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CYTOTOXIC *ENT*-KAURENE DITERPENOIDS FROM *ISODON GESNEROIDES*

HAN-DONG SUN, ZHONG-WEN LIN, FANG-DI NIU, QI-TAI ZHEN,* BIN WU,* LONG-ZE LIN† and GEOFFREY A. CORDELL‡

Laboratory of Phytochemistry, Kunming Institute of Botany, Academia Sinica, Kunming 650 204, Yunnan, P.R. China; *Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100 050, P.R. China; †Program for Collaborative Research in Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, U.S.A.

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Key Word Index—*Isodon gesneroides*; Labiatae; *ent*-kaurene diterpenoids; gesneroidins A-C; Dawoensin A; structure determination; NMR assignments; two-dimensional NMR techniques; biological evaluation.

Abstract—From *Isodon gesneroides*, three new cytotoxic diterpenoids, gesneroidins A, B and C, together with one known diterpenoid, dawoensin A, were isolated, and the structure determination and unambiguous assignment of their stereochemistry and NMR spectral data were made by a combination of one- and two-dimensional NMR techniques, computer modelling calculations and X-ray analysis.

INTRODUCTION

In previous studies of the chemical constituents of the plants from the *Isodon* genus (family Labiatae), we have reported on the isolation of several new diterpenoids, and some bioactive compounds [1–6]. Our continuing studies on the plants of this genus led to the isolation of three new *ent*-kaurene diterpenoids from *Isodon gesneroides* (J. Sincl.) Hara (Labiatae), a previously uninvestigated perennial herb, which grows to a height of 30 cm in the southwestern area of Sichuan Province, P. R. China. It has been used in traditional folk medicine for its antibacteral and anti-inflammatory activity by local practitioners. In this paper, we present the isolation, structure elucidation and assignment of stereochemistry of these three new cytotoxic diterpenoids (gesneroidins A–C) by a combination of spectral and X-ray analyses and computer modelling calculations.

RESULTS AND DISCUSSION

An ether extract from the leaves of *I. gesneroides* was subjected to column chromatography on silica gel followed by recrystallization to yield gesneroidins A–C and dawoensin A.

Gesneroidin A (**1**), $C_{22}H_{32}O_4$ [high resolution mass spectrometry (HRMS)], colourless crystals, mp 167°, showed IR and UV absorptions for the existence of a five-membered ring ketone conjugated with an *exo*-methylene and an acetyl function (239 nm; 1725 and 1645 cm^{-1}) [3]. The ^1H, ^{13}C, DEPT and HMQC [7] spectra of **1** showed signals for a diterpenoid with an acetyl function, i.e. four methyls (C-18, C-19, C-20 and the methyl of the acetyl), seven methylenes (C-1, C-2, C-3, C-7, C-12, C-14 and C-17), five methines (C-5, C-6, C-9, C-11 and C-13), four quaternary carbons (C-4, C-8, C-10 and C-16), and two carbonyls (C-15 and the carbonyl of the acetyl). As described in several previous papers dealing with triterpenoids and polyoxypregnanes [8–11], DQF-COSY [12, 13], HOHAHA [7, 13–15] and ROESY [16–18], experiments were used to establish information concerning the coupling correlations and coupling constants between the geminal and vicinal protons, the long-range connectivity of the protons four bonds away, and the spatial distances between two protons. The relative stereochemical and long-range correlation relationships of the protons from these experiments are shown in Table 1. Thus, the existence of three fragments, $-CH_2CH_2CH_2-$ (C-1 to C-3), $-CHCHCH_2-$ (C-5 to C-7), and $-CHCHCH_2CHCH_2-$ (C-9 and C-11 to C-14) was disclosed. The stereotopical assignments of these protons were established as shown in Table 2, and the skeleton was further determined by the use of FLOCK [19] and selective INEPT [20, 21] experiments, which served to divulge the long-range (three- or two-bond) correlations between protons and carbons, and afforded the skeletal connectivity; the results from these experiments are presented in Table 1. Isolate **1** was considered to have a diterpenoid skeleton with two geminal

‡Author to whom correspondence should be addressed.

