HETEROCYCLES, Vol. 53, No10, 2000, pp. 2225 - 2232, Received, 12th June, 2000

PRIEURIANIN-TYPE TETRANORTRITERPENOIDS FROM THE BARK OF *DYSOXYLUM HAINANENSE*

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Abstract - From the ethanolic extract of the bark of *Dysoxylum hainanense* Merr., three new *seco*-tetranortriterpenoids structurally related to prieurianin, dysoxylumins A (**1**), B (**2**) and C (**3**) were isolated. Their structures were elucidated on the basis of 1D and 2D NMR techniques. The structure and stereochemistry of compound **1** were demonstrated by X-Ray crystallography.

In a continuing study on tetranortriterpenoids from Meliaceae,[1] we focused on the genus *Dysoxylum* with about 200 species growing naturally in India, Southeast Asia, about 10 of them distributed in Yunnan province.[2] Many of them have been used as traditional medicine by the indigenous people. *D. richii* is such an example which has been used by the indigenous Fiji people as a traditional medicine plant to treat many diseases.[3] Tetranortriterpenoids were obtained from this genus in the previous publications.[4, 5] *D. hainanense* Merr. distributed in Guangxi Zhuang Autonomous Region, Hainan province, and Xishuangbanna, Yunnan province.[2] Due to the absence of chemical study on this species, we examined the ethanolic extracts of bark from *D. hainanense*. In this note, we describe the isolation and structure elucidation of three new tetranortriterpenoids with prieurianin-type skeleton, named dysoxylumins A (**1**), B (**2**) and C (**3**) (Figure 1). The relative configuration of **1** was determined by X-ray crystallographic analysis.

Compound (**1**) was obtained as cubic crystalline (Me_2CO), analyzed for $C_{42}H_{56}O_{17}$ by negative-ion HRFABMS. Its IR spectrum showed absorption bands assigned to a hydroxyl (3571 cm^{-1}), carbonyl group (1747 cm^{-1}), and double bond (1644 cm^{-1}). The ^{1}H and ^{13}C NMR spectra showed the presence of two

acetyl [δ_C 170.3, 169.9, 21.2, 20.6; δ_H 2.05 (3H, s), 2.02 (3H, s)], a formyl [δ_C 160.1 (d), δ_H 7.95 (s)], and a methoxyl groups. The 1D and 2D NMR spectra of **1** indicated the presence of two ester substituents

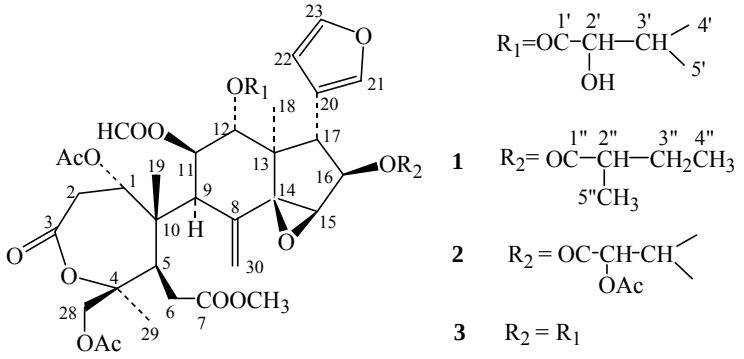

Figure 1 Structures of **1-3**

containing ten carbons. Besides above signals, compound (**1**) comprised 26 carbons and thus was assumed to be a tetranortriterpenoid. In the ^{1}H NMR spectrum, proton signals appeared as broad peaks due to the slow interconversion of rotational and multiple conformational isomer on the NMR time scale at room temperature, and signals sharpened when measured in higher temperature (60 °C) (Table 1). This evidence suggested that **1** was prieurianin derivative.[6-9] Comparing ^{1}H NMR spectrum of **1** with that of 14,15β-epoxyprieurianin[9] showed good agreements for H-1, 9, 11, 12, 15, 30. Instead of a $C_6H_{11}O_2$-moiety in 14,15β-epoxyprieurianin, two ester substituting groups presented in **1**. The ester group attached to C-12 was determined as 2-hydroxy-3-methylbutyrate on the basis of the ^{1}H-^{1}H COSY spectrum of **1**, with correlation between δ_H 5.83 (br s, H-12) with 5.54 (br s, H-11), δ_H 1.70 (m, H-3') with 0.85 (3H, d, J = 6.8 Hz, H-4'), as well as H-3' with δ_H 0.66 (3H, d, J = 6.8 Hz, H-5') and 3.32 (br s, H-2').

