Pseudoirroratin A, a New Cytotoxic *ent*-Kaurene Diterpene from *Isodon pseudo-irrorata*

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A new *ent*-kaurene diterpene, pseudoirroratin A (**1**), and a known diterpene, pseurata A, were isolated from *Isodon pseudo-irrorata*. Compound **1** showed significant cytotoxicity against the Lu1, SW626, LNCaP, KB, and HOS cancer cell lines with IC_{50} values of 0.26 (0.75), 0.20 (0.57), 0.90 (2.59), 0.90 (2.59), and 0.50 (1.44) μg/mL (μM), respectively. The structure of **1** was elucidated by spectroscopic means including 1D and 2D NMR techniques.

The genus *Isodon* of the Lamiaceae (formerly Labiatae) is well-known for producing biologically active *ent*-kaurane diterpenoids.[1,2] The first study on *Isodon* species was conducted in 1954 in a search for antibiotics,[3] and the first compound (enmein) was isolated from *I. japonica and I. Trichocarpus* in 1958.[4] Enmein was reported to be an antitumor *ent*-kaurane diterpene,[5] and the structure was determined by X-ray analysis in 1966.[6] Since then, more than 400 *ent*-kaurane diterpenes have been reported from *Isodon* species.

In our continuing search for new antitumor and antiviral agents, a new cytotoxic *ent*-kaurane diterpenoid, pseudoirroratin A (**1**), and the known diterpene, pseurata A,[7] were isolated from the leaves of *I. pseudo-irrorata* C. Y. Wu, a perennial plant distributed mainly in Tibet and in the Sichuan Province of China.[7−10] The present paper reports the isolation, structure elucidation, and biological evaluation of **1**.

Pseudoirroratin A (**1**) showed a molecular formula of $C_{20}H_{28}O_5$ by HRFABMS ($[M + H]^+$ *m/z* 349.20314). The 20 carbons found in **1** were characterized by DEPT-135 and DEPT-90 spectra as composed of two non-oxymethyl carbons (δ 32.5 and 21.6), five non-oxymethylene carbons (δ chemical shifts between 20 and 50 ppm), an oxymethylene carbon (δ 69.4), an olefinic methylene carbon (δ 116.6), three non-oxymethine carbons (δ chemical shifts between 40 and 64 ppm), two oxymethine carbons (δ 75.1 and 74.3), three non-oxyquaternary carbons (δ chemical shifts between 30 and 60 ppm), a quaternary carbon attached by two oxy groups (δ 103.7), an olefinic quaternary carbon (δ 153.2), and a carbonyl carbon (δ 209.1). The two methyl carbons were assigned as tertiary groups due to their singlet signals (δ 0.83 and 0.64) in the ^{1}H NMR spectrum. The downfield shift of the olefinic quaternary carbon (δ 153.2) and the upfield shift of the olefinic methylene (δ 116.6) coupled with the carbonyl carbon (δ 209.1) in the ^{13}C NMR spectra showed the presence of an α,β-conjugated keto group. On the basis of these data and the chemotaxonomic considerations, compound **1** was presumed to be an *ent*-kaurene diterpenoid.

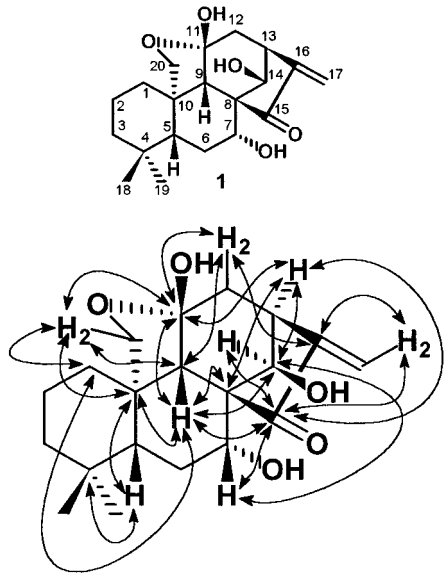

Figure 1. Selected HMBC correlations for pseudoirroratin A (**1**) (Bruker DPX 300 MHz, pyridine-d_5).