Table 1. Principal results from the ROESY, FLOCK and selective INEPT spectra of gesneroidin A (**1**)*†

Proton	ROESY (proton)	FLOCK (carbon)	Selective INEPT (carbon)
1α	2β, 11α	(2), 5, 9, (10), 20	(2), 3, 5, (10)
1β	2β	3, 20	3, 5, 9, (10), 20
2α	19, 20	(1), 4	(1), 4, 10
2β	1β	n.o.	(1), (3), 4, 10
3α	18, 19	1, (2)	1, (2), (4), 18, 19
3β	18	(2), (4), 18	1, (4), 5, 19
5β	6β, 7β, 9β, 18	3, (4), 7, 10, 18, 19, 20	1, (4), 9, (10), 19, 20
6β	5β, 7α, 7β, 18, 19	(7), 8, 10	4, (5), (7), 8, 10
7α	6β, 14β	(6), 9	5, (6), (8), 9, $OCOC\underline{H}_3$
7β	5β, 6β, 9β	(6), (8), 14	(8), 15, $OCOC\underline{H}_3$
9β	5β, 7β, 11α	1, 7, (8), (10), (11), 12, 20	1, 5, 7, (8), (10), (11), 12, 14, 15
11α	1α, 9β, 12α, 12β, 20	8, 13	8, (9), 10, 13
12α	11α, 13α, 14α, 20	(11), (13), 14, 16	9, (13), 14, 16
12β	11α, 13α	(11), 16	9, (11), (13), 16
13α	12α, 12β, 14α, 14β, 17b	11, (14)	8, 11, (12), (14), 15, (16), 17
14α	12α, 20	(8), 9, 12, 15	(8), 9, 12, (13), 15, 16
14β	7β, 13α	9, 12	7, (8), 9, (13)
17a	17b	13, 15, (16)	15, (16)
17b	13α, 17a	13, 15	13, 15, (16)
18	3α, 3β, 5β, 6β	(4), 5, 19	3, (4), 5
19	2α, 3α, 6β, 20	(4), 5, 18	3, (4), 5, 18
20	2α, 11α, 12α, 14α, 19	1, 5, 9, (10)	1, 5, 9, (10)
$OCOC\underline{H}_3$	19, 20	6	$OC\underline{O}CH_3$

*The ROESY experiment was performed at 500.1 MHz with a spin-lock time of 300 msec and a spin-lock field strength of 5 kHz [7, 14].

†FLOCK and selective INEPT were performed with $J = 6$ Hz at 125.8 and 90.8 MHz, respectively [7, 14]; two-bond correlations are indicated in parentheses; n.o. indicates no clear correlations with this proton.

