Phytochemistry, Vol 29, No 1, pp 209–213, 1990
Printed in Great Britain

0031-9422/90 $3.00+0.00

TWO TRITERPENE GLYCOSIDES FROM *ISERTIA HAENKEANA*

F. JAVIER ARRIAGA, ANGEL RUMBERO and PURIFICACION VAZQUEZ*

Departamento de Química, Universidad Autónoma de Madrid, Cantoblanco, 28049-Madrid, Spain

(*Received in revised form* 12 *May* 1989)

Key Word Index—*Isertia haenkeana*, Rubiaceae; quinovic acid glycosides, cincholic acid glycosides, quinovic acid, ^{1}H, ^{13}C NMR

Abstract—Two new triterpene glycosides, quinovic acid 3β-*O*-6-deoxy-D-glucopyranoside-28-*O*-β-D-glucopyranoside and cincholic acid 3β-*O*-β-6-deoxy-D-glucopyranoside-28-*O*-β-D-glucopyranoside, were isolated from aerial parts of *Isertia haenkeana*. Their structures were established on the basis of spectral data and chemical transformations.

INTRODUCTION

As a part of our investigations of *Isertia haenkeana*, we have previously reported the isolation and identification of secoiridoids from the leaves of this plant [1]. Many interesting glycosides of quinovic and cincholic acids have been obtained from the Rubiaceae family [2–4] We now describe the isolation and characterization of two new triterpene glycosides (**5** and **10**) and five known triterpene acids (**1**, **2**, **4**, 7 and **9**).

RESULTS AND DISCUSSIONS

The known triterpene glycosides **4**, **7** and **9** were identified by means of physical constants and spectral data [5, 6]. The two new triterpene glycosides, **5** and **10**, are saponins in which the aglycones are quinovic acid (**3**) and cincholic acid (**8**), respectively

Acid hydrolysis of compounds **4**–**7** gave the same aglycone which was characterized as quinovic acid by comparison with an authentic sample. The ^{1}H NMR spectrum of the triacetate derivative **4a** indicated that **4** was composed of a D-quinovose subunit linked to C-3 of the aglycone (Tables 1 and 2) [6, 7]. Confirmation of the structure **4** was provided by ^{13}C NMR spectroscopy (Table 3) which established a β-configuration for the proton attached to C-3 in the aglycone [8], the other carbon chemical shifts being in accordance with those reported in the literature for quinovic acid [9]. In the present study, the ^{1}H and ^{13}C NMR spectra are reported for the first time.

Alkaline hydrolysis of compound **7** gave compound **6** which was identified by ^{1}H and ^{13}C NMR spectroscopy as quinovin glycoside C [5, 6, 10] The ^{13}C NMR spectrum of the acetate of compound **7** (Table 3) was identical to quinovic acid 3β-*O*-[β-D-glucopyranosil-(28 → 1)-β-D-glucopyranosil] ester peracetyl methyl ester [6].

Compound **9** is a triterpene with an oleanane skeleton. Thus in its ^{1}H NMR spectrum H-18 appears at δ2.89 (*dd*, J=4.3 and 13.9 Hz), a typical value for this triterpene series, cf ursane skeleton which gives rise to a signal at δ2.20 (*d*, J=12 Hz) [11] Alkaline hydrolisis gave a compound whose spectral data and physical constants are in accordance with those for quinovin glycoside B (**9**) previously reported [5]

Quinovic acid 3β-O-β-6-*deoxy*-D-*glucopyranoside*-28-O-β-D-*glucopyranoside* (**5**)

The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) indicated the presence of two sugar subunits: quinovose and glucose. The signal at δ4.49 (*d*, J=7.8 Hz) was attributed to the anomeric hydrogen of quinovose attached to C-3 of the aglycone and the chemical shift at δ5.59 (*d*, J=7.8 Hz) was consistent with the anomeric proton of glucose in an ester linkage The glycosyl ester linkage was proposed to be at C-28 in view of ^{13}C NMR spectrum which showed for C-27 in compounds **4** and **5**, a chemical shift at δ179.0 and 179.3, respectively, although the signal for C-28 was shifted upfield by 3.9 ppm, a difference attributed to esterification The signal for C-3 was at δ90.7, compared with δ77.8 in quinovic acid (**3**) demonstrating an ether linkage at this carbon

