Pergamon

S0031-9422(96)00029-5

Phytochemistry, Vol. 42, No. 4, pp. 1135–1139, 1996

0031-9422/96 $15.00 + 0.00

PREGNANE GLYCOSIDES FROM *CARALLUMA RETROSPICIENS*

AHMED F. HALIM and ASHRAF T. KHALIL*

Faculty of Pharmacy, Mansoura University, Mansoura, Egypt

(*Received in revised form* 20 *November* 1995)

Key Word Index—*Caralluma retrospiciens*; Asclepiadaceae; pregnane glycosides; new diacylated pregnane trioside.

Abstract—Two pregnane ester glycosides were isolated and identified from the alcohol extract of the aerial parts of *Caralluma retrospiciens*. Their structures were established as 12β-benzoyloxy-20-isovaleroyloxy-8β,14β-dihydroxypregnane-3-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-β-D-(3-*O*-methyl-6-deoxy)-galactopyranoside] (caretroside A) and the bioside 12β-benzoyloxy-8β,14β-dihydroxypregn-20-one-3-*O*-[β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranoside]. They were characterized through physical and chemical methods in addition to standard spectroscopic techniques especially 2D NMR (COSY, HMQC and HMBC). This is the first report of the isolation of these compounds from a natural source.

INTRODUCTION

Caralluma retrospiciens (Ehrenb.) N.E.Br. is a rare succulent plant that grows wild on the stony grounds in the Elba region [1]. Many Asclepiadaceae plants are rich in pregnane glycosides [2], which may possess prominent antitumour activities [3, 4] and function as precursors of cardenolides [5]. Certain pregnane glycosides were recently proved to induce differentiation of the mouse myeloid leukaemia cells into phagocytic cells [6]. Several pregnane ester glycosides were isolated from a few *Caralluma* species [7–10]. In a previous work by one of the authors [11], two pregnane aglycones were isolated after acid hydrolysis of a complex mixture of glycosides obtained from a column chromatographic fraction of the ether extract of *C. retrospiciens*. These aglycones were identified as 12β-benzoyloxy-3β,8β,14β-trihydroxy-pregn-20-one and 11α,12β-benzoyloxy and isovaleroyloxy-3β,8β,14β-trihydroxy-pregn-20-one.

The present study is concerned with the characterization of certain glycosides in some of the less complicated column fractions of the same extract.

RESULTS AND DISCUSSION

The diethyl ether soluble fraction of the ethanol extract of the aerial part of the plant was fractionated on a silica gel column to afford compounds **1** and **2**. Compound **1** gave positive Liebermann–Burchard and Salkowski tests (steroidal nature) and a positive Keller–Killiani test (2-deoxy sugar). The mass spectrum of **1** (determined by liquid chromatography) displayed a $[M+1]^+$ ion peak at m/z 759, suggesting a molecular formula $C_{42}H_{62}O_{12}$ and a double bond equivalence = 12. The sugar moieties were represented by two anomeric carbons at δ 95.4 and 101.2, indicating the presence of two sugar residues. These sugars were identified as D-cymarose and D-oleandrose by TLC after acid hydrolysis. The aglycone was proved to be 12β-benzoyloxy-3β,8β,14β-trihydroxy-pregn-20-one based on cochromatography, UV, mass spectral, IR and ^{1}H NMR data [11]. The ^{13}C NMR data for the aglycone (Table 1) revealed a glycosylation site at C-3 as indicated by the downfield shift of C-3 (δ + 6) and the upfield shifts of C-2 and C-4 (δ −2.3 and −4, respectively), proving that the sugar moieties are attached to C-3. In addition, the H-17 signal of the aglycone moiety appeared as a double doublet at δ 2.90 (J = 11 and 6 Hz) and the ^{13}C signals of both C-12 and C-18 indicated that **1** has H-17α and its C-17 side chain was in a β-configuration [12]. The ^{13}C NMR signals of the sugar moieties confirmed the identities of D-cymarose and D-oleandrose (Table 1). The D-oleandrose was identified as the terminal sugar based on the values for C-1 and C-3 (δ 101.2 and 80.3) [13]. The β-glycosidic linkage of each sugar was revealed by the high coupling constants of the anomeric protons in the ^{1}H NMR spectrum. Thus, the structure of **1** was established as 12β-benzoyloxy-8β,14β-dihydroxy-pregn-20-one-3-*O*-[β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranoside].

