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ent-Pimarane derivatives from *Dysoxylum hainanense*

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Abstract

Four *ent*-pimarene diterpenoids, *ent*-18-acetoxy-8(14)-pimarene-15*S*,16-diol, *ent*-18-acetoxy-16-hydroxy-8(14)-pimaren-15-one, *ent*-16,18-dihydroxy-8(14)-pimaren-15-one and *ent*-19-nor-4,16,18-trihydroxy-8(14)-pimaren-15-one, together with three known damarane triterpenoids, richenoic acid, eichlerianic acid and shoreic acid were isolated from the bark of *Dysoxylum hainanense* Merr. Their structures were elucidated on the basis of spectroscopic techniques. The absolute configurations of four diterpenoids were assigned as *ent*-pimarene type by chemical transformation and by co-occurrence in the plant as well as by negative optical rotations for four compounds.

Keywords: Dysoxylum hainanense; Meliaceae; *ent*-Pimarene diterpenoids

1. Introduction

The genus *Dysoxylum*, comprising about 200 species grows naturally in India and southeast Asia. Fourteen of them are distributed in China. About 10 species of this genus have been found in the Yunnan province and *D. hainanense* Merr. is distributed in the Guangxi, Hainan, and Xishuangbanna County, Yunnan provinces (Yunnan Institute of Botany, 1977). While diterpenoids have been isolated from this genus (Onan et al., 1985; Aladesanmi and Adewunmi, 1990), the chemical constituents of this species have not been reported previously. As a continuing study on chemical constituents from Meliaceae plants (Luo et al., 1999), we examined the EtOH extract of the bark of *D. hainanense*. Four new diterpenoids, together with three known damarane triterpenoids, richenoic acid, eichlerianic acid and shoreic acid (Aalbersberg and Singh, 1991) were obtained. This paper describes the isolation and structural elucidation of these compounds.

2. Results and discussion

Compound **1** was assigned the molecular formula of $C_{22}H_{36}O_4$ by negative-ion HRFABMS. The IR spectrum showed absorption bands at 3318 cm^{-1} (–OH), 1714 cm^{-1} (C=O) and 1622 cm^{-1} (olefinic linkage). The ^1H NMR spectrum exhibited signals for an olefinic proton (δ_H 5.19, s), three upfield tertiary (δ_H 0.94, 0.84, 0.77) and downfield (δ_H 2.03) methyl singlets. The EIMS spectrum exhibited a strong fragment peak at m/z 303 (the loss of $-CHOHCH_2OH$ from the molecular ion peak). In addition, the ^{13}C and DEPT NMR spectra displayed the presence of nine methylenes, two of which were oxymethylenes, three methines, one of which was oxygenated, three quaternary carbons, two olefinic carbons (δ_C 127.3, 139.7), and an acetate group. All these data suggested that **1** was either a pimarene or isopimarene diterpenoid having a 1,2-dihydroxyethyl side chain and a double bond between C-8 and C-14 (Cambie et al., 1975; Jakupovic et al., 1987; Tsichritzis and Jakupovic, 1990). This was confirmed by analysis of the HMBC spectrum, with long range coupling for H-14 (δ_H 5.19, *s*) to C-13 (δ_C 36.6, *s*), H-15 (δ_H 3.40, *dd*, $J = 9.1$, 2.1 Hz) to C-16 (δ_C 62.7, *t*), and H-15 to C-13.

The chemical shift value (δ_C 139.7) of the olefinic carbon (C-8) suggested a pimar-8(14)-ene skeleton and not its C-13 epimer (about 136 ppm) (Cambie et al., 1975) for compound **1**. The ^{13}C NMR spectrum of **1** showed a resonance at δ_C 79.4, almost identical with the value of 15*R* (i.e. *ent*-15*S*), but very different from that reported for the 15*S* epimer (Wenkert et al., 1979) which also provided proof of the stereochemistry at C-15. Comparison of the chemical shift of the other oxymethylene (δ_C 73.0)

