PERGAMON

Phytochemistry 59 (2002) 791–794

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Triterpenoids from *Gentiana scabra*

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Recieved in revised form 15 November 2001

Abstract

Five triterpenoids, (20*S*)-dammara-13(17),24-dien-3-one, (20*R*)-dammara-13(17),24-dien-3-one, chirat-16-en-3-one, chirat-17(22)-en-3-one and 17β,21β-epoxyhopan-3-one, were isolated from the rhizomes and roots of *Gentiana scabra* together with five known ones, chiratenol, hop-17(21)-en-3-one, hop-17(21)-en-3β-ol, lupeol and α-amyrin. The structures of new compounds were elucidated on the basis of spectroscopic studies.

Keywords: *Gentiana scabra*; *Gentianaceae*; Triterpenoid

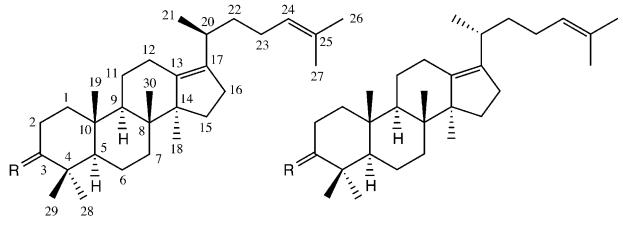
1. Introduction

The rhizomes and roots of *Gentiana scabra* Bunge (Gentianaceae) are used in Japan as a crude drug, Gentianae Scabrae Radix, e.g. as an appetite stimulant (Ikeshiro and Tomita, 1983). The constituents of this crude drug have been previously investigated and shown to contain secoiridoid glucosides (Inouye and Nakamura, 1971; Ikeshiro and Tomita, 1983; Ikeshiro et al., 1990). In this paper, we describe the isolation and structure elucidation of five new triterpenoids, (20*S*)-dammara-13(17),24-dien-3-one (**1**), (20*R*)-dammara-13(17),24-dien-3-one (**2**), chirat-16-en-3-one (**3**), chirat-17(22)-en-3-one (**4**) and 17β,21β-epoxyhopan-3-one (**5**), together with five known ones (**6–10**) from the rhizomes and roots of *G. scabra*.

2. Results and discussion

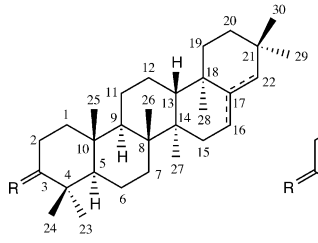
The known compounds **6–8** were identified as chiratenol (**6**) (Chakravarty et al., 1990), hop-17(21)-en-3-one (**7**) (Hui and Li, 1977) and hop-17(21)-en-3β-ol (**8**) (Arthur et al., 1964) on the basis of their spectral and physical data. Compounds **9** and **10** were identified as lupeol (**9**) (Yaoita and Kikuchi, 1993) and α-amyrin (**10**) (Kurihara et al., 1976), respectively, by direct comparison with authentic samples.

Compound **1** was isolated as an amorphous powder, $[\alpha]_D$ +29.4° and the IR spectrum suggested the presence of carbonyl group (1699 cm^{-1}). The molecular formula was determined to be $C_{30}H_{48}O$ by HR–MS and the EI–MS gave fragment ion peaks at *m/z* 355 [loss of part of side-chain (s.c.) by cleavage of C-22–C-23], 313 (loss of s.c.), 311 (313–2H), 219 (A, B rings formed by cleavage of C-8–C-14 and C-11–C-12) and 205 (A, B rings formed by cleavages of C-8–C-14 and C-9–C-11), suggesting **1** to has 13(17),24-diene type of dammarane skeleton (Arai et al., 1982). The ^1H NMR spectrum (see Experimental) gave signals due to five tertiary methyl groups [δ_H 0.85 (3H, H_3-30), 0.93 (3H, H_3-19), 1.04 (3H, H_3-29), 1.090 (3H, H_3-18), 1.093 (3H, H_3-28)], a secondary methyl group [δ_H 0.97 (3H, H_3-21)], a terminal