Compound **2** was identified as 12β-benzoyloxy-20-isovaleroyloxy-8β,14β-dihydroxypregnane-3-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-β-D-(3-*O*-methyl-6-deoxy)-galactopyranoside]. The identification was based on thorough investigation of

*Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR data (75 MHz, $CDCl_3$) for compound **1**

C atom no.	δ	C atom no.	δ
1	38.0	D-Cymarose	
2	28.8	1′	95.4
3	77.2	2′	35.6*
4	33.9	3′	77.6
5	45.3	4′	82.8
6	23.2*	5′	68.3
7	35.1†	6′	18.2
8	75.6	$-OCH_3$	57.9
9	47.4		
10	36.4	D-Oleandrose	
11	24.7*	1″	101.2
12	77.2	2″	35.9*
13	54.6	3″	80.3
14	86.2	4″	76.4
15	36.2†	5″	71.3
16	25.1*	6″	18.5
17	57.7	$-OCH_3$	56.0
18	12.6		
19	12.6		
20	217.5		
21	33.1		
Benzoyl			
1	166.3		
2	130.2		
3	129.5		
4	128.6		
5	133.2		
6	128.5		
7	129.5		

*,†Assignments are exchangeable in the same column.

chemical and spectral data including IR, FAB mass spectra, ^1H NMR, ^{13}C NMR, APT, DEPT, COSY, HMQC and HMBC.

The ^{13}C NMR data (Table 2), as well as the APT and DEPT spectra, indicated the presence of 52 carbons of which seven were methyls, 11 methylenes, 27 methines and seven quaternaries. The same spectral data revealed the absence of any steroidal olefinic bond signals as well as any signal characteristic for a carbonyl group at C-20 (δ 217.5). Instead, two carbonyl ester signals were evident at δ 167.2 and 171.4. The former was proved to be due to a benzoyl ester as indicated by an IR band at 1708 cm^{-1} and the NMR signals of the aromatic ring protons and carbons (see Experimental and Table 1). The latter was of an isovaleroyl ester as confirmed by an IR band at 1720 cm^{-1}, the COSY spectral data, which revealed two CH_3 signals at δ 0.88, each coupled to a CH multiplet at δ 1.98, which in turn was coupled to a CH_2 signal at δ 2.10 attributed to H-5, H-4, H-3 and H-2 of the isovaleroyl and in the HMQC correlated to the corresponding carbons at δ 22.1, 22.0, 25.0 and 43.1 [3, 11].

One ester moiety was attached to C-12 (δ 74.2) as evident from the downfield shift of H-12, δ 5.36 (1H, *dd*, J = 12.6 and 4.4 Hz). This large coupling constant proved the α (axial)-configuration of this proton. The other ester moiety was connected to C-20 (δ 72.2) as indicated by the downfield shift of H-20 at δ 4.90 (1H, *dq*, J = 7.7 and 6.0 Hz).

The oxygenated quaternary carbons at δ 75.9 and 82.7 were assigned to C-8 and C-14, respectively, in accordance with reported data [14]. The last oxygenated carbon in the aglycone moiety was that at C-3 to which the sugar moiety was attached.

The relative stereochemistry at the chiral centres in the aglycone moiety was deduced from comparison of the coupling constants of the protons and/or the chemical shifts of the carbons with those reported for related pregnanes [3, 9, 12, 14]. However, the geometry at C-17 and C-20 could not be assigned.

The sugar moiety was represented by three monosaccharides as demonstrated in the HMQC spectrum (^1H–^{13}C correlations) by three anomeric carbon signals at δ 100.7, 102.9 and 103.4 crossed with the anomeric protons at δ 4.14 (1H, *d*, J = 7.60 Hz), 4.35 (1H, *d*, J = 7.65 Hz) and 4.28 (1H, *d*, J = 7.74 Hz), respectively. The large coupling constants indicated β-glycosidic linkages in all cases.