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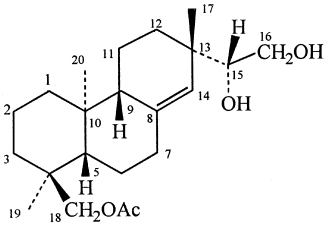
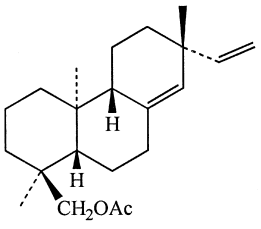
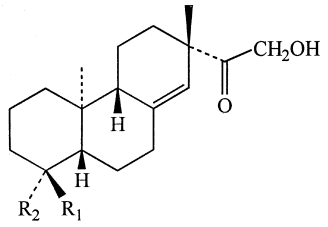
in **1** with that of 18-hydroxy (about 72 ppm) and 19-hydroxy (about 65ppm) diterpenic substances (Jakupovic et al., 1987) suggested that an acetoxyl or a hydroxyl group was connected at C-18 rather than at C-19 in compound **1**. The assumption was further supported by NOE interactions between H-18 [3.83 (1H, *d*, 10.9), 3.61 (1H, *d*, 10.9)] and H-5 [δ_H 1.30 (m)], and δ_H 0.77 (H-20) and 0.84 (H-19) in the ROESY spectrum. In the HMBC spectrum, cross-peaks between oxymethylene protons (H-18) to δ_C 17.9 (*q*, C-19), 37.9 (*s*, C-4), and 171.2 (*s*, OAc) confirmed an acetoxyl attached to C-18.



1



1a



2. $R_1 = CH_2OAc$, $R_2 = CH_3$

3. $R_1 = CH_2OH$, $R_2 = CH_3$

4. $R_1 = CH_2OH$, $R_2 = OH$

In order to establish the absolute configuration of compound **1**, a chemical transformation was performed according to the literature (Hiyama and Nozaki, 1973). Compound **1** was converted to **1a** whose optical rotation {$[\alpha]_D^{23}$ −82.3° (*c* 0.15, $CHCl_3$)} was similar in magnitude, but of opposite sign to that of pimarinol ($[\alpha]_D^{22}$ −94°) (Erdtman and Westfelt, 1963). The optical rotation value of **1a** was the same sign and similar in magnitude to those of (−)-pimara-8(14),15-diene ($[\alpha]_D$ −90°) and its derivatives (Hanson and White, 1970). Hence **1a** was determined to be an *ent*-pimarene type rather than a pimarene type. Thus, **1** was elucidated as *ent*-18-acetoxy-8(14)-pimarene-15*S*,16-diol.

Because of the co-occurrence in the plant as well as the negative optical rotations for all four compounds, compounds **2**–**4** were also regarded as *ent*-pimarenes.

Compound **2** was analyzed for $C_{22}H_{34}O_4$ by negative-ion HRFABMS. The ^{13}C and ^1H NMR spectral data of **2** and **1** were similar. The main difference was that a ketonic carbonyl group was present in the ^{13}C NMR spectrum of **2**, instead of the oxymethine in **1**. The ketonic carbonyl group was assigned to be C-15 since the proton signal for the H-15 was missing in that of **2**. A singlet peak appearing at δ_H 4.30 (2H, s) in the ^1H NMR spectrum of **2** was attributed to H-16 which was free from coupling with the neighboring proton. Compound **2** was regarded as the 15-oxo derivative of **1**. Therefore, **2** was deduced to be *ent*-18-acetoxy-16-hydroxy-8(14)-pimaren-15-one.

Compound **3** possessed the molecular formula $C_{20}H_{32}O_3$ as revealed by negative-ion HRFABMS and analysis of its ^{13}C NMR spectra. The NMR spectral data of **3** were very similar to those of **2** except for the absence of the acetyl data in **3**, which was consistent with its molecular formula comprising twenty carbons. The chemical shift value of H-18 shifted upfield to δ_H 3.36, 3.07 (each 1H) in the ^1H NMR spectrum of **3**, which suggested that **3** was the 18-deacetylated derivative of **2**. Thus **3** was elucidated as *ent*-16,18-dihydroxy-8(14)-pimaren-15-one.

