Isolation, Characterization and Crystal Structure of Cytotoxic *ent*-Kaurane Diterpenoids from *Isodon weisiensis* C. Y. Wu

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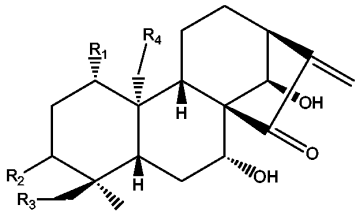
The new *ent*-kaurane diterpenoid weisiensin C **(1)** (1α,7α,14β,18,20-pentahydroxy-*ent*-kaur-16-en-15-one), together with four known *ent*-kaurene diterpenoids, glaucocalyxin A **(2)**, kamebanin **(3)**, macrocalyxin D **(4)**, and excisanin K **(5)**, was isolated from the leaves of *Isodon weisiensis* C. Y. Wu. The structure of the new constituent **(1)** was elucidated by spectroscopic methods and confirmed by single-crystal X-ray diffraction.

Key words: Isodon Weisiensis C. Y. Wu, *Ent*-kaurane Diterpenoid, Weisiensin C, Crystal Structure

Introduction

The genus *Isodon* (Labiatae) are known to be rich in tetracyclic *ent*-kaurane diterpenoids [1 – 2] which display various biologic activities, *i.e*. *anti*-bacterial [2], NF-*k*B activation-inhibiting [3], saccharomyces growth-inhibiting [4], cytotoxic [2, 5], and apoptosis-inducing activities [6], *etc*. *Isodon weisiensis* C. Y. Wu, has been used in Chinese folk medicine to treat gastric ulcer and enteritis and is distributed in the south of Gansu Province and Yunnan Province, P. R. China. Only one *ent*-kaurene diterpenes has been isolated from it [7]. The present phytochemical investigation on *Isodon Weisiensis* C. Y. Wu has led to isolation of five *ent*-kauranoids, a new diterpene, weisiensin C **(1)**, along with four known compounds, glaucocalyxin A **(2)**, kamebanin **(3)**, macrocalyxin D **(4)** and excisanin K **(5)**. The new compound, weisiensin C had the basic skeleton of C-20-non-oxygenated-*ent*-kaur-16-en-15-one and its structure was confirmed by X-ray crystallographic analysis.

Four major structural types, C-20-non-oxygenated-*ent*-kaurane type, C-20-oxygenated-*ent*-kaurane type, *ent*-8,9-seco-kaurane type and *ent*-6,7-seco-kaurane type, have been found among *Isodon* tetracyclic diterpenoids. Our pharmacological research indicated that C-20-non-oxygenated-*ent*-kauronoid showed most significant cytotoxicity activity among the major structural types. Although more than 160 C-20-non-oxygenated-*ent*-kauronoids have been isolated from the genus *Isodon*, only the structures of four compounds, mebadonin, xindongnin B, leucophyllin F and leucophyllin C, have been determined by X-ray diffraction analysis [2]. The biological activity of the compounds seems to depend on certain key structure and conformational features. The present work, therefore, provides further confirmation of the structures in this class of compounds, in addition to the preliminary evaluation of the cytotoxicity of *ent*-kauranoids from *Isodon Weisiensis* C. Y. Wu.



1 R_2 = H, R_1 = R_3 = R_4 = OH
2 R_1 = R_3 = R_4 = H, R_2 = oxo
3 R_1 = OH, R_2 = R_3 = R_4 = H
4 R_3 =CHO, R_4 =OH, R_1 = R_2 = H
5 R_1 = R_3 = OH, R_2 = R_4 = H

Fig. 1. Structures of the compounds.

Results and Discussion

Compound **1** was isolated as colorless crystals and the molecular formula was established as $C_{20}H_{30}O_6$ by

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz), HMBC and ^{1}H–^{1}H COSY spectral data of compound **1** (δ, ppm, TMS, C_5D_5N).

