2H, dd, *J* = 6.7, 2.0 Hz, H-3' and H-7'), 6.65 (2H, dd, *J* = 6.7, 2.0 Hz, H-4' and H-6), 2.04 (3H, s, 5-OCOCH_3), 8.13 (2H, m, *o*-OCOC_6H_5), 7.62 (2H, m, *m*-OCOC_6H_5), 7.73

(1H, m, *p*-$OCOC_6H_5$). Positive ESIMS *m/z* (%): 691 $[M + Na]^+$ (100). Positive HR-ESIMS: 691.2736 (Calcd for $C_{36}H_{44}O_{12}Na$ 691.2731).

Cytotoxic activity: The compounds (**1-2**) were examined for their cytotoxic activity against humane cell lines (T-24, QGY and MCF-7). Cancer cells were incubated for 48 h at 37℃ in the presence of various concentrations of compounds from DMSO-diluted stock. The growth inhibitory property was determined by in vitro treatment of respective cell lines using the 3-(4,5-dimethylthiazo-2-yl) -2,5diphenyltetrazolium bromide (MTT) assay.

ACKNOWLEDGEMENTS

The authors are grateful to the Bioscience and Biotechnique Foundation of Chinese Academy of Sciences (Project No. STZ-01-15) for financial support, and the members of analytical group for the spectral measurements in the Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

REFERENCES

1. E. Baloglu and D. G. I. Kingston, *J. Nat. Prod*., 1999, **62**, 1448.
2. F. S. Wang, L. Y. Peng, Y. Zhao, K. Gu, Q. S. Zhao, and H. D. Sun, *J. Nat. Prod.*, 2004, **67**, 905.
3. S. J. Yang, J. M. Fang, and Y. S. Cheng, *Phytochemistry*, 1999, **50**, 127.
4. Y. C. Shen, Y. T. Chang, S. S. Wang, Y. C. Lin, and C. Y. Chen, *Chem. Pharm. Bull.*, 2002, **50**, 1561.
5. S. H. Li, H. J. Zhang, P. Yao, H. D. Sun, and F. H. S. Harry, *J. Nat. Prod.*, 2000, **63**, 1488.