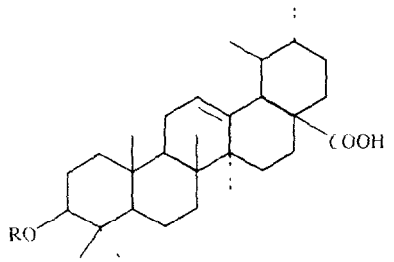
The presence of a quinovose subunit was confirmed by comparative NMR spectroscopy analysis of the alkaline hydrolysis product of compound **5** with the ^{13}C NMR data of compound **4** and the ^{1}H NMR data of compound **4a**. Moreover, the disappearance of the glucose signals in the ^{1}H and ^{13}C NMR spectra of the alkaline hydrolysis product of this compound was consistent with the presence of a glucose molecule in a ester linkage

The ^{13}C NMR spectral data (Table 3) corresponding to the glucose carbons are in accordance with other similar triterpenes esterified with D-glucose at C-28. The other signals in the ^{13}C NMR spectrum are identical to those of quinovic acid All these considerations, led us to assign to compound **5** the structure of quinovic acid 3β-*O*-β-6-deoxy-D-glucopyranoside-28-*O*-β-D-glucopyranoside.

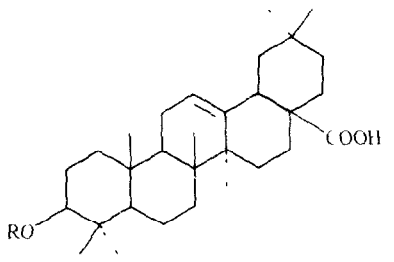
Cincholic acid 3β-O-β-6-*deoxy*-D-*glucopyranoside*-28-O-β-D-*glucopyranoside* (**10**)

The nature of the sugars subunits for this glycoside were determined in a similar manner to that just described above. The major difference between the two

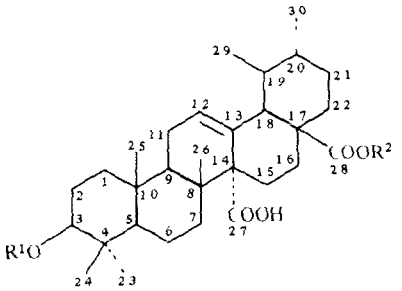
*Author to whom correspondence should be addressed.



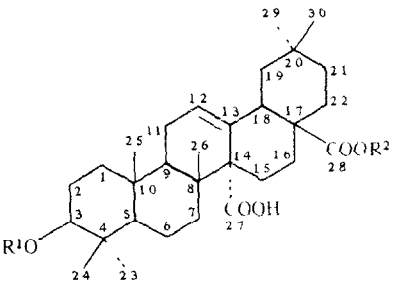
1 R = H
1a R = Ac



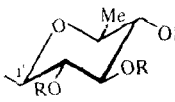
2 R = H
2a R = Ac



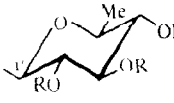
3 $R^1 = R^2 = H$
3a R^1 = Ac R^2 = H



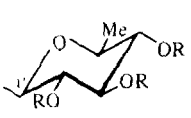
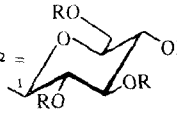
8 $R^1 = R^2 = H$
8a R^1 = Ac R^2 = H

4 R^1 =  R = R^2 = H

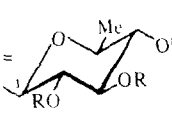
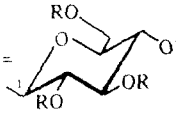
4a R = Ac R^2 = H

