Pergamon

0031-9422(95)00078-X

Phytochemistry, Vol. 40, No. 2, pp. 537–541, 1995

0031 9422/95 $9.50 + 0.00

ACETOPHENONES FROM *CYNANCHUM TAIWANIANUM*

PAO-LIN HUANG, CHAI-MING LU,* MING-HONG YEN,* RU-RONG WU† and CHUN-NAN LIN*‡§

Ta-Jen Pharmaceutical Junior College, Ping Tung Hsieng, Taiwan 907; *School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan 807; †Instrumental Center, National Cheng Kung University, Tainan, Taiwan 701; ‡National Research Institute of Chinese Medicine, Taipei Hsieng, Taiwan 231, R.O.C.

(*Received in revised form* 6 *December* 1994)

Key Word Index—*Cynanchum taiwanianum*; Asclepiadaceae; biacetophenone; biacetophenone dimers; 4,3′-diacetyl-2,3,2′,6′-tetrahydroxybiphenyl; cynandione A–C.

Abstract—A novel biacetophenone, cynandione A, and two of its dimers, cynandione B and C, were isolated from the rhizome of *Cynanchum taiwanianum*. The structures of these new compounds have been characterized as 4,3′-diacetyl-2,3,2′,6′-tetrahydroxybiphenyl and its two steric isomeric 4,3′-diacetyl-2,3,2′,6′-tetrahydroxybiphenyl dimers.

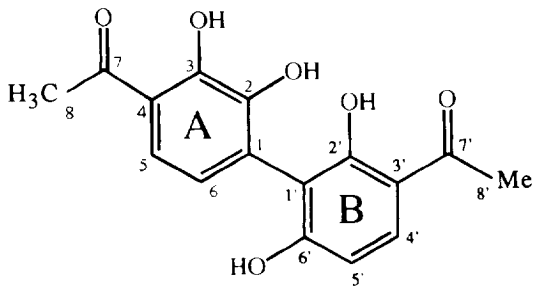
INTRODUCTION

Studies on the constituents of *Cynanchum* species have been reported [1, 2]. However, no work has been done on the constituents of the rhizome of *C. taiwanianum* L. [3]. As part of a study of the cytotoxic principles of Formosan medicinal plants [4, 5], the constituents of *C. taiwanianum*, one of the anticancer folk medicines in Taiwan, were studied. A novel biacetophenone, cynandione A (**1**), and two of its dimers, cynandione B (**2**) and C (**3**), and four known compounds, germanicol acetate, cycloartenol, β-sitosterol and β-sitosterol-β-D-glucoside, were isolated and characterized from the rhizome of this plant. In this paper, we report on the structure characterization of the novel acetophenones.