Table 2. ^{1}H NMR data for gesneroidin A (**1**) and B (**2**)*

Proton	**1**	**2**
1α	1.82 (*ddd*, 13.0, 3.0, 3.0)	1.78 (*ddd*, 13.5, 3.0, 3.0)
1β	0.89 (*ddd*, 13.0, 13.0, 4.0)	1.12 (*ddd*, 13.5, 13.0, 4.0)
2α	1.26 (*ddddd*, 12.5, 13.0, 13.0, 4.0, 3.0)	1.25 (*ddddd*, 12.5, 13.0, 13.0, 4.0, 3.0)
2β	1.67 (*ddddd*, 12.5, 4.0, 4.0, 3.0, 3.0)	1.44 (*ddddd*, 12.5, 4.0, 4.0, 3.0, 3.0)
3α	1.22 (*ddd*, 14.0, 4.0, 3.0)	1.19 (*ddd*, 15.0, 4.0, 3.0)
3β	0.98 (*ddd*, 14.0, 13.0, 4.0)	1.07 (*ddd*, 15.0, 13.0, 4.0)
5β	1.01 (*d*, 3.0)	1.02 (*d*, 2.0)
6β	5.66 (*ddd*, 3.5, 3.0, 2.0)	5.67 (3.5, 3.5, 3.0)
7α	1.67 (*dd*, 15.5, 3.5)	1.76 (*dd*, 15.5, 3.5)
7β	2.35 (*dd*, 15.5, 3.0)	2.20 (*dd*, 15.5, 3.5)
9β	1.81 (*d*, 2.0)	2.02 (*br.s*)
11α	4.23 (*dd*, 4.3, 2.0)	
12α	2.12 (*ddd*, 13.5, 4.3, 3.0)	2.73 (*dd*, 15.5, 4.5)
12β	2.21 (*dddd*, 13.5, 4.0, 2.0, 2.0)	2.55 (*ddd*, 15.5, 4.5, 3.0)
13α	2.96 (*ddd*, 4.0, 3.0, 3.0)	3.12 (*dt*, 4.5, 4.5)
14α	2.59 (*br d*, 12.0)	2.93 (*d*, 12.5)
14β	1.28 (*dddd*, 12.0, 3.0, 2.0, 2.0)	1.50 (*dddd*, 12.5, 3.0, 2.0, 2.0)
17a	5.95 (*br s*)	6.01 (*br s*)
17b	5.21 (*br s*)	5.23 (*br s*)
18	0.85 (*s*)	0.86 (*s*)
19	0.93 (*s*)	0.90 (*s*)
20	1.31 (*s*)	1.34 (*s*)
OAc	2.03 (*s*)	2.07 (*s*)

*Recorded in pyridine-d_5; chemical shift values are reported as δ values (ppm) from TMS at 500.1 MHz; signal multiplicity and coupling constants (Hz) are shown in parentheses.

methyls at C-4, and one methyl at C-10, consistent with many of the diterpenoids from plants of the *Isodon* genus. The signals of the two methyl groups at δ0.85 and 0.93 showed correlations with one methylene (δ43.65), one quaternary carbon (δ34.01) and one methine (δ55.00) carbon, and the latter also showed a correlation with the methyl at δ1.31 and should be assigned to C-5. Thus, the methyl signal at δ1.31 is H_3-20, the other two methyls (δ0.85 and 0.93) are H_3-18 and H_3-19, the methylene is C-3 and the quaternary carbon is C-4. The H_3-20 signal also showed a correlation with another methine carbon (δ63.65, C-9), as well as a methylene carbon (δ41.80, C-1) and a quaternary carbon (δ38.95). H-7β, H-9β, H-14α and H-17 were coupled with C-15 (δ208.45), H-13α was coupled with C-16, H-12 protons were coupled with C-16, H-14 protons were coupled with C-12, and H-17 protons were coupled with C-13 and C-15, which served to confirm the location of the double bond (C-16 and C-17) connected to C-13, and the carbonyl function at C-15. Based on the results shown in Table 1, all of the carbons could be assigned unambiguously, as shown in Table 3.

According to the results (Table 1) from the DQF-COSY and ROESY experiments, C_{11}-OH was assigned to occupy a β-orientation, and the C_6-OAc group an α-orientation. The β-orientation for the C_{11}-OH was based on the coupling constants for H-11α with H-9β (0.65 Hz), H-12α (2.30 Hz) and H-12β (4.18 Hz), and also the interspatial distances of H-11 with H-1α (2.22 Å) and the α-H-20 methyl (2.54 Å). In the same way, H-6 was assigned to a β-orientation, and thus, the C_6-OAc should occupy an α-orientation. Most of the coupled protons show complex peaks in the ^{1}H spectrum, but could be assigned with the assistance of computer modelling calculations, which generated a chair, a chair and a chair conformation for the three six-membered rings, respectively. For example, H-2α has four coupling constants (J values) with its four different vicinal protons, i.e. J = 3.37 Hz (57°) with H-1α, 13.10 Hz (171°) with H-1β, 3.96 Hz (54°) with H-3α, 12.97 Hz (169°) with H-3β, and one more coupling constant with its geminal partner H-2β. Thus, the multiplet signal at δ1.26 was recognized as a *ddddd* peak for H-2α with J = 12.5, 13.0, 13.0, 4.0 and 3.0 Hz. Similarly, the signal of its geminal partner H-2β appeared at δ1.67 as a very complex resonance, which was resolved as a *ddddd* peak with J = 12.5, 4.0, 4.0, 3.0 and 3.0 Hz based on the following computer calculations: J = 3.24 Hz (58°) with H-1α, 3.89 Hz (55°) with H-1β,

