Pergamon

PII: S0031-9422(96)00460-8

Phytochemistry, Vol. 43, No. 6, pp. 1307–1311, 1996

0031-9422/96 $15.00 + 0.00

TRITERPENOIDS AND A TRITERPENE GLYCOSIDE FROM *SCHEFFLERA BODINIERI* LEAVES

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(*Received in revised form* 29 *May* 1996)

Key Word Index—*Schefflera bodinieri*; Araliaceae; triterpenoids.

Abstract—Two novel triterpenoids and a triterpene glycoside have been isolated from the leaves of *Schefflera bodinieri*. They are 3-oxo-20-demethylisoaleuritolic-14(15)-ene-28,29-dioic acid, 28-*O*-[α-L-rhamnopyranosyl (1→4)-*O*-β-D-glucopyranosyl(1→6)-]-*O*-β-D-glucopyranoside of 3-oxo-20-demethylisoaleuritic-14(15)-ene-28,29-dioic acid and 3α-hydroxyl-20-demethylisoaleuritolic-14(15)-ene-28,30-dioic acid. The known compounds, D-sorbitol, stigmasterol-3-*O*-β-D-glucose and two trisaccharides, were also isolated from the leaves. The structures were established on the basis of chemical and spectral evidence. Copyright © 1996 Elsevier Science Ltd

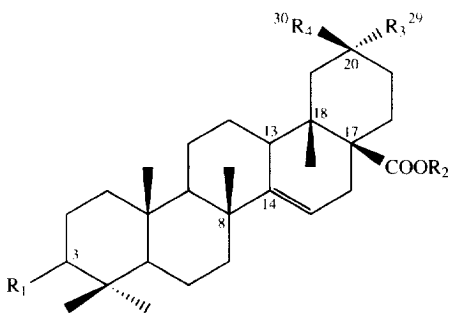
INTRODUCTION

Some plants of *Schefflera* are recorded as folk remedies for the treatment of pain, rheumatic arthritis, and lumbago in P.R. China [1]. Previous animal tests by other workers showed that an ethanol leaf extract of *S. arboricola* had sedative, hypnotic, analgesic, anticonvulsant and smooth muscle relaxant effects [2]. Screenings by our ligand receptor binding assays indicated that the extract of leaves of *Schefflera bodinieri* (Levi.) Rehd. was able to bind to 5HT, dopamine and GABA receptors. Therefore, a chemical investigation was undertaken on the leaf extract guided by ligand receptor binding assays. This paper reports the isolation and structure identification of the two novel triterpenoids and a triterpene glycoside.

RESULTS AND DISCUSSION

Repeated column chromatography on the 70% ethanol extract from the dried leaves of *S. bodinieri* afforded compounds **1**, **3** and **4**. The further purification of polar fractions by reverse phase HPLC provided compound **2**. Compounds **5**, **6**, and **7** were purified by Sephadex column chromatography.