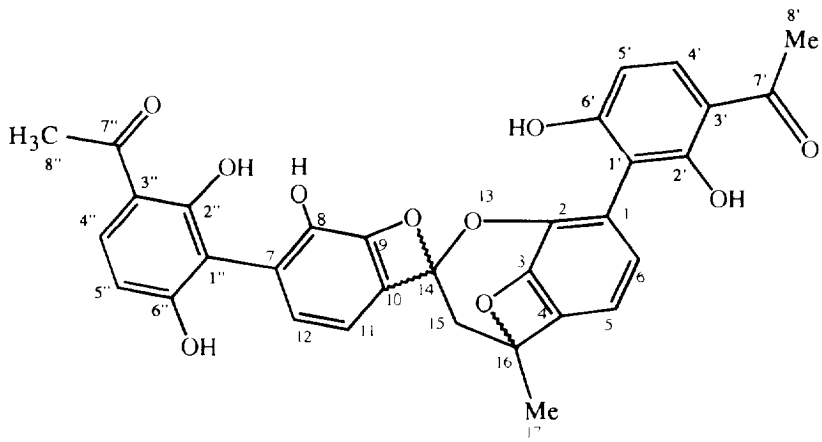
RESULTS AND DISCUSSIONS

Compound **1**, $C_{16}H_{14}O_6$, had similar UV maxima to σ-hydroxyacetophenone or 2,4-dihydroxyacetophenone [6]. Its IR spectrum showed bands for hydroxyl groups at 3550, 3395 and 3125 cm^{-1} and for two chelated carbonyls at 1675 and 1638 cm^{-1}. Its EIMS spectrum exhibited a $[M]^+$ at *m/z* 302 and a base peak at *m/z* 284 $[M - H_2O]^+$. In the ^1H NMR spectrum of **1**, two acetyl signals at δ2.18 and 2.56 and two pairs of *ortho* coupled aromatic protons at δ6.49 (1H, *d*, $J = 8.9$ Hz) and 7.78 (1H, *d*, $J = 8.9$ Hz), and δ6.79 (1H, *d*, $J = 8.8$ Hz) and 6.94 (1H, *d*, $J = 8.8$ Hz), were visible. After acetylation of **1**, four additional acetyl signals at δ2.06, 2.08, 2.12 and 2.30 were observed in the ^1H NMR spectrum of the peracetate (**1a**). The EIMS of **1a** showed a $[M]^+$ at *m/z* 470 and significant peaks at *m/z* 428, 386 344, and 302 ($[M]^+$ of **1**) indicating the existence of four acetoxy groups in **1a** and hence four hydroxy groups in **1**. The above findings suggested that **1** was a biphenyl with two sets of unsymmetrically tri-substituted benzene rings. By comparing the aromatic proton chemical shifts of **1** and **1a** (Table 1), it was found that the signal at δ6.49 experienced a significant downfield shift of $\Delta + 0.82$ ppm whereas the signal at δ7.78 only showed a slight downfield shift by $\Delta + 0.17$ ppm. The other aromatic proton signals of **1** at δ6.94 and 6.79 showed almost the same significant downfield shift of $\Delta + 0.43$ and $\Delta + 0.46$ ppm (Table 1), respectively, which is in accord with the acetylation of hydroxyl groups in *para*- or *ortho*-positions to the respective protons [7]. Based on the above evidence, **1** was characterized as 4,3′-diacetyl-2,3,2′,6′-tetrahydroxybiphenyl or 2,3′-diacetyl-3,4,2′,6′-tetrahydroxybiphenyl. In the HMBC spectrum of **2** (Figs 1, 2), the proton signals of H-5 and H-11 at δ6.97 and 7.29 showed cross-peaks with the carbon signals of C-16 and C-14 at δ75.6 and 101.3, respectively. Therefore, **1** was characterized as **1**. The 2D spectra of **1a** (Fig. 1) also supported the characterization of cynandione A (**1**) as 4,3′-diacetyl-2,3,2′,6′-tetrahydroxybiphenyl.

§Author to whom all correspondence should be addressed.



1



The ^{13}C NMR chemical shifts assignment of **1** were compared with those of **1a** (Table 1). The data for **1a** were obtained by a combination of ^{1}H–^{1}H COSY, HMBC, HMQC and NOESY spectra.

Compound **2**, yellow–orange powder, mp > 300°. Its UV spectrum showed similar absorption maxima to **1** and to 2,4-dihydroxyacetophenone [6]. The ^{1}H NMR spectrum showed four pairs of *ortho* coupled aromatic protons at δ6.80 and 7.83 (J = 8.8 Hz), δ6.84 and 7.78 (J = 8.8 Hz), δ6.97 and 7.17 (J = 8.8 Hz) and δ7.29 and 7.39 (J = 8.8 Hz), two acetyl signals at δ2.53 and 2.57, a methyl singlet at δ2.00, and a pair of geminal CH_2 doublets at δ3.30 and 4.50 (J = 14.2 Hz). The above findings suggested that **2** was a biacetophenone derivative with four aromatic rings. The signals at δ6.80 and 7.83, and δ6.84 and 7.78 were similar to those of H-4′ and H-5′ of **1**, and the signals at δ6.97 and 7.17, and δ7.29 and 7.39 were also similar to those of H-5 and H-6, or H-6 and H-5 of **1** and **1a** (Table 1) which indicated that **2** was a dimer of **1**. Comparison of the corresponding acetyl signals and the signals of the aromatic protons in the ^{1}H NMR spectra of **1** and **2** suggested that the connection site of the monomer was on the A ring of **1**. The EI mass spectrum of **2** showed a $[M]^+$ at m/z 568, an intense peak at m/z 152 due to the dihydroxyacetophenone fragment, and like **1** a base peak at m/z 284 and significant peaks at m/z 285, 269, 266, 251, 237, 227 and 213. These findings also supported the proposal that **2** was a dimer of **1** (Scheme 1).

In the HMBC spectrum of **2** (Fig. 2), the signals at δ6.97 and 7.29 showed $^{3}J_{CH}$ cross-peaks with the two oxygenated quaternary carbons at δ75.6 and 101.3 and thus established that H-5 and H-11 were substituted at the *ortho* position of C-4 and C-10, respectively. The geminal CH_2 signals at δ3.30 and 4.50 both showed $^{2}J_{CH}$ correlation to the two oxygenated quaternary carbons at δ75.6 and 101.3, and $^{3}J_{CH}$ to the carbon signals at δ24.8 (Me); the geminal CH_2 signals at δ3.30 showed $^{3}J_{CH}$ correlation to the quaternary carbon at δ120.9; and the methyl signal at δ2.00 showed $^{3}J_{CH}$ correlation to the carbon signals at δ41.1 and 120.9, and $^{2}J_{CH}$ correlation to the carbon signal at δ75.6. The above evidence revealed that the CH_2 bridge connected two oxygenated quaternary carbons at δ75.6 and 101.3, and that the CH_2 and methyl groups were derived from the acetyl group of ring A of **1** and the carbonyl of ring A of **1**, transformed into the two oxygenated carbons (C-14 and C-16), as shown in Fig. 2. In addition, the other HMBC and HMQC spectra of **2** also supported the structure elucidation of cynandione B (**2**) and established the ^{13}C NMR spectral assignments (Table 1).