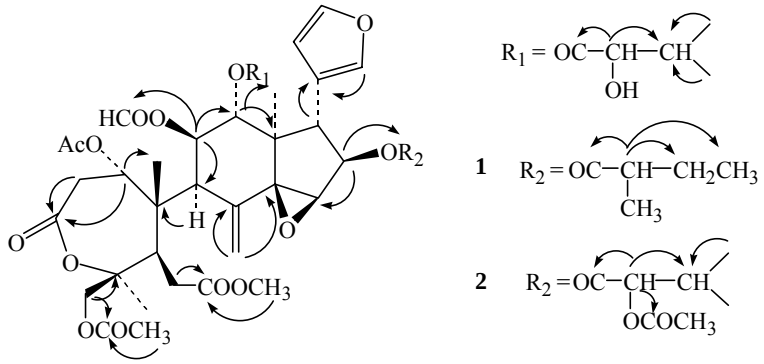

Figure 2 selected HMBC of compounds **1** and **2** (from H to C)

The other ester group was established as 2-methylbutyrate at C-16 by the HMBC spectrum (Figure 2), in which cross signals between δ_H 5.24 (d, J = 9.2 Hz, H-16) and δ_C 59.1 (d, C-15), H-16 and δ_C 176.7 (s, C-

1”), δ_H 2.32 (m, H-2”) and C-1’’, δ_H 1.35, 1.45 (each 1H, m, H-3”) and C-1’’, and δ_H 1.03 (3H, d, J = 6.8 Hz, H-5”) and C-1’’ were found. Correlation between δ_H 0.59 (3H, t, J = 7.4 Hz, H-4”) and 1.35, 1.45 (each 1H, m, H-3”), 5.24 (H-16) and 3.10 (d, J = 9.2 Hz, H-17) in the ^{1}H-^{1}H COSY spectrum also supported the assignment. NOE interactions between H-16 with H-15 and H-18, in the NOESY spectrum placed 2-methylbutyrate at 16β position. The remaining moieties and stereochemistry at the other chiral centers in **1** were identical to those of 14,15β-epoxyprieurianin based on comparing ^{1}H NMR of two compounds, and detailed analysis of ^{1}H-^{1}H COSY, HMBC, HMQC and NOESY spectral data of **1**. Finally, the structure of **1** was demonstrated unambiguously by X-Ray crystallographic analysis, which confirmed its proposed configuration, the results of which are shown in Figure 3.

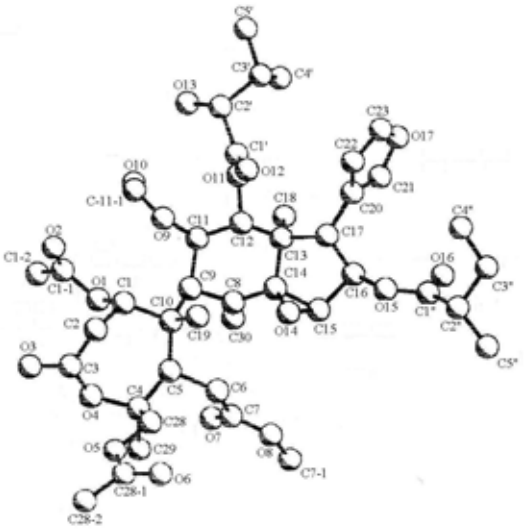

Figure 3. The crystal structure of compound **1**.

Compound (**2**) possessed the molecular formula of $C_{44}H_{58}O_{19}$ as determined by negative-ion HRFABMS. The ^{1}H NMR spectrum of **2** also showed broad signals as in that of **1**, which also resulted from a rotational barrier about the bond between C-9 and 10, suggesting that **2** was a prieurianin-type tetranortriterpenoid.[6-9] The ^{1}H and ^{13}C NMR of **2** exhibited similarities to those of **1**, with the apparent differences being in substituting group. Instead of a triplet methyl proton signal in the ^{1}H NMR spectrum of **1**, a doublet methyl and one more acetoxyl signals presented in the ^{1}H NMR spectrum of **2**, which was consistent with the evidence that molecular weight of **2** was 58 amu more than that of **1**. The correlation between δ_H 2.15 (m, H-3’) and 4.74 (d, J = 4.7 Hz, H-2”), H-3’ and 0.88 (3H, d, J = 6.8 Hz, H-4”), and H-3’ and 0.90 (3H, d, J = 6.8 Hz, H-5”) in the ^{1}H-^{1}H COSY unambiguously determined a 2-acetoxyl-3-methyl-butyrate as substituting group in **2** instead of 2-methylbutyrate in **1**. A prominent fragment ion peak at *m/z* 159 $[R_2O]^-$ in the negative-ion FABMS spectrum also supported the presence of a 2-acetoxyl-3-methylbutyrate substituting group. The position of the 2-acetoxyl-3-methylbutyrate was determined to

be at C-16 by HMBC (Figure 2).

