Pergamon 0031-9422(95)00432-7 Phytochemistry, Vol. 40, No. 5, pp. 1489–1492, 1995
Elsevier Science Ltd
Printed in Great Britain
0031-9422/95 $9.50 + 0.00

TRITERPENOID SAPONINS FROM THE AERIAL PARTS OF *ASTER BELLIDIASTRUM**

THOMAS SCHÖPKE, CHALED AL-TAWAHA, VICTOR WRAY,† MANFRED NIMTZ,† ANKE MEYER† and KARL HILLER

Humboldt-Universität zu Berlin, Mathematisch-Naturwissenschaftliche Fakultät I, Institut für Pharmazie, Goethestr, 54, D-13086, Berlin, Germany; †Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-38124 Braunschweig, Germany

(*Received in revised form* 11 *April* 1995)

Key Word Index—*Aster bellidiastrum*; Asteraceae; triterpenoid saponins; bellidiastroside C_2; bellissaponins; besysaponin C_{12}; polygalacic acid; chemotaxonomy.

Abstract—Four major triterpenoid saponins were isolated from the aerial parts of *Aster bellidiastrum*. The structures were elucidated from their NMR and mass spectral data, and from derivatization. One is a new compound with the structure 3-*O*-β-D-glucopyranosyl-2β,3β,16α,23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl(1 → 4)-α-L-rhamnopyranosyl(1 → 2)-β-D-fucopyranoside, while the three others have been previously identified in extracts from various *Bellis* species.

INTRODUCTION

Aster bellidiastrum is a small perennial herb growing in the European Alps on soil with a high lime content. In outward appearance it resembles members of the *Bellis* species more than other asters. Thus, while most species of the genus *Aster* possess a branched or, at least, a leafy stem with blue–violet ligulate flowers, *A. bellidiastrum* only possesses a single stem, with basal leaves, that carries white to white–reddish ligulate flowers.

In previous studies we have investigated the triterpenoid saponins of various species of the genus *Bellis* [1–4]. The present paper describes the isolation and structure elucidation of four major deacylsaponins obtained from the aerial parts of *A. bellidiastrum*. Significantly, three of these have already been identified in *Bellis* species while the fourth is a new saponin.

RESULTS AND DISCUSSION

Four compounds, bellidiastrosides B (**1**), C_1 (**2**), C_2 (**3**) and D_1 (**4**), were isolated from the mild alkaline hydrolysate of the saponin mixture obtained from the aerial parts of *A. bellidiastrum* as described in Experimental. There were considerable similarities in the TLC behaviour of these compounds to the deacylsaponins obtained from *Bellis perennis* and *B. sylvestris*.

Electrospray ionization–mass spectrometry (ESI MS) of compound **1** afforded a molecular ion at *m/z* 1075 $[M + H]^+$. MS/MS of the ion at *m/z* 1075 afforded fragment ions generated by the respective loss of monosaccharide residues leaving the OH group on the respective fragments at *m/z* 943 $[M - pent + H]^+$, 929 $[M - dhex + H]^+$, 797 $[M - pent - dhex + H]^+$, 651 $[M - pent - dhex - dhex + H]^+$ and 505 $[M - pent - dhex - dhex - dhex + H]^+$, indicating that **1** is a bisdesmoside having a terminal pentose and a terminal deoxyhexose. A further ion at *m/z* 425 $[pent + dhex + dhex - H_2O + H]^+$ suggests that three of the sugars are present as a trisaccharide unit. Methanolysis and GC identification yielded fucose, rhamnose and xylose as sugar constituents. The ^1H NMR spectrum showed signals of four anomeric protons, six tertiary methyl groups and three secondary methyl groups. The ^{13}C NMR spectrum of **1** was identical with that of besysaponin C_{12}. GC of the L-cysteine methyl ester derivatives of the sugars [5] showed that xylose and fucose are present as the D-enantiomers, and the rhamnoses as L-enantiomers. Hence, **1** has the structure of a 3-*O*-α-L-rhamnopyranosyl-2β,3β,16α,23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl(1 → 4)-α-L-rhamnopyranosyl(1 → 2)-β-D-fucopyranoside.