Position (H)	δ_H	δ_C	HMBC (carbon)	^{1}H–^{1}H COSY
1β	3.68 (t, J = 10.0)	81.3 CH	9, 20	2β
2α	2.10 (m)	30.4 CH_2	1, 3, 4, 18, 19	2β, 9β
2β	1.94 (m)		–	2α
3α	1.43 (m)	33.1 CH_2	1, 4, 5, 18, 19	3β
3β	1.93 (m)		–	3α
4	–	37.4 C		
5β	1.88 (dd, J = 11.8, 1.6)	45.3 CH	4, 7, 9, 10	6β, 7β
6α	2.17 (dd, J = 10.8, 7.2)	29.9 CH_2	5, 7, 8	19 (Me)
6β	2.36 (dd, J = 12.0, 8.0)		5	5β, 19 (Me)
7β	5.06 (dd, J = 12.0, 4.4)	74.7 CH	8, 9, 14	5β
8	–	62.1 C		
9β	2.06 (d, J = 8.4)	56.9 CH	5, 8, 10, 11, 12, 14, 20	1β
10	–	48.1 C		
11α	3.33 (m)	21.6 CH_2	9	11β, 12β
11β	1.74 (m)		8, 9, 10, 12, 13	11α, 12α
12α	1.98 (m)	31.5 CH_2	–	11β
12β	1.71 (m)		11, 13, 14, 16	11α
13α	3.31 (br s)	47.6 CH	12, 14, 15	11β, 12β, 14α, 17a
14α	5.34 (s)	76.7 CH	8, 15, 16, 17	13α
15	–	209.5 C		
16	–	150.9 C		
17-Ha, Hb	6.27, 5.68 (s)	115.2 CH_2	–	13α, 17b, 17a
18-Ha, Hb	3.63, 3.30 (AB d, J = 10.8)	70.7 CH_2	4, 19	18a, 18b
19	0.93	18.6 CH_3	3, 4, 5, 18	–
20-Ha, Hb	4.77(d, J = 12.0), 4.45 (m)	62.5 CH_2	1, 9, 10	20a, 20b

Table 2. Atomic coordinates and equivalent isotropic displacement parameters (Å^2).

Atom	x	y	z	*U*(eq)
O(1)	0.66232(12)	0.49477(5)	1.14459(10)	0.0427(2)
O(2)	0.45349(15)	0.41889(5)	0.33150(11)	0.0583(2)
O(3)	0.45140(12)	0.69421(4)	0.37591(9)	0.0392(2)
O(4)	0.95419(11)	0.67417(5)	0.59365(11)	0.0466(2)
O(5)	0.48883(11)	0.77751(4)	0.65802(11)	0.0390(2)
O(6)	0.42954(11)	0.61134(5)	1.06579(9)	0.0401(2)
C(1)	0.45749(16)	0.41681(6)	0.89963(14)	0.0368(3)
C(2)	0.39138(17)	0.39584(6)	0.69432(15)	0.0393(3)
C(3)	0.28658(16)	0.45612(6)	0.56165(13)	0.0353(3)
C(4)	0.43383(14)	0.52056(5)	0.61242(12)	0.0272(2)
C(5)	0.35028(15)	0.58477(6)	0.48631(12)	0.0297(2)
C(6)	0.52725(15)	0.63438(5)	0.49618(12)	0.0288(2)
C(7)	0.65258(13)	0.65959(5)	0.69552(12)	0.0260(2)
C(8)	0.56970(14)	0.72038(5)	0.78506(13)	0.0287(2)
C(9)	0.76778(15)	0.74547(6)	0.94178(14)	0.0364(3)
C(10)	0.82962(17)	0.69123(7)	1.10122(14)	0.0391(3)
C(11)	0.86658(15)	0.61879(6)	1.02856(14)	0.0339(3)
C(12)	0.71223(13)	0.59486(5)	0.83418(12)	0.0260(2)
C(13)	0.52347(13)	0.54481(5)	0.82384(12)	0.0240(2)
C(14)	0.60849(15)	0.47891(6)	0.94744(13)	0.0321(3)
C(15)	0.2597(2)	0.43214(7)	0.35942(16)	0.0511(4)
C(16)	0.06359(18)	0.46940(8)	0.56075(18)	0.0491(4)
C(17)	0.86064(14)	0.69195(6)	0.69381(14)	0.0327(3)
C(18)	0.92463(16)	0.74729(6)	0.84253(16)	0.0381(3)
C(19)	1.08972(19)	0.78763(8)	0.8749(2)	0.0560(4)
C(20)	0.35183(14)	0.57808(6)	0.88627(12)	0.0308(2)