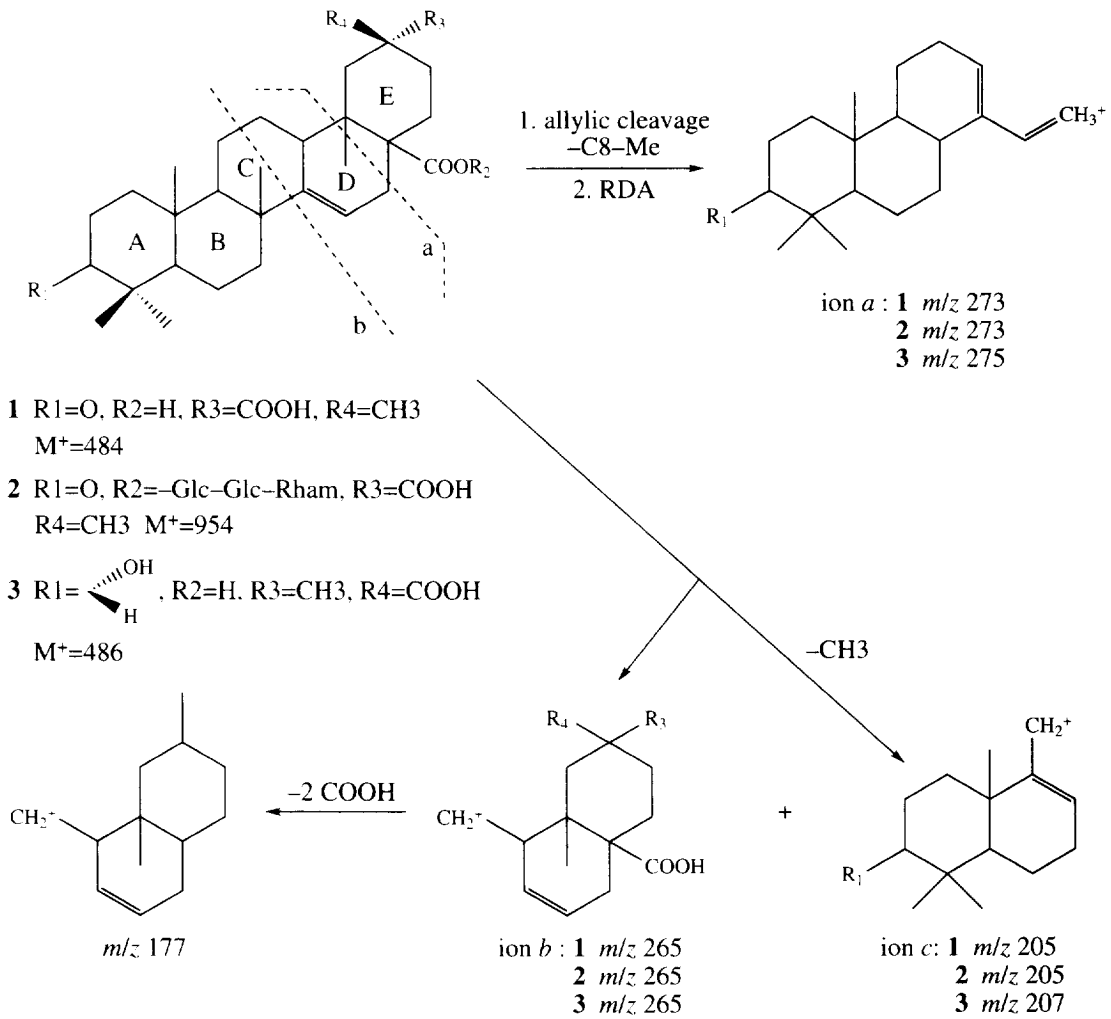
Compound **1** was obtained as a white amorphous powder. The high resolution mass spectrum established the molecular weight with the molecular ion at *m/z* 484.3185 (requires 484.3189) and the molecular formula as $C_{30}H_{44}O_5$. The ^{1}H NMR data indicated that the compound was a terpenoid with six tertiary methyl groups at δ 0.84–1.05 (*s*, 3H each) and one olefinic proton (δ 5.73). The ^{13}C NMR-DEPT spectrum exhibited the presence of two carboxyl groups (δ 178.7 and 181.0), one carbonyl group (δ 220.2), two double bond carbons (δ 126.3, 137.1) and a total of thirty carbon atoms including six methyl, ten methylene, three methine and six quaternary carbons. These data suggested that the compound might have a β-amyrane structure. In the EI-mass spectrum, there was a peak due to the RDA fragmentation at *m/z* 273 (ion *a*, Scheme 1) indicating the presence of a Δ^{14}-β-amyrane skeleton. The fragments formed by collapse of ring C were also observed at *m/z* 265 (ion *b*) and 205 (ions *c*, lost C_8-Me by allylic cleavage at the same time), which suggested the allocation of functional groups on the two parts of the molecule. The signal of one carboxylic group at δ 178.1 in the ^{13}C NMR spectrum was



1 R1=O, R2=H, R3=COOH, R4=CH3

2 R1=O, R2=–Glc–Glc–Rham, R3=COOH, R4=CH3

3 R1=  R2=H, R3=CH3, R4=COOH



Scheme 1. Fragment ions obtained in EI-mass spectra of compounds **1–3**.

assigned to the C-17 carboxyl, and the other carboxyl signal at δ 181.0 might be assigned to either C-29 or C-30 due to the fragment of ion *b* in the EI-mass spectrum. Comparing with the related compounds [3], the carboxylic group (δ 181.0) should be located at the C-29 position being α-equatorial oriented. From these results and compared with the related data in refs 4, 6, the new compound was identified as 3-oxo-20-demethylisoaleuritolic-14(15)-ene-28,29-dioic acid.

