Two New *ent*-Kaurane Diterpenoids from *Isodon xerophilus*

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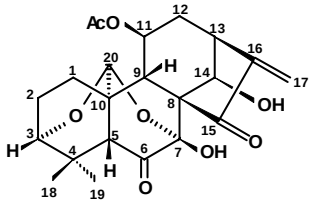
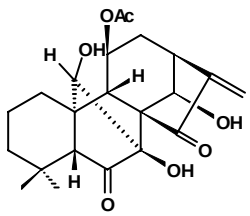
Abstract: Two new *ent*-kaurane diterpenoids, xerophilusin E (**1**) and xerophilusin F (**2**), were isolated from the leaves of *Isodon xerophilus*. Their structures were determined as 3, 20: 7, 20-diepoxy-*ent*-kaur-16-en-15-one and 7β, 14β, 20 (*R*)-trihydroxy-11β-acetoxy-7, 20-cyclo-*ent*-kaur-16-en-6, 15-dione, respectively, by spectral methods and X-ray crystallographic analysis.

Keywords: *Isodon xerophilus*, Labiatae, *ent*-kaurane diterpenoids, xerophilusin E, xerophilusin F.

In continuation of our research on diterpenoids in the *Isodon* species, several new compounds[1,2] were obtained from the leaves of *Isodon xerophilus* (C. Y. Wu et H. W. Li) H. Hara (Labiatae), a perennial shrub native to Yunnan province. Further fractionation of the EtOAc extract led to the isolation of two new *ent*-kauranoids, xerophilusin E (**1**) and xerophilusin F (**2**). This paper deals with the structural elucidation of the new compounds.



1



2: R = OAc
3: R = OH

Xerophilusin E (**1**), a minor constituent, was obtained as prismatic crystals, and produced $[M+1]^+$ quasi-molecular ion in EIMS at *m/z* 419. In conjunction with the analysis of the ^{13}C NMR and DEPT spectra, its molecular formula was deduced to be $C_{22}H_{26}O_8$. The signals at δ1.95 (3H, s), δ170.4 (s) and 21.1 (q) exhibited the presence of

an acetoxyl group. An *exo*-methylene group conjugated with a carbonyl group on a five-membered ring was revealed by the following spectral data: UV (MeOH) λ_{max} (log ε) 229.2 (3.82) nm; IR (KBr) ν 1738 (br) and 1644 cm^{-1}; ^1H NMR: δ 5.49 and 5.93 (each 1H, s); ^{13}C NMR: δ 118.8 (t), 151.5 (s) and 201.3 (s). The NMR spectra also gave the signals owing to two methyls, three methylenes, an acetal group [δ 5.78 (1H, br s) and δ 97.0 (d)], six methines including three oxygen-bearing ones, and five quaternary carbons including a carbonyl at δ 203.5 and a ketalic carbon at δ 98.8. All the evidence combined with the degree of unsaturation indicated an *ent*-kaurane skeleton, in which two ether linkages, a carbonyl group, a secondary hydroxyl, and a secondary acetoxyl existed.

According to the results of ^1H-^1H COSY and HMQC, the unambiguous assignment of all protons and related carbons was achieved, which clearly displayed the positions of the carbonyl groups and three oxygenated methines. The singlet at δ 2.12 (H-5) and the carbon signal at δ 57.8 (d, C-5) directed that the carbonyl group was located at C-6. The signals at δ 65.2 (d, C-11) and δ 5.10 (1H, m, H-11) disclosed that the acetoxyl group should be at C-11. The other two oxygenated methines originated from C-3 (d, 77.2) and C-14 (d, 73.0). The problems concerning the position of the hydroxyl (C-3 or C-14) and the linkages of the two ether bridges were settled by HMBC spectrum. The following long-range couplings: H-3 with C-20; H-20 with C-3 and C-7 revealed the 3α, 20: 7α, 20-diepoxy units in **1** (**Figure 1**). Obviously, C-14 was substituted by the hydroxyl group.