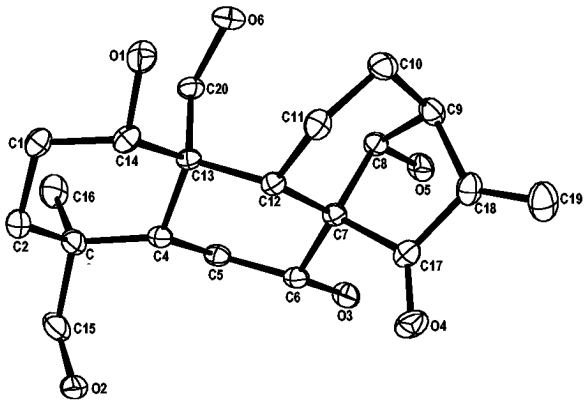


Fig. 2. Molecular structure of compound **1** at 30% ellipsoid probability.

HRESIMS. The IR absorption spectrum indicated the presence of hydroxy groups (3445, 3340 cm^{-1}) and α,β-unsaturated carbonyl groups (1728, 1648 cm^{-1}), and the assignments were confirmed by its ^{13}C and ^{1}H NMR spectral data ($\delta = 81.3$, $\delta = 74.7$, $\delta = 76.7$, $\delta = 70.7$ and $\delta = 62.5$ ppm; $\delta = 209.5$, $\delta = 150.9$, $\delta = 115.2$ ppm and $\delta = 6.27$, $\delta = 5.68$ ppm). Compound **1** showed 20 carbon signals, which indicated

Table 3. Selected bond lengths (Å) and bond angles (°).

Bond	Dist	Bond	Dist	Bond	Dist	Bond	Dist
O(1)-C(14)	1.4565(13)	C(1)-C(2)	1.5310(15)	C(5)-C(6)	1.5147(15)	C(9)-C(10)	1.5417(16)
O(2)-C(15)	1.4338(19)	C(2)-C(3)	1.5354(15)	C(6)-C(7)	1.5466(12)	C(10)-C(11)	1.5386(17)
O(3)-C(6)	1.4430(12)	C(3)-C(16)	1.5418(17)	C(7)-C(8)	1.5471(14)	C(11)-C(12)	1.5662(12)
O(4)-C(17)	1.2032(14)	C(3)-C(4)	1.5477(15)	C(7)-C(17)	1.5531(14)	C(12)-C(13)	1.5827(13)
O(5)-C(8)	1.4356(12)	C(3)-C(15)	1.5627(16)	C(7)-C(12)	1.5843(13)	C(13)-C(20)	1.5456(14)
O(6)-C(20)	1.4381(12)	C(4)-C(5)	1.5392(13)	C(8)-C(9)	1.5429(13)	C(13)-C(14)	1.5570(13)
C(1)-C(14)	1.5274(15)	C(4)-C(13)	1.5888(12)	C(9)-C(18)	1.5122(18)	C(17)-C(18)	1.5005(16)
Angle	(°)	Angle	(°)	Angle	(°)		
C(14)-C(1)-C(2)	112.56(10)	C(6)-C(7)-C(17)	108.19(8)	C(20)-C(13)-C(14)	107.80(8)		
C(1)-C(2)-C(3)	112.96(9)	C(8)-C(7)-C(17)	100.56(8)	C(20)-C(13)-C(12)	115.47(8)		
C(2)-C(3)-C(16)	111.12(10)	C(6)-C(7)-C(12)	110.33(7)	C(14)-C(13)-C(12)	109.05(7)		
C(2)-C(3)-C(4)	108.07(8)	C(8)-C(7)-C(12)	109.44(8)	C(20)-C(13)-C(4)	111.48(7)		
C(16)-C(3)-C(4)	115.30(10)	C(17)-C(7)-C(12)	106.76(7)	C(14)-C(13)-C(4)	108.53(8)		
C(2)-C(3)-C(15)	107.84(10)	O(5)-C(8)-C(9)	108.83(8)	C(12)-C(13)-C(4)	104.32(8)		
C(16)-C(3)-C(15)	105.33(9)	O(5)-C(8)-C(7)	112.30(8)	O(1)-C(14)-C(1)	108.50(9)		
C(4)-C(3)-C(15)	108.92(10)	C(9)-C(8)-C(7)	102.63(8)	O(1)-C(14)-C(13)	111.80(8)		
C(5)-C(4)-C(3)	113.69(7)	C(18)-C(9)-C(10)	111.37(9)	C(1)-C(14)-C(13)	113.37(7)		
C(5)-C(4)-C(13)	109.77(8)	C(18)-C(9)-C(8)	101.54(9)	O(2)-C(15)-C(3)	113.53(9)		
C(3)-C(4)-C(13)	118.36(8)	C(10)-C(9)-C(8)	109.68(9)	O(4)-C(17)-C(18)	126.91(10)		
C(6)-C(5)-C(4)	110.20(7)	C(11)-C(10)-C(9)	110.32(9)	O(4)-C(17)-C(7)	125.61(9)		
O(3)-C(6)-C(5)	110.73(7)	C(10)-C(11)-C(12)	117.57(8)	C(18)-C(17)-C(7)	107.46(9)		
O(3)-C(6)-C(7)	109.90(8)	C(11)-C(12)-C(13)	119.53(8)	C(19)-C(18)-C(17)	123.99(13)		
C(5)-C(6)-C(7)	113.81(8)	C(11)-C(12)-C(7)	110.16(8)	C(19)-C(18)-C(9)	128.96(12)		
C(6)-C(7)-C(8)	120.39(7)	C(13)-C(12)-C(7)	114.61(6)	C(17)-C(18)-C(9)	107.05(9)		