The high resolution negative-ion FABMS spectrum of compound **4** indicated a molecular formula of $C_{19}H_{30}O_4$. Comparison of the ^1H and ^{13}C NMR spectral data of **4** with those of **3** revealed that there were two tertiary methyl groups present in **4** instead of three in **3**. Taking the similar spectral data and molecular formula ($C_{19}H_{30}O_4$) into account, compound **4** was an *ent*-norpimaren-15-one bearing a double bond between C-8 and C-14.

The HMBC spectrum showed correlations from δ_H 3.40 (2H, *d*, 10.8, H-18) to δ_C 73.7 (*s*, C-4), and 35.8 (*t*, C-3), which suggested that the methyl group attached to C-4 (C-19) was lost and replaced by a hydroxyl in **4**. The remainder of the structure was identical to that of **3** based on comparing the ^1H and ^{13}C NMR spectral data of two compounds, and detailed analysis of the HMBC and ROESY spectra of **4**. So compound **4** was determined to be *ent*-19-nor-4,16,18-trihydroxy-8(14)-pimaren-15-one.

Three known damarane triterpenoids, richenoic acid, eichlerianic acid and shoreic acid were identified by direct comparison of their spectral data with authentic samples (Aalbersberg and Singh, 1991).

3. Experimental

3.1. General

Mps: uncorr.; IR: KBr; ^1H, ^{13}C and 2D NMR spectra were recorded on a Brüker AM-400 and a DRX-500 NMR spectrometer with TMS as an internal standard. Chemical shift values were reported in δ (ppm) and coupling constants in Hz; FAB and EIMS data were recorded on a VG Autospec-3000 spectrometer; EIMS: 70 eV

3.2. Plant material

The bark of *D. hainanense* Merr. was collected from Xishuangbanna, Yunnan province, PR China, in December 1996. The plant was identified by Professor Tao, G.-D., Xishuangbanna Botany Garden, Academia Sinica. A voucher specimen (No. 7188) was deposited in the herbarium of Taxonomy Department, Kunming Institute of Botany, Academia Sinica, Kunming, PR China.

3.3. Extraction and isolation

Dried and powdered bark (4.2 kg) of *D. hainanense* was extracted three times with EtOH under reflux conditions, the extracts were combined and solvent was evaporated in vacuo. The resulting residue was suspended in H_2O and partitioned with EtOAc. The EtOAc layer was concentrated in vacuo to give a residue (72 g), which was subjected to column chromatography (silica gel), using $CHCl_3$–Me_2CO (from $CHCl_3$ to $CHCl_3$–Me_2CO 1:1) as eluent. Fractions were combined on the basis of their TLC profiles. Fractions 1–6 were further purified on silica gel CC with petroleum ether-EtOAc (from 5:1 to 1:1) as eluent and finally on reversed-phase C_{18} silica gel CC, eluted with CH_3OH–H_2O (3:1-3:2) to yield **1** (118 mg), **2** (66 mg), **3** (106 mg), **4** (27 mg), and three known damarane triterpenoids, richenoic acid, eichlerianic acid and shoreic acid.

3.4. ent-18-Acetoxy-8(14)-pimarene-15S,16-diol (1)

Viscous oil; $[\alpha]_D^{26}$ −3.5° (*c* 0.50, CH_3OH); EIMS *m/z* (70 eV): 364 $[M]^+$ (1.4), 303 (88), 289 (37), 243 (73), 229 (25), 213 (8), 181 (10), 161 (21), 147 (29), 135 (95), 121 (100), 107 (81), 95 (86), 81 (84), 69 (45); negative-ion HRFABMS *m/z*: 363.2500 $[M\text{-}1]^-$ (calc. for $C_{22}H_{35}O_4$, 363.2535); IR (KBr) ν_{max} cm^{-1}: 3418, 2931, 2870, 1714, 1622, 1469, 1381, 1240, 1036, 947; ^1H NMR ($CDCl_3$, 400 MHz): δ 5.19 (1H, *s*, H-14), 3.83 (1H, *d*, *J* = 10.9 Hz, H-18a), 3.61 (1H, *d*, *J* = 10.9 Hz, H-18b), 3.70 (1H, *brd*, *J* = 10.6 Hz, H-16a), 3.48 (1H, *J* = 11.0, 9.1 Hz, H-16b), 3.40 (1H, *dd*, *J* = 9.1, 2.1 Hz, H-15), 2.03 (3H, *s*, OAc), 0.94 (3H, *s*, H-17), 0.84 (3H, *s*, H-19), 0.77 (3H, *s*, H-20); for ^{13}C NMR spectral data: see Table 1.