TLC and HPLC of **2** and **4** indicated that they are identical with bellissaponins BS1 and BS2, respectively. These were unambiguously confirmed by the same techniques as those used above. Hence, **2** is 3-*O*-α-L-rhamnopyranosyl-2β,3β,16α,23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl(1 → 3)-β-D-xylopyranosyl(1 → 4)-α-L-rhamnopyranosyl(1 → 2)-β-D-fucopyranoside, while **4** is 3-*O*-β-D-glucopyranosyl-2β,3β,16α,23-tetrahydroxyolean-12-en-28-oic acid 28-α-L-rhamnopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl(1 → 4)-α-L-rhamnopyranosyl(1 → 2)-β-D-fucopyranoside.

*Dedicated to Prof. H. Schilcher on the occasion of his 65th birthday.

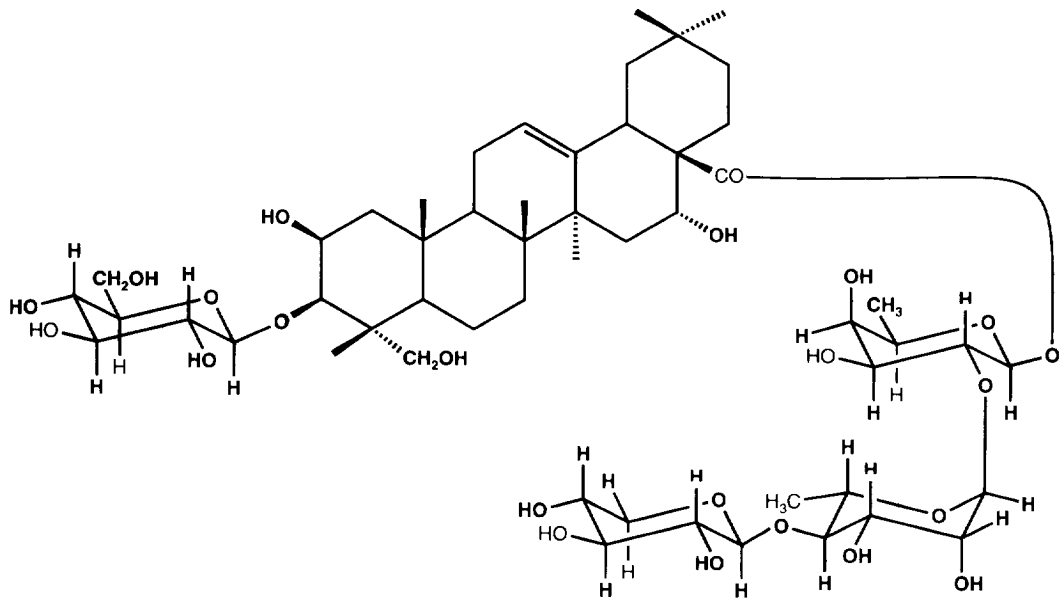

Table 1. ^{13}C NMR data from compound **3** in CD_3OD

Aglycone				Sugars					
C-1	44.5	C-16	74.7	Glc 1‡	C-1	105.5	Fuc 1,2	C-1	95.2
C-2	71.2	C-17	50.1		C-2	75.4		C-2	74.1
C-3	83.9	C-18	42.4		C-3	77.7		C-3	76.7
C-4	43.2	C-19	48.1*		C-4	71.2		C-4	73.6
C-5	48.0	C-20	31.3		C-5	78.2		C-5	71.9
C-6	18.7	C-21	36.5		C-6	62.3		C-6	16.5
C-7	33.8	C-22	32.0	Rha 1.4	C-1	101.1	Xyl 1	C-1	107.7
C-8	40.9	C-23	65.6		C-2	72.3		C-2	76.1
C-9	48.5*	C-24	14.7		C-3	72.7		C-3	78.2
C-10	37.5	C-25	18.3†		C-4	84.4		C-4	71.1
C-11	24.7	C-26	17.7		C-5	68.8		C-5	67.3
C-12	123.6	C-27	27.3		C-6	17.8†			
C-13	144.7	C-28	177.3						
C-14	42.9	C-29	33.4						
C-15	36.5	C-30	24.9						

*Taken from the DEPT spectrum.
†Assignments may be interchanged.
‡Sugar bound to C-3 of aglycone.