9 R^1 =  R = R^2 = H

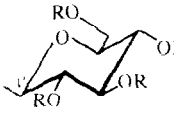
9a R = Ac R^2 = H

5 R^1 =  R^2 =  R = H

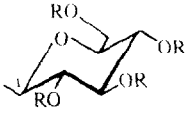
5a R = Ac

10 R^1 =  R^2 =  R = H

10a R = Ac

6 R^1 =  R = R^2 = H

6a R = Ac R^2 = H

7 $R^1 = R^2$ =  R = H

7a R = Ac

glycosides is the aglycone part. Acid hydrolysis of compound **10** gave cincholic acid (**8**). A comparative spectroscopy study between pentacyclic triterpenes of the α-amyrin type (ursa-12-en-3β-ol) and β-amyrin type (oleana-12-en-3β-ol) [12] revealed significant differences between both series. The ^{1}H NMR spectrum showed a signal at δ 2.82 (*dd*, *J* = 4.3 and 13.9 Hz) for H-18, a typical shift in the oleanane series [11]. However, the major differences were found in the ^{13}C NMR spectra, especially in the chemical shifts for carbon atoms in ring E. A comparison in the Δδ between the olefinic carbon signals (C-12 and C-13) for both series indicated a higher Δδ value for the β-amyrin type [1, 13]. Compound **5** (α series) showed a Δδ = 2.5 (CD_3OD) and 3.7 pyridine-d_5 whereas compound **10** (β series) showed a Δδ = 9.8 (CD_3OD) and 11.2 pyridine-d_5. The other carbon chemical shifts for ring

Table 1 ^{1}H NMR spectral data of compounds **4a–7a**, **9a** and **10a** (200 MHz; $CDCl_3$, TMS as internal standard)

H	4a	5a	6a	7a	9a	10a
1′	4 50 *d*	4 49 *d*	4 53 *d*	4.53 *d*	4.50 *d*	4.44 *d*
2′	5 00 *dd*	5 00 *dd*	5 02 *dd*	5 03 *dd*	5 00 *dd*	4 93 *dd*
3′	5 15 *dd*	5 15 *dd*	5 20 *dd*	5.25 *dd*	5.15 *dd*	5 09 *dd*
4′	4.80 *dd*	4.80 *dd*	5 05 *dd*	5.06 *dd*	4.80 *dd*	4 74 *dd*
5′	3 50 *dq*	3 54 *dq*	3.69 *ddd*	3 68 *ddd*	3.50 *dq*	3 49 *dq*
CH_3-6′	1 30 *d*	1 22 *d*			1.30 *d*	1 17 *d*
$6'_A$			4 24 *dd*	4 25 *dd*		
$6'_B$			4 12 *dd*	4 13 *dd*		
1″		5 59 *d*		5 59 *d*		5 53 *d*
2″		5.13 *dd*		5.16 *dd*		5 11 *dd*
3″		5 25 *dd*		5.25 *dd*		5.20 *dd*
4″		5 11 *dd*		5 11 *dd*		5 06 *dd*
5″		3 80 *ddd*		3 81 *ddd*		3 75 *ddd*
$6''_A$		4.27 *dd*		4 27 *dd*		4 21 *dd*
$6''_B$		4 03 *dd*		4 04 *dd*		3 97 *dd*

Table 2 Coupling constants for the protons in compounds **4a–7a**, **9a**, and **10a**

	4a	5a	6a	7a	9a	10a
$J_{1',2'}$	7 8	7 8	7 9	7 9	7 8	7 8
$J_{2',3'}$	9 6	9 5	9 3	9 4	9 6	9 5
$J_{3',4'}$	9 6	9.5	9.3	9.4	9.6	9 5
$J_{4',5'}$	9 6	9 5	9 3	9 4	9 6	9 5
$J_{5',6'}$	6 3	6 3			6 3	6 2
$J_{5',6'_A}$			4.9	5.0		
$J_{5',6'_B}$			2 4	2 4		
$J_{6'_A,6'_B}$			12 2	12 4		
$J_{1'',2''}$		7 8		7.9		7.8
$J_{2'',3''}$		9 5		9 4		9.5
$J_{3'',4''}$		9 5		9 4		9.5
$J_{4'',5''}$		9 5		9 4		9.5
$J_{5'',6''_A}$		4 5		4 4		4 3
$J_{5'',6''_B}$		2 1		2 0		2 0
$J_{6''_A,6''_B}$		12 7		12.5		12.4

E are in accordance with the values reported in the literature for oleanane skeleton-based compounds [14, 15]. Finally, alkaline hydrolysis gave a compound which was identified with the quinovin glycoside B (**9**). Consequently, the structure of the compound **10** was concluded to be cincholic acid 3*β*-*O*-*β*-6-*deoxy*-D-glucopyranoside-28-*O*-*β*-D-glucopyranoside.

EXPERIMENTAL

Mps, uncorr MS· Hewlett-Packard GC-MS 5985B instrument ^{1}H and ^{13}C NMR 200 and 50 3 MHz, respectively, TMS (δ=0) as int standard, the DEPT technique was used in the ^{13}C NMR spectra. HPLC Polygosil 60-C_{18} column.

