Pergamon

PII: S0031-9422(97)00090-3

*Phytochemistry*, Vol. 45, No. 5, pp. 1023–1029, 1997

0031-9422/97 $17.00+0.00

# TRITERPENOIDS FROM THE FERN *GONIOPHLEBIUM MENGTZEENSE*

MSAYOSHI HIROHARA, YUH YASUOKA, YÔKO ARAI, KENJI SHIOJIMA, HIROYUKI AGETA* and HSIEN-CHANG CHANG†

Shôwa College of Pharmaceutical Sciences, Machida, Tokyo 194, Japan; † Brion Research Institute of Taiwan, 116 Chung-Ching South RD. Sec., 3, Taipei, Taiwan 10734, Republic of China

(*Received* 5 *November* 1996)

**Key Word Index**—*Goniophlebium mengtzeense*; Polypodiaceae; triterpenoid; fern-9(11)-en-20α-yl palmitate; 8βH-fern-9(11)-en-7-one.

**Abstract**—Two new triterpenoids, fern-9(11)-en-20α-yl palmitate and 8βH-fern-9(11)-en-7-one were isolated from the fresh fronds of *Goniophlebium mengtzeense*. Their structures were established by spectroscopic techniques and chemical correlation. Thirty-seven other compounds were identified from this fern. 

## INTRODUCTION

In order to get an insight into the chemotaxonomic aspect of polypodiaceous ferns, a number of ferns belonging to this family has been investigated in this laboratory and a number of novel triterpenoids has been reported [1–4]. In continuation of this study, we have now investigated *Goniophlebium mengtzeense* (Christ) Rödl-Linder [5] (*Polypodium taiwanianum* Hayata, 'taiwan-uraboshi' in Japanese), belonging to the same family and distributed in Taiwan, resulting in the isolation of two new triterpenoids, characterized as fern-9(11)-en-20α-yl palmitate (**1**) and 8βH-fern-9(11)-en-7-one (**2**), along with many other known compounds. We report herein the isolation of the constituents and structure elucidation of **1** and **2**.

## RESULTS AND DISCUSSION

The fresh rhizomes and leaves of *G. mengtzeense* were extracted separately with *n*-hexane and the extracts were chromatographed over silica gel to provide two sets of five fractions containing mainly triterpenoid hydrocarbons, fatty acid esters, acetates, alcohols, or sterolds. The individual fractions were subjected to preparative HPLC to obtain a total of thirty seven compounds which are listed in Tables 1 and 2 along with their $RR_t$ (GC) values and yields. It can be seen that the hydrocarbon fraction consists of triterpenoids belonging to the malabaricane, euphane, tirucallane, lupane, hopane, migrated hopane, and oleanane skeleta; fatty acid ester fraction consists of esters of triterpenoids having 31-norcycloartane and cycloartane skeleta while the acetate fraction is composed of triterpenoids possessing 24-methyl or 24-ethyl-31-norlanostane, 31-norcycloartane, cycloartane and lupane skeleta. The two new compounds were isolated from the fatty acid fraction of the leaves.

Compound **1**, amorphous, $[\alpha]_D+19°$, showed a strong absorption of 1725 $cm^{-1}$ in its IR spectrum indicating the presence of an ester function in the molecule. Its EI-mass spectrum showed, besides the molecular ion at *m/z* 664, the fragment ions at *m/z* 257, 243 and 231 diagnostic [16] of triterpenoids having a $\Delta^7$, $\Delta^8$ or $\Delta^{9(11)}$-double bond without of any oxygen function in the A, B or C-ring (Scheme 1). Its $^1H$ NMR spectrum (Table 3) displayed signals for six tertiary and two secondary methyl groups, one carbinyl proton and one trisubstituted vinylic proton. The chemical shifts and the characteristic splitting pattern [8] of the vinylic proton signal at $\delta$ 5.280 (*ddd*, $J = 5.3$, 2.5, 2.5 Hz) demonstrated that the trisubstituted double bond was located at the $\Delta^{9(11)}$-position rather than $\Delta^7$. The splitting pattern of the carbinyl proton signal as *ddd* with $J = 7.7$, 7.7 and 4.1 Hz indicated that the OCOR grouping must be located at a carbon flanked by a CH and a $CH_2$ group in a five-membered ring.

A comparison of the $^{13}C$ NMR data (Table 3) of **1** with those of fern-9(11)-ene [8] showed very close chemical shifts for all the skeletal carbons except C-18 to C-22. The deshielding of C-19 ($CH_2$) and C-21 (CH) by ~11 and ~3 ppm, and shielding of C-22 (CH) by ~5 ppm clearly demonstrated that **1** must be

*Author to whom correspondence should be addressed.