Compound **3** gave a molecular ion at *m/z* 1091 $[M + H]^+$ and at 1113 $[M + Na]^+$ in the ESI MS. MS/MS of the protonated molecular ion afforded ions at *m/z* 959 $[M - pent + H]^+$, 929 $[M - hex + H]^+$, 813 $[M - pent - dhex + H]^+$, 797 $[M - pent - hex + H]^+$, 667 $[M - pent - dhex - dhex + H]^+$, 651 $[M - pent - hex - dhex + H]^+$ and 505 $[M - pent - hex - dhex - dhex + H]^+$, indicating that **3** has a terminal pentose, a terminal hexose and two inner deoxyhexoses. Appearance of an ion at *m/z* 425 $[pent + dhex + dhex - H_2O + H]^+$ suggests that three of the sugars are present as a trisaccharide unit. GC of the monosaccharides obtained by methanolysis afforded glucose, rhamnose and xylose. Consequently, there are four anomeric signals and two secondary methyl groups in the ^1H NMR spectrum belonging to the sugar moieties. Comparison of the ^{13}C NMR data (Table 1) with those for **1**, **2** and **4** clearly indicates that polygalacic acid is also the aglycone in **3**. However, the ^{13}C NMR data for fucose, rhamnose and xylose are very similar to those for **1**, and the data for glucose are similar to those for **4**, suggesting that **3** is comparable with **1**, with the exception that glucose replaces rhamnose at C-3 of the aglycone. To confirm this assumption, GC/MS analysis of the partially methylated alditol acetates [6] was performed and indicated the presence of a 1,2-branched and a 1,4-branched deoxyhexose, a terminal hexose and a terminal pentose. Finally, the L-cysteine methyl ester derivatives

were prepared and analysed by GC, proving that glucose, fucose and xylose have D-configurations and rhamnose has the L-configuration. Hence, **3** has the structure 3-*O*-*β*-D-glucopyranosyl-2*β*,3*β*,16*α*,23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-*β*-D-xylopyranosyl(1 → 4)-*α*-L-rhamnopyranosyl(1 → 2)-*β*-D-fucopyranoside.

While compound **3** is a new triterpenoid saponin, compound **1** has already been obtained from *B. sylvestris* [3], **2** from *B. sylvestris* [3] and *B. perennis* [1] and **4** from *B. perennis* [1] and *Solidago virgaurea* [7]. Bellidiastroside C_2 has also been obtained, during the course of this work, as a product of the enzymic hydrolysis of virgaureasaponin 1.

A common chemical feature both of *Bellis* and *Aster* plants is the presence of triterpenoid saponins as major constituents. While *Bellis* sp. usually contain glycosides of polygalacic acid [1, 3], *Aster* sp. contain glycosides of astgerogenic, oleanolic and echinocystic acids [8–10]. The present paper indicates that, from a chemical point of view, *A. bellidiastrum* is more closely related to *Bellis* than to *Aster*. The similarities not only include the structures of the major deacylsaponins, but also their relative proportions. That means, like *Bellis* sp., the aerial parts of *A. bellidiastrum* contain **2** as the major deacylsaponin while we have found that the underground parts contain compounds **2** and **4** in a ratio of 1:1 (unpublished results).*

EXPERIMENTAL

General experimental procedure. NMR spectra of **1** and **2** were recorded in CD_3OD at 300 K on a Bruker AM 300 NMR spectrometer (^1H: 300.13 MHz; ^{13}C: 75.47 MHz), those of **3** and **4** on a Bruker ARX 400 NMR spectrometer (^1H: 400.13 MHz; ^{13}C: 100.61 MHz), and mass spectra on a Kratos MS 50 RF mass spectrometer (high resolution FAB-MS), on a Finnigan TSQ 700 equipped with a Finnigan electrospray source (ESI-MS and MS/MS) and on a Kratos MS 50 FS connected to a Carlo Erba Mega Series gas chromatograph (GC/MS). GC was carried out on a Hewlett Packard HP 5890 Series II gas chromatograph, HPLC on a Hitachi/Merck, model D-6000, equipped with a L-4000 UV detector, TLC on silica gel 60 plates or foils (Merck) and CC on Sephadex LH-20 (Pharmacia), Diaion HP-20 and silica gel 60, 0.063–0.2 *μ*m (Merck).

Plant material. Plants were collected on 26th/27th May 1993 in the Swiss Alps (Kanton Graubünden) at an altitude of *ca* 2000 m. The material was dried at 50–60°. A voucher specimen is deposited at the herbarium of the Institute of Pharmacy, Humboldt-University, herbal number Scho-21.

