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DITERPENOIDS FROM *RABDOSIA PSEUDO-IRRORATA*

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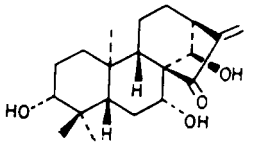
ABSTRACT.—From the leaves of *Rabdosia pseudo-irrorata*, three new *ent*-kaurene-type diterpenoids, pseurata A [**1**], pseurata B [**2**], and pseurata C [**3**], along with a known *ent*-kaurene-type diterpenoid, isodomedin, have been isolated. Their structures have been established by spectroscopic means and by comparison with known compounds.

The plants of the genus *Rabdosia* contain *ent*-kaurene-type diterpenoids with cytotoxic activity (1). As a part of our biochemical investigation of *Rabdosia* (Labiatae), we report here the isolation and structural elucidation of three new *ent*-kaurene-type diterpenoids: pseurata A [**1**], pseurata B [**2**], and pseurata C [**3**], along with a known diterpenoid isodomedin [**4**] (2) from *Rabdosia pseudo-irrorata* C.Y. Wu. This species has not been studied previously.

RESULTS AND DISCUSSION

Pseurata A [**1**], mp 165–167°, was isolated as colorless crystals from the Et_2O extract of the dried leaves of *Rabdosia pseudo-irrorata* by conventional chromatography and reversed-phase hplc. Its ir absorption indicated the presence of hydroxyl groups (3416), a carbonyl group (1729), and a double bond (1645 cm^{-1}). The uv spectrum indicates an absorption at λ max (EtOH) 246 nm (ε 6950). The 400 MHz ^{1}H-nmr spectrum showed signals due to three tertiary methyl groups at δ 0.88, 1.02, and 1.10, three methine protons on oxygenated carbon atoms at δ 3.17 (1H, dd, J = 11.5, 5.4 Hz), 4.19 (1H, dd, J = 13.2, 4.3 Hz), and 4.83 (1H, br s), and one terminal methylene group at δ 5.34 and 5.99 ppm (each 1H, br s). The DEPT spectra showed the presence of a C=O, one $C=CH_2$, six CH_2, three CH-OH, three CH, and three quaternary carbon atoms (Table 1). These spectral data, together with the consideration of the structure of diterpenoids isolated so far from the genus *Rabdosia* (1) and the co-occurrence of isodomedin [**4**], suggested that pseurata A has the basic skeleton *ent*-kaur-16-en-15-



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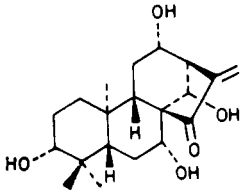

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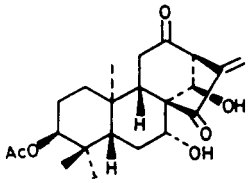

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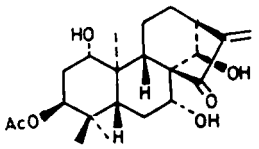

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TABLE 1. The ^{13}C-nmr Spectral Data of Diterpenoids **1–4** (400 MHz, TMS).[a]

Carbon	Compound							
	1		**2**		**3**		**4**	
	ppm	DEPT[b]	ppm	DEPT	ppm	DEPT	ppm	DEPT
C-1	38.65	(CH_2)	38.30	(CH_2)	33.52	(CH_2)	75.80	(CH)
C-2	28.00	(CH_2)	28.05	(CH_2)	23.02	(CH_2)	34.32	(CH_2)
C-3	78.05	(CH)	77.76	(CH)	77.61	(CH)	78.44	(CH)
C-4	39.36	(C)	39.37	(C)	36.21	(C)	36.83	(C)
C-5	53.06	(CH)	52.46	(CH)	47.74	(CH)	47.35	(CH)
C-6	29.29	(CH_2)	29.95	(CH_2)	28.83	(CH_2)	29.32	(CH_2)
C-7	74.98	(CH)	74.79	(CH)	73.74	(CH)	74.40	(CH)
C-8	61.67	(C)	61.58	(C)	61.50	(C)	62.59	(C)
C-9	55.28	(CH)	56.95	(CH)	50.23	(CH)	56.36	(CH)
C-10	39.36	(C)	38.74	(C)	39.80	(C)	45.60	(C)
C-11	18.22	(CH_2)	26.74	(CH_2)	36.21	(CH_2)	20.23	(CH_2)
C-12	31.81	(CH_2)	72.39	(CH)	207.10	(C)	31.84	(CH_2)
C-13	47.03	(CH)	52.48	(CH)	64.23	(CH)	47.07	(CH)
C-14	75.56	(CH)	71.23	(CH)	73.15	(CH)	75.50	(CH)
C-15	208.31	(C)	209.44	(C)	[c]		208.59	(C)
C-16	149.85	(C)	147.80	(C)	145.30	(C)	[c]	
C-17	116.30	(CH_2)	117.05	(CH_2)	119.21	(CH_2)	115.75	(CH_2)
C-18	28.83	(Me)	28.77	(Me)	28.16	(Me)	27.82	(Me)
C-19	16.35	(Me)	16.39	(Me)	21.90	(Me)	21.82	(Me)
C-20	18.36	(Me)	16.63	(Me)	16.66	(Me)	14.86	(Me)
OCOMe	—		—		170.54	(C)	170.33	(C)
$OCOCH_3$	—		—		21.04	(Me)	20.97	(Me)