Table 1. Hydrocarbons of *Goniophlebium mengtzeense*

| Compound | $RR_t$ | Yield (% × $10^3$) (rhizomes)* | Yield (% × $10^3$) (Fronds)* | Ref. |
|---|---|---|---|---|
| 13β-Malabaicatriene (**3**) | 1.16 | 0.6 | | [6] |
| Isodammara-18(28)-diene (**4**) | 2.03 | 0.4 | Trace | [7] |
| Eupha-7,21-diene (**5**) | 1.58 | Trace | 1.0 | [7] |
| Tirucalla-7,21-diene (**6**) | 1.76 | 0.6 | 0.9 | [7] |
| Hop-22(29)-ene (**7**) | 2.64 | 2.3 | 3.7 | [8] |
| Hop-17(21)-ene (**8**) | 1.67 | Trace | Trace | [8] |
| Hop-16-ene (**9**) | 2.11 | 0.4 | | [8] |
| Neohop-13(18)-ene (**10**) | 1.91 | 0.5 | 0.6 | [8] |
| Neohop-12-ene (**11**) | 2.25 | 1.9 | 0.7 | [8] |
| Fern-7-ene (**12**) | 2.30 | 1.0 | 8.4 | [8] |
| Fern-8-ene (**13**) | 1.88 | 1.0 | | [8] |
| Fern-9(11)-ene (**14**) | 1.98 | 0.4 | 40.9 | [8] |
| Ferna-7,9(11)-diene (**15**) | 1.86 | Trace | Trace | [8] |
| Adian-5-ene (**16**) | 2.15 | 0.4 | 2.2 | [8] |
| Filic-4(23)-ene (**17**)† | 2.64 | 0.4 | | |
| Filic-3-ene (**18**) | 2.70 | 16.2 | 1.9 | [8] |
| Squalene (**19**) | 0.91 | | Trace | [9] |
| Hop-21-ene (**20**) | 2.67 | | Trace | [8] |
| Olean-18-ene (**21**) | 1.59 | Trace | 1.4 | [10] |

* Yields: in relation to dried materials, below 0.3 (% × $10^3$) were treated as trace.
† See Experimental for $^1$H NMR data.

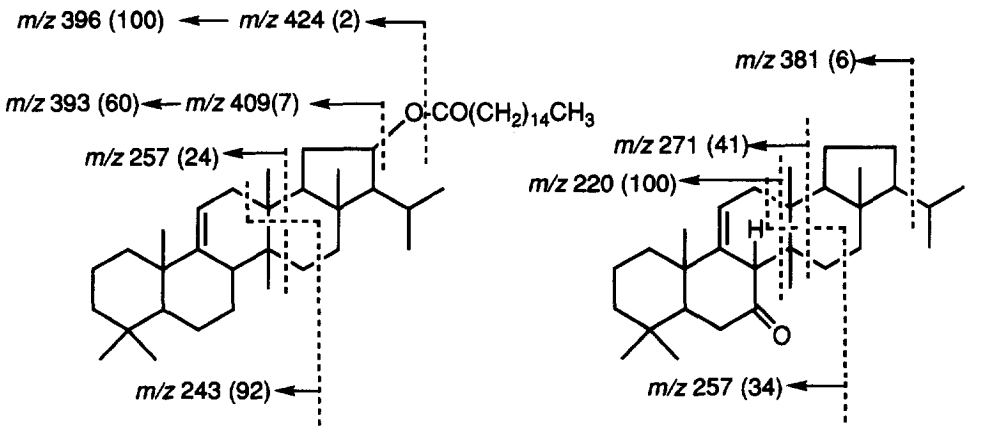
Table 2. Triterpenoid acetates from *G. mengtzeense*

| | $RR_t$ | Rhizomes yield (% × $10^3$)* | Fronds yield (% × $10^3$)* | Ref. |
|---|---|---|---|---|
| Sitosteryl acetate (**22**) | 3.63 | 1.0 | 0.7 | [11] |
| 24-Ethyl-31-norlanosta-8,25-dien-3β-yl acetate (**23**) | 4.01 | 10.8 | 1.0 | |
| 31-Norcycloartenyl acetate (**24**) | 3.37 | | 0.3 | [12] |
| 31-Norcyclolaudenyl acetate (**25**) | 3.82 | 1.0 | | [12] |
| Cycloeucalenylacetate (**26**) | 3.82 | 0.8 | 0.6 | [12] |
| 31-Norcyclomargenyl acetate (**27**) | 4.65 | 3.4 | 0.6 | |
| Cycloartenyl acetate (**28**) | 3.77 | 0.4 | 3.8 | [13] |
| Cyclolaudenyl acetate (**29**) | 4.43 | 6.9 | 1.3 | [12] |
| 24-Methylenecycloartanyl acetate (**30**) | 4.43 | 2.5 | 0.6 | [12] |
| Cyclomargenyl acetate (**31**) | 5.41 | 4.8 | | [12] |
| Phytyl acetate (**32**) | | | Trace | [14] |
| 24-Methyl-31-norlanost-8,25-dien-3β-yl acetate (**33**) | 3.37 | 0.8 | | |
| 24,24-Dimethylcycloart-25-en-3β-yl acetate (**34**) | 5.55 | 0.5 | | [12] |
| Dryocrassyl acetate (**35**) | 6.29 | 0.5 | | [8] |
| 22-Acetoxyhopane (**36**) | 2.66 | 0.4 | 0.3 | |
| Lupenyl acetae (**37**) | 6.29 | | 0.5 | [15] |

* Yields: related to dried materials.