Table 4. Evaluation of the cytotoxicity of compounds **1**–**5** against human cancer cell lines.

		Cytotoxicity	
Test substance	MW	IC_{50} (μ M)[a] (Bel-7402 cells)	IC_{50} (μ M) (HO-8910 cells)
1	366	96.7±2.6	133±3.4
2	332	1.41±0.9	1.58±0.1
3	334	1.46±0.42	3.2±0.83
4	362	2.25±0.1	2.9±0.5
5	350	22.0±1.0	37.0±6.2

[a] IC_{50}: 50% inhibition concentration.

one methyl groups, eight methylenes groups including an *exo*-methylene group and two oxymethylenes, six methine groups including there oxymethines, five quaternary carbons. The ^{1}H NMR spectrum exhibited two signals at $\delta = 6.27$ (s, 1H) and $\delta = 5.68$ (s, 1H), attributable to *exo*-methylene protons, four doublets at $\delta = 4.77$ (d, $J = 12.0$, 1H), $\delta = 4.45$ (m, 1H), $\delta = 3.63$ (d, $J = 10.8$, 1H) and $\delta = 3.30$ (d, $J = 10.8$, 1H) assignable to oxygen-bearing methylene protons, and one singlet signal at $\delta = 0.93$ (3H, s) due to one methyl group attached to a quaternary carbon. Since two out of the six degrees of unsaturations were accounted for, compound **1** was inferred to contain four rings. The above data corresponded to that of the basic skeleton of C-20-non-oxygenated-*ent*-kaurane diterpenoids previously described from the genus *Isodon* [9–12]. The cross-peaks observed in the HMBC revealed that five hydroxy groups were located at C-1, C-7, C-14, C-18 and C-20.

The aforementioned inferences were confirmed by the X-ray diffraction analysis. Furthermore, the X-ray crystal structure showed that the hydroxyl groups at C-1 and C-7 is situated in the α-orientation, and the hydroxyl at C-14 in the β-orientation, respectively, and three six-membered rings are in a chair-like conformation. A five-membered ring adopts a twist envelope-like conformation in the compound, respectively, as shown in Fig. 2. The molecules formed extensive networks through the intra-molecular hydrogen bonds O(1)–H(1C)…O(6), 2.673 Å; O(3)–H(3A)…O(5), 2.619 Åand intermolecular hydrogen bonds O(2)–H(2C)…O(1), 2.751 Å; O(5)–H(5C)…O(2), 2.714 ÅO(6)–H(6A)…O(3), 2.810 Å and arrange along *a* axis in the crystal.