The HR-FAB-mass spectrum of compound **2** established the molecular formula as $C_{48}H_{74}O_{19}$ and the molecular weight was found to be 954.4828 (requires 954.4824). The ^{13}C and ^{1}H NMR spectra showed that compound **2** was a triterpenoid glycoside with three sugar moieties. The anomeric carbon signals appeared at δ 95.6, 104.0, 102.5 and the anomeric proton signals at δ 5.36, 4.41, and 4.86. Hydrolysis of the compound gave glucose and rhamnose as sugar components (co-TLC, in three solvent systems, with glucose, rhamnose, arabinose, galactose, mannose and xylose as references). There was only one methyl group signal at δ 1.29 (3H, *d*, $J = 6.5$ Hz) with the anomeric signal at δ 4.86 (*br s*) indicating the presence of one rhamnose. The anomeric proton signals at δ 5.36 (Glc) suggested the glucose was connected to the genin via a carboxylic acid, since the chemical shift of the proton was 1 ppm downfield compared to that of the glucose linked with a hydroxyl group [7]. The coupling constants of the anomeric protons in the ^{1}H NMR spectrum indicated that the glucoses were β-linked ($J = 8$ Hz), and the rhamnose was α-linked (*br s*) [8]. The ^{13}C NMR-DEPT spectrum suggested that the sequence of the sugar moieties was α-L-rhamnopyranosyl(1 $\rightarrow$ 4)-*O*-β-D-glucopyranosyl(1 $\rightarrow$ 6)-*O*-β-D-glucopyranoside, because the chemical shift of the carbon at C-6 on the glucose linked with the genin and the C-4 of the second glucose were both 7 ppm downfield comparing with those of the free sugar. Moreover, the sugar residue of compound **2** was found to be the same as those of triterpene glucosides isolated from *Schefflera* [9–11].

The EI-mass spectrum of compound **2** exhibited similar fragments to those in the spectrum of compound **1** (Scheme 1) except for the ion at *m/z* 484, which did not appear due to the loss of the C-29 carboxyl group

(α-cleavage). Such loss readily occurred under the same condition as for loss of the sugar moieties. There was a clear peak at m/z 440 corresponding to the fragment of [aglycone − COOH + 1]$^+$. Apart from the sugar signals, the ^{1}H NMR spectrum of **2** presented a similar spectral pattern as **1**, showing six tertiary methyl groups at δ 0.89–1.08 (*s*. 3H each) and one olefinic proton at δ 5.70. The ^{13}C NMR-DEPT spectrum also indicated the presence of two carboxyl groups (δ 178.0, 180.0), one carbonyl group (δ 218.8), two double bond carbons (δ 127.6, 137.5) and a total of thirty carbon atoms. These data suggested the skeleton of the aglycone was the same as compound **1**, which was further substantiated by comparing the hydrolysed aglycone with **1** (co-TLC). From the ^{1}H NMR, ^{1}H–^{1}H COSY, ^{13}C NMR and ^{13}C–^{1}H COSY data, and the comparison with related data in references [8–10], the assignment of protons and carbons in compound **2** was achieved as indicated in Table 1. The structure of the new compound was then identified as 28-*O*-[α-L-rhamnopyranosyl (1 → 4)-*O*-β-D-glucopyranosyl(1 → 6)-*O*-β-D-glucopyranoside of 3-oxo-20-demethylisoaleuritic-14(15)-ene-28,29-dioic acid.