20-*O*-acyloxyfern-9(11)-ene. Though no HMBC correlation was observed for the carbinyl proton (H-20) with the neighboring carbons (Table 4), H-20 showed correlations with H-21 and $H_2$-19 in its $^1$H-$^1$H COSY spectrum. The orientation of the OCOR group at C-20 could be easily assigned as on the basis of two large couplings (7.7 Hz, H-20β vs H-19β and H-21β), and one small coupling (4.1 Hz, H-20β vs H-19α). Having established the structure of the triterpenoid alcohol moiety of **1** as fern-9(11)-en-20α-ol, the fatty acid part could easily be identified as palmitic acid by GC analysis of the fatty acid methyl ester obtained from **1** on hydrolysis followed by methylation. Thus, compound **1** was the palmitate of a new triterpenoid fern-9(11)-en-20α-ol.

Compound **2**, $C_{30}H_{48}O$ (HR-MS $M^+$ at *m/z* 424.3721), mp 277–283°, $[\alpha]_D$ −6°, showed a strong absorption band at 1710 $cm^{-1}$ for a six-member ring C=O group in its IR spectrum. Its $^1$H NMR spectrum displayed, besides the signals for six tertiary and two secondary methyl groups, a trisubstituted vinylic proton signal at δ 5.319 (*ddd* $J$ = 3.3, 2.8, 2.8 Hz) and, comparatively downfield signals for a CH at δ 2.780 (*d*, $J$ = 2.8 Hz) and two $CH_2$ protons at δ 2.374 (*dd*,




1 2

Scheme 1. Mass spectral fragmentations of compounds **1** and **2**.

$J$ = 14.0, 14.0 Hz) and $\delta$ 2.297 (*dd*, $J$ = 14.0, 3.8 Hz). Their chemical shifts, multiplicity and coupling constant indicated that the CH proton must be located between the trisubstituted double bond and the C=O group, and the $CH_2$ group is located in between the C=O group and other ring juncture CH with an axial hydrogen. The EI-mass spectrum of **2** exhibited ion peaks at *m/z* 271, 257 and 245 diagnostic [16] of $\Delta^7$ or $\Delta^{9(11)}$-triterpenoid with a keto group in the A, B or C ring (Scheme 1).

On the basis of the above observations, a $\Delta^{9(11)}$-7-keto triterpenoid structure could be assigned for **2**. However, the $^{13}C$ NMR data were found to be inconclusive in determining the skeleton of the molecule. The skeleton of the compound was ascertained from its HMBC spectrum. Thus, the two and three-bond correlation observed for the methyl protons, $H_2$-6 and H-8 with the neighboring carbons (Table 5) revealed the presence of the part structure, as shown by a heavy line in Fig. 1, clearly demonstrating a fern-9(11)-en-7-one structure for **2**. However, no NOE interaction between H-8 and $H_3$-27 was observed in the NOESY spectrum. On the contrary, the H-8 signal showed strong NOE interaction with both $H_3$-25 and $H_3$-26, thereby confirming the $\beta$-configuration of H-8. The structure of **2** was finally confirmed by comparison with one of the products obtained by an oxidation of fern-8-ene with $CrO_3$-AcOH. Thus, compound **2** was identified as 8$\beta$H-fern-9(11)-en-7-one.

The structure of **2** is interesting in regard to its biogenesis, because **2** has the unusual 8$\beta$H-fern-9(11)-ene skeleton. The chemotaxonomic study of *Goniophlebium* and *Polypodiodes* [1–4, 17] ferns will be continued through the study of triterpenoids from the type species.

## EXPERIMENTAL

*General.* Mps: uncorr.; EI Ms: 30 eV; TLC: on precoated Kiesel gel 60; $^1H$ NMR (500 MHz) and $^{13}C$ NMR (125 MHz) spectra: in $CDCl_3$ (TMS as int. standard); HPLC: reversed-phase $C_{18}$ column, RI detector, with MeOH–$CHCl_3$ (7:3) for hydrocarbons, $CH_3CN$–$CHCl_3$ (7:3) for esters and, $CH_3CN$–$CHCl_3$ (17:3) for acetates as a mobile phase; CC: $SiO_2$ 60 (230–400 mesh, Merck) and 20% $AgNO_3$-impregnated silica gel (Mallincrodt); GC: 1.4% SE-30 on Chromosorb G, Oven: 260°. (cholestane as int. standard).