Table 1. ^{1}H NMR Spectral Data for Compounds (**1, 2** and **3**) (in $CDCl_3$, 400 MHz).[a]

H	**1** (60 °C)[b]	**1**	**2**	**3**
1	5.54 dd (7.6, 6.8)	5.54 br s	5.57 br s	5.55 br s
2	3.12, 3.14 (m)	3.10 br	3.14 br	3.07 br
5	3.13 dd (5.2, 3.8)	3.10 br	3.14 br	3.11 br
6	2.23 dd (13.6 5.2),	2.20, 2.95 m	2.18, 3.00 m	2.22, 3.07 m
9	3.66 d (9.6)	3.61 br	3.64 br	3.60 br
11	5.54 dd (9.6, 9.5)	5.54 br s	5.57 br s	5.55 br s
12	5.87 d (9.5)	5.83 br s	5.86 br s	5.84 br s
15	3.98 s	3.94 s	3.97 s	3.96 s
16	5.26 d (9.2)	5.24 d (9.2)	5.30 d (9.2)	5.27 d (9.2)
17	3.12 d (9.2)	3.10 d (9.2)	3.14 d (9.2)	3.12 d (9.2)
18	1.07 s	1.04 s	1.04 s	1.03 s
19	1.51 s	1.48 s	1.51 s	1.48 s
21	7.31 s	7.29 s	7.35 s	7.32 s
22	6.17 d (1.3)	6.14 s	6.18 s	6.14 d (1.2)
23	7.14 s	7.10 s	7.16 s	7.12 s
28	4.25 d (12.3)	4.22 d (11.2)	4.26 d (12.1)	4.22 d (12.1)
29	1.53 s	1.48 s	1.51 s	1.48 s
30	5.37, 5.61 s	5.31, 5.53 br s	5.35, 5.57 br s	5.33, 5.55 br s
12-R_1				
2’	3. 40 brs	3. 32 br s	3.37 br s	3.33 br s
3’	1.75 m	1.70 m	1.73 m	1.72 br
4’	0.90 d (6.9)	0.88 d (6.8)	0.88 d (6.8)	0.88 d (6.8)
5’	0.71 d (6.9)	0.66 d (6.8)	0.70 d (6.8)	0.67 d (6.8)
16-R_2				
2”	2.35 m	2.32 m	4.74 d (4.7)	3.96 d (5.9)
3”	1.35, 1.40 m	1.35, 1.45 m	2.15 m	2.10 m
4”	0.67 t (7.4)	0.59 t (7.4)	0.88 d (6.8)	0.81 d (6.8)
5”	1.06 d (6.7)	1.03 d (6.8)	0.90 d (6.8)	0.94 d (6.8)
$\underline{CH_3}COO$	2.04 s	2.02 s	2.09 s	2.01 s
	2.07 s	2.05 s	2.06 s	2.03 s
$\underline{H}COO$	7.94 s	7.91 s	7.95 s	7.91 s
OCH_3	3.64 s	3.61 s	3.64 s	3.60 s

[a] Chemical shifts are in ppm and coupling constants in Hz, with TMS as internal standard.
[b] Compound (**1**) was measured on a DRX-500 spectrometer at 60 °C.

Dysoxylumin C (**3**), assigned the molecular formula as $C_{42}H_{56}O_{18}$ by negative-ion HRFABMS. The ^{1}H and ^{13}C NMR of **3** were very similar to those of **2**, except for two acetates in **3** instead of three in **2**, which was consistent with the molecular formula of **3**. According to almost identical ^{1}H and ^{13}C NMR spectral data of two compounds, compound (**3**) was assigned as a deacetylated derivative of **2**. A signal for H-2” at δ_H 4.74 (d, J = 4.7 Hz) in the ^{1}H NMR spectrum of **2** shifted upfield to δ_H 3.96 (d, J = 5.9 Hz, H-2”) in

that of **3**, which indicated the absence of acetyl group at the 2'' position in **3**. Thus **3** had a 2-hydroxy-3-methylbutyrate attached to C-16.