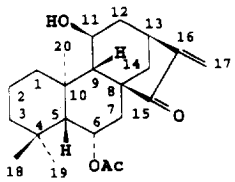
Table 3. ^{13}C NMR data for genseroidins A (**1**), B (**2**) and C (**3**), dawoensin A (**4**) and the acetate (**5**)*

Carbon	1	2	3	4	5
1	41.80	42.50	76.97	35.45	79.97
2	18.68	18.44	33.74	22.70	29.43
3	43.65	43.26	79.09	78.26	78.20
4	34.01	34.19	37.14	36.90	37.11
5	55.00	54.24	42.39	43.52	42.19
6	69.11	68.14	70.46	70.25	70.06
7	38.44	36.16	71.03	71.43	70.57
8	48.93	51.12	48.82	48.40	48.39
9	63.65	68.32	55.99	59.23	54.71
10	38.95	40.07	44.11	38.18	43.08
11	65.04	209.03	71.49	64.74	69.72
12	41.59	51.95	38.22	40.79	38.56
13	38.01	37.25	36.97	37.39	36.68
14	38.44	36.55	35.71	35.63	35.60
15	208.45	206.42	205.21	205.10	204.44
16	151.08	147.87	150.84	151.16	150.33
17	111.22	118.25	112.38	111.05	112.84
18	33.40	33.37	28.03	28.01	27.83
19	23.21	23.21	23.45	23.19	24.14
20	19.08	20.54	15.07	19.19	15.48
1α-COCH$_3$	—	—	—	—	20.69
1α-COCH$_3$	—	—	—	—	170.17
3β-COCH$_3$	—	—	20.84	20.76	21.14
3β-COCH$_3$	—	—	170.20	169.97	170.03
6α-COCH$_3$	21.60	21.51	21.35	21.16	21.24
6α-COCH$_3$	170.11	170.02	169.80	169.64	169.57
7β-COCH$_3$	—	—	21.23	21.31	21.66
7β-COCH$_3$	—	—	169.72	169.48	169.43
11β-COCH$_3$	—	—	21.23	—	20.98
11β-COCH$_3$	—	—	169.55	—	169.20

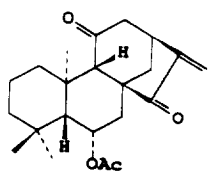
*Recorded in pyridine-d_5, at 125.8 MHz.

2.85 Hz (60°) with H-3α, and 4.07 Hz (54°) and H-3β. All of the remaining coupled signals were resolved in the same manner, and the coupling patterns and *J* values are also indicated in Table 2. Thus, this isolate should be 6α-acetoxy-11β-hydroxy-*ent*-kaur-16-en-15-one. The structure of **1** deduced from NMR spectral analyses was consistent with the result derived from a single crystal X-ray analysis of its oxidation product **2**.