*Plant material. Goniophlebium mengtzeense* was collected at Ali-shan, Taiwan in August 1992. The voucher specimen has been deposited in the herbarium of Showa College of Pharmaceutical Sciences, Tokyo.

*Extraction and separation.* Sliced fresh rhizomes (A: 357 g) and fronds (B: 238 g) were extracted, respectively, with *n*-hexane and the extracts [2.31 g with $H_2O$ (277 ml) from rhizomes and 1.73 g with $H_2O$ (135 ml) from fronds] were chromatographed over silica gel to give six frs from each extract (Table 7). Each fr. was subjected to prep. HPLC. The solvents used as the mobile phase for both CC and HPLC are summarized in Table 7. The compounds purified and identified by GC and $^1H$ NMR are listed in Tables 1 and 2.

*Isolation of fern-9(11)-en-20α-yl palmitate* (**1**) *and 8βH-fern-9(11)-en-7-one* (**2**). Fr. 2 (B) was purified through $AgNO_3$–silica gel followed by HPLC to give **1** (8 mg) and **2** (2.5 mg).

*Hydrolysis of* **1**. Compound **1** was hydrolyzed with 5% KOH–EtOH, resulting in an alcoholic and an acidic fr. The acidic fr. was esterified with an ether solution of diazomethane MS: ($M^+$ *m/z* 270).

*Synthesis of* **2**. An acetic acid soln (7 ml) of $CrO_3$ (24 mg) was added to a soln (20 ml) of fern-8-ene (50 mg) in benzene–AcOH (1:4) at room temp., stood over night, and then treated in the usual manner to give a solid (44 mg), purified over silica gel CC followed by HPLC to give **2** (4 mg), fern-9(11)-en-7-one (2.7 mg), $^1H$ NMR ($CDCl_3$): $\delta$ 0.848 (3H, *s*, H-23), 0.900 (6H, *s*, H-24, 26), 1.041 (3H, *s*, H-25), 0.890 (3H, *s*, H-27), 0.745 (3H, *s*, H-28), 0.866 (3H, *d*, $J$ = 6.7 Hz, H-29), 0.829 (3H, *d*, $J$ = 6.7 Hz, H-30), 5.410 (1H, *ddd*, $J$ = 5.0, 2.6, 2.6 Hz, H-11) and fern-7-en-11-one (2.4 mg), $^1H$ NMR ($CDCl_3$): $\delta$ 0.844 (3H, *s*, H-23), 0.885 (3H, *s*, H-24), 0.990 (3H, *s*, H-25), 0.846 (3H, *s*,

Table 3. $^1H$ NMR and $^{13}C$ NMR spectral data for compounds **1** and **2**

| H and C | **1** $^1H$ | **1** $^{13}C$ | **2** $^1H$ | **2** $^{13}C$ |
|---|---|---|---|---|
| 1 | 1.16 | 41.47 | — | 37.19 |
| | 1.89 | | | |
| 2 | 1.40 | 19.53 | — | — |
| | 1.56 | | | |
| 3 | 1.13 | 42.40 | 1.17 | 48.81 |
| | 1.38 | | 1.46 | |
| 4 | | 33.64 | | 33.88 |
| 5 | 1.26 | 44.86 | 1.50 | 52.95 |
| 6 | 1.72 | 19.48 | 2.30 | 41.18 |
| | 1.56 | | 2.38 | |
| 7 | 1.61 | 17.84 | | 208.86 |
| | 1.33 | | | |
| 8 | 2.07 | 39.98 | 2.78 | 58.65 |
| 9 | | 151.67 | | 140.02 |
| 10 | | 38.07 | | 39.56 |
| 11 | 5.280 | 115.43 | 5.319 | 115.96 |
| | (*ddd*, 5.3, 2.5, 2.4) | (*ddd*, 3.3, 2.8, 2.8) | | |
| 12 | 1.50 | 36.49 | 1.63 | 36.95 |
| | 1.58 | | 1.65 | |
| 13 | | 36.51 | | 36.26 |
| 14 | | 37.65 | | 40.03 |
| 15 | 1.33 | 29.04 | 1.72 | 29.09 |
| | 1.43 | | 3.21 | |
| 16 | 1.41 | 36.20 | | 39.94 |
| | 1.69 | | | |
| 17 | | 43.19 | | 43.59 |
| 18 | 1.48 | 50.15 | 1.29 | 52.86 |
| 19 | 2.16 | 31.94 | | |
| | 1.23 | | | |
| 20 | 5.169 | 75.21 | | |
| | (*ddd*, 7.7, 7.7, 4.1) | | | |
| 21 | 1.07 | 63.16 | | 59.62 |
| 22 | 1.93 | 25.10 | | 30.77 |
| 23 | 0.845 | 32.79 | 0.827 | 32.62 |
| 24 | 0.888 | 21.67 | 0.859 | 29.92 |
| 25 | 1.048 | 25.04 | 1.206 | 19.91 |
| 26 | 0.734 | 15.49 | 0.935 | 22.97 |
| 27 | 0.825 | 15.82 | 0.852 | 18.34 |
| 28 | 0.927 | 15.08 | 0.852 | 13.97 |
| 29 | 0.937 | 21.64 | 0.903 | 22.17 |
| | (*d*, 6.4) | | (*d*, 6.4) | |
| 30 | 0.818 | 22.61 | 0.832 | 23.02 |
| | (*d*, 6.4) | | (*d*, 6.4) | |
| $OCO(CH_2)_{14}CH_3$ | | | | 170.88 |

Coupling constants are shown in parentheses.