Compound **3** is an amorphous powder. The HR-mass spectrum indicated the molecular weight was 486.3342 (requires 486.3345) and the molecular formula was established as $C_{30}H_{46}O_5$. The ^{1}H NMR spectrum suggested that the compound was a triterpenoid with six tertiary methyl groups, one olefinic proton (δ 5.55), one proton adjacent to a hydroxyl group (δ 4.18, *br s*) and two carboxylic acids (δ 12.15, 2H, *br s* $W_{1/2}$ = 70 Hz). ^{13}C NMR spectrum exhibited the presence of two carboxylic carbons (δ 176.8, 178.7), two olefinic carbons (δ 136.8, 125.4), one carbon linked with a hydroxyl group (δ 73.76), three methines, ten methylenes and six quaternary carbons, corresponding to the formula $C_{30}H_{46}O_5$. These data suggested that the compound had the structure of a β-amyrane with one double bond, a C_3-OH, and two carboxyl groups in the molecule. Comparing its ^{13}C NMR data with those of compound **1**, there was a 3-OH carbon (δ 73.76) in compound **3** rather than a 3-ketonic carbon (δ 220.2) as in **1**. The chemical shift of C-3 at δ 73.8 suggested that the hydroxyl group was α-axially oriented in comparison with the corresponding signal of the β-equatorial isomer [12]. In the EI-mass spectrum the C-8

Table 1. ^{13}C and ^{1}NMR assignments of compound **2**

Aglycone	δ_C	δ_H	Sugar C	δ_C	δ_H
1	35.1	1.98, 1.50	Glc1*		
2	35.2	2.47, 2.51	1	95.6	5.36 (*d*, *J* = 8 Hz)
3	218.8	—	2	73.8	3.33 (*m*)
4	44.7	—	3	79.5	3.67 (*t*, *J* = 6 Hz)
5	48.5	2.10	4	71.0	3.45 (*t*, *J* = 6 Hz)
6	20.7	1.73, 2.08	5	76.9	3.57 (*m*)
7	35.0	1.58, 1.47	6	69.4	3.85, 4.13 (*dd*, *J* = 8, 1 Hz)
8	47.6	1.20			
9	56.4	1.37	Glc2		
10	40.6	—	1	104.0	4.41 (*d*, *J* = 8 Hz)
11	24.2	1.29, 1.31	2	74.3	3.29 (*t*, *J* = 3 Hz)
12	44.7	1.41, 1.18	3	76.7	3.51 (*t*, *J* = 8 Hz)
13	44.7	2.92 (*dd*, *J* = 10, 2 Hz)	4	78.2	3.43 (*t*, *J* = 6 Hz)
14	137.5	—	5	78.1	3.31 (*m*)
15	127.6	5.70 (*t*, *J* = 1 Hz)	6	61.9	3.83, 3.68 (*dd*, *J* = 8, 1 Hz)
16	25.7	1.98, 2.00			
17	57.1	—	Rham		
18	39.4	—	1	102.5	4.86 (*br s*)
19	40.6	1.57, 1.31	2	72.5	3.88 (*br s*)
20	31.6	—	3	72.1	3.67 (*dd*, *J* = 5, 1 Hz)
21	32.9	1.58, 1.45	4	73.9	3.43 (*t*, *J* = 3 Hz)
22	25.7	2.08, 1.73	5	71.0	4.03 (*m*)
23	33.7	0.94 (3H, *s*)	6	17.9	1.29 (*d*. *J* = 6.5 Hz)
24	19.0	0.93 (3H, *s*)			
25	17.9	1.08 (3H, *s*)			
26	21.8	0.89 (3H, *s*)			
27	24.1	1.06 (3H, *s*)			
28	178.0	—			
29	180.0	—			
30	27.3	1.04 (3H, *s*)			

*Glc1: the first glucose linked with the aglycone, Glc2: the second glucose linked with Glc1, Rham: rhamnose linked with Glc2.