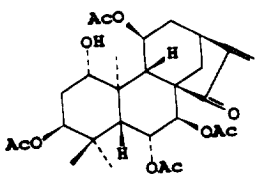
Gesneroidin B (**2**), $C_{22}H_{30}O_4$ (HRMS), was obtained from this plant as colourless needles. Its mass spectrum showed a molecular ion (*m/z* 358) 2 amu less than that of **1**. The ^1H, ^{13}C and DEPT NMR spectra of **2** were very similar to those of **1**, and the only difference between **2** and **1** was that **2** had one more carbonyl and one less hydroxy function. Inspection of the ^1H and ^{13}C NMR data and COSY spectra of **2** and **1**, indicated that **2** had a $-CH_2CHCH_2-$ fragment for C-12 to C-14, which was supported by the observation that H-9β appeared as a broad singlet without any vicinal correlation relationships, but with W-type coupling to H-14β. Thus, **2** has C-11 as a carbonyl carbon instead of a hydroxyl group as in **1**. Compound **2** was obtained by oxidation of **1** with $K_2Cr_2O_7$-acid. The ^1H and ^{13}C data for **2** were completely assigned by the use of COSY, ROESY, HETCOR and FLOCK experiments, and are shown in Tables 2 and 3, respectively. Therefore, **2** should be 6α-acetoxy-*ent*-kaur-16-ene-11,15-dione.



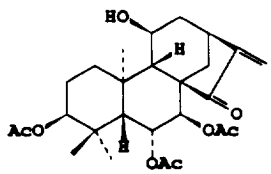
Gesneroidin A (1)



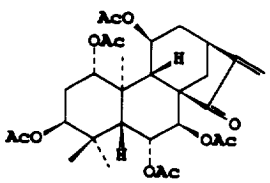
Gesneroidin B (2)



Gesneroidin C (3)



Dawoensin A (4)



3-Acetylcalciolin A (5)

Gesneroidin C (**3**), $C_{28}H_{40}O_{10}$ (HRMS), was obtained from this plant as a crystals. Like **1** and **2**, its UV and IR spectra showed absorptions for the existence of acetyls and a five-membered ring ketone conjugated with an *exo*-methylene. The ^1H, ^{13}C, and DEPT NMR spectra showed that this isolate had three more acetyl functions than had **1**. Furthermore, analysis of the COSY, ROESY, HETCOR and FLOCK spectra of **3** indicated that this compound should be 1α-hydroxy-3β,6α,7β,11β-tetra-acetoxy-*ent*-kaur-16-en-15-one, and the unambiguous assignments of its ^1H and ^{13}C spectra are shown in Tables 2 and 3. The FLOCK experiment is indicative of correlations of the acetyl methyl hydrogen signals to C-3, C-6, C-7 and C-11. The ROESY spectrum of **3** showed correlations between H-1 and H-9β, the α-H_3-19 and H-3, β-H_3-18 and H-6, H-5β and H-6, H-14β and H-7, and H-11 and α-H-20, which served to assign H-1 and H-6 in a β-configuration, and H-3, H-7 and H-11 in an α-configuration. Acetylation of **3** afforded the known compound, 3-acetyl-calcicolin A (**5**) [3], the structure and stereochemistry of which were deduced by single crystal X-ray diffraction analysis as 1α,3β,6α,7β,11β-penta-acetoxy-*ent*-kaur-16-en-15-one. This conclusion is consistent with the structure of **3** derived from the above spectral analysis.

Compounds **2–5** were subjected to tests for cytotoxicity, antimalarial and HIV-inhibitory activities; they were found to be active in some of the cytotoxicity tests [22–24].

EXPERIMENTAL

Mps (uncorr.) were determined on a Kofler hot-stage apparatus. The optical rotations were measured with a JASCO-20C polarimeter. UV spectra were recorded in MeOH on a Shimadzu UV-210A spectrometer. IR spectra were recorded in KBr pellets on a Perkin-Elmer 577 interferometer. Solns in pyridine-d_5 were used for all the NMR studies. ^1H NMR, DQF-COSY, HOHAHA and ROESY spectra were recorded at 500.1 MHz with a GE OMEGA 500 instrument, using GE standard programs; ^{13}C NMR and DEPT spectra were recorded at 125.8 MHz with a GE OMEGA 500 instrument; HETCOR and FLOCK spectra were obtained at 500.1/125.8 MHz with a GE OMEGA 500 instrument, using standard programs from the GE library, and $^nJ_{CH} = 6$ Hz was used in the FLOCK and HMBC experiments. Selective INEPT spectra were recorded at 90.8 MHz with a Nicolet NT-360 NMR instrument with $J = 6$ Hz. EI mass spectra and HR mass spectra were recorded on a Finnigan MAT-90 instrument. X-ray data were collected on an R3m/E diffractometer, and the major programs used were the SHELXTL system for elucidation of the structure.