H-26), 1.142 (3H, *s*, H-27), 0.715 (3H, *s*, H-28), 0.909 (3H, *d*, $J$ = 6.7 Hz, H-29), 0.837 (3H, *d*, $J$ = 6/7 Hz, H-30), 5.502 (1H, *ddd*, $J$ = 3.7, 3.7, 3.4 Hz, H-7).

*Triterpenoid esters.* Frs 2 A and B were sepd by HPLC ($CHCl_3$–$CH_3CN$, 3:7) directly to give the long chain fatty acid esters (Table 6). Each compound was estimated by comparison of the signal intensities of the $^1H$ NMR spectrum with those of the corresponding alcohols. 24-Ethyl-31-norlanosta-8,25-diene-3$\beta$-ol, $^1H$ NMR ($CDCl_3$): $\delta$ 0.697 (3H, *s*, H-18), 0.978 (3H, *s*, H-19), 4.664, 4.730 (2H, *m*, H-26), 1.568 (3H, *s*, H-27), 0.803 (3H, *t*, $J$ = 7.3 Hz, H-29), 0.870 (3H, *s*, H-32), 4.382 (1H, *ddd*, $J$ = 10.8 Hz, 10.8, 4.9, H-3). 31-Norcyclomargenol, $^1H$ NMR ($CDCl_3$): $\delta$ 0.957 (3H, *s*, H-18), 0.144, 0.391 (2H, *d*, $J$ = 4.0 Hz, H-19), 0.851 (3H, *d*, $J$ = 6.4 Hz, H-21), 4.652, 4.730 (2H, *m*, H-26), 1.567 (3H, *s*, H-27), 0.803 (3H, *t*, $J$ = 7.3 Hz, H-29), 0.835 (3H, *d*, $J$ = 6.1 Hz, H-30), 0.883 (3H, *s*, H-32), 4.511 (1H, *ddd*, $J$ = 10.3, 10.3, 4.9 Hz, H-3).

*Triterpenoid acetates.* Frs 3 A and B were purified by HPLC ($CHCl_3$–$CH_3CH$, 3:7) to give **22**–**37**, respectively. 24-Methyl-31-norlanosta-8,25-dien-3$\beta$-yl acetate (**33**), $^1H$ NMR ($\delta$, $CDCl_3$): 0.698 (3H, *s*, H-18), 0.983 (3H, *s*, H-19), 0.858 (6H, *d*, $J$ = 6.4 Hz, H-21,

Table 4. One bond (H—C COSY) and multiple bond (HMBC) H—C correlation data of compound **1**

| One bond correlation | | Multiple bond correlation | | | |
|---|---|---|---|---|---|
| $\delta_H$ ppm | $\delta_C$ ppm | $\delta_C$ ppm | | | |
| 0.845 ($H_3$-23) | 32.79 (C-23) | 21.67 (C-24) | 33.64 (C-4) | 42.40 (C-3) | 44.86 (C-5) |
| 0.888 ($H_3$-24) | 21.67 (C-24) | 32.79 (C-23) | 33.64 (C-4) | 42.40 (C-3) | 44.86 (C-5) |
| 1.048 ($H_3$-25) | 25.04 (C-25) | 38.07 (C-10) | 41.47 (C-1) | 44.86 (C-5) | 151.67 (C-9) |
| 0.734 ($H_3$-26) | 15.49 (C-26) | 29.09 (C-15) | 36.51 (C-13) | 37.65 (C-14) | 39.98 (C-8) |
| 0.825 ($H_3$-27) | 15.82 (C-27) | 36.49 (C-12) | 36.51 (C-13) | 37.65 (C-14) | 50.15 (C-18) |
| 0.927 ($H_3$-28) | 15.08 (C-28) | 36.20 (C-16) | 43.19 (C-17) | 50.15 (C-18) | 63.16 (C-21) |
| 0.937 ($H_3$-29) (*d*, 6.4 Hz) | 21.64 (C-29) | 22.61 (C-30) | 25.10 (C-22) | 63.16 (C-21) | |
| 0.810 ($H_3$-30) (*d*, 6.4 Hz) | 22.61 (C-30) | 21.64 (C-29) | 25.10 (C-22) | 63.16 (C-21) | |
| 5.280 (H-11) (*ddd*, 5.3, 2.5, 2.4) | 115.43 (C-11) | | | | |
| 5.169 (H-20) (*ddd*, 7.7, 7.7, 4.1) | 75.21 (C-20) | | | | |
| 1.48 (H-18) | 50.15 (C-18) | 15.08 (C-28) | 15.82 (C-27) | 36.49 (C-12) | 36.51 (C-13) |
| | 43.19 (C-17) | | | | |
| 2.169 (H-19) | 31.94 (C-19) | 43.19 (C-17) | | | |