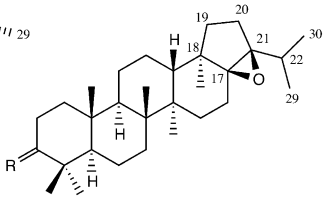


1 R = O
11 R = α-H, β-OH

2 R = O
12 R = α-H, β-OH



3 R = O, Δ^{16}
4 R = O, $\Delta^{17(22)}$
6 R = α-H, β-OH, Δ^{16}



4 R = O
13 R = α-H, β-OH

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0031-9422/02/$ - see front matter
PII: S0031-9422(02)00021-3

isopropylidene group [δ_H 1.56 (3H, H_3-27), 1.67 (3H, H_3-26)] and a trisubstituted olefinic proton [δ_H 5.08 (1H, H-24)]. The ^{13}C NMR spectrum (Experimental), obtained with the aid of DEPT spectral analysis, revealed 30 carbon signals that included four olefinic carbons [δ_C 125.0 (CH), 131.0 (C), 135.1 (C), 138.8 (C)] and a carbonyl carbon (δ_C 218.4). The ^1H and ^{13}C NMR spectra of **1** were similar to those of (20*S*)-dammara-13(17),24-dien-3β-ol (**11**), previously isolated from camellia and sasanqua oils from the seeds of *Camellia japonica* L. and *C. sasanqua* Thunb., respectively (Akihisa et al., 1997), except that the C-3 hydroxyl group in **11** was replaced by a carbonyl group in **1**. The position of this carbonyl group was confirmed by HMBC spectrum, in which cross-peaks were observed between H_3-28 and C-3, and H_3-29 and C-3, so that the carbonyl group is attached at C-3. The stereochemistry at C-20 was next determined to be *S* by comparison of the ^1H NMR spectral data with that of **11**. The *S* and *R* configuration of the secondary methyl group at C-20 of the side-chain can be distinguished by ^1H NMR spectroscopy mainly by the chemical shifts of the secondary methyl group at C-20 and the trisubstituted olefinic proton at C-24 (Akihisa et al., 1997). Therefore, the structure of **1** was determined to be (20*S*)-dammara-13(17),24-dien-3-one. No trace of the *R* isomer was present, indicating that the 20*S* isomer was not an artifact.

Compound **2** was isolated as an amorphous powder, $[\alpha]_D$ +14.3°. The molecular formula was determined to be $C_{30}H_{48}O$ by HR–MS. The ^1H NMR spectrum of **2** was quite similar to that of **1**, except for the chemical shifts of signals due to the secondary methyl group at C-20 [δ_H 0.93 (3H, H_3-21)] and the trisubstituted olefinic proton at C-24 [δ_H 5.12 (1H, H-24)]. This indicated that the difference between **1** and **2** was due to differences in the stereochemistry of the secondary methyl group at C-20. The stereochemistry at C-20 was determined to be *R* by comparison of the ^1H NMR spectral data with that of (20*R*)-dammara-13(17),24-dien-3β-ol (**12**), previously isolated from camellia and sasanqua oils from the seeds of *Camellia japonica* L. and *C. sasanqua* Thunb., respectively (Akihisa et al., 1997). Thus, **2** was deduced to be (20*R*)-dammara-13(17),24-dien-3-one.