Extraction and isolation. The dried plant material (545 g) was defatted with petrol and extracted with EtOAc in a Soxhlet extraction apparatus. The plant material was dried and then refluxed 3× for 1 hr with 80% MeOH (3 × 1400 ml). MeOH was removed under red. pres. The residue was dissolved in 1 l of H_2O and extracted 5× with *n*-BuOH (5 × 500 ml). The dried *n*-BuOH extract was dissolved in MeOH and dropped into an excess of Et_2O to give 50.5 g of a brown, powdery crude glycoside mixture.

The crude glycoside mixt. was subjected to Sephadex LH-20 CC (MeOH) to give 34.2 g of a saponin containing fr. This fr. was dissolved in H_2O and subjected to CC on Diaion HP-20 using a H_2O, 50% MeOH, MeOH gradient. The MeOH eluate was dried to give 26.3 g of a saponin fr.

The saponin fr. (13 g) was hydrolysed with 500 ml of 1% KOH for 2 hr at room temp. After neutralization with HCl the deacylated saponins were extracted 3× with *n*-BuOH (3 × 150 ml) to give 10.5 g of deacylated saponins. These were separated by CC on silica gel ($CHCl_3$–MeOH–H_2O 10:3:1, lower layer) to give 880 mg of fr. B, 3.4 g of fr. C and 200 mg of fr. D.

Fr. B (90 mg), when sepd by HPLC on LiChrosorb RP-18 (7 *μ*m, 250 × 10 mm) with MeOH–H_2O (7:3), gave 59 mg of **1**, 300 mg of fr. C gave 219 mg of **2** and 20 mg of **3**, and 20 mg of fr. D gave 11 mg of **4**.

Identification of the sugars. Each compound (1 mg) was dissolved in 0.5 ml of 1 N HCl–MeOH and hydrolysed at 100° for 2 hr. After neutralization with Ag_2CO_3 the ppt was removed by centrifuging and the supernatant was dried with N_2. The residue was trimethylsilylated with trimethylsilylimidazole and then analysed by GC. GC conditions: column, J&W Scientific DB-17 (30 m × 0.25 mm i.d., film thickness 0.25 *μ*m); oven temp. 170° for 10 min, increased by 2° min^{-1} to 190°; injection port and detector temp., 280°; carrier gas, He (linear velocity 13.6 cm sec^{-1}), split ratio 1:50.

Determination of the absolute configuration of the sugars. The determination was performed according to ref. [5] using *ca* 1 mg of the compounds. GLC conditions: column, J&W Scientific DB-17 (30 m × 0.25 mm i.d., film thickness 0.25 *μ*m); 250°, oven temp.; 280°, injection port and detector temp.; carrier gas, He (linear velocity 12.5 cm sec^{-1}), split ratio 1:55.

Bellidiastroside B_1 (**1**). Crystals (from MeOH); mp 231–233°; $[\alpha]_D^{22} - 22.9°$ ($c = 0.24$). TLC; R_f 0.55 ($CHCl_3$–MeOH–H_2O, 7:4:1); HPLC: R_t 6.4 min (LiChrosorb RP-18, 7 *μ*m, 250 × 4 mm i.d., MeOH–H_2O, 7:3). ^1H NMR: aglycone *δ*0.82, 0.92, 0.94, 0.99, 1.37, 1.43 (6 × CH_3), 2.98 (*dd*, J = *ca* 4, 14 Hz, H-18), 5.36 (*t*, J = *ca* 3 Hz, H-12), sugar methyl protons *δ*1.26 (*d*, J = 6.4 Hz), 1.28 (*d*, J = 6.2 Hz), 1.36 (*d*, J = 6.0 Hz), sugar anomeric protons *δ*4.53 (*d*, J = 7.2 Hz), 4.98 (*bs*), 5.34 (*d*, J = 8.2 Hz), 5.40 (*d*, J = 1.6 Hz). ^{13}C NMR: Aglycone C-1 45.2, C-2 71.9, C-3 82.5, C-4 43.4, C-5 48.4, C-6 18.8, C-7 33.8, C-8 40.8, C-9 48.7, C-10 37.7, C-11 24.6, C-12 123.6, C-13 144.8, C-14 42.9, C-15 36.5, C-16 74.7, C-18 42.3, C-19 48.1, C-20 31.3, C-21 36.5, C-22 32.0, C-23 65.6, C-24 14.7, C-25 18.3, C-26 17.8, C-27 27.3, C-28 177.2, C-29 33.4, C-30 24.8, Xyl 1,3 C-1 107.1, C-2 76.1, C-3 78.2, C-4 71.1, C-5 67.3, Rha1, 4 C-1 101.1, C-2 71.9, C-3 72.3,

*Triterpenoid saponins from *Aster bellidiastrum* (L.) Scop. II. Structures of the deacylsaponins of the underground parts. (Th. Schöpke, Ch. Al-Tawaha, V. Wray, M. Nimtz and K. Hiller, manuscript in preparation.)