[a]The solvent for diterpenoid **2** is pyridine-d_5; for **1**, **3**, and **4**, CD_3COCD_3.
[b]One-dimensional Distortionless Enhancement Polarization Transfer (DEPT) spectrum.
[c]These signals were overlapped with those of CD_3COCD_3.

one, to which three hydroxyl groups were attached. Thus, pseurata A may have a molecular formula $C_{20}H_{30}O_4$, which was confirmed by its eims (*m/z* 334 $[M]^+$).

The proton signals at δ 4.19 and 4.83 were assigned to H-7β and H-14α according to the magnitude of the chemical shifts, the coupling pattern, and the following observations. The eims spectrum showed intense fragment ions at *m/z* 194 and 176, which were formed by cleavage of the B ring (3), and its reaction product with 2,4-dimethylphenylboric acid (DMPBA) showed a strong peak at *m/z* 448 $[M]^+$ (32%), which was the molecular ion peak of the condensed product of 7α-OH and 14β-OH with DMPBA. The ^{1}H-nmr (see Experimental) and ^{13}C-nmr spectral data (Table 1) were very similar to those of wangzaozin A (*ent*-3β,7α,14β-trihydroxykaur-16-en-15-one) (4) except for those signals due to the A ring. The spectral data mentioned above indicated that another hydroxyl group should be equatorially attached to either C-1 or C-3. The downfield shift (ca. 6 ppm) of C-4 and the upfield shifts of C-18 and C-19 (ca. 5 ppm) suggested that the hydroxyl group should be attached to C-3. Consequently, the structure of pseurata A should be presented as **1**.

Structure **2**, $C_{20}H_{30}O_5$, was assigned for the new diterpenoid pseurata B. Its uv, ir, eims, cims, its reaction with DMPBA [eims *m/z* 464 $[M]^+$ (100%)], and ^{1}H-nmr spectrum along with its ^{13}C-nmr spectrum (Table 1) suggested that pseurata B had *ent*-kaur-16-en-15-one as a basic skeleton with four hydroxyl groups. The presence of 7α-OH and 14β-OH was determined as described for **1**. The B-ring α cleavage formed the

most prominent fragments at *m/z* 192 and 174 (3), which suggested the possibility of one hydroxyl group on the C ring and another on the A ring. The hydroxyl group on the A ring was assigned as 3α by comparing its nmr spectral data with those of **1**. Further comparison of its nmr spectral data with those of exisanin A (*ent*-1α,7α,12α,14β-tetrahydroxykaur-16-en-15-one) (5) revealed that the hydroxyl group on the C ring should be located at C-12 in the α orientation. Thus, the structure of pseurata B was established as **2**.