Compound **3** was isolated as colorless needles, mp 160–162 °C, $[\alpha]_D$ +73.0°, and the IR spectrum suggested the presence of carbonyl group (1698 cm^{-1}). The molecular formula was determined to be $C_{30}H_{48}O$ by HR–MS and in the EI–MS, **3** showed fragment ion peaks at *m*/*z* 205 (A, B rings formed by cleavages of C-8−C-14 and C-9−C-11) and 189 (D, E rings formed by cleavages of C-8−C-14 and C-12−C-13). Further, the EI–MS displayed significant fragment ion peaks at *m*/*z* 204, 203, 187, 150 and 135, diagnostic of triterpenoids with a Δ^{16} double bond (Shiojima et al., 1992). The ^1H and ^{13}C NMR spectral data of **3** closely resembled those of **6**, except for the appearance of a carbonyl carbon signal at δ_C 218.3 instead of the hydroxy-bearing methine (C-3) signal of **6**, and the downfield shifts ($\Delta\delta$ +6.8, +8.4, +5.6, respectively) for C-2, C-4 and C-24 compared with those of **6**. Thus, **3** was deduced to be chirat-16-en-3-one. Compound **3** has been synthesized by Chakravarty et al. (1990), but its isolation from natral sources has not previously been reported.

Compound **4** was isolated as colorless needles, mp 170–172 °C, $[\alpha]_D$ +61.9°, and the IR spectrum suggested the presence of carbonyl group (1698 cm^{-1}). The molecular formula was determined to be $C_{30}H_{48}O$ by HR–MS and the ^1H and ^{13}C NMR data for **4** were very similar to those of **3** except for the signals ascribed to the rings D and E. The HMBC correlations of H_2-16 to C-17 and C-22; H-22 to C-16, C-18, C-20, C-21, C-29 and C-30; and H_3-30 to C-20 and C-22 implied the presence of a $\Delta^{17(22)}$ double bond in **4**. This was evident from fragment ion peaks at *m*/*z* 245, 149, 136 and 135 in the EI–MS (Shiojima et al., 1992). Therefore, **4** was determined to be chirat-17(22)-en-3-one. The natural occurrence of chiratane triterpenoids is extremely rare, and only one such triterpenoid, viz., chiratenol (**6**), has so far been reported from *Swertia chirata* (Gentianaceae) (Chakravarty et al., 1990). Compound **4** is the first example of a chiratane triterpenoid having a $\Delta^{17(22)}$ double bond.

Compound **5** was isolated as colorless needles, mp 250–253 °C, $[\alpha]_D$ +39.2°, and the IR spectrum suggested the presence of carbonyl group (1698 cm^{-1}). The molecular formula was determined to be $C_{30}H_{48}O_2$ by HR–MS and in the EI–MS, **5** gave a fragment ion peak due to cleavage of ring C at *m*/*z* 205, together with ion peaks at *m*/*z* 245, 152 and 43, suggesting **5** to has a hopane skeleton (Shiojima et al., 1992). The ^1H and ^{13}C NMR spectral data of **5** closely resembled those of 17β,21β-epoxyhopan-3β-ol (**13**) (Tanaka et al., 1990), except for the appearance of a carbonyl carbon signal at δ_C 217.5 instead of the hydroxy-bearing methine (C-3) signal of **13**, and the downfield shifts ($\Delta\delta$ +6.8, +8.5, +5.7, respectively) for C-2, C-4 and C-24 compared with those of **13**. Thus, **5** was deduced to be 17β,21β-epoxyhopan-3-one. This is the first isolation of **5** from natural source, although **5** has already been synthesized by Tanaka et al. (1990).

3. Experimental

3.1. General

^1H and ^{13}C NMR spectra were recorded using a Jeol JNM-LA 600 spectrometer (600 and 150 MHz, respectively) and chemical shifts are given relative to TMS as int. standard. Optical rotations were determined using a JASCO DIP-360 digital polarimeter. IR spectra were recorded with a Perkin-Elmer FT–IR 1725X IR spectrophotometer. EI LR- and HR–MS were recorded on a Jeol JMS-DX 303 mass spectrometer. CC was carried out on Kieselgel 60 (230–400 mesh, Merck). HPLC was

carried out on a Tosoh HPLC system (pump, CCPS; detector, RI-8020) using a TSK gel ODS-120T (7.8 mm i.d. × 30 cm) column (Tosoh). HPLC conditions: flow rate, 1.0 ml min^{-1}; column temperature, 40 °C.