Compounds **2**, **3**, **4**, and **5** were identified as the diterpenoids glaucocalyxin A (**2)**, kamebanin (**3)** macrocalyxin D (**4)**, and excisanin K (**5)**, respectively, as shown in Fig. 1.

Compounds **1**–**5** were evaluated against human tumor Bel-7402 and HO-8910 cells and found to be significantly cytotoxic, exhibiting IC_{50} values ranging from $1.41 \pm 0.9 - 133 \pm 3.4$ μM. The most potent activity was observed with the IC_{50} values of 1.41 ± 0.9, 1.58 ± 0.1; 1.46 ± 0.42, 9.2 ± 1.3; $2.25 \pm$

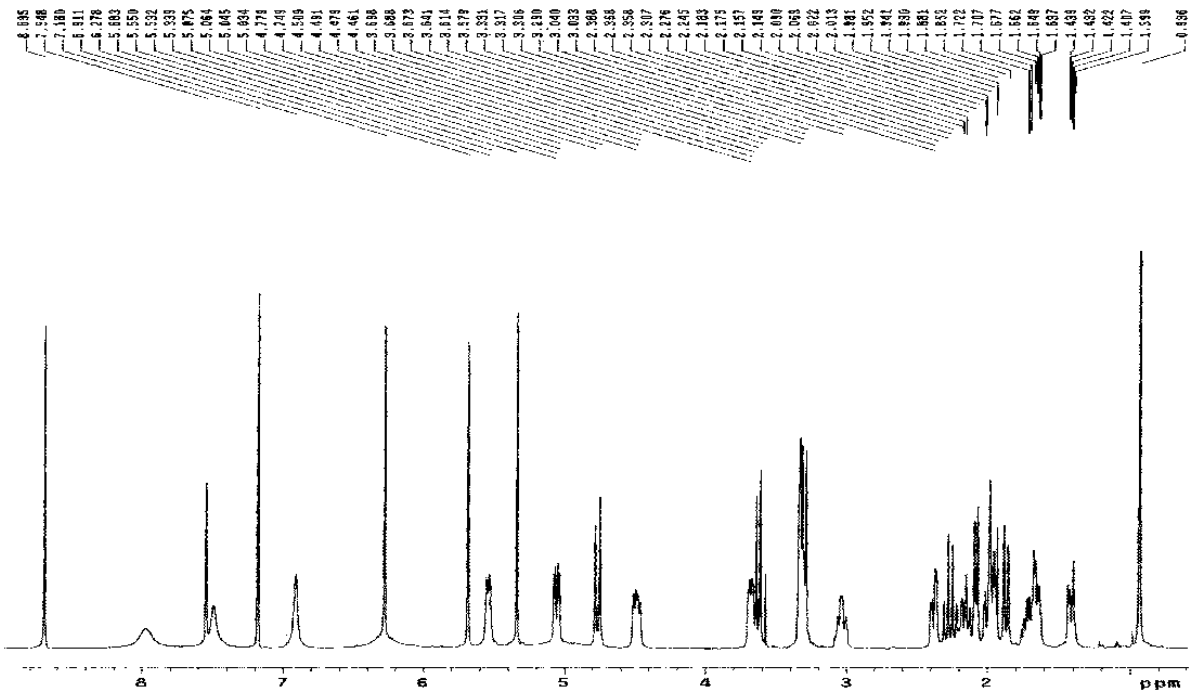


Fig. 3. The ^{1}H NMR spectrum of compound **1**.

0.1, 2.9 ± 0.5 μM for compounds **2**, **3**, and **4**, respectively.

Experimental Section

General methods

Melting points were obtained on Kofler-microscope apparatus. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Bruker IFS-120H IR spectrometer with KBr pellets. 1D and 2D NMR spectra were recorded on an INOVA-400 (Varian) spectrometer with TMS as internal standard. HR-ESI-MS, EI-MS spectra and FAB-MS were obtained on a Bruker APEXIIFT-MS, HP 5988 MS and ZAB-HS mass spectrometers respectively.

Plant material

The leaves of *Isodon weisiensis* C. Y. Wu were collected in Li county of Gansu Province, China, August 2004, and identified by Prof. Sun Kun and a voucher specimen (XCC-04-8-20) was deposited in College of Life Sciences, Northwest Normal University.