Pseurata C has a mol wt of 390 ($C_{22}H_{30}O_6$) on the basis of its eims and cims and the eims of its reaction product with DMPBA, which showed a molecular ion at *m/z* 504 $[M]^+$ (25%). The uv, ir, ^{1}H-nmr, and ^{13}C-nmr (Table 1) spectral data suggested that pseurata C also has a basic skeleton of *ent*-kaur-16-en-15-one, to which an acetoxyl group and two hydroxyl groups were attached. In addition, one of the methylenes in the skeleton was oxidized to C=O. The singlet signal at δ 5.04 and the dd peak at δ 4.43 (J = 12.5, 4.2 Hz) in its ^{1}H-nmr spectrum along with the eims of its reaction product with DMPBA indicated that pseurata C has 7α-OH and 14β-OH. Among three carbinyl carbon atoms (C-5, C-9, and C-13), one showed a large downfield shift at δ 64.23 ppm (CH) in ^{13}C-nmr spectrum, which suggested that the carbonyl carbon should be C-6, C-11, or C-12. The dd appearance of H-7β excluded the possibility of a C-6 carbonyl group. The ^{13}C-nmr spectral data for the C ring of pseurata C were quite different from those of henryine A (*ent*-7α,14β,19-trihydroxykaur-16-ene-11,15-dione) (6). This evidence indicated that the carbonyl carbon should be at C-12. The proton signal at the carbon bearing an acetoxyl group appeared as dd pattern at δ 4.62 (J = 4.9, 2.0 Hz), which revealed that the acetoxyl group should be axially attached to either C-1 or C-3. The downfield shift of C-4 (3 ppm) and upfield shift of C-18 (ca. 5 ppm) excluded the former. These facts indicated that pseurata C should be presented as **3**.

The uv, ir, ms, ^{1}H-nmr (see Experimental), and ^{13}C-nmr (Table 1) spectral data of another diterpenoid were in good agreement with those reported for isodomedin [**4**] (2).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined with a Kofler micromelting point apparatus and are uncorrected. Uv spectra were measured with a Beckman Du-7 spectrophotometer. Ir spectra were measured with a Nicolet 5D-X FT-ir spectrophotometer. Eims were obtained with a ZAB-HS mass spectrometer; cims and eims of each diterpenoid's reaction product with DMPBA were obtained with a MAT-44 mass instrument. Nmr spectra were measured with a Bruker AM-400 FT-NMR spectrometer. All reagents used were of analytical quality (Beijing Chemical Plant). Si gel (200–300 mesh) was used for cc, and Si gel (GF_{254}) (Haiyang Chemical Industry Factory, Qingdao) was used for tlc. Gilson HPLC instrument was used for reversed-phase hplc with a Whatman ODS column (20 × 0.46 cm i.d.).

PLANT MATERIALS.—*R. pseudo-irrorata* was collected by Mr. Yongmin Yuan and the author Yuanzong Li from Beina Community, Dazi County of Tibet on August 9, 1987. It was identified by Prof. Guoliang Zhang and Mr. Yongmin Yuan of the Biology Department of Lanzhou University, where a voucher specimen is deposited.

ISOLATION AND PURIFICATION OF THE DITERPENOIDS.—The air-dried leaves of *R. pseudo-irrorata* were powdered (2 kg) and macerated in Et_2O at room temperature for 2 months. Evaporation of solvent left a viscous dark green residue (15 g), which was dissolved in 2000 ml MeOH; then H_2O (800 ml) was slowly dropped into it with stirring. The viscous dark green material on the upper layer was removed by filtration. The filtrate was concentrated under reduced pressure to yield a light yellow powder (10 g), which was subjected to chromatography on a Si gel column (200–300 mesh). Elution with petroleum ether gave alkanes (4 g); elution was continued with a gradient mixture of petroleum ether-Me_2CO (9:1, 8:2, 7:3, 6:4, and 5:5). Fractions of 100 ml each were collected and monitored by tlc and uv light (set at 254 nm). Those fractions of similar composition were combined, and each of them was rechromatographed on a Si gel column. After these treatments we obtained six fractions. Part of each fraction was further purified by reversed-phase hplc (uv monitored at 254 nm) with flow rate 1 ml/min and elution solvent MeOH-H_2O (11:9),

which led to the isolation of pure compounds **1** (40 mg), **2** (20 mg), **3** (26 mg), and isodomedin [**4**] (30 mg).

PROCEDURE FOR EIMS.—Equimolar amounts of diterpenoid and DMPBA were dissolved in EtOH. After standing at room temperature for a few minutes (reaction completed within 10 min), an aliquot of the reaction mixture was subjected directly to mass spectrometric analysis.