Table 5. One bond (H-C COSY) and multiple bond (HMBC) H-C correlation data of compound **2**

| One bond correlation | | Multiple bond correlation | | | |
|---|---|---|---|---|---|
| $\delta_H$ ppm | $\delta_C$ ppm | $\delta_C$ ppm | | | |
| 0.827 ($H_3$-23) | 32.62 (C-23) | 20.92 (C-24) | 33.88 (C-4) | 41.81 (C-3) | 52.95 (C-5) |
| 0.859 ($H_3$-24) | 20.94 (C-24) | 32.62 (C-23) | 33.88 (C-4) | 41.81 (C-3) | 52.95 (C-5) |
| 1.206 ($H_3$-25) | 19.91 (C-25) | 37.19 (C-1) | 39.56 (C-10) | 52.95 (C-5) | 145.02 (C-9) |
| 0.935 ($H_3$-26) | 22.97 (C-26) | 29.09 (C-15) | 36.26 (C-13) | 40.03 (C-14) | 58.05 (C-8) |
| 0.852 ($H_3$-27) | 18.34 (C-27) | 46.95 (C-12) | 36.26 (C-13) | 40.03 (C-14) | |
| 0.852 ($H_3$-28) | 13.97 (C-28) | 35.94 (C-16) | 43.59 (C-17) | 52.86 (C-18) | |
| 0.903 ($H_3$-29) (*d*, 6.7 Hz) | 22.17 (C-29) | 23.02 (C-30) | 30.77 (C-22) | 59.62 (C-21) | |
| 0.832 ($H_3$-30) (*d*, 6.7 Hz) | 23.03 (C-30) | 22.17 (C-29) | 30.77 (C-22) | 59.62 (C-21) | |
| 5.319 (H-11) (*ddd*, 3.3, 2.8, 2.8) | 115.96 (C-11) | | | | |
| 2.30, 2.38 (H-6) | 41.18 (C-6) | 52.95 (C-5) | 208.08 (C-7) | | |
| 2.78 (H-8) | 29.09 (C-15) | 145.02 (C-9) | 208.08 (C-7) | | |

30), 4.665 (2H, *m*, H-26), 1.639 (3H, *s*, H-27), 0.996 (3H, *d*, $J = 7.3$ Hz, H-28), 0.870 (3H, *s*, H-32), 2.057 (3H, *s*, $COCH_3$), 4.378 (1H, *ddd*, $J = 10.8, 10.8, 5.1$ Hz, H-3). Dryocrassyl acetate (**35**). $^1$H NMR ($CDCl_3$): $\delta$ 0.846 (3H, *s*, H-23), 0.791 (3H, *s*, H-24), 0.813 (3H, *s*, H-25), 0.951 (3H, *s*, H-26, 27), 0.723 (3H, *s*, H-28), 1.016 (3H, *d*, $J = 5.6$ Hz, H-29), 2.036 (3H, *s*, $COCH_3$), 3.753 (1H, *dd*, $J = 10.7, 6.7$ Hz), 4.091 (1H, *dd*, $J = 9.9, 1.7$ Hz $CH_2OAc$). 22-Acetoxyhopane (**36**), $^1$H NMR ($CDCl_3$): $\delta$ 0.846 (3H, *s*, H-23), 0.791 (3H, *s*, H-24), 0.816 (3H, *s*, H-25), 0.951 (3H, *s*, H-26), 0.958 (3H, *s*, H-27), 0.770 (3H, *s*, H-28), 1.492 (3H, *s*, H-29), 1.432 (3H, *s*, H-30), 1.936 (3H, *s*, $COCH_3$).