Plant material. The plant material of *I. gesneroides* was collected in Miannin County, Sichuan Province, China, in August, 1986, and identified by Prof. H.-W. Li, Kunming Institute of Botany, Academia Sinica, Kunming, where the herbarium specimen has been deposited.

Extraction and separation. The dried powdered leaves (4 kg) of *I. gesneroides* were extracted with Et_2O and the solvent was removed *in vacuo*. The residue was dissolved in MeOH, and decolorized with activated charcoal. The MeOH soln was evapd and the residue (96 g) was subjected to CC on silica gel, eluted with petrol, petrol–Me_2CO mixts with increasing proportions of Me_2CO. Frs were collected, and combined by monitoring with TLC, followed by recrystallization to yield gesneriodins A (**1**, 700 mg, 0.018%), B (**2**, 500 mg, 0.013%), and C (**3**, 500 mg, 0.013%) and dawoensin A (**4**, 2.4 g, 0.06%), respectively. Compound **4** was identified by direct comparison with an authentic sample, on mix. mp., TLC, IR and ^1H measurement (1).

Gesneroidin A (**1**). Compound **1** was obtained as crystals, mp 167°; $[\alpha]_D$ −130.9° (*c* 0.84, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε): 239 (4.26); IR (KBr) ν_{max} cm^{-1}: 3280, 1735, 1725, 1645, 1250, 1040 and 935; ^1H NMR, see Table 2; ^{13}C NMR: see Table 3; EIMS: *m/z* (rel. int. %): 360 (M^+, 36), 318 (31), 300 (93), 285 (50), 267 (74), 257 (18), 239 (24), 231 (27), 229 (36), 225 (64), 196 (53), 177 (37), 154 (61), 136 (61), 123 (47), 105 (51), 91 (65), 81 (52), 69 (81), and 55 (100); HRMS: *m/z* 360.2296 for $C_{22}H_{32}O_4$, calc. 360.2300.

Gesneroidin B (**2**). Compound **2** was obtained as crystals, mp 186°, $[\alpha]_D$ +17.9° (*c*, 1.12, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε): 232 (4.41); IR (KBr) ν_{max} cm^{-1}; 1725, 1703, 1640, 1255 and 1030 cm^{-1}; ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 3; EIMS *m/z* (rel. int. %): 358 (M^+, 12), 330 (34), 316 (57), 298 (84), 283 (45), 270 (27), 225 (25), 242 (63), 193 (64), 153 (56), 147 (55), 135 (57), 123 (62), 109 (67), 91 (70), 81 (67), 69 (87) and 55 (100); HRMS: *m/z* 358.2144 for $C_{22}H_{30}O_4$, calc. 358.2144.

Gesneroidin C (**3**). Compound **3** was obtained as needles, mp 204°, $[\alpha]_D$ −69.5° (*c* 0.92, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε): 239 (4.07); IR (KBr) ν_{max} cm^{-1}: 3485, 1740, 1725, 1648, 1250 and 1032, ^1H NMR, see Table 4; ^{13}C NMR, see Table 3; EIMS *m/z* (rel. int. %): 534 (17), 474 (32), 432 (57), 420 (63), 414 (47), 390 (76), 372 (54), 354 (55), 312 (98), 294 (100), 279 (54), 269 (66), 268 (86), 257 (62), 239 (69), 215 (78), 197 (37), 159 (37), 121 (48), 91 (51), 69 (76), and 55 (65), HRMS: *m/z* 534.2467 for $C_{28}H_{38}O_{10}$, calc. 534.2464.