Compound **3**, yellow needles, mp 255 ~ 257°. Its UV spectrum showed similar absorption maxima to **1**, **2** and 2,4-dihydroxyacetophenone [6]. Its EIMS spectrum also showed a $[M]^+$ at m/z 568, a base peak at m/z 43 $[COMe]^+$ and significant peaks at m/z 285, 284, 269, 266, 251, 237, 227 and 213, similar to **1** and **2**. This indicated that **3** also was a dimeric compound of **1**. In the ^{1}H NMR

Table 1. ^{13}C NMR and ^{1}H NMR chemical shift assignments for **1**, **1a**, **2** and **3**

	1(CD_3OD)*		**1a**($CDCl_3$)*†		**2**(pyridine-d_5)*†		**3**(pyridine-d_5)*†	
C	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	113.4[a]		122.4[a]		113.3		113.1	
2	152.6		145.9		143.1		142.3	
3	149.3		144.1		143.1		142.3	
4	128.1		135.8		120.9		126.2	
5	122.0	6.94 (*d*)	125.0	7.37 (*d*)	118.9	6.97 (*d*)	120.1	6.79 (*d*)
6	118.5	6.79 (*d*)	124.3	7.25 (*d*)	121.0	7.17 (*d*)	120.6	7.20 (*d*)
7	207.7		199.4		117.5		117.8	
8	31.2	2.18 (*s*)	30.7	2.07 (*s*)	149.1		148.9	
9					147.8		147.9	
10					122.1		122.0	
11					119.6	7.29 (*d*)	119.5	7.31 (*d*)
12					122.1	7.39 (*d*)	122.2	7.41 (*d*)
14					101.3		100.9	
15					41.1	3.30, 4.50 (*d*)	46.1	3.43, 5.08 (*d*)
16					75.6		75.2	
17					24.8	2.00 (*s*)	26.2	1.79 (*s*)
1′	114.8[a]		122.5[a]		112.6		112.4	
2′	164.0		152.0		159.2		159.0	
3′	120.6		128.7		115.7		115.5	
4′	134.3	7.78 (*d*)	131.0	7.95 (*d*)	133.2	7.78 (*d*)	133.0	7.74 (*d*)
5′	109.0	6.49 (*d*)	120.1	7.31 (*d*)	110.8	6.84 (*d*)	111.2	6.85 (*d*)
6′	164.0		147.8		157.9		157.8	
7′	204.9		196.4		205.1		205.0[a]	
8′	26.6	2.56 (*s*)	29.0	2.59 (*s*)	26.4	2.53 (*s*)	26.3	2.55 (*s*)
1″					114.1		114.0	
2″					160.7		161.3	
3″					116.8		116.6	
4″					132.6	7.83 (*d*)	132.4	7.81 (*d*)
5″					111.6	6.80 (*d*)	111.6	6.47 (*d*)
6″					158.7		158.9	
7″					205.1		205.1[a]	
8″					26.7	2.57 (*s*)	26.7	2.58 (*s*)

(**1a**) OCOMe 20.6, 20.7 (2), 20.8 2.06, 2.08, 2.12, 2.30
OCOMe 168.0, 168.4, 168.8, 169.1

* The number of directly attached protons to each individual carbons was verified with DEPT pulse sequence.

† These signals were obtained by HMQC and HMBC techniques.

[a] These signals may be reversed in each column.

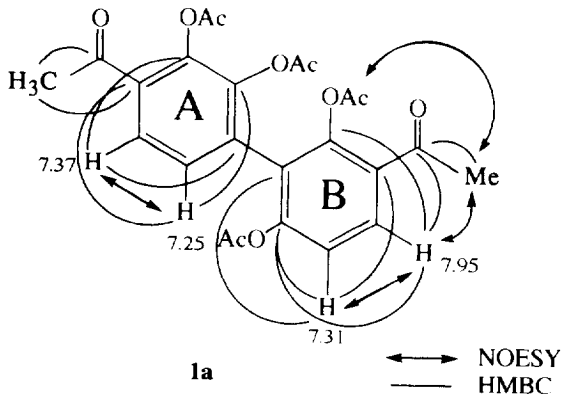


Fig. 1.

spectrum of **3**, there were four pairs of *ortho* coupled aromatic protons signals which were almost the same as those of **2** (Table 1), except that the signal at δ6.47 exhibited a Δ − 0.33 ppm highfield shift. The acetyl signals appeared at δ2.55 and 2.58, the methyl signal exhibited a high field shift to δ1.79 and the geminal CH_2 signals a lowfield shift to δ3.43 and 5.08. These findings suggested that **3** was a steric isomer of **2**. The ^{13}C NMR spectrum of **3** also showed similar chemical shifts to those of **2**, except C-5 and C-15 exhibited significant lowfield shifts of Δ + 5.3 and Δ + 5.0 ppm, respectively (Table 1). The HMBC spectrum of **3** also showed similar correlations between the geminal CH_2 and methyl signals to that of **2** (Fig. 2). In addition to the above evidence, **2** and **3** showed different specific rotation values (**2** + 30° and **3** − 18.2°). Therefore, **3** was characterized as the