*Alcohols.* Frs 4 and 5 A were chromatographed through silica gel to give $\alpha$-tocopherol [18]. 23.0 mg, $RR_t$ 1.80, and triacylglycerol of unsaturated fatty acids [19] from $C_6H_6$ eluants. 140.5 mg, $^1$H NMR ($\delta$, $CDCl_3$): 1.254, 1.302, 1.309 [$(CH_2)_n$], 2.049, 2.311, 2.770 ($COCH_2$), 4.121 (1H, *d*, $J = 6.1$ Hz), 4.165 (1H, *d*, $J = 6.1$ Hz), 4.274 (1H, *d*, $J = 4.3$ Hz), 4.318 (1H, *d*, $J = 4.3$ Hz, $CH_2$-CH-$CH_2$) from benzene, respectively. The detailed composition ratio of the fatty acids of the triacylglycerol was not clear. Fr. 4 B was purified by alumina CC to give free phytol, 22.7 mg. From both alcoholic frs A and B, sterols were sepd (21.5 mg from A and 31.6 mg from B), the composition ratio of sitosterol, stigmasterol and campesterol was evaluated by GC and GC-MS. sitosterol: 67.1%, $RR_t$ 2.73, LR-MS *m/z* (rel.int.): 414 $[M]^+$ (100), 396 (46), 381 (23), 255 (23), 231 (14), 213 (19), stigmasterol: 10.7%, $RR_t$ 2.26, LR-MS *m/z* (rel.int.): 412 $[M]^+$ (16), 394 (2), 255 (23), 231 (14), 213 (19). campesterol: 22.1%, $RR_t$ 2.41,

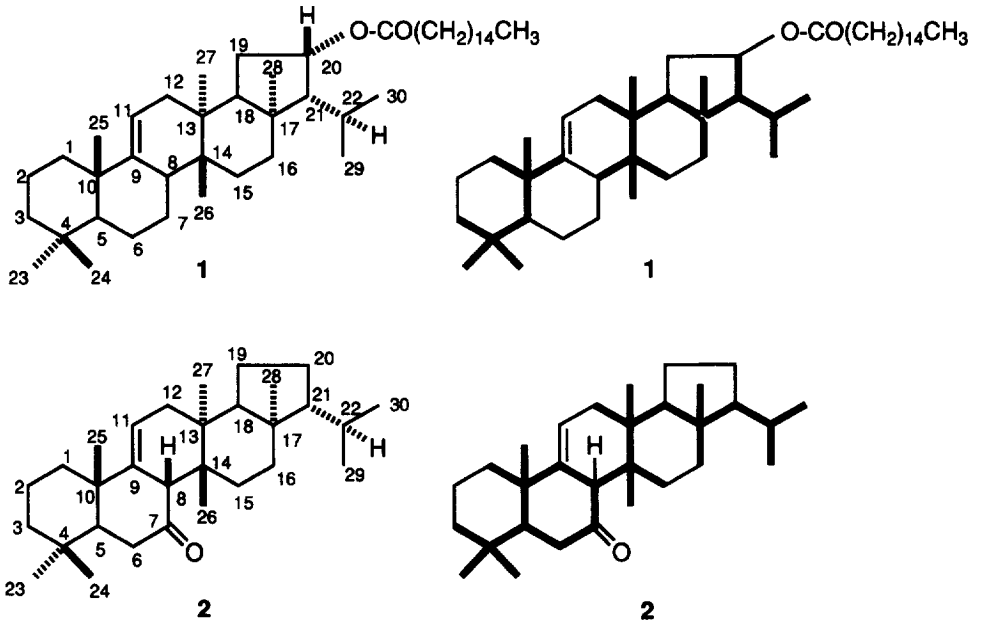



Fig. 1. Compounds **1** and **2**, and partial structures of **1** and **2** deduced from the HMBC spectrum.

Table 6. Sterols and their fatty acid esters isolated from *G. mengtzeense*

| Alcohol/fatty acid | Rhizomes (357 g) P($M^+$) | O($M^+$) | Li($M^+$) | Le($M^+$) | Fronds (238 g) P($M^+$) | O($M^+$) | Li($M^+$) | Le($M^+$) |
|---|---|---|---|---|---|---|---|---|
| Sitosterol | — | — | (676; 54) | — | (680; 2) | (678; 12) | (676; 12) | — |
| 24-Ethyl-31-norlanost-8,25-dien-3$\beta$-ol | (678; 4) | — | (676; 5) | (674; 6) | — | — | — | — |
| 31-Norcycloartenol | — | — | (676; 30) | — | — | — | — | — |
| 31-Norcyclolaudenol | (664; 4) | — | (688; 40) | (686; 8) | — | — | — | — |
| Cycloeucalenol | — | — | (688; 100) | (686; 6) | — | — | (702; 2) | — |
| 31-Norcyclomargenol | — | — | (702; 30) | (700; 7) | — | — | — | (702; 4) |
| Cycloartenol | — | — | (688; 40) | — | (664; 9) | — | (688; 2) | — |
| Cyclolaudenol | (678; 10) | (704; 4) | (702; 40) | (700; 9) | (678; 4) | (704; 2) | — | — |
| 24-Methylene-cycloartanol | (678; 10) | (704; 4) | (702; 100) | — | (678; 4) | — | — | — |
| Cyclomargenol | — | — | (716; 70) | — | — | (718; 2) | (716; 2) | — |
| Phytol | — | — | — | — | (534; 5) | (560; 4) | — | — |
| 24,24-Dimethylcycloart-25-en-3$\beta$-ol | — | — | — | — | — | — | — | (716; 3) |

Fatty acid esters of *G. mengtzeense* were fractionated by HPLC and estimated from signal intensities of the $^1$H NMR spectra. Configurations of the side chain of the alcohols were not determined. P, palmitate; O, oleate; Li, linoleate; Le, linolenate. The molecular ion observed in the EI-MS and yield (mg) are in parentheses.