Figure 1. Selected HMBC correlations of **1**

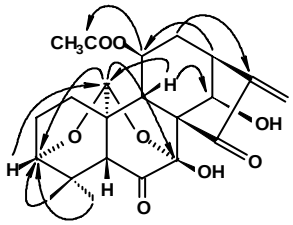
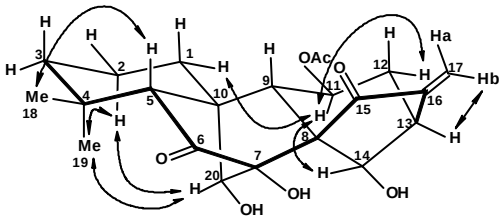


Figure 2. Key NOESY correlations of **2**



Each substituent in **1** had β-orientation based on X-ray crystallographic analysis[3], by which the conformations of ring A (C_1, C_2, C_3, C_4, C_5, C_{10}), B (C_5, C_6, C_7, C_8, C_9, C_{10}) and C (C_9, C_{11}, C_{12}, C_{13}, C_{14}, C_8) were all concluded to be boat forms. Consequently, xerophilusin E (**1**) was characterized as 7β, 14β-dihydroxy-11β-acetoxy-3α, 20: 7α, 20-diepoxy-*ent*-kaur-16-en-6, 15-dione.

Xerophilusin F (**2**), colorless crystals, mp: 193-195 °C, $[\alpha]_D^{23}$ +12.2 (*c* 0.31, C_5H_5N); UV (MeOH) λ_{max} (log ε) 227.5 (3.95) nm; IR (KBr) ν 3445, 2970, 2940, 1748, 1725, 1645, 1449, 1389, 1299, 1236, 1104, 1046, 1030 cm^{-1}. It has a molecular formula of $C_{22}H_{28}O_7$ established by positive HRFABMS (obsd 405.1981, calcd 405.1913). A singlet at δ 1.97 (3H, s) in the ^1H NMR spectrum and two signals at δ 170.1 (s) and 21.3 (q) in the ^{13}C NMR spectrum exhibited the presence of an acetoxyl group. Comparison of other NMR data with those of phyllostachysin A **3**[4] indicated that **2** had the same skeleton of 7β, 14β, 20-trihydroxy-7, 20-cyclo-*ent*-kaur-16-en-6, 15-dione as **3**. However, some

marked differences were also observed. The signals at δ5.30 (1H, m, H-11α) and δ64.9 (d, C-11) in **3** were shifted downfield to δ5.67 (1H, m, H-11α) and δ68.7 (d, C-11) in **2**, respectively. Moreover, the signals at δ62.3 (d, C-9) and 43.2 (t, C-12) in **3** were shifted upfield to δ 59.7 (d, C-9) and 38.8 (t, C-12) in **2**. These changes suggested that the position of the acetoxyl group should be at C-11. In the HMBC spectrum, the signal at δ 4.10 (1H, d, J = 4.2 Hz, H-20) showed long range couplings with two quaternary carbons at δ59.2 (C-8) and 210.0 (C-6), and the proton at δ5.67 (1H, m, H-11α) was correlated with the ester carbonyl group at δ 170.1 (OAc), which further proved 7, 20-cyclo part and the location of the acetoxyl group.

The β-orientation of the acetoxyl group was decided by the NOESY correlations: H-11α with H-14α, H-12α and H-1α. The stereochemistry at C-20 in **2** was assigned as *R* from the NOE effects of H-20 with Me-19 and H-2α (**Figure 2**). So, xerophilusin F (**2**) was characterized as 7β, 14β, 20(*R*)-trihydroxy-11β-acetoxy-7, 20-cyclo-*ent*-kaur-16-en-6, 15-dione, which is the third 7, 20-cyclo-*ent*-kauranoid after **3**[2,4] and rubescensin D[5].