3.5. Preparation of ent-18-acetoxyl-pimara-8(14),15-diene (1a)

Compound **1** (22 mg), ethyl orthoformate (100 mg), and benzoic acid (3 mg) were mixed and heated at 90–100°C for 2 h. After concentration in vacuo, benzoic acid (5 mg) was added and the mixture was heated at 175–180°C for 1.5 h. After cooling, CH_2Cl_2 (2 ml) was added, and the solution was washed with saturated Na_2CO_3. The CH_2Cl_2 fraction was concentrated and purified by CC on silica gel with CH_2Cl_2 as eluent to give **1a** (5 mg) (yield 25). Viscous oil; $[\alpha]_D^{23}$ −82.3° (*c* 0.15, $CHCl_3$); EIMS *m/z* (70eV): 330 $[M]^+$ (78), 316 (64), 301 (45), 270 (32), 257 (87), 241 (33), 225 (66), 211 (34), 181 (44), 149 (61), 135 (91), 121 (100), 105 (78), 55 (87); ^1H NMR ($CDCl_3$, 400 MHz): δ 5.75 (1H, *dd*, *J* = 17.6, 10.5 Hz, H-15), 5.20 (1H, *s*, H-14), 4.89 (1H, *dd*, *J* = 17.6, 1.0 Hz, H-16a), 4.86 (*dd*, *J* = 10.5, 2.2 Hz, H-16b), 3.84 (1H, *d*, *J* = 10.9 Hz, H-18a), 3.63 (1H, *d*, *J* = 10.9 Hz, H-18b), 2.04 (3H, *s*, OAc), 1.02 (3H, *s*, H-17), 0.85 (3H, *s*, H-19), 0.81 (3H, *s*, H-20).

Table 1
^{13}C NMR spectral data for compounds **1–4** (in $CDCl_3$, 100 MHz)

C	1	2	3	4
1	38.8 *t*	38.8 *t*	38.8 *t*	38.5 *t*
2	18.1 *t*	18.1 *t*	18.2 *t*	18.6 *t*
3	35.5 *t*	35.6 *t*	35.3 *t*	35.8 *t*
4	37.9 *s*	38.3 *s*	37.7 *s*	73.7 *s*
5	48.5 *d*	48.5 *d*	47.5 *d*	55.2 *d*
6	22.3 *t*	22.3 *t*	22.1 *t*	21.2 *t*
7	35.9 *t*	36.7 *t*	35.6 *t*	36.1 *t*
8	139.7 *s*	140.8 *s*	141.0 *s*	140.4 *s*
9	51.0 *d*	50.2 *d*	50.1 *d*	49.7 *d*
10	38.0 *s*	38.3 *s*	38.2 *s*	39.0 *s*
11	18.3 *t*	18.4 *t*	18.4 *t*	19.6 *t*
12	29.1 *t*	31.2 *t*	31.2 *t*	31.1 *t*
13	36.6 *s*	46.7 *s*	46.6 *s*	46.6 *s*
14	127.3 *d*	123.0 *d*	122.6 *d*	123.3 *d*
15	79.4 *d*	214.3 *s*	214.4 *s*	214.2 *s*
16	62.7 *t*	64.8 *t*	64.7 *t*	64.6 *t*
17	22.9 *q*	23.5 *q*	23.5 *q*	23.6 *q*
18	73.0 *t*	72.9 *t*	71.9 *t*	62.8 *t*
19	17.9 *q*	17.8 *q*	17.9 *q*	
20	15.3 *q*	15.5 *q*	15.5 *q*	15.6 *q*
OAc	20.9 *q*	20.9 *q*		
	171.2 *s*	171.1 *s*		

3.6. ent-18-Acetoxy-16-hydroxy-8(14)-pimaren-15-one (2)