Table 7. Separation of groups of compounds from the extract of *G. mengtzeense*

| Fr. | Used solvent | A (Yield) | B (Yield) | Main contents | Solvent for HPLC |
|---|---|---|---|---|---|
| 1 | *n*-$C_6H_{14}$ | 150 mg | 167 mg | Hydrocarbons | MeOH(7)–$CHCl_3$(3) |
| 2 | *n*-$C_6H_{14}$(9)–$C_6H_6$(1) | 1.01 g | 144 mg | Esters | MeCN(7)–$CHCl_3$(3) |
| 3 | *n*-$C_6H_{14}$(7)–$C_6H_6$(3) | 209 mg | 257 mg | Acetates | MeCN(8.5)–$CHCl_3$(1.5) |
| 4 | *n*-$C_6H_{14}$(1)–$C_6H_6$(1) | 66 mg | 261 mg | Alcohols | |
| 5 | $C_6H_6$ | 210 mg | 180 mg | Triacyl glycerols | |
| 6 | $Et_2O$ | 440 mg | 432 mg | Others | |

LR-MS *m/z* (rel.int.): 400 $[M]^+$ (33), 382 (16), 367 (2), 255 (23), 231 (14), 213 (19) from A and sitosterol: 69.4% stigmasterol: 14.6% campesterol: 16.0% from B.

*Filic*-4(23)-*ene* (**17**). $^1H$ NMR ($CDCl_3$): $\delta$ 4.491 (2H, *br s*, H-23), 1.026 (3H, *s*, H-24), 0.906 (3H, *s*, H025), 0.906 (3H, *s*, H-26), 0.906 (3H, *s*, H-27), 0.774 (3H, *s*, H-28), 0.871 (3H, *d*, $J = 6.4$ Hz, H-29), 0.823 (3H, *d*, $J = 6.6$ Hz, H-30).

## REFERENCES


1. Ageta, H. and Arai, Y., *Phytochemistry*, 1983, **22**, 1801.
2. Arai, Y., Hirohara, M. and Ageta, H., *Tetrahedron Letters*, 1989, **30**, 7209.
3. Arai, Y., Hirohara, M., Ageta, H. and Hsü, H., *Tetrahedron Letters*, 1992, **33**, 1325.
4. Arai, Y., Hirohara, M., Ogawa, R., Masuda, K., Shiojima, K., Ageta, H., Chang, H.-C. and Chen, Y.-P., *Tetrahedron Letters*, 1996, **37**, 4381.
5. Lödl-Linder, G., *Blumea*, 1990, **34**, 277.
6. Masuda, K., Shiojima, K. and Ageta, H., *Chemical and Pharmaceutical Bulletin*, 1989, **37**, 1140.
7. Masuda, K., Shiojima, K. and Ageta, H., *Chemical and Pharmaceutical Bulletin*, 1983, **31**, 2430.
8. Ageta, H., Shiojima, K., Suzuki, H. and Nakanura, S., *Chemical and Pharmaceutical Bulletin*, 1993, **41**, 1939.
9. Shiojima, K., Arai, Y., Masuda, K., Kamada, T. and Ageta, H., *Tetrahedron Letters*, 1983, **24**, 5738.
10. Ageta, H., Arai, Y., Suzuki, H. and Kiyotani, T., *Chemical and Pharmaceutical Bulletin*, 1995, **43**, 198.
11. Arai, Y., Koide, N., Ohki, F. and Ageta, H., *Chemical and Pharmaceutical Bulletin*, 1994, **42**, 228.
12. Ageta, H. and Arai, Y., *Phytochemistry*, 1984, **23**, 2875.
13. Ageta, H. and Arai, Y., *Journal of Natural Products*, 1990, **53**, 325.
14. Rasool, N., Ahmad, V. D. and Malik, A., *Phytochemistry*, 1991, **30**, 1333.
15. Shiojima, K., Masuda, K., Suzuki, H., Lin, T., Ohishi, Y. and Ageta, H., *Chemical and Pharmaceutical Bulletin*, 1995, **43**, 1634.
16. Shiojima, K., Arai, Y., Masuda, K., Ageta, T. and Ageta, H., *Chemical and Pharmaceutical Bulletin*, 1992, **40**, 1683.
17. Ching, R. C., *Acta Phytotaxia Sinica*, 1978, **16**, 16.
18. Urano, S., Hattori, Y., Yamanoi, S. and Matsuo, M., *Tetrahedron*, 1980, **28**, 1992.
19. Arai, Y., Yamaide, M., Yamazaki, S. and Ageta, H., *Phytochemistry*, 1991, **30**, 3369.