PSEURATA A [**1**].—Colorless crystals from MeOH: mp 165–167°; uv λ max (EtOH) 246 nm (ε 6950); ir (KBr) ν max 3416, 1729, 1645, 1535, 1370 cm^{-1}; eims (70 eV) *m/z* (rel. int.) $[M]^+$ 334 (4), $[M-H_2O]^+$ 316 (11), $[M-2H_2O]^+$ 298 (4), 194 (18), 176 (36); ^1H nmr (400 MHz, CD_3COCD_3) δ (ppm) 5.99, 5.34 (each 1H, s, H-17a, H-17b), 4.83 (1H, br s, H-14α), 4.19 (1H, dd, J = 13.2, 4.3 Hz, H-7β), 3.17 (1H, dd, J = 11.5, 4.5 Hz, H-3β), 2.99 (1H, br s, H-13), 1.77 (1H, q, J = 12.6 Hz, H-6α), 1.10, 1.02, 0.88 (each 3H, s, 3 × Me); ^{13}C nmr see Table 1.

PSEURATA B [**2**].—White crystals from MeOH: mp 238–241°; uv λ max (EtOH) 241 nm (ε 5930); ir (KBr) ν max 3409, 1715, 1645, 1441, 1377 cm^{-1}; eims (70 eV) *m/z* (rel. int.) $[M-H_2O]^+$ 332 (23), $[M-2H_2O]^+$ 314 (6), 192 (22), 174 (37); cims (200 eV) *m/z* (rel. int.) $[M+1]^+$ 351 (6), 333 (66), 315 (100); ^1H nmr (400 MHz, pyridine-d_5) δ (ppm) 5.89 (1H, br s, H-14α), 5.44, 6.36 (each 1H, s, H-17a, H-17b), 4.94 (1H, dd, J = 11.8, 4.7 Hz, H-7β), 4.40 (1H, t, J = 2.9 Hz, H-12β), 3.64 (1H, d, J = 3.2 Hz, H-13), 3.41 (1H, dd, J = 11.7, 4.7 Hz, H-3β), 2.32 (1H, dd, J = 14.2, 4.0 Hz, H-6β), 2.18 (1H, q, J = 12.4 Hz, H-6α), 1.68 (3H, s, Me-20), 1.22, 1.09 (each 3H, s, Me-18, Me-19); ^{13}C nmr see Table 1.

PSEURATA C [**3**].—Colorless crystals from MeOH: mp 119–121°; uv λ max (EtOH) 238 nm (ε 5980); ir (KBr) ν max 3395, 1729, 1645, 1447, 1370 cm^{-1}; eims (70 eV) *m/z* (rel. int.) $[M-H_2O]^+$ 372 (23), $[372-HOAc]^+$ 312 (35), 43 (100); cims (200 e V) *m/z* (rel. int.) $[M+1]^+$ 391 (14), 373 (17), 313 (100); ^1H nmr (400 MHz, CD_3COCD_3) δ (ppm) 6.12, 5.16 (each 1H, s, H-17a, H-17b), 5.04 (1H, br s, H-14α), 4.62 (1H, dd, J = 4.9, 2.0 Hz, H-3α), 4.43 (1H, dd, J = 12.5, 4.2 Hz, H-7β), 3.58 (1H, br s, H-13), 2.03 (3H, s, OAc), 1.73 (1H, q, J = 12.2 Hz, H-6α), 0.96, 0.93, 0.92 (each 3H, s, 3 × Me); ^{13}C nmr see Table 1.

ISODOMEDIN [**4**].—Colorless flakes from MeOH: mp 216–218°; uv λ max (EtOH) 240 nm (ε 7238); ir (KBr) ν max 3402, 1722, 1645, 1462, 1377, cm^{-1}; eims (70 eV) *m/z* (rel. int.) $[M]^+$ 392 (3), 374 (37), 176 (45), 43 (100); ^1H nmr (400 MHz, CD_3COCD_3) δ (ppm) 6.30, 5.36 (each 1H, br s, H-17a, H-17b), 5.29 (1H, br s, H-14α), 4.97 (1H, t, J = 3.0 Hz, H-3α), 4.83 (1H, t, J = 8.0 Hz, H-7β), 3.95 (1H, dd, J = 10.9, 5.0 Hz, H-1β), 3.61 (1H, dd, J = 13.4, 5.5 Hz, H-11α), 3.30 (1H, br s, H-13), 2.02 (3H, s, OAc), 1.44 (3H, s, Me-20), 0.93, 0.89 (each 3H, s, 2 × Me); ^{13}C nmr see Table 1.

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