3.2. Plant material

The dried rhizomes and roots of *Gentiana scabra* (from Jilin, China) were purchased from Uchida Wakanyaku Co., Ltd., Tokyo, Japan in 1999. A voucher specimen (No. 8) is deposited in the laboratory of M. Kikuchi.

3.3. Extraction and isolation

The dried rhizomes and roots of *G. scabra* (1.5 kg) were extracted with MeOH at room temp. The MeOH extract (160.0 g dry wt) was successively extracted with $CHCl_3$, EtOAc and *n*-BuOH. The $CHCl_3$-soluble fr. was conc. under red. pres. to afford a residue (66.0 g). A part of this residue (29.0 g) was subjected to silica gel column chromatography using $CHCl_3$–MeOH–H_2O (30:10:1), and the eluate was sepd. into 24 frs. (frs. 1–24). Fr. 5 (3.0 g) was applied to a silica gel column using $CHCl_3$–MeOH (29:1) as eluent, and the eluate was sepd. into 15 frs. (frs. 5–1 – 5–15). Fr. 5–4 (19.0 mg) was purified by prep. HPLC (mobile phase, MeOH) to give **1** (1.4 mg), **2** (0.7 mg), **3** (7.8 mg), **5** (0.5 mg) and the mixture of **4** and **7** (4.0 mg). The mixture of **4** and **7** was purified by prep. HPLC [mobile phase, MeOH–H_2O (97:3)] to give **4** (2.0 mg) and **7** (1.0 mg). Fr. 5–6 (6.0 mg) was purified by prep. HPLC (mobile phase, MeOH) to give **6** (1.2 mg), **8** (1.2 mg), **9** (0.5 mg) and **10** (0.5 mg).

3.4. (20S)-Dammara-13(17),24-dien-3-one (*1*)

Amorphous powder; $[\alpha]_D^{28}$ +29.4° ($CHCl_3$; *c* 0.1); IR ν_{max} ($CHCl_3$) cm^{-1}: 1699; HR–MS *m/z*: 424.3698 (M^+, calc. for $C_{30}H_{48}O$; 424.3705); EI–MS *m/z* (rel. int.): 424 (M^+, 81), 409 (4), 355 (100), 313 (37), 311 (22), 219 (12), 205 (75); ^1H NMR ($CDCl_3$): δ 0.85 (3H, *s*, H_3-30), 0.93 (3H, *s*, H_3-19), 0.97 (3H, *d*, *J* = 7.0 Hz, H_3-21), 1.04 (3H, *s*, H_3-29), 1.090 (3H, *s*, H_3-18), 1.093 (3H, *s*, H_3-28), 1.56 (3H, *s*, H_3-27), 1.67 (3H, *br s*, H_3-26), 5.08 (1H, *t*, *J* = 7.3 Hz, H-24); ^{13}C NMR ($CDCl_3$): δ 16.4 (C-19), 16.6 (C-30), 17.6 (C-27), 19.7 (C-6), 20.1 (C-21), 21.1 (C-29), 22.5 (C-11), 22.9 (C-18), 23.1 (C-12), 25.7 (C-26), 26.4 (C-23, C-28), 29.1 (C-16), 30.7 (C-15), 31.6 (C-20), 34.1 (C-2), 35.7 (C-7, C-22), 37.1 (C-10), 39.9 (C-1), 41.3 (C-8), 47.3 (C-4), 50.9 (C-9), 55.2 (C-5), 56.4 (C-14), 125.0 (C-24), 131.0 (C-25), 135.1 (C-17), 218.4 (C-3).