Viscous oil; $[\alpha]_D^{26}$ −0.4° (*c* 0.85, CH_3OH); EIMS *m/z* (70eV): 362 $[M]^+$ (2) 318 (5), 303 (55), 289 (56), 275 (10), 259 (7), 243 (51), 229 (49), 213 (16), 201 (10), 181 (29), 197 (24), 161 (39), 147 (43), 135 (65), 121 (100), 105 (63), 93 (57), 79 (64), 69 (40), 55 (45); negative-ion HRFABMS *m/z*: 361.2329 $[M\text{-}1]^-$ (calc. for $C_{22}H_{33}O_4$, 361.2379); IR (KBr) ν_{max} cm^{-1}: 3478, 2934, 2870, 1713, 1620, 1453, 1382, 1242, 1149, 1102, 1037, 1001, 946, 857, 756; ^1H NMR ($CDCl_3$, 400 MHz): δ 5.38 (1H, *s*, H-14), 4.30 (2H, *s*, H-16), 3.84 (1H, *d*, *J* = 10.9 Hz, H-18a), 3.62 (1H, *d*, *J* = 10.9 Hz, H-18b), 2.04 (3H, *s*, OAc), 1.17 (3H, *s*, H-17), 0.84 (3H, *s*, H-19), 0.79 (3H, *s*, H-20); for ^{13}C NMR spectral data: see Table 1.

3.7. ent-16,18-Dihydroxy-8(14)-pimaren-15-one (3)

Viscous oil; $[\alpha]_D^{25}$ −9.1° (*c* 0.55, CH_3OH); EIMS *m/z* (70eV): 320 $[M]^+$ (1.2), 261 (100), 243 (15), 229 (16), 187 (5), 175 (8), 159 (10), 153 (11), 135 (26), 121 (55), 109 (69), 95 (66), 81 (44), 67 (25), 59 (77); negative-ion HRFABMS *m/z*: 319.2235 $[M\text{-}1]^-$ (calcd. for $C_{20}H_{31}O_3$, 319.2273).IR (KBr) ν_{max} cm^{-1}: 3425, 2934, 2870, 1711, 1460, 1384, 1275, 1197, 1102, 1040, 1010, 886, 752; ^1H NMR ($CDCl_3$, 400 MHz): δ 5.36 (1H, *s*, H-14), 4.29 (2H, *d*, *J* = 1.7 Hz, H-16), 3.36 (1H, *d*, *J* = 10.9 Hz, H-18a), 3.07 (1H, *d*, *J* = 10.9 Hz, H-18b), 1.16 (3H, *s*, H-17), 0.79 (3H, *s*, H-19), 0.76 (3H, *s*, H-20); for ^{13}C NMR spectral data: see Table 1.

3.8. ent-19-Nor-4,16,18-trihydroxy-8(14)-pimaren-15-one (**4**)

Colorless needles (Me_2CO); mp 121–123°C; $[\alpha]_D^{19}$ −25.6° (*c* 0.31, CH_3OH); EIMS *m/z* (70eV): 322 $[M]^+$ (0.4), 291 (13), 273 (5), 263 (100), 245 (36), 227 (17), 217 (10), 189 (5), 171 (5), 159 (11), 145 (13), 133 (16), 121 (63), 107 (61), 95 (53), 81 (59), 67 (27), 55 (41); negative-ion HRFABMS *m/z*: 321.2079 $[M-1]^-$ (calc. for $C_{19}H_{29}O_4$, 321.2066); IR (KBr) ν_{max} cm^{-1} 3524, 3339, 2928, 2870, 1720, 1621, 1463, 1446, 1383, 1344, 1310, 1278, 1253, 1103, 1063, 1048; ^1H NMR ($CDCl_3$, 400 MHz): δ 5.38 (1H, *s*, H-14), 4.28 (2H, *d*, *J* = 1.3 Hz, H-16), 3.61 (1H, *d*, *J* = 10.8 Hz, H-18a), 3.40 (1H, *d*, *J* = 10.8 Hz, H-18b), 1.14 (3H, *s*, H-17), 0.64 (3H, *s*, H-20); for ^{13}C NMR spectral data: see Table 1.

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