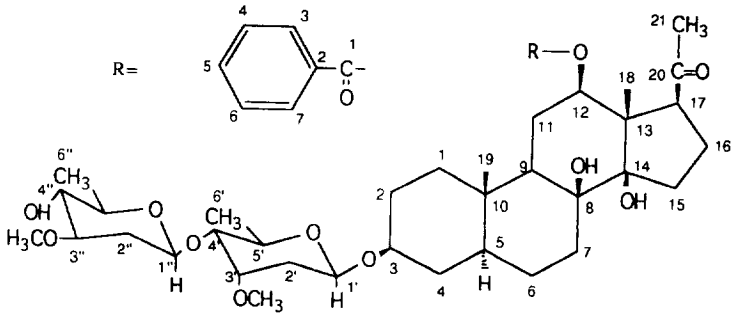
On acid hydrolysis, compound **2** yielded glucose (R_f 0.55) and another sugar (R_f 0.73) [7]. The latter was not a 2-deoxysugar, as indicated by a negative Keller–Killiani test, and it contained one methoxyl group at δ 3.35 (3H, *s*) crossed with the carbon signal at δ 57.7 and a secondary methyl group at δ 1.11 (3H, *d*, J = 6.2 Hz) crossed with δ 18.8. This monosaccharide was proved to be 6-deoxy-3-*O*-methyl-D-galactose and not 6-deoxy-3-*O*-methyl-D-allose, which is commonly present in Asclepiadaceous pregnanes. The H-3′ proton absorbed as a double doublet at δ 3.03 ($J_{2',3'}$ = 9.6 and $J_{3',4'}$ = 2.80 Hz), which indicated that H-3′ was axial. The distinctly smaller $J_{3',4'}$ showed that H-4′ was equatorial. The latter absorbed as a broad doublet at δ 4.01; the $J_{4',5'}$ was very small and could not be calculated.

The sequence of attachment of the sugars was finally achieved from the HMBC spectrum of **2** (Fig. 1) where C-H long-range coupling was observed between H-1‴ of the terminal D-glucose (δ 4.28) and C-6″ of the middle D-glucose (δ 68.6) and the H-1″ of the latter (δ 4.35) and C-4′ of 6-deoxy-3-*O*-methyl-D-galactose [δ 73.4) [10].

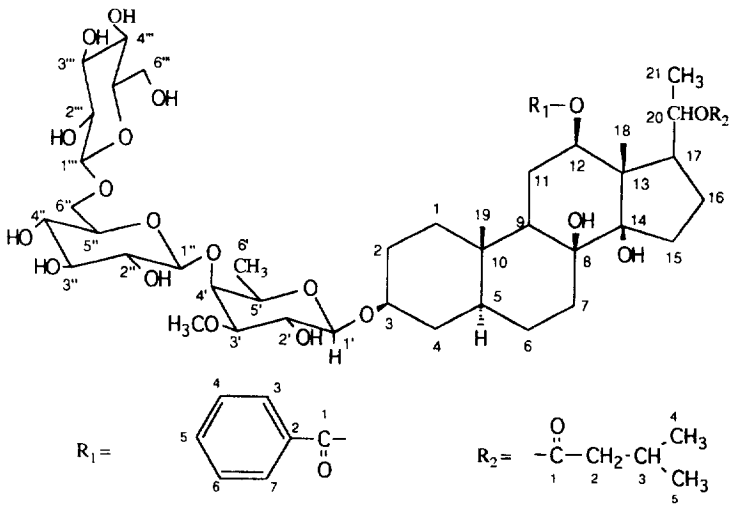
Furthermore, HMBC measurements (Fig. 1) helped in the unambiguous confirmation of the sites of attachment of certain moieties to the steroidal nucleus. For example, glycosylation occurred at C-3 as observed from the coupling between H-1′ of 6-deoxy-3-*O*-methyl-D-galactose (δ 4.14) and C-3 of the aglycone moiety (δ 76.1). Acylation of C-12 occurred with the benzoyl moiety as demonstrated by the coupling between H-12 (δ 5.36) and the benzoyl carbonyl (δ 167.2). This new compound was designated caretroside A.