The data were obtained from 500 MHz NMR in CD_3OD. The assignments are based on ^{1}H NMR, ^{13}C NMR,^{1}H–^{1}H COSY and ^{13}C–^{1}H COSY spectral data.

methyl group readily lost due to allylic cleavage and the fragment arising from RDA cleavage (ion *a*, Scheme 1) was similar to that of compound **1**, except for ions *a* and *b* being 2 mass units more than those of **1** due to the presence of a hydroxyl group at C-3. The characteristic peaks at *m/z* 275, 265, 207 and 177 in the EI-mass spectrum indicated the double bond position at C-14, a methyl group at C-8, a hydroxyl group at C-3 and a carboxylic group at either C-29 or C-30 (Scheme 1) [13, 14]. In the FAB-mass spectrum *m/z* 289 (ion $a + CH_3$) was observed as base peak, thus confirming the presence of C-8 methyl. The carboxylic carbon signal at δ 178.6 was typical for a C-17 carboxyl group therefore, the other carboxylic group (signal at δ 176.8) was then assigned to C-30 due to this carboxyl group being β-axial orientated [3]. Based on these results, the structure of the new compound was determined to be 3α-hydroxyl-20-demethylisoaleuritolic-14(15)-ene-28,30-dioic acid.

The ^{13}C HMR, ^1H NMR and EI-mass spectra suggested that compounds **4** and **5** were stigmasterol-3-*O*-β-D-glucoside and D-sorbitol which were confirmed with authentic samples by co-TLC. Compounds **6** and **7** were a mixture of epimeric trisaccharides including two glucoses and one rhamnose in an approximate ratio of 0.8(α):1.0(β) as indicated by the ^{13}C NMR spectrum. Hydrolysis of this mixture produced glucose and rhamnose (co-TLC). Their ^{13}C and ^1H NMR spectral data were identical to those of the sugar moieties of the triterpenoid glycosides isolated from this plant. The linkage of the sugars was further confirmed by ^1H–^1H COSY and ^{13}C–^1H COSY spectral data and the molecular weight was given by the FAB-mass spectrum of this mixture (*m/z* 511, $M^+ + Na^+$). Thus compound **6** was identified as α-L-rhamnopyranosyl (1 → 4)-*O*-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside and **7** as its 1α-epimer. These epimeres have also been isolated from *Schefflera octophylla* [15].

Previous phytochemical studies on several species of *Schefflera* by other workers have resulted in the identifications of triterpenoids and their glycosides as major components. The findings of this study indicate that the major ingredients of *S. bodinieri* leaves are also triterpenoids and the glycosides, but the triteropenoids have a Δ_{14}-β-amyrane structure. This type of structure does not commonly occur in the plants of the Araliaceae. In our radioligand receptor binding assays, compounds **1**, **2** and **5** were able to bind to muscarinic receptor, **4** bound to 5HT-2 receptor and the mixture of **6** and **7** bound to the Ca^{2+} channel receptor with an IC_{50} in the μM level indicating the efficiency of the bioassay guided isolation.

EXPERIMENTAL

General experimental procedure. ^1H NMR, ^1H–^1H COSY and ^{13}C–^1H COSY spectra were measured at 500 MHz and the ^{13}C NMR spectrum (DEPT) was measured at 127.7 MHz on an AMX-500, at room temp, with either CD_3OD or DMSO soln. TMS was used as internal standard. EI-MS (70 eV), FAB-MS and HR-FAB were taken with a direct probe on an Analytical ZAB-2F, (VG, Micromass Ltd). The isolation was conducted on the following chromatographic columns: silica gel C-60 (40–60A, May and Baker); sephadex LH-20 (Sigma); reverse phase ODS column (dp 5 μ, 25 cm × 4.6 mm, Beckman) in HPLC (Waters 991) with photodiode array detector.

Plant material. Schefflera bodinieri was collected on Jinfo Mountain, Sichuan province, P.R. China in November, 1990 and identified by Professor Z. Liu. The voucher specimens are kept in the herbarium of the Institute of Medicinal Plant Cultivation, Sichuan province, P.R. China.