Extraction and isolation of compounds

The dried and powdered leaves (7.5 kg) of *I. weisiensis* C. Y. Wu were extracted three times with 60% Me_2CO at room temperature and filtered. The filtrate was concentrated under reduced pressure and partitioned with EtOAc. The EtOAc layer was evaporated in vacuum to give a residue (120 g). The dried extract was adsorbed on silica gel (1600 g, 200 – 300 mesh) and eluted with a gradient of $CHCl_3$–Me_2CO (60:1 – 1:1) and finally with MeOH. According to the differences in composition indicated by TLC, 12 crude fractions were obtained. F9 (8:1 – 1:1) was chromatographed and eluted with mixtures of Me_2CO in $CHCl_3$ (50% – 100%) and afforded compound **2** (32 mg), compound **3** (65 mg) and compound **4** (35 mg), respectively. The MeOH eluant was concentrated and the residue (25 g) was chromatographed on silica gel (400 g) with $CHCl_3$–MeOH (10:1, 7:1) to obtain compound **5** (67 mg) and compound **1** (42 mg).

Weisiensin C (**1**)

Colorless crystals. M. p. 236 – 238 °C. – $[\alpha]_D^{20} - 96°$ (c = 0.15, MeOH). – IR (KBr): $\tilde{\nu}$ = 3445, 3340, 2935, 2883, 1728, 1648, 1442, 1261, 1058, 937 cm^{-1}. – MS(EI, 70 eV): *m/z* (%) = 366 $[M]^+$(6), 348 (12), 332 (45), 314 (9), 296 (28), 283 (11), 245 (100). – HRESIMS: *m/z* obsd. = 384.2381 ($M+NH_4$) for $C_{20}H_{30}O_6+NH_4$ calcd. = 384.2386. ^{1}H NMR and ^{13}C NMR see Table 1.

Glaucocalyxin A (**2**)

Colorless needles. M. p. 230 – 232 °C. – $[\alpha]_D^{20} - 158°$ (c = 0.35, $CHCl_3$). – IR (KBr): $\tilde{\nu}$ = 3252, 2940, 2870, 1725,

1708, 1651, 1459, 1385, 1252, 1127, 1082, 946 cm^{-1}. – ^1H NMR (400 MHz, $CDCl_3$): $\delta = 6.19$ and 5.44 (s, each 1H, 17-H_2), 4.85 (d, $J = 1.0$ Hz, 1H, 14αH), 4.37 (dd, $J = 12.0$ Hz, $J = 4.0$ Hz, 1H, 7β-H), 3.11 (br s, 1H, 13β-H), 1.14 (s, 3H, 20-Me), 1.09 and 1.09 (s, 6H, 18-Me and 19-Me). – $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): $\delta = 38.1$ (C-1), 33.5 (C-2), 216.6 (C-3), 46.6 (C-4), 51.5 (C-5), 28.9 (C-6), 74.1 (C-7), 61.5 (C-8), 52.6 (C-9), 38.7 (C-10), 18.0 (C-11), 30.6 (C-12), 45.8 (C-13), 74.7 (C-14), 207.5 (C-15), 147.1 (C-16), 118.4 (C-17), 27.7 (C-18), 20.8 (C-19), 18.2 (C-20). – MS(EI, 70 eV): *m/z* = 332(M^+)(5), 315 (11), 299 (28), 281 (43), 176 (16), 138 (100).

Kamebanin (**3**)