EXPERIMENTAL

Instruments. mp: hot-stage melting point microscope (Sybron, U.S.A.). UV: Perkin-Elmer 550 S. IR: Pye



1



2

Unicam Sp. 1000 (Cambridge, U.K.). MS: Vestec 201 Thermospray MS system for LC-MS and JEOL JMS DX-303 for FAB-MS. Optical rotation: JASCO DIP-370 digital polarimeter at 25° in MeOH. HPLC: system equipped with M-6000A Waters Associates Chromatography pump; U6K injector; Waters 440 Absorbance Detector operating at 254 nm and HP3390 recording integrator (Hewlett Packard); Bondapak (250 mm × 13 mm i.d.) C_{18} column (Waters Associates); MeCN–H_2O (1:1) as mobile phase at a flow rate of 2 ml min^{-1}. NMR: Varian VXR-300 FT Spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C including APT, DEPT, COSY and HETCOR and Bruker AM 500 for HMQC and HMBC in $CDCl_3$ or DMSO-d_6 with TMS as int. standard.

Extraction and isolation of the glycosides. The fresh plant material was collected and elaborated as described in ref. [11]. The Et_2O extract yielded a bitter residue (5.5 g) which was fractionated by silica gel CC using gradient mixts of $CHCl_3$–MeOH. The frs eluted with 4% MeOH in $CHCl_3$ afforded **1** while frs eluted with 7% MeOH in $CHCl_3$ afforded **2**. Each compound was further purified by HPLC as mentioned above.

Acid hydrolysis. Each glycoside (40 mg) was treated with 0.05 N H_2SO_4 in 50% MeOH (25 ml) at 70° for 60 min. H_2O (10 ml) was then added and the mixt. was warmed at 60° for a further 30 min. After cooling, the mixt. was extracted with $CHCl_3$ (3 × 25 ml) to obtain the aglycone. The aq. phase of the hydrolysate was neutralized with satd $Ba(OH)_2$ soln. After the inorganic ppt was filtered off, the filtrate was concd, then used for TLC examination of the sugars against authentic samples using silica gel, $CHCl_3$–MeOH–H_2O [(18:3:1) lower layer] in the case of **1** and cellulose, EtOAc–MeOH–H_2O–HOAc (13:3:3:4) in the case of **2**. The plates were sprayed with aniline phthalate reagent in each case and the R_f values were as follows: 0.68 (cymarose), 0.62 (oleandrose) in the case of the first solvent system and 0.73 (6-deoxy-3-*O*-methyl-D-galactose) and 0.55 (glucose) in the case of the second.

12β-*Benzoyloxy*-8β,14β-*dihydroxypregn*-20-*one*-3-O-[β-D-*oleandropyranosyl*-(1 → 4)-β-D-*cymaropyranoside*] (**1**). Amorphous powder (80 mg); $[\alpha]_D$ −3.25 (*c* 0.65; MeOH) UV: λ_{max}^{MeOH} nm: 230, 282; LC-MS *m/z*: 759 $[M+1]^+$, $C_{42}H_{62}O_{12}$; IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 2980 (CH), 1710 (C=O of benzoyl ester), 1690 (C=O), 1600 (C=C aromatic), 1270 (ester), 715 (C-H aromatic); ^1H NMR (300 MHz, $CDCl_3$): δ 0.99 (3H, *s*, H-19), 1.22 (3H, *d*, *J* = 6.2 Hz, H-6′), 1.25 (3H, *d*, *J* = 6.2 Hz, H-6″), 1.30 (3H, *s*, H-18), 2.15

Table 2. ^{13}C NMR (75 MHz, DMSO-d_6) data for compound **2**

C atom no.	δ	C atom no.	δ
1	40.8	Isovaleroyl	
2	28.5	1	171.4
3	76.1	2	43.1
4	33.9	3	25.0
5	44.3	4	22.0
6	24.4	5	22.1
7	37.4	6-Deoxy-3-methyl galactose	
8	75.9	1′	100.7
9	50.2	2′	70.3
10	36.1	3′	84.1
11	31.2	4′	73.4*
12	74.2	5′	68.9
13	46.6	6′	18.8
14	82.7	$-OCH_3$	57.7
15	33.3		
16	17.3	Glucose-1	
17	50.4	1″	102.9
18	16.9	2″	73.4*
19	12.9	3″	76.6†
20	72.2	4″	70.0‡
21	17.0	5″	75.0†
Benzoyl		6″	68.6
1	167.2		
2	128.7	Glucose-2	
3	129.6	1‴	103.4
4	128.5	2‴	73.6*
5	133.5	3‴	76.8†
6	128.5	4‴	69.2‡
7	129.6	5‴	76.2†
		6‴	61.0

*,†,‡Assignments are exchangeable in the same column.