Extraction and isolation. The air-dried leaves of *S. bodinieri* (1 kg) were extracted by 70% EtOH at room temp. to yield an extract (160 g). The extract (20 g) was subjected to a flash column on silica gel eluted with solvents of increasing polarity. The frs eluted with $CHCl_3$–MeOH (9:1) afforded compound **1** (100 mg). Compounds **2** (10 mg) and **4** (10 mg) were from the frs eluted with $CHCl_3$–MeOH (8:2) and purified by reverse-phase HPLC. Compound **2** (50 mg) was obtained from the frs of $CHCl_3$–MeOH (7:3) and also purified by the HPLC. Compounds **5** (20 mg), **6** and **7** (100 mg) were isolated from the MeOH frs and purified by Sephadex columns.

Hydrolosis of compound **2**. Compound **2** (5 mg) was dissolved in 10 ml MeOH with 10% HCl and heated at 80° for 1 hr. The reaction soln was diluted with H_2O, and extracted with $CHCl_3$. The $CHCl_3$ fr. was dried over anhydrous Na_2SO_4 and concd to obtain the aglycone. The H_2O layer was neutralized by NaOH and dried *in vacuo* to give the sugar moieties of the glycoside. The dry material was dissolved in MeOH and identified by co-TLC with authentic samples. TLC examination was carried out with silica gel 60 F254 plates (Merck). Spray reagent: vanillin–H_2SO_4 for glycosides, aglycones and sugars. Solvent systems for examining sugars: $CHCl_3$–MeOH–HAc (2:2:1); BuOH–HAc–H_2O (5:1:2); EtOAc–MeOH–HAc (3:2:1). Reference compounds for sugar detection included glucose, rhamnose, arabinose, galactose, mannose, and xylose.

Compound **1**. Amorphous powder, soluble in $CHCl_3$–MeOH (4:1). TLC (silica gel): R_f 0.67 ($CHCl_3$–MeOH, 9:1). Found: M_r 484.3185, $C_{30}H_{44}O_5$, requires 484.3189. $[\alpha]_D$ (25°C): +126.4 (*c* 0.5, $CHCl_3$–MeOH 1:1). ^1H NMR (DMSO-d_6, 500 MHz): δ 5.73 (1H, *t*, *J* = 1 Hz, H-15), 2.92 (1H, *dd*, *J* = 10, 2 Hz, H-13), 0.84, 0.89, 0.90, 1.00, 1.01, 1.05, (3H, each, *s*, *tert*-Me). ^{13}C NMR (DMSO, 125.7 MHz): δ 16.27, 18.19, 21.45, 23.30, 26.96, 33.15 (6C, *tert*-Me); δ 178.7 (C-17-COOH); δ 181.0 (C-20-COOH); δ 220.2 (C-3); 137.1 (C-14); 126.3 (C-15). CH_2: 19.04, 23.30, 24.17, 24.69, 32.38, 33.94, 34.36, 36.08, 39.49, 43.56; CH: 43.63, 46.41, 55.04, quaternary carbon: 30.95, 37.00, 39.51, 47.61, 56.03. EI-MS *m/z* (rel. int.): 484 (5), 440 (27), 425 (55), 394 (7), 379

(23), 273 (15), 265 (13), 234 (18), 205 (47), 189 (19), 177 (29), 55 (100).

Compound **2**. Amorphous powder, soluble in MeOH. TLC (silica gel): R_f 0.48 ($CHCl_3$–MeOH–H_2O, 8:2:1). Found M_r 954.4828, $C_{48}H_{74}O_{19}$, requires 954.4824. $[\alpha]_D$ (25°C): +37.8 (*c*0.5, MeOH) ^1H NMR and ^{13}C NMR: see Table 1. EI-MS *m/z* (rel. int.): 440 (20), 425 (44), 394 (5) 379 (42), 273 (18), 265 (7), 220 (5), 205 (17), 201 (19), 189 (23), 177 (36). FAB-MS *m/z*: 977 $[M + Na]^+$.