Plant material Isertia haenkeana collected in February 1986 in Costa Rica (Palmar Norte). A voucher of the plant is deposited in the herbarium of the Natural History Museum of San Jose, N 3046

Extraction and isolation of triterpene glycosides The air-dried plant material (2 25 kg) was extracted with MeOH in a Soxhlet-type extractor Evaporation of the solvent *in vacuo* left a semi-solid dark-green residue (379 g) The residual extract was extracted with $NaHCO_3$ (5%) and, upon acidification with HCl (5%), a ppt consisting mainly of triterpene glycosides was separated (147 g) A portion of the ppt was chromatographed in silica gel column and eluted successively with $CHCl_3$–EtOAc (1 1) to give ursolic acid (**1**) and oleanolic acid (**2**), and $CHCl_3$–MeOH (4 1) to give a mixture of triterpene glycosides which gave the following R_f data, TLC [(silica gel) (**4**) and (**9**): R_f 0 65, (**5**) and (**10**). R_f 0 34, and (**7**). R_f 0.23, $CHCl_3$–MeOH (4.1)]. This mixture was separated by semipreparative HPLC [column· Polygosil 60-C_{18} (5 μm); solvent MeCN–H_2O (3 7), 9 ml/min., detection UV].

Quinovic acid 3*β*-O-*β*-6-*deoxy*-D-*glucopyranoside* (**4**). Mp 237–238° (lit), $[\alpha]_D^{20}$ +57° (EtOH; *c* 1 00), IR ν^{KBr} cm^{-1} 2925, 1680; ^{13}C NMR (50 3 MHz, CD_3OD, pyridine-d_5, DEPT) see Table 3. Compound **4** was acetylated with Ac_2O–pyridine at room temp, work-up in the usual manner afforded **4a** Mp 115–118°, ^{1}H NMR (200 MHz, $CDCl_3$). δ5 75 (1H, *m*, H-12), 3 05 (1H, *dd*, *J* = 9 8 and 5 Hz, H-3), sugar protons (see Tables 1 and 2)

Table 3 ^{13}C NMR spectral data of compounds **4**–**7** and **10** (50 32 MHz. TMS as int standard)

C	4*	4†	5*	5†	6*	7a‡	10*	10†
1	40 1	38 5	39 9	38 7	39 9	38 6	39 9	38 7
2	26 5	26 1	26 4	26 4	26 4	25 6	25 6	25 1
3	90 6	88 0	90 7	88 2	90 6	90 2	90 6	88 3
4	39 9[a]	38 5[a]	40 1[a]	39 1[a]	40 1[a]	39 7	40 1[a]	39 2[a]
5	56 9	55 2	56 8	55 5	56 9	55 1	56 9	55 6
6	19 3	18 1	19 2	18 2	19 3	18 1	19 3	18 5
7	38 0	36 9	38 0	37 1	38 0	36 8	37 8	36 9
8	40 7[a]	39 4[a]	40 8[a]	39 8[a]	40 7[a]	36 7	40 6[a]	39 8[a]
9	47 9	46 6	48 0	46 9	48 1	46 4	48 1	47 3
10	37 8	36 4	37 8	36 6	37 8	36 7	37 8	36 9
11	23 8	22 7	23 9	23 0	23 8	22 4	24 0	23 3
12	130 4	128 4	130 8	129 2	130 4	130 4	127 7	126 1
13	133 9	133 4	133 3	132 9	133 8	130 8	137 5	137 3
14	57 3	56 2	57 2	56 3	57 3	55 4	57 3	56 5
15	27 0	25 7	27 1	25 7	27 0	24 2[a]	27 1	26 5
16	25 7	24 9	25 8	25 1	25 7	24 9[a]	25 1	24 6
17	—	48 1	—	48 6		48 3		47 8
18	55 5	54 3	55 3	54 3	55 5	53 5	44 6	43 8
19	40 3	38 8	40 2	38 7	40 3	38 6	44 6	43 5
20	38 3	37 1	38 2	37 1	38 3	36 5	31 5	30 5
21	31 2	30 0	31 1	29 9	31 2	29 4	34 7	33 6
22	37 6	36 4	37 0	36 0	37 6	35 2	32 7	31 9
23	28 5	27 4	28 5	27 6	28 5	27 5	28 5	27 8
24	16 8[b]	15 9[b]	17 0[b]	16 3[b]	16 9[b]	16 2	16 9[b]	16 3[b]
25	17 0[b]	16 4[b]	17 1[b]	16 7[b]	17 1[b]	16 2	17 0[b]	16 8[b]
26	18 1	18 1[c]	18 2	18 4[c]	18 1	18 1	18 2[c]	18 7[c]
27	179 0	177 4	179 3	177 8	179 0	179 8	180 1	178 7
28	181 8	179 6	177 9	176 2	181 5	175 2	178 0	176 5
29	19 0	17 6	19 2	17 7	19 1	17 0	33 6	32 9
30	21 4	20 7	21 5	20 8	21 5	20 9	24 0	23 5
1′	106 4	105 9	106 5	106 2	106 6	102 7	106 5	106 3
2′	75 9	75 1	75 8	75 3	75 6	70 8	75 8	75 5
3′	78 0	77 6	77 8	77 8	78 2	72 6	77 9	78 0
4′	77 0	76 1	76 9	76 4	71 7	68 5	77 0	76 5
5′	72 9	71 9	72 9	72 2	77 6	71 5	72 9	72 3
6′	18 1	18 3[c]	18 2	18 8[c]	62 8	61 9	19 0[c]	18 5[c]
1″			95 5	95 2		91 3	95 6	95 4
2″			73 8	73 6		69 8	73 9	73 9
3″			78 5[c]	78 8[d]		72 3[b]	78 6[d]	79 0[d]
4″			71 1	70 7		67 8	71 1	70 9
5″			78 1[c]	78 3[d]		72 6[b]	78 2[d]	78 5[d]
6″			62 5	61 9		61 5	62 5	62 0