(3H, *s*, H-21), 2.90 (1H, *dd*, $J = 11$, 6 Hz, αH-17), 3.38 (3H, *s*, $-OCH_3$ of cymarose), 3.44 (3H, *s*, $-O-CH_3$ of oleandrose), 3.62 (1H, *m*, H-3), 4.45 (1H, *dd*, $J = 9.5$, 2 Hz, H-1′ anomeric), 4.60 (1H, *dd*, J = 9, 1.5 Hz, H-1″ anomeric), 4.86 (1H, *dd*, $J = 10.5$, 4 Hz, H-12), 7.48 (2H, *t*, $J = 7.8$ Hz, H-4,6 of benzoyl), 7.61 (1H, *dd*, $J = 7.8$, 1.2 Hz, H-5 of benzoyl), 8.07 (2H, *dd*, $J = 7.8$, 1.2 Hz, H-3,7 of benzoyl). ^{13}C NMR: Table 1.

2β - Benzoyloxy - 20 - isovaleroyloxy - 8β,14β - dihydroxypregnane-3-O-[*β*-D-*glucopyranosyl*-(1→6)-*β*-D-*glucopyranosyl*-(1→4)-*β*-D-(3-O-*methyl*-6-*deoxy*)-*galactopyranoside*] (*caretroside A*) (**2**). Crystals (200 mg), mp 238–242°; $[\alpha]_D$ −2.47 (*c* 0.96; MeOH); FAB-MS *m/z*: 1063 $[M + Na]^+$, $C_{52}H_{80}O_{21}$. IR ν_{max}^{KBr} cm^{-1}: 3550 (OH), 2970 (CH), 1720 (C=O of aliphatic ester), 1708 (C=O of aromatic ester), 1600 (C=C aromatic), 1270 (ester), 713 (C-H aromatic). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.88 (6H, *d*, $J = 7$ Hz, H-4,5 of isovaleroyl), 0.89 (3H, *s*, H-19), 1.05 (3H, *d*, $J =$ 6 Hz, H-21), 1.11 (3H, *d*, $J = 6.2$ Hz, H-6′), 1.16 (3H, *s*, H-18), 1.98 (1H, *m*, H-3 of isovaleroyl), 2.10 (2H, *d*, $J = 5.5$ Hz, H-2 of isovaleroyl), 3.03 (1H, *dd*, $J = 9.6$, 2.8 Hz, H-3′), 3.35 (3H, *s*, $-OCH_3$ of 6-deoxy-3-*O*-methyl-D-galactose), 3.45 (1H, *m*, H-3), 3.55 (1H, *m*, H-6″b), 3.68 (1H, *m*, H-6″a), 4.14 (1H, *d*, $J = 7.6$ Hz, H-1′ anomeric), 4.28 (1H, *d*, $J = 7.74$ Hz, H-1‴ anomeric), 4.35 (1H, *d*, $J = 7.65$ Hz, H-1″ anomeric), 4.90 (1H, *dq*, $J = 7.7$, 6 Hz, H-20), 5.36 (1H, *dd*, $J = 12.6$, 4.4 Hz, H-12), 7.54 (2H, *t*, $J = 7.9$ Hz, H-4,6 of benzoyl), 7.65 (1H, *dd*, $J = 7.9$, 1.2 Hz, H-5 of benzoyl), 8.12 (2H, *dd*, $J = 7.9$, 1.2 Hz, H-3,7 of benzoyl); ^{13}C NMR: Table 2; HMBC: Fig. 1.