Compound **3**. Amorphous powder, soluble in DMSO or mixture of $CHCl_3$ and MeOH. TLC (silica gel): R_f 0.55 ($CHCl_3$–MeOH, 9:1). Found M_r 486.3342, $C_{30}H_{46}O_5$, requires 486.3345. $[\alpha]_D$ (25°C): +95.7 (*c* 0.125, $CHCl_3$–MeOH, 1:1). ^1H NMR (DMSO-d_6, 500 MHz): *tert* Me (*s*): δ 0.75, 0.80, 0.81, 0.82, 0.86, 0.87. δ 5.55 (1H, *br s*, H-15), 4.18 (1H, *br s* H-3β), 3.15 (1H, *br s*, H-13), 12.15 (2H, *br s*, $W_{1/2}$ = 70 Hz, COOH-28, 30). ^{13}C NMR (DMSO, 125.7 MHz): 33.11 (C-1), 23.98 (C-2), 73.76 (C-3), 37.11 (C-4), 46.40 (C-5), 25.45 (C-6), 33.54 (C-7), 36.90 (C-8), 48.40 (C-9), 36.90 (C-10), 18.00 (C-11), 40.18 (C-12), 46.42 (C-13), 136.8 (C-14), 125.4 (C-15), 23.90 (C-16), 55.35 (C-17), 43.01 (C-18), 31.85 (C-19), 30.57 (C-20), 36.17 (C-21), 22.58 (C-22), 33.25 (C-23), 18.20 (C-24), 16.10 (C-25), 22.50 (C-26), 23.60 (C-27), 178.6 (C-28), 28.80 (C-29), 176.8 (C-30). EI-MS *m/z* (rel. int.): 486 (M^+, absent), 442 $[M—COOH]^+$ (10), 427 (26), 409 (51), 381 (15), 363 (48), 317 (2), 287 (24), 275 (29), 273 (27), 265 (11), 243 (13), 241 (30), 227 (45), 219 (11), 201 (42), 190 (62), 189 (65), 175 (58), 135 (95), 105 (100). FAB-MS *m/z* 487 $[M + 1]^+$ (7), 469 (18), 441 (10), 307 (97), 289 (100), 275 (10), 273 (24).

Acknowledgements—The authors thank Professor Z. Liu, Head of the Department of Medicinal Plant, Institute of Cultivation of Medicinal Plant, Sichuan province, China, for helping to collect the plant material and identify the plant species. Thanks are also due to the mass spectrum laboratory in the School of Pharmacy and NMR service in King's College, University of London. The project was supported by Pfizer Ltd.

REFERENCES

1. Li, G. X. (1992) in *Pharmacology, Toxicity and Clinic of Traditional Chinese Medicine*, p. 111. Tianjin Science and Technique Translation Publishing House, Tianjin, P.R. China.
2. Liao, N. (1986) in *Pharmacology and Applications of Chinese Materia Medica* (Chang H. M. and But P. P. H., eds), Vol. I, p. 6. World Scientific Press, Singapore.
3. Miyakoshi, M., Ida, Y. Isoda, S. and Shoji, J. (1993) *Phytochemistry* **34**, 1599.
4. Woo, W. S. and Wagner, H. (1977) *Phytochemistry* **16**, 1845.
5. Djerassi, C., Budzikiewicz, H. and Wilson, J. M. (1962) *Tetrahedron Letters*, 263.
6. Paul, B. D., Rao, G. B. and Kapadia, G. J. (1974) *J. Pharm Sci.* **63**, 958.
7. Tripapathi, V. K., Pandey, K. N., Udupa, K. N. and Rücker, G. (1992) *Phytochemistry* **23** 1695.
8. Agrawal, P. K. (1992) *Phytochemistry* **31**, 3307.
9. Sung, T. V., Kataling, J. P. and Adam, G. (1991) *Phytochemistry* **30**, 3717.
10. Sung, T. V., Lavaud, C., Porzel, A., Steglich, W. and Adam, G. (1992) *Phytochemistry* **31**, 227.
11. Sung, T. V. and Adam, G. (1991) *Phytochemistry* **30**, 2717.
12. Tanaka, R. and Matsunaga, S. (1991) *Phytochemistry* **30**, 4093.
13. Razdan, T. K., Harkar, S., Kachroo, V. and Koul, G. L. (1982) *Phytochemistry* **21**, 2339.
14. Budzikiewicz, B. H., Wilson, J. M. and Djearassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
15. Sung, T. V., Steglich, W. and Adam, G. (1991) *Phytochemistry* **30**, 2349.