Table 2. ^{13}C NMR Spectral Data for Compounds (**1, 2** and **3**) (in $CDCl_3$, 100 MHz)

C	**1**	**2**	**3**
1	72.0	71.9 d	72.0 d
2	36.4	36.6 t	36.4 t
3	174.6	174.6 s	174.6 s
4	84.7	84.6 s	84.6 s
5	46.2	46.2 d	46.0 s
6	33.8	33.7 t	33.6 t
7	172.8	172.7 s	172.7 s
8	133.9	133.8 s	133.7 s
9	52.0	52.0 d	51.9 d
10	49.1	49.0 s	49.0 s
11	70.2	70.0 d	69.6 d
12	75.0	75.0 d	75.0 d
13	45.2	45.3 s	45.3 s
14	69.5	69.4 s	69.6 s
15	59.1	58.7 d	58.6 d
16	76.5	76.8 d	75.0 d
17	42.3	42.2 d	42.3 d
18	16.1	15.4 q	15.3 q
19	20.6	20.6 q	20.5 q
20	119.1	118.8 s	118.7 s
21	143.2	143.2 d	143.3 d
22	110.9	110.8 d	110.7 d
23	141.6	141.5 d	141.5 d
28	65.9	66.0 t	66.0 t
29	27.1	27.0 q	27.0 q
30	124.7	124.8 t	124.5 t
12-R_1			
1'	169.2	169.2 s	169.1 s
2'	75.0	75.0 d	74.9 d
3'	31.8	31.1 d	32.2 d
4'	16.1	17.0 q	15.8 q
5'	19.0	19.0 q	18.9 q
16-R_2			
1''	176.7	174.6 s	174.6 s
2''	40.7	77.6 q	78.0 d
3''	26.7	29.9 d	32.2 d
4''	10.8	18.3 q	18.4 q
5''	15.3	20.4 q	18.9 q
$\underline{C}H_3COO$	21.2	21.1 q	21.0 q
	20.6	20.6 q	20.5 q
$CH_3\underline{C}OO$	170.3	170.5 s	170.2 s
	169.9	169.8 s	169.8 s
HCOO	160.1	160.1 d	160.0 d
$\underline{OCH_3}$	52.0	52.0 q	51.9 q

EXPERIMENTAL

General Experimental Procedures -- Melting points were determined using an XRC-1 Micromelting apparatus and were uncorrected. Optical rotations were taken with a Horiba SEAP-300 spectropolarimeter. IR spectra (KBr) were obtained on a Bio-Rad FTS-135 infrared spectrophotometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Bruker AM-400 NMR spectrometer and a Bruker DRX-500 NMR spectrometer in $CDCl_3$ solution, with TMS as an internal standard. MS spectral data were obtained on a VG Autospec-3000 spectrometer, 70 eV for EI. Silica gel (200-300 mesh) for CC and GF_{254} for analytical TLC were got from the Qindao Marine Chemical Factory, Qindao, People's Republic of China.

Plant Materials -- The bark of *D. hainanense* was collected from Xishuangbanna, Yunnan province, People's Republic of China, in December 1996. It was identified by Prof. Tao G. D., Xishuangbanna Botany Garden, *Academia Sinica*. A voucher specimen has been deposited in the herbarium of the Department of Taxonomy, Kunming Institute of Botany, no. 7188.

Extraction and Isolation -- The dried and powdered bark (4.2 kg) of *D. hainanense* was extracted with EtOH (10 L) three times under reflux (each process lasting three hours), and the combined extracts were evaporated *in vacuo*. The residue (650 g) was partitioned in H_2O (2 L) and extracted with EtOAc (1.5 L×3). The EtOAc extracts were concentrated *in vacuo* to afford 72 g of residue, which was subjected to column chromatography on silica gel, using $CHCl_3$-Me_2CO (from $CHCl_3$ to $CHCl_3$-Me_2CO 1:1) as eluent. Combining the fractions with TLC (GF_{254}) monitoring, twelve fractions were obtained. Then, the third fraction (3.6 g) was further purified on silica gel chromatography with petroleum ether-acetone (2:1) and recrystallized from acetone to yield **1** (1.28 g) and **2** (135 mg). Fifth fraction (2.9 g) was subjected to silica gel chromatography eluted with $CHCl_3$-EtOAc (2:1), as well as recrystallized in acetone to afford **3** (168 mg).

Dysoxylumin A (**1**). Cubic crystalline (Me_2CO): mp 188-190 °C; $[\alpha]_D^{27}$ +60.4° (*c* 0.54, $CHCl_3$); IR (KBr) ν_{max} 3571, 3451, 2964, 2937, 2877, 1747, 1644, 1468, 1380, 1279, 1259, 1235, 1168, 1074, 1031, 1006, 979, 931, 877 cm^{-1}; ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2; EIMS *m/z* 733, 716, 672, 656, 600, 388, 344, 283, 241, 225, 167, 111, 107, 85, 57; HRFAB-MS *m/z* $[M-H]^-$ (calcd for $C_{42}H_{55}O_{17}$, 831.3439).