Oxidation of **1**. To a soln of **1** (50 mg) in Me_2CO (10 ml), 15 drops of Beckmann's mixture ($K_2Cr_2O_7$–H_2SO_4–H_2O) [25] was added, with stirring at room temp. for 1 hr. Sepn of the reaction mixt. by CC yielded an oxidation product (48 mg), identified as **2** by direct comparison of its TLC, mix. mp. and ^1H NMR spectrum with an authentic sample.

Acetylation of **3**. Acetylation of **3** (50 mg) was carried out with Ac_2O–pyridine (10 ml each, 1:1) at room temp. overnight, and after work-up in the usual manner, gave a product (50 mg), which was confirmed to be **5** by direct comparison with a standard sample of **5** [3].

X-ray diffraction data for **2**. $C_{22}H_{30}O_4$; the crystal was monoclinic, space group $P2_1$ with $a = 6.661(2)$, $b = 10.402(4)$, $c = 14.399(5)$ Å, $\beta = 98.75(3)°$, $V = 986.19$ Å^3

Table 4. ^1H NMR data for compounds **3–5***

Proton	3	4	5
1α	—	1.63 (*ddd*, 13.5, 3, 3)	—
1β	4.23 (*dd*, 12, 3.5)	1.44 (*ddd*, 13.5, 13.5, 3)	5.37 (*dd*, 9.5, 6)
2α	2.31 (*ddd*, 15, 12, 4.5)	1.88 (*dddd*, 15, 13.5, 4, 3)	2.08 (*m*)
2β	2.09 (*ddd*, 15, 4.5, 3.5)	1.55 (*dddd*, 15, 6, 3, 3)	2.06 (*m*)
3α	4.87 (*t*, 4.5)	4.70 (*t*, 3)	4.81 (*t*, 3)
5β	2.35 (*d*, 2)	2.28 (*d*, 2)	2.41 (*d*, 2)
6β	5.35 (*dd*, 4, 2)	5.33 (*dd*, 4, 2)	5.32 (*dd*, 4, 2)
7α	5.47 (*d*, 4)	5.46 (*d*, 4)	5.44 (*d*, 4)
9β	2.64 (*br.* s)	2.47 (*br s*)	2.62 (*br s*)
11α	6.38 (*d*, 4.5)	4.76 (*t*, 3.5)	5.94 (*t*, 4)
12α	2.19 (*ddd*, 13.5, 4.5, 3)	2.16 (*m*)	2.22 (*ddd*, 15, 4, 4)
12β	2.03 (*dddd*, 13.5, 4.5, 4, 2.5)	2.11 (*m*)	1.96 (*m*)
13α	2.85 (*dd*, 4, 3)	2.93 (*dd*, 4, 3)	2.86 (*dd*, 4.5, 3)
14α	2.67 (*d*, 13)	2.62 (*d*, 12)	2.62 (*d*, 12.5)
14β	1.40 (*dddd*, 13, 3.5, 2.5, 2)	1.34 (*m*)	1.41 (*dddd*, 12.5, 4.5, 3.2)
17a	6.38 (*s*)	5.84 (*s*)	5.86 (*s*)
17b	5.88 (*s*)	5.14 (*s*)	5.13 (*s*)
18	0.93 (*s*)	0.91 (*s*)	0.90 (*s*)
19	1.04 (*s*)	0.97 (*s*)	1.05 (*s*)
20	1.64 (*s*)	1.34 (*s*)	1.67 (*s*)
1α-OAc	—	—	2.19 (*s*)
3β-OAc	2.20 (*s*)	2.21 (*s*)	2.12 (*s*)
6α-OAc	2.13 (*s*)	2.09 (*s*)	2.11 (*s*)
7β-OAc	1.97 (*s*)	1.91 (*s*)	1.95 (*s*)
11β-OAc	1.70 (*s*)	1.91 (*s*)	1.64 (*s*)

*Recorded in pyridine-d_5; chemical shift values are reported as δ values (ppm) from TMS at 500.1 MHz; signal multiplicity and coupling constants (Hz) are shown in parentheses.