On the basis of this assumption, elucidation of the substituent groups proceeded accordingly. A hydroxy group was assigned to C-7 due to the presence of the ^3J HMBC correlations (Figure 1) between H-7 (δ 4.83) and C-15 (δ 209.1). Another hydroxy group was assigned to C-14 due to the ^3J HMBC correlations between H-14 (δ 5.44) and C-15 and the ^2J HMBC correlations between C-14 (δ 74.3) and H-13 (δ 3.30). On the basis of the molecular formula of $C_{20}H_{28}O_5$, it was calculated that the compound had seven double-bond equivalents. When the four rings and the two double bonds ($\Delta^{16,17}$ and the carbonyl group) were considered, one undefined double-bond equivalent remained in the *ent*-kaurene skeleton. This was assumed to belong to an additional ring since no other double bond was observed in the NMR spectrum. The presence of the ^3J HMBC correlations of a quaternary carbon at δ 103.7 to H-13 and H_2-12, respectively, led to its assignment as C-11. The fact that C-11 correlated to H_2-20 in the HMBC spectra suggested that the additional ring was constituted through the formation of a hemiacetal bridge between C-20 and C-11, which caused downfield chemical shifts of C-9 (δ 62.8) and C-12 (δ 46.3) in the ^{13}C NMR spectra.

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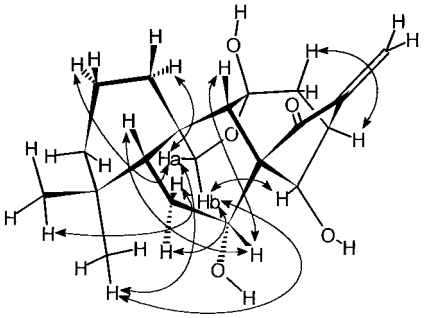

Figure 2. Selected ROESY correlations for pseudoirroratin A (**1**) (Bruker DRX 500 MHz, pyridine-d_5).

The configuration of **1** was determined according to coupling constants and ROESY experiments. The hydroxy group at C-14 was determined to be of β-orientation due to the singlet signal of H-14 (δ 5.44) and the 90° dihedral angle between H-14 and H-13α. The hydroxy group at C-7 was assigned as the α-orientation due to the observation of the ROEs between H-7 and H-9β and between H-7 and H-5β (Figure 2). The presence of the ROESY correlations of H-20b to H-14α, H-6α, and Me-19 and of H-20a to H-1α, H-2α, and Me-19, respectively, revealed an α-position for C-20. In addition, the ROEs between H-20b and H-14α established the hemiacetal hydroxy group at C-11 as being β-oriented. These stereochemical assignments are supported by a literature report of the isolation of macrocalyxin F, a structurally similar diterpene having the same configuration as **1**, from *Isodon macrocalyx*.[11] Accordingly, the structure of **1** was elucidated as *ent*-7β,11α,14α-trihydoxy-11β,20-epoxykaur-16-en-15-one and given the trivial name pseudoirroratin A.

Pseudoirroratin A (**1**) lacked significant inhibitory activity against the replication of HIV-1[12,13] at nontoxic concentrations. However, the toxicity observed in the HOG.R5 reporter cell-based anti-HIV assay prompted us to evaluate **1** in a panel of human cancer cell lines[14] for potential antitumor activity.[12] Compound **1** showed significant cytotoxicity against the Lu1 (human lung cancer), SW626 (ovarian cancer), LNCaP (hormone-dependent human prostate cancer), KB (human oral epidermoid carcinoma), and HOS (human osteosarcoma) cell lines, with IC_{50} values of 0.26 (0.75), 0.20 (0.57), 0.90 (2.59), 0.90 (2.59), and 0.50 (1.44) μg/mL (μM), respectively. The positive control compound utilized was camptothecin, which demonstrated IC_{50} values of 0.01 (28.7), 0.01 (28.7), 0.03 (86.1), 0.01 (28.7), and 0.01 (28.7) μg/mL (nM) in Lu1, SW626, LNCaP, KB, and HOG.R5 cells, respectively. These values are in agreement with those published in the literature utilizing the microtetrazolium (MTT) assay.[15] It is noteworthy that HOS cells constitute the parental line from which the HOG.R5 reporter cell line was constructed specifically for the convenient quantitation of HIV replication.[13] However, no cytotoxic selectivity was apparent against any of the cell lines that comprise the screening panel adopted.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter. UV spectra were obtained with a Beckman DU-7 spectrometer. IR spectra were run on a Jasco FT/IR-410 spectrometer as a film on a KBr plate. 1D and 2D NMR experiments were performed on either a Bruker AM-400, a DPX-300, or a DRX-500 spectrometer. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. FABMS and HRFABMS were taken on a VG Auto Spec-3000 instrument. Column chromatography was performed on Si gel (200−300 mesh, Qingdao Marine Chemical, China). Fractions were monitored by TLC, and spots were visualized by heating Si gel plates sprayed with 10% H_2SO_4 in H_2O.