Dysoxylumin B (**2**). Cubic crystalline (Me_2CO): mp 122-125 °C; $[\alpha]_D^{19}$ +74.2° (*c* 0.83, CH_3OH); IR (KBr) ν_{max} 3526, 2970, 2880, 1745, 1642, 1504, 1469, 1437, 1375, 1234, 1134, 1032, 990, 917, 874, 793, 604 cm^{-1}; ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2; FAB-MS *m/z* 889 $[M-H]^-$, 861, 843, 801, 159, 117, 59; HRFAB-MS *m/z* $[M-H]^-$ (calcd for $C_{44}H_{57}O_{19}$, 889.3542).

Dysoxylumin C (**3**). Crystalline solid (Me_2CO): mp 118-120 °C; $[\alpha]_D^{19}$ +59.5° (*c* 0.57, CH_3OH); IR (KBr) ν_{max} 3506, 2967, 2939, 2879, 1744, 1649, 1559, 1506, 1469, 1437, 1388, 1373, 1233, 1179, 1030, 987, 916, 874, 794 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; FAB-MS *m/z* 847 $[M-H]^-$, 819, 759, 209, 159, 117; HRFAB-MS *m/z* $[M-H]^-$ (calcd for $C_{42}H_{55}O_{18}$, 847.3409).

X-Ray Crystal Structure Analysis of **1**. Crystal data: $C_{42}H_{56}O_{17}$, MW = 832.38; orthorhombic, space group $P2_12_12_1$; a = 10.745 (1) Å, b = 19.622 (1) Å, c = 20.230 (1) Å. V = 4265.3 (5) $Å^3$, Z = 4, D_{calc} = 1.293 g/cm^3. The data were collected on a MAC DIP-2030K diffractmeter, with graphite-monochromater, Mo-Kα radiation using a colorless crystal of dimensions of 0.10 × 0.30 × 0.50 mm, maximum 2θ value of 50.0°, independent reflections: 4131, observed number of reflections: 4052 $[/F/^2 \geq 8\sigma(/F/^2)]$. The structure was solved by the direct method SHELX-86[10] and expanded using difference Fourier techniques, refined by the program and method NOMCSDP[11] and full-matrix least-squares calculations. Hydrogen atoms were fixed at calculated positions. The final indices were R = 0.057, R_w = 0.053. $(\Delta/\sigma)max$ = 0.147, $(\Delta\rho)max$ = 0.200 e/ $Å^3$, $(\Delta\rho)min$ = -0.220 e/ $Å^3$.

ACKNOWLEDGEMENTS

This work was supported by Natural Science Foundation of China (NSFC) and Yunnan Committee of Sciense and Technology (NSFY). The authors are grateful to the analytical group of Laboratory of Phytochemistry, Kunming Institute of Botany, for the spectral measurements.

REFERENCES AND NOTES

1. X. D. Luo, Y. B. Ma, S. H. Wu, and D. G. Wu, *J. Nat. Prod.*, 1999, **62**, 1022.
2. Yunnan Institute of Botany, 'Flora Yunnanica,' Vol. 1, Science Press, Beijing, 1977, p. 250.
3. W. Aalbersberg, and Y. Singh, *Phytochemistry*, 1991, **30**, 921.
4. M. K. Jogia, and R. J. Andersen, *Phytochemistry*, 1987, **26**, 3309.
5. S. Singh, H. S. Garg, and N. M. Khanna, *Phytochemistry*, 1976, **15**, 2001.
6. V. P. Gullo, I. Miura, K. Nakanishi, A. F. Cameron, J. D. Connolly, F. D. Duncanson, A. E. Harding, R. McCrindle, and D. A. H. Taylor, *J. Chem. Soc., Chem. Commun.*, 1975, 345.
7. J. D. Connolly, D. A. Okorie, L. D. D. Wit, and D. A. H. Taylor, *J. Chem. Soc., Chem. Commun.*, 1976, 909.
8. J. A. Akinniyi, J. D. Connolly, D. S. Rycroft, B. L. Sondengam, and N. P. Ifeadike, *Can. J. Chem.* 1980, **58**, 1865.
9. V. Lukaćova, J. Polonsky, C. Moretti, G. R. Pettit, and J. M. Schmidt, *J. Nat. Prod.*, 1982, **45**, 288.

10. Sheldrick, G. M. University of Gottingen, Federal Republic of Germany, 1985.
11. Lu, Y.; Wu, B. M. *Chinese Chem. Lett.*, 1992, **3**, 637.