*In CD_3OD
†In pyridine-d_5
‡In Cl_3CD
[a–d]Assignments may be interchanged in each vertical column

Quinovic acid 3β-O-β-6-*deoxy*-D-*glucopyranoside*-28-O-β-D-*glucopyranoside* (**5**) 9 02 g, mp 208–210°, $[\alpha]_D^{20}$ 20° (EtOH, *c* 1 00) IR ν^{KBr} cm^{-1} 3400, 2925, 1730, 1690, 1630, ^{13}C NMR (50 3 MHz, CD_3OD, pyridine-d_5, DEPT) see Table 3 (Found C, 63 51, H, 8 28 $C_{42}H_{68}O_{14}$ requires C, 63 48, H, 8 31%) Compound **5** was acetylated in the usual manner to give **5a**, mp 143–145°; ^{1}H NMR (200 MHz, $CDCl_3$) δ5 73 (1H, *m*, H-12), 3 03 (1H, *dd*, *J* = 9 8 and 5 Hz, H-3), sugar protons (see Tables 1 and 2)

Quinovic acid 3β-O-D-*glucopyranoside* (**6**) Mp 247–250° (lit), $[\alpha]_D^{20}$ +62° (MeOH, *c* 1 00), IR ν^{KBr} cm^{-1} 2925, 1680, ^{13}C NMR (50 3 MHz, CD_3OD, DEPT) (see Table 3) Compound **6a** Mp 270–272°, ^{1}H NMR (200 MHz, $CDCl_3$) δ5 75 (1H, *m*, H-12), 3 04 (1H, *dd*, *J* = 9 8 and 5 Hz, H-3), sugar protons (see Tables 1 and 2)

Quinovic acid 3β-O-β-D-*glucopyranoside*-28-O-β-D-*glucopyranosyl ester* (**7**) Mp 163–165 $[\alpha]_D^{20}$ +23° (EtOH, *c* 1 00), IR ν^{KBr} cm^{-1} 2970, 1740, 1690, 1620 Compound **7a** Mp 134–136°, ^{1}H NMR (200 MHz, $CDCl_3$) δ5 73 (1H, *m*, H-12), 3 05 (1H, *dd*, *J* = 9 8 and 5 Hz, H-3), sugar protons (see Tables 1 and 2), ^{13}C NMR (50 3 MHz, $CDCl_3$, DEPT) see Table 3

Cinchola acid 3β-O-β-6-*deoxy*-D-*glucopyranoside* (**9**) Mp 193–196° (lit), $[\alpha]_D^{20}$ +78 (EtOH, *c* 0 78), IR ν^{KBr} cm^{-1} 2925, 1680 Compound **9a** Mp 190–192°, ^{1}H NMR (200 MHz,