Plant Material. The leaves of *Isodon pseudo-irrorata* C. Y. Wu (Lamiaceae) was collected in Lasa, People's Republic of China. A voucher specimen has been deposited at Xizang Plateau Institute of Biology, Lasa, 850001, China.

Extraction and Isolation. Dried and milled leaves (4.1 kg) were extracted by maceration in EtOH (95%) over a 3-week period. Removal of the solvent afforded an extract (605 g), which was redissolved in 600 mL of EtOH (95%), filtered, and concentrated in vacuo to afford a residue (219 g). This material was solubilized, absorbed on 210 g of Si gel, and chromatographed on a pre-packed (1.25 kg) Si gel column. Gradient elution was accomplished with petroleum ether−$CHCl_3$ and $CHCl_3$−Me_2CO. The combined fractions (35 g) eluted by 8:2 $CHCl_3$−Me_2CO were subjected to additional pre-packed (500 g) Si gel column chromatography and eluted with petroleum ether−Me_2CO to yield pseudoirroratin A (**1**) (2.5 g) and pseurata A (0.3 g).

Pseudoirroratin A (1): white powder; $[\alpha]_D^{20}$ −132.21° (*c* 0.65, MeOH); UV (MeOH) λ_{max} (log ϵ) 234 (4.28) nm; IR (KBr) ν_{max} 3550, 3418, 3330, 3210, 2985, 2978, 2910, 2895, 2870, 1709, 1635, 1489, 1465, 1450, 1440, 1385, 1370, 1352, 1337, 1290, 1270, 1252, 1238, 1208, 1190, 1170, 1142, 1112, 1095, 1070, 1063, 1000, 985, 975, 960, 955, 942, 925, 845, 820, 745, 600 cm^{-1}; ^1H NMR (400 MHz, py-d_5, *J* in Hz) δ 6.23 (1H, s, H-17a), 5.44, (1H, brs, H-14α), 5.41 (1H, s, H-17b), 4.83 (1H, dd, *J* = 12.1, 3.2, H-7β), 4.17 (1H, ABd, *J* = 8.6, H-20a), 4.05 (1H, ABd, *J* = 8.0, H-20b), 3.36 (1H, brd, *J* = 13.5, H-1α), 3.30 (1H, brd, *J* = 9.2, H-13α), 3.25 (1H, dd, *J* = 13.7, 9.1, H-12α), 2.29 (1H, s, H-9β), 2.20 (1H, d, *J* = 13.7, H-12β), 2.17 (1H, brd, *J* = 15.7, H-6β), 1.94 (1H, q, *J* = 12.4, H-6α), 1.54 (1H, m, H-2α), 1.48 (1H, dd, *J* = 12.4, 1.7, H-5β), 1.38 (1H, brd, *J* = 13.8, H-2β), 1.25 (1H, brd, *J* = 13.2, H-3β), 1.12 (1H, dd, *J* = 13.0, 4.3, H-3α), 1.08 (1H, brd, *J* = 14.8, H-1β), 0.83 (3H, s, Me−), 0.64 (3H, s, Me−); ^{13}C NMR (100 MHz, py-d_5) δ 39.4 (t, C-1), 20.3 (t, C-2), 41.6 (t, C-3), 34.3 (s, C-4), 51.3 (d, C-5), 28.8 (t, C-6), 75.1 (d, C-7), 57.9 (s, C-8), 62.8 (d, C-9), 50.5 (s, C-10), 103.7 (s, C-11), 46.3 (t, C-12), 45.0 (d, C-13), 74.3 (d, C-14), 209.1 (s, C-15), 153.2 (s, C-16), 116.6 (t, C-17), 32.5 (q, C-18), 21.6 (q, C-19), 69.4 (t, C-20); FABMS *m/z* 349 $[M + H]^+$ (4), 331 (100), 313 (9), 283 (5); HRFABMS *m/z* 349.20314 (calcd for $C_{20}H_{29}O_5$ 349.20150).

Bioassay Evaluation. Compound **1** was evaluated for cytotoxicity against a panel of human cancer cell lines according to previously established protocols.[14] This panel was comprised of Lu1, SW626, LNCaP, and KB cells. The effect of **1** on HIV-1 replication was assessed using a green fluorescent protein (GFP)-based reporter cell line according to established protocols.[12,13] This microtiter assay is based on the transactivation of a stably integrated HIV-1 LTR-GFP transcription unit by the viral Tat protein that is produced intracellularly following an infection.

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