Acknowledgements—The authors are grateful to the Research Institute of Pharmaceutical Sciences (RIPS), MS, U.S.A., for spectral facilities, to Dr K. Hayashi, Faculty of Pharmaceutical Sciences, Hokkaido University, Japan, for providing samples of authentic

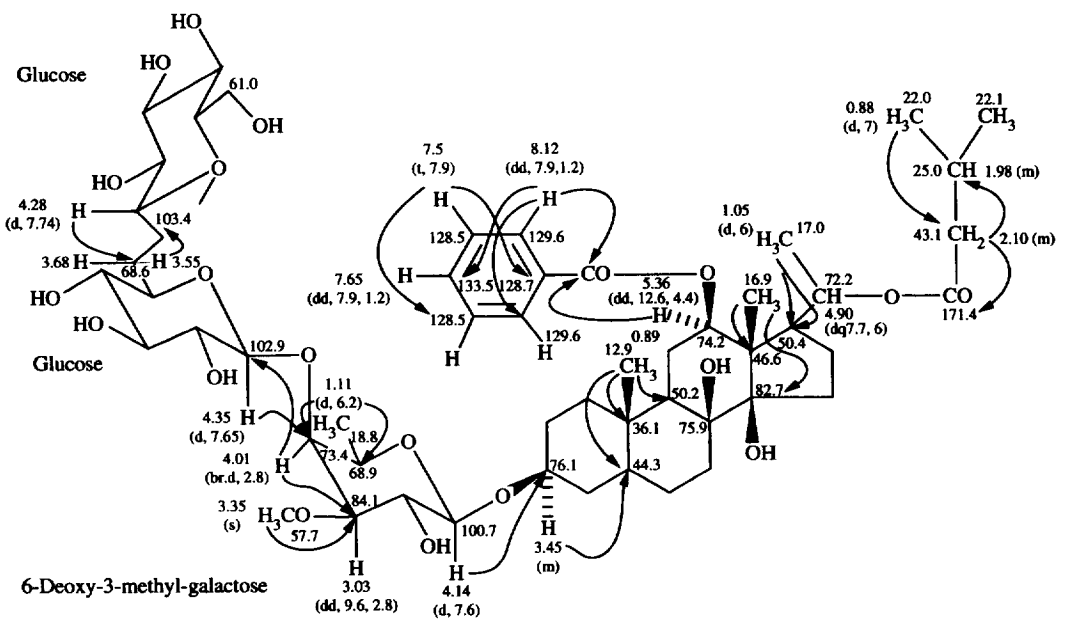


Fig. 1. Important HMBC correlations of compound **2**.

sugars, and to Dr K. Takeya, Tokyo College of Pharmacy, Japan, for running the HMQC and HMBC spectra of compound **2**.

REFERENCES

1. Tackholm, V. (1974) *Students' Flora of Egypt*, 2nd Edn, p. 416. Cairo University, Cooperative Printing Co., Beirut.
2. Hegnauer, M. (1986) *Chemotaxonomie Der Pflanzen*, Band 7, p. 89. Birkhauser, Verlag, Basel, Boston, Stuttgart.
3. Yoshimura, S. I., Narita, H., Hayashi, K. and Mitsuhashi, H. (1983) *Chem. Pharm. Bull.* **31**, 3971.
4. Itokawa, H., Xu, J. and Takeya, K. (1987) *Chem. Pharm. Bull.* **35**, 4524.
5. Deepak, D., Khare, A. and Khare, M. P. (1989) *Phytochemistry* **28**, 3255.
6. Umehara, K., Endoh, M., Miyase, T., Kuroyanagi, M. and Ueno, A. (1994) *Chem. Pharm. Bull.* **42**, 611.
7. Ahmad, V. U., Usmanghani, K. and Rizwani, G. H. (1988) *J. Nat. Prod.* **51**, 1092.
8. Hayashi, K., Iida, I., Nakao, Y. and Kaneko, K. (1988) *Phytochemistry* **27**, 3919.
9. Tanaka, T., Tsukamoto, S. and Hayashi, K. (1990) *Phytochemistry* **29**, 229.
10. Lin, L. J., Lin, L. Z., Gil, R. R., Cordell, G. A., Ramesh, M. Srilatha, B., Reddy, B. and Rao, A. V. N. A. (1994) *Phytochemistry* **35**, 1549.
11. Khalil, A. T. (1995) *Fitoterapia* **66**, 261.
12. Luo, S. Q., Lin, L. Z., Cordell, G. A., Xue, L. and Johnson, M. E. (1993) *Phytochemistry* **34**, 1615.
13. Warashina, T. and Noro, T. (1994) *Chem. Pharm. Bull.* **42**, 322.
14. Berger, S., Junior, P. and Kopanski, L. (1988) *Phytochemistry* **27**, 1451.