C-4 84.3, C-5 68.7, C-6 18.0, Fuc1,2 C-1 95.1, C-2 74.1, C-3 76.7, C-4 73.6, C-5 72.7, C-6 16.5, Rha1 C-1 104.2, C-2 72.4, C-3 72.3, C-4 74.0, C-5 70.3, C-6 17.8.

Bellidiastroside C_1 (**2**). Crystals (from MeOH); mp 225–227°; $[\alpha]_D^{22} - 36.5°$ ($c = 1.00$). TLC; R_f 0.52 ($CHCl_3$–MeOH–H_2O, 7:4:1); HPLC: R_t 6.4 min (LiChrosorb RP-18, 7 μm, 250 × 4 mm i.d., MeOH–H_2O, 7:3). ^{1}H NMR: aglycone δ0.83, 0.92, 0.96, 0.99, 1.37, 1.43 (6 × CH_3), 2.98 (*dd*, J = *ca* 4, 14 Hz, H-18), 5.37 (*t*, J = *ca* 3 Hz, H-12), sugar methyl protons δ1.26 (*d*, J = 6.5 Hz), 1.28 (*d*, J = 6.4 Hz), 1.29 (*d*, J = 6.2 Hz), 1.36 (*d*, J = 4.8 Hz), sugar anomeric protons δ4.53 (*d*, J = 7.5 Hz), 4.98 (*bs*), 5.19 (*d*, J = 1.6 Hz), 5.35 (*d*, J = 8.1 Hz), 5.42 (*d*, J = 1.6 Hz). ^{13}C NMR: aglycone C-1 45.1, C-2 71.8, C-3 82.4, C-4 43.4, C-5 48.0, C-6 19.0, C-7 33.6, C-8 40.9, C-9 48.4, C-10 37.7, C-11 24.6, C-12 123.6, C-13 144.8, C-14 43.0, C-15 36.4, C-16 74.7, C-17 50.1, C-18 42.3, C-20 31.3, C-21 36.5, C-22 32.0, C-23 65.8, C-24 14.9, C-25 18.0, C-26 17.9, C-27 27.2, C-28 177.3, C-29 33.4, C-30 24.8, Rha1 C-1 102.5, C-2 72.2, C-3 72.2, C-4 74.0, C-5 70.0, C-6 18.0, Xyl1,3 C-1 107.1, C-2 76.4, C-3 84.2, C-4 68.8, C-5 67.2, Rha1,4 C-1 101.3, C-2 72.2, C-3 72.3, C-4 84.5, C-5 69.8, C-6 18.0, Fuc 1,2 C-1 95.0, C-2 74.5, C-3 76.7, C-4 73.5, C-5 71.9, C-6 16.5, Rha1 C-1 104.2, C-2 72.6, C-3 72.3, C-4 74.0, C-5 70.3, C-6 17.9.

Bellidiastroside C_2 (**3**). Crystals (from MeOH); mp 214–217°; $[\alpha]_D^{22} - 15.3°$ ($c = 0.17$). HR-FAB-MS: *m/z* $[M + Na]^+$ 1113.5477 (calc. 1113.5458 for $C_{53}H_{86}O_{23}Na$). TLC: R_f 0.52 ($CHCl_3$–MeOH–H_2O, 7:4:1); HPLC: R_t 4.7 min (LiChrosorb RP-18, 7 μm, 250 × 4 mm i.d., MeOH–H_2O, 7:3). ^{1}H NMR: aglycone δ0.82, 0.92, 0.99, 0.99, 1.34, 1.43 (6 × CH_3), 2.98 (*dd*, J = *ca* 4, 13 Hz, H-18), 5.37 (*t*, J = *ca* 3 Hz, H-12), sugar methyl protons δ1.26 (*d*, J = 6.4 Hz), 1.37 (*d*, J = 6.0 Hz), sugar anomeric protons δ4.48 (*d*, J = 7.7 Hz), 4.53 (*d*, J = 7.1 Hz), 5.34 (*d*, J = 8.2 Hz), 5.46 (*bs*); ^{13}C NMR: see Table 1.