3.5. (20R)-Dammara-13(17),24-dien-3-one (*2*)

Amorphous powder; $[\alpha]_D^{23}$ +14.3° ($CHCl_3$; *c* 0.07); IR ν_{max} ($CHCl_3$) cm^{-1}: 1699; HR–MS *m/z*: 424.3716 (M^+, calc. for $C_{30}H_{48}O$; 424.3705); EI–MS *m/z* (rel. int.): 424 (M^+, 25), 409 (1), 355 (18), 313 (10), 311 (7), 219 (4), 205 (15); ^1H NMR ($CDCl_3$): δ 0.85 (3H, *s*, H_3-30), 0.93 (3H, *s*, H_3-19), 0.93 (3H, *d*, *J* = 7.0 Hz, H_3-21), 1.04 (3H, *s*, H_3-29), 1.07 (3H, *s*, H_3-18), 1.09 (3H, *s*, H_3-28), 1.59 (3H, *s*, H_3-27), 1.69 (3H, *br s*, H_3-26), 5.12 (1H, *m*, H-24).

3.6. Chirat-16-en-3-one (*3*)

Colorless needles, mp 160–162 °C; $[\alpha]_D^{28}$ +73.0° ($CHCl_3$; *c* 0.8); IR ν_{max} ($CHCl_3$) cm^{-1}: 1698; HR–MS *m/z*: 424.3711 (M^+, calc. for $C_{30}H_{48}O$; 424.3705); EI–MS *m/z* (rel. int.): 424 (M^+, 100), 409 (20), 205 (53), 204 (37), 203 (20), 189 (48), 187 (29), 150 (19), 135 (20); ^1H NMR ($CDCl_3$): δ 0.77 (3H, *s*, H_3-30), 0.92 (3H, *s*, H_3-29), 0.946 (3H, *s*, H_3-25), 0.950 (3H, *s*, H_3-28), 0.99 (3H, *s*, H_3-27), 1.01 (3H, *s*, H_3-26), 1.03 (3H, *s*, H_3-24), 1.08 (3H, *s*, H_3-23), 2.43 (1H, *ddd*, *J* = 15.8, 7.7, 4.8 Hz, H-2α), 2.50 (1H, *ddd*, *J* = 15.8, 9.5, 7.7 Hz, H-2β), 5.23 (1H, *ddd*, *J* = 5.5, 1.8, 1.8 Hz, H-16); ^{13}C NMR ($CDCl_3$): δ 16.1 (C-25), 16.2 (C-26), 16.7 (C-28), 17.5 (C-27), 19.6 (C-6), 21.1 (C-24), 22.0 (C-11), 23.4 (C-12), 24.6 (C-30), 26.8 (C-23), 32.4 (C-29), 32.5 (C-21), 32.9 (C-15), 33.1 (C-7), 34.2 (C-2), 35.2 (C-20), 36.8 (C-10), 36.9 (C-18), 38.3 (C-19), 39.6 (C-1), 40.5 (C-14), 41.0 (C-8), 45.3 (C-13), 46.2 (C-22), 47.3 (C-4), 50.0 (C-9), 54.8 (C-5), 119.9 (C-16), 139.2 (C-17), 218.3 (C-3) .