Colorless needles. M. p. 238-240 °C. – $[\alpha]_D^{20} - 86°$ (c = 0.5, C_5H_5N). – IR (KBr): $\tilde{\nu} = 3248$, 1730, 1649, 1467, 1099, 1023, 929 cm^{-1}. – ^{1}H-NMR (400 MHz, C_5D_5N): $\delta = 6.19$ and 5.25 (br s, each 1H, 17-H_2), 5.95 (br s, 1H, 14α-H), 4.66 (m, 1H, 7β-H), 4.41(dd, $J = 11.2$ Hz, 6.8 Hz, 1H, 1β-H), 3.16 (m, 1H, 13α-H), 1.36 (s, 3H, 20-Me), 0.83 (s, 3H, 18-Me), 0.80 (s, 3H, 19-Me). – $^{13}C\{^1H\}$ NMR (100 MHz, C_5D_5N): $\delta = 79.1$ (C-1), 29.0 (C-2), 38.8 (C-3), 32.6 (C-4), 51.0 (C-5), 29.1 (C-6), 73.3 (C-7), 61.2 (C-8), 56.0 (C-9), 44.5 (C-10), 19.8 (C-11), 31.5 (C-12), 46.9 (C-13), 74.6 (C-14), 207.6 (C-15), 149.1 (C-16), 115.6 (C-17), 32.9 (C-18), 21.3 (C-19), 14.5 (C-20). – MS(EI, 70 eV): *m/z* = 334 (M^+)(9), 316 (13), 298 (26), 283 (45), 195 (32), 176 (16), 121 (100).

Macrocalyxin D ***(4)***

White powder. M. p. 218-220 °C. – $[\alpha]_D^{20} - 78°$ ($c = 0.37$, C_5H_5N). – IR (KBr): $\tilde{\nu} = 3445$, 3350, 3311, 2910, 1714, 1640, 1210 cm^{-1}. – ^{1}H NMR (400 MHz, C_5D_5N): $\delta = 9.26$ (s, 1H, CHO), 6.34 and 5.65 (br s, each 1H, 17-H_2), 5.38 (s, 1H, 14α-H), 4.97 (dd, $J = 12.0$ Hz, 4.8 Hz, 1H, 7β-H), 4.28 and 4.16 (ABd, $J = 12.0$ Hz, each 1H, 20-H_2), 3.29 (br s, 1H, 13α-H), 1.21 (s, 3H, 19-Me). – $^{13}C\{^1H\}$ NMR (100 MHz, C_5D_5N): $\delta = 34.2$ (C-1), 17.5 (C-2), 32.1 (C-3), 49.5 (C-4), 45.7 (C-5), 32.3 (C-6), 73.9 (C-7), 61.9 (C-8), 54.9 (C-9), 41.9 (C-10), 18.5 (C-11), 30.8 (C-12), 47.4 (C-13), 76.5 (C-14), 208.2 (C-15), 150.3 (C-16), 115.9 (C-17), 205.6 (C-18), 14.9 (C-19), 60.4 (C-20). – MS (FAB): *m/z* = 371 $[M+Na]^+$, 349 $[M+H]^+$, 331, 313, 57.

Excisanin K (**5**)

Colorless needles. M. p. 138 – 140 °C. – $[\alpha]_D^{20} - 101°$ ($c = 0.32$, MeOH). – IR (KBr): $\tilde{\nu} = 3328$, 1725, 1645, 1448, 1255, 1026 cm^{-1}. – ^{1}H NMR (400 MHz, C_5D_5N): $\delta = 6.25$ and 5.30 (br s, each 1H, 17-H_2), 5.30 (s, 1H, 14α-H), 4.93 (t, $J = 12.0$ Hz, 1H, 7β-H), 3.66 and 3.31 (ABd, $J = 10.8$ Hz, each 1H, 18-H_2), 3.58 (m, 1H, 11α-H), 3.54(t, $J = 8.4$ Hz, 1H, 1β-H), 3.27 (br s, 1H, 13α-H), 1.49 (s, 3H, 20-Me), 0.89 (s, 3H, 19-Me). – $^{13}C\{^1H\}$ NMR (100 MHz, C_5D_5N): $\delta = 80.2$ (C-1), 30.0 (C-2), 33.7 (C-3), 37.8 (C-4), 45.4 (C-5), 29.4 (C-6), 74.4 (C-7), 62.7 (C-8), 56.7 (C-9), 45.4 (C-10), 20.3 (C-11), 31.9 (C-12), 47.4 (C-13), 75.9 (C-14), 208.9 (C-15), 150.5 (C-16), 115.4 (C-17), 70.9 (C-18), 17.9 (C-19), 15.6 (C-20). – MS (FAB): *m/z* = 373 $[M+Na]^+$, 351 $[M+H]^+$, 332.