Bellidiastroside D_1 (**2**). Crystals (from MeOH); mp 219–222°; $[\alpha]_D^{22} + 19.5°$ ($c = 0.64$). TLC: R_f 0.48 ($CHCl_3$–MeOH–H_2O, 7:4:1); HPLC: R_t 4.7 min (LiChrosorb RP-18, 7 μm, 250 × 4 mm i.d., MeOH–H_2O, 7:3). ^{1}H NMR: aglycone δ0.83, 0.92, 0.99, 1.01, 1.36, 1.42 (6 × CH_3), 2.99 (*dd*, J = *ca* 4, 14 Hz, H-18), 5.37 (*t*, J = *ca* 3 Hz, H-12), sugar methyl protons δ1.26 (*d*, J = 6.4 Hz), 1.29 (*d*, J = 6.2 Hz), 1.33 (*d*, J = 6.9 Hz), sugar anomeric protons δ4.47 (*d*, J = 7.7 Hz), 4.52 (*d*, J = 7.5 Hz), 5.18 (*d*, J = 1.2 Hz), 5.34 (*d*, J = 8.2 Hz), 5.42 (*d*, J = 1.4 Hz). ^{13}C NMR: C-1 44.5, C-2 71.1, C-3 84.1, C-4 43.2, C-5 48.4, C-6 19.0, C-7 33.7, C-8 40.9, C-9 48.9, C-10 37.6, C-11 24.6, C-12 123.6, C-13 144.7, C-14 43.0, C-15 36.5, C-16 74.8, C-17 50.2, C-18 42.3, C-19 48.0, C-20 31.3, C-21 36.5, C-22 32.0, C-23 66.0, C-24 14.9, C-25 17.9, C-26 17.7, C-27 27.2, C-28 177.3, C-29 33.4, C-30 24.8, Rha1 C-1 102.5, C-2 72.3, C-3 72.4, C-4 74.8, C-5 70.0, C-6 18.4, Xyl1,3 C-1 107.2, C-2 76.5, C-3 84.3, C-4 68.8, C-5 67.2, Rha1,4 C-1 101.4, C-2 72.2, C-3 72.6, C-4 84.7, C-5 69.9, C-6 17.8, Fuc1,2 C-1 95.1, C-2 74.5, C-3 76.8, C-4 73.6, C-5 72.0, C-6 16.5, Glc1 C-1 105.5, C-2 75.4, C-3 77.8, C-4 71.1, C-5 78.2, C-6 62.3.

Acknowledgements—We thank H. Döhnert, Humboldt-University, Institute of Pharmacy, for GC measurements, B. Jaschok-Kentner and C. Kakoschke (GBF) for NMR measurements, and R. Christ and C. Proppe (GBF) for FAB and GC/MS measurements. We are particularly indebted to Dr G. Bader (Humboldt-University, Institute of Pharmacy) for detailed information regarding compound **3**.

REFERENCES

1. Hiller, K., Schöpke, T., Wray, V. and Schulten, H.-R. (1988) *Pharmazie* **43**, 850.
2. Schöpke, T., Wray, V., Rzazewska, B. and Hiller, K. (1991) *Phytochemistry* **30**, 627.
3. Schöpke, T., Wray, V., Yamasaki, K., Kasai, R. and Hiller, K. (1994) *J. Nat. Prod.* **57**, 1279.
4. Schöpke, T., Thiele, H., Wray, V., Nimtz, M., and Hiller, K. *J. Nat. Prod.* (in press).
5. Hara, S. and Okabe, H., Mihashi, K. (1987) *Chem. Pharm. Bull.* **35**, 501.
6. Jansson, P.-E., Kenne, L., Liedgren, H., Lindberg, B. and Lönngren, J. (1976) *Chem. Commun. Univ. Stockholm* **8**, 1.
7. Hiller, K., Bader, G. and Schulten, H. R. (1987) *Pharmazie* **42**, 541.
8. Nagao, T., Tanaka, R. Shimokawa, H. and Okabe, H. (1991) *Chem. Pharm. Bull.* **39**, 1719.
9. Nagao, T., Hachiyama, S., Okabe, H. and Yamauchi, T. (1989) *Chem. Pharm. Bull.* **37**, 1977.
10. Nagao, T., Tanaka, R. and Okabe, H. (1991) *Chem. Pharm. Bull.* **39**, 1699.