3.7. Chirat-17(22)-en-3-one (*4*)

Colorless needles, mp 170–172 °C; $[\alpha]_D^{28}$ +61.9° ($CHCl_3$; *c* 0.2); IR ν_{max} ($CHCl_3$) cm^{-1}: 1698; HR–MS *m/z*: 424.3710 (M^+, calc. for $C_{30}H_{48}O$; 424.3705); EI–MS *m/z* (rel. int.): 424 (M^+, 30), 409 (8), 245 (100), 205 (36), 189 (22), 149 (16), 136 (18), 135 (35); ^1H NMR ($CDCl_3$): δ 0.92 (3H, *s*, H_3-30), 0.93 (3H, *s*, H_3-25), 0.94 (6H, s, H_3-28, H_3-29), 0.97 (3H, *s*, H_3-26), 1.03 (3H, *s*, H_3-24), 1.08 (3H, *s*, H_3-23), 1.09 (3H, *s*, H_3-27), 1.33 (1H, *dd*, *J* = 11.0, 2.6 Hz, H-5), 1.83 (1H, *ddd*, *J* = 13.6, 4.0, 2.9 Hz, H-16β), 1.94 (1H, *ddd*, *J* = 13.2, 7.7, 4.4 Hz, H-1α), 2.30 (1H, *dddd*, *J* = 13.6, 13.6, 4.4, 1.8 Hz, H-16α), 2.42 (1H, *ddd*, *J* = 15.8, 7.3, 4.4 Hz, H-2α), 2.50 (1H, *ddd*, *J* = 15.8, 9.9, 7.7 Hz, H-2β), 5.03 (1H, *br s*, H-22); ^{13}C NMR ($CDCl_3$): δ 15.6 (C-27), 16.0 (C-25), 16.1 (C-26), 19.8 (C-6), 20.2 (C-28), 21.1 (C-24), 21.9 (C-11), 22.5 (C-12), 26.6 (C-23), 28.8 (C-30), 29.6 (C-16), 31.5 (C-29), 31.6 (C-18), 32.5 (C-15), 33.1 (C-7), 33.5 (C-20), 34.2 (C-2), 36.8 (C-10), 35.7 (C-21), 36.2 (C-19), 39.6 (C-1), 41.8 (C-8), 42.0 (C-14), 47.4 (C-4), 49.3 (C-13), 49.9 (C-9), 54.9 (C-5), 130.4 (C-22), 142.0 (C-17), 218.2 (C-3).

3.8. 17β,21β-Epoxyhopan-3-one (*5*)

Colorless needles, mp 250–253 °C; $[\alpha]_D^{24}$ +39.2° ($CHCl_3$; *c* 0.05); IR ν_{max} ($CHCl_3$) cm^{-1}: 1698; HR–MS *m/z*: 440.3672 (M^+, calc. for $C_{30}H_{48}O_2$; 440.3654); EI–

MS *m*/*z* (rel. int.): 440 (M^+, 100), 425 (9), 422 (10), 397 (18), 245 (3), 205 (13), 152 (52), 43 (60); ^{1}H NMR ($CDCl_3$): δ 0.84 (3H, *s*, H_3-28), 0.94 (3H, *s*, H_3-25), 0.96 (3H, *d*, $J = 7.0$ Hz, H_3-30), 1.03 (3H, *s*, H_3-24), 1.057 (3H, *s*, H_3-27), 1.063 (3H, *d*, $J = 6.6$ Hz, H_3-29), 1.07 (3H, *s*, H_3-26), 1.08 (3H, *s*, H_3-23), 1.94 (1H, *ddd*, $J = 13.2$, 7.3, 4.4 Hz, H-1α), 2.05 (1H, *ddd*, $J = 13.6$, 13.6, 4.4 Hz, H-16α), 2.40 (1H, *ddd*, $J = 15.8$, 7.3, 4.4 Hz, H-2α), 2.54 (1H, *ddd*, $J = 15.8$, 10.3, 7.3 Hz, H-2β); ^{13}C NMR ($CDCl_3$): δ 15.78 (C-25), 15.80 (C-27), 16.4 (C-26), 18.0 (C-28), 18.4 (C-30), 19.2 (C-29), 19.7 (C-6), 20.2 (C-16), 21.1 (C-24), 21.5 (C-11), 23.3 (C-12, C-20), 26.6 (C-23), 28.5 (C-22), 29.2 (C-15), 32.6 (C-7), 34.2 (C-2), 34.6 (C-19), 36.9 (C-10), 39.7 (C-1), 41.9 (C-8), 42.1 (C-14), 43.38 (C-13), 43.42 (C-18), 47.4 (C-4), 49.8 (C-9), 55.0 (C-5), 75.9 (C-21), 76.3 (C-17), 217.5 (C-3).

Acknowledgements

We are grateful to Mr. S. Sato and Mr. T. Matsuki of this University for providing the mass and NMR spectra.

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