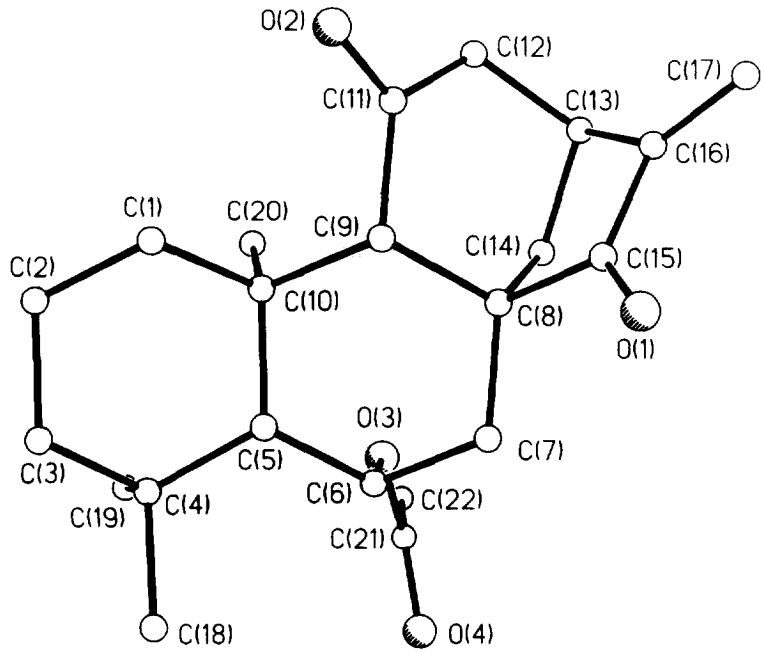


Fig. 1. Molecular structure of gesneroidin B (2).

Table 5. Cytotoxic activity (ED_{50}, $\mu g\ ml^{-1}$) of compounds 2–5*

	Cell lines					
	LU-1	KB	KB-V (+ VLB)	KB-V (− VLB)	LNCap	ZR-75-1
Test compounds						
2	5.5	5.5	7.9	5.1	1.7	0.52
3	4.1	2.8	> 20	> 20	2.7	1.8
4	2.6	2.3	> 20	> 20	1.8	1.4
5	4.3	6.9	> 20	> 20	2.0	1.9
Control compounds						
Colchicine	0.2	0.2	0.6	3.5	0.06	0.1
Ellipticine	0.2	0.04	0.2	0.3	0.8	0.9

*LU-1 = human lung cancer; KB = human oral epidermoid carcinoma; KB-V = vinblastine-resistant KB tested in the presence (+ VLB) or absence (− VLB) of 1 μM vinblastine; LNCap = hormone-dependent human prostatic cancer; ZR-75-1 = hormone-dependent human breast cancer; $ED_{50} < 5.0\ \mu g\ ml^{-1}$ regarded as active.

for $Z = 2$ (M_r 358). A crystal was chosen for determination of the cell parameters and for data collection. All reflection intensities were measured with w scans, graphite-monochromatized CuK_α radiation in the range $0° < \theta < 57°$. The structural model was carried out by a direct method (SHELXTL) and all the C and O atoms were positioned from the difference Fourier method and full-matrix least squares. The positional parameters were refined by full-matrix least squares to the final $R = 0.0524$ for 1272 observed reflections [$I \geqslant 3\sigma(I)$] out of 1398 collected. A perspective view of the molecule is shown in Fig. 1. The crystal data will be deposited with the Cambridge X-ray Crystallographic Center, Cambridge, U.K.

Cytotoxicity, antimalarial and HIV-1 RT inhibitory assays. The biological evaluation for cytotoxic, antimalarial and HIV-1 RT inhibitory activities of these compounds were carried out according to established protocols [22–24]. Cytotoxicity data are shown in Table 5.

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