X-ray crystallographic analysis of **1**

A crystal of dimensions $0.50 \times 0.40 \times 0.40$ mm^3 was mounted on a glass fiber. X-ray diffraction intensity data were collected on a Siemens P4 diffractometer equipped with a graphite-monochromated Mo-K_α radiation (λ = 0.71073 Å) by an ω scan mode at 293 K. A total of 1855 reflections were measured within $3.03° \leq \theta \leq 25.03°$, yielding 1684 unique reflections ($R_{int} = 0.0241$). The compound crystallizes in a monoclinic space group $P2_1$ with $a = 6.8316(14)$ Å, $b = 19.016(3)$ Å, $c = 7.6541(10)$ Å, $\tilde{\nu} = 933.6(3)$ $Å^3$, $Z = 2$ and with a calculated density of 1.304 Mg/m^3, $F(000) = 396$, $\mu = 0.095$ mm^{-1}. The crystal structure was solved by direct methods with the Siemens SHELXTL^{TM} Version 5 package of crystallographic software [13], and refined by full-matrix least-square refinement on F^2. All non-hydrogen atoms were refined anisotropically. The final refinement corerged at $R = 0.0362$, $wR = 0.1035[w = 1/(\backslash s^2(F_0^2) + (0.0804P)^2 + 0.1769P)$, where $P = (F_0^2 + 2F_c^2)/3]$, $(\Delta/\sigma)_{max} = 0.001$. The largest peak and deepest hole on the final difference Fourier map are 0.283 and -0.274 e/$Å^3$, respectively.

Crystallographic data for the structure have been deposited with the Cambridge Crystallographic Data Centre, CCDC-261962. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: int. code + (1223) 336-033; e-mail for inquiry: fileserv@ccdc.cam.ac.uk).

Tests of cytotoxicity against human tumor Bel-7402 and HO-8910 cells

Compounds **1** – **5** were evaluated for cytotoxic potential against human cancer cell lines Bel-7402 and HO-8910 as previously described [8].

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[1] Y. Takeda, H. Otsuka, Recent advances in the chemistry of deterpenoids from *Rabdosia* species. In Atta-ur-Rahman (ed.): Studies in Natural Products Chemistry, Vol. 15, p. 111, Elsevier, Amsterdam (1995).
[2] H. D. Sun, Y. L. Xu, B. Jiang, Diterpenoids from *Isodon* species, p. 93, Science Press, Beijing (2001).
[3] A. Castrillo, M. De las Heras, S. Hortelano, B. Rodriguez, A. Villar, L. J. Bosva, Biol. Chem. **276**, 15854 (2001).
[4] A. Abbaskhan, M. L. Choudhary, Y. Tsuda, M. Parvez, A. Rahman, F. Shaheen, Z. Parween, R. B. Tareen, M. A. Zaidi, Planta Med. **69**, 94 (2003).
[5] X. M. Niu, S. H. Li, M. L. Li, Q. S. Zhao, S. X. Mei, Z. Na, S. J. Wang, Z. W. Lin, H. D. Sun, Planta Med. **68**, 528 (2002).
[6] C. L. Zhang, L. J. Wu, S. I. Tashiro, S. Onodera, T. Ikejima, Acta Pharmacol Sin. **25**, 691 (2004).
[7] Y. L. Xu, M. Wu, Phytochemistry **28**, 1978 (1989).
[8] K. T. Paoazisis, G. D. Geromichalos, K. A. Dimitriadia, A. H. Kortsaris, J. Immunol Methods **208**, 151 (1997).
[9] Y. Z. Li, Y. Z. Chen, Phytochemistry **31**, 221 (1992).
[10] Y. H. Wang, Y. Z. Chen, J. Nat. Prod. **60**, 1161 (1997).
[11] N. Zhi, B. Jiang, X. M. Niu, Z. W. Lin, C. M. Li, H. D. Sun, Acta. Botanica Sinica **44**, 477 (2002).
[12] M. Y. Gui, Y. Aoyagi, Y. R. Jin, X. W. Li, T. Hasuda, K. Takeya, J. Nat. Prod. **67**, 373 (2004).
[13] Siemens (1994), SHELXTL^{TM} Version 5 Reference Manual, Siemens Energy & Automation Inc., Madison, Wisconsin, USA.