$CDCl_3$) δ5 80 (1H, *m*, H-12), 3 05 (1H, *dd*, J=9.8 and 5 Hz, H-3), 2.89 (1H, *dd*, J=13.9 and 4 3 Hz, H-18), sugar protons (see Tables 1 and 2)

Cincholic acid 3β-O-β-6-*deoxy*-D-*glucopyranoside*-28-O-β-D-*glucopyranoside* (**10**) 2 25 g, mp 187–190°, $[\alpha]_D^{20}$ +31° (EtOH, *c* 1 00); IRν_{max}^{KBr} cm^{-1} 3400, 2925, 1730, 1690, 1630, ^{13}C NMR (50.3 MHz, CD_3OD, pyridine-d_5, DEPT) see Table 3 (Found. C, 63 50, H, 8 29 $C_{42}H_{66}O_{14}$ requires C, 63 48, H, 8 31%) Compound **10a** Mp 123–125°, ^{1}H NMR (200 MHz, $CDCl_3$) δ5 78 (1H, *m*, H-12), 3.02 (1H, *dd*, J=9 8 and 5 Hz, H-3), 2 82 (1H, *dd*, J=13 9 and 4 3 Hz, H-18), sugar protons (see Tables 1 and 2).

Alkaline hydrolysis of **5**, **7** and **10** Compound **5** (100 mg) in 5 ml MeOH and 15 ml 1 M KOH was heated for 3 hr at 100° The soln was acidified with HCl (6%) and twice extracted with EtOAc The organic layer was evapd to dryness to give **4** (87 mg). Hydrolysis of compounds **7** and **10** to give from **6** and **9**, respectively, was performed in the same manner

Acid hydrolysis of **4**–**7** *and* **10**. Compound **5** (500 mg) in 125 ml H_2SO_4 (7% EtOH) was heated for 4 hr at 100° The soln was evapd to 20 ml, H_2O was added to give a white ppt of quinovic acid (**3**) (250 mg) (identified by TLC, ^{1}H and ^{13}C NMR) Quinovic acid was isolated in a similar way from compounds **4**, **6** and **7**, and cincholic acid from **10**

Acknowledgements—Samples of *Isertia haenkeana* were kindly provided by Mr L Poveda (National Museum, San Jose, Costa Rica) We are grateful to Dr P Hoet for his active co-operation and to the late Dr J Borges for his encouragement at the beginning of this work Financial support by Rio Ródano, S A is also gratefully acknowledged

REFERENCES

1. Rumbero-Sánchez, A and Vázquez, P (1988) *Heterocycles* **27**, 2863
2. Raffauf, R F, Le Chuesne, P W and Ghosh, P C. (1978) *J Nat. Prod* **41**, 432
3. Banerji, N. (1978) *J Indian Chem Soc* **LV**, 275
4. Hui, W H. and Yee, C W (1968) *Aust. J Chem* **21**, 543.
5. Tschesde, R, Duphorn, I and Snatzke, G (1963) *Ann* **667**, 151.
6. Matos, M E. O, Sousa, M. P, Machado, M. I L. and Filho, R. B (1986) *Phytochemistry* **25**, 1419
7. Sahu, N P, Mahato, S B, Banerji, N and Chakravarti, R N (1974) *Indian J Chem.* **12**, 284
8. Book, K and Pedersen, C (1983) in *Advances in Carbohydrate Chemistry and Biochemistry* Vol 41, p 55 (Tipson, R S and Horton, D, eds), Academic Press, New York
9. Miana, G A and Al-Hazimi, H M G (1987) *Phytochemistry* **26**, 225
10. Adeoye, A. O. and Waigh, R D (1983) *Phytochemistry* **22**, 975
11. Furuya, T, Orihara, Y and Hayashi, C (1987) *Phytochemistry* **26**, 715
12. Knight, S A (1974) *Org Magn. Reson* **6**, 603.
13. Seo, S, Tomita, Y and Tori, K (1975) *Tetrahedron Letters* 7
14. Schteingart, C. D and Pomilio, A B. (1984) *Phytochemistry* **23**, 2907.
15. Liu, C N., Chung, M I, Gau, K H and Chiang, J R. (1987) *Phytochemistry* **26**, 2381