Xerophilusin E (**1**): $C_{22}H_{26}O_8$, colorless prismatic crystals, mp: 223-225 °C, $[\alpha]_D^{20}$ –32.0 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 229.2 (3.82) nm; IR (KBr) ν 3437, 2956, 1738 (br), 1644, 1456, 1371, 1240, 1190 cm^{-1}; EIMS *m/z* 419 $[M+1]^+$ (4), 390 $[M-CO]^+$ (42), 374 (50), 358 $[M-AcOH]^+$ (13), 346 (48), 330 (16), 314 (51), 296 (61), 268 (30); ^{1}H NMR (400 MHz) δ1.92 (1H, overlap, H-1α), 1.68 (1H, m, H-1β), 1.70 (1H, m, H-2α), 1.32 (1H, m, H-2β), 3.50 (1H, t, J = 2.2 Hz, H-3β), 2.12 (1H, s, H-5β), 1.91 (1H, d, J = 9.0 Hz, H-9β), 5.10 (1H, m, H-11α), 3.05 (1H, overlap, H-12α), 1.30 (1H, dd, J = 9.0, 13.7 Hz, H-12β), 3.06 (1H, d, J = 9.2 Hz, H-13α), 4.85 (1H, s, H-14α), 5.93 (1H, s, H-17a), 5.49 (1H, s, H-17b), 1.09 (3H, s, Me-18), 1.08 (3H, s, Me-19), 5.78 (1H, br s, H-20), 1.95 (3H, s, OAc); ^{13}C NMR (100 MHz) δ23.0 (t, C-1), 21.1 (t, C-2), 77.2 (d, C-3), 38.0 (s, C-4), 57.8 (d, C-5), 203.5 (s, C-6), 98.8 (s, C-7), 59.0 (s, C-8), 49.1 (d, C-9), 37.6 (s, C-10), 65.2 (d, C-11), 37.2 (t, C-12), 43.1 (d, C-13), 73.0 (d, C-14), 201.3 (s, C-15), 151.5 (s, C-16), 118.8 (t, C-17), 30.4 (q, C-18), 25.9 (q, C-19), 97.0 (d, C-20), 170.4 (s, OAc) and 21.1 (q, OAc).

Xerophilusin F (**2**), $C_{22}H_{28}O_7$, colorless crystals, mp: 193-195 °C, $[\alpha]_D^{23}$ +12.2 (*c* 0.31, C_5H_5N); UV (MeOH) λ_{max} (log ε) 227.5 (3.95) nm; IR (KBr) ν 3445, 2970, 2940, 1748, 1725, 1645, 1449, 1389, 1299, 1236, 1104, 1046, 1030 cm^{-1}; HRFABMS *m/z* 405.1981 $[M+1]^+$ (calcd 405.1913); ^{1}H NMR (400 MHz) δ1.90 (1H, d, J = 13.8 Hz, H-1α), 1.52 (1H, dd, J = 4.3, 13.8 Hz, H-1β), 1.45 (2H, m, H_2-2α, β), 1.37 (1H, m, H-3α), 1.18 (1H, overlap, H-3β), 2.05 (1H, s, H-5β), 1.94 (1H, d, J = 11.4 Hz, H-9β), 5.67 (1H, m, H-11α), 2.88 (1H, ddd, J = 9.0, 9.0, 13.5 Hz, H-12α), 1.32 (1H, dd, J = 9.0, 13.5 Hz, H-12β), 2.95 (1H, d, J = 9.0 Hz, H-13α), 5.23 (1H, s, H-14α), 5.92 (1H, s, H-17a), 5.46 (1H, s, H-17b), 1.20 (3H, s, Me-18), 0.82 (3H, s, Me-19), 4.10 (1H, d, J = 4.2 Hz, H-20), 1.97 (3H, s, OAc); ^{13}C NMR (100 MHz) δ24.1 (t, C-1), 19.4 (t, C-2), 41.3 (t, C-3), 33.9 (s, C-4), 61.2 (d, C-5), 210.0 (s, C-6), 89.2 (s, C-7), 59.2 (s, C-8), 59.7 (d, C-9), 47.2 (s, C-10), 68.7 (d, C-11), 38.8 (t, C-12), 43.1 (d, C-13), 72.9 (d, C-14), 203.0 (s, C-15), 151.6 (s, C-16), 118.1 (t, C-17), 34.9 (q, C-18), 23.8 (q, C-19), 80.9 (d,

C-20), 170.1 (s, OAc) and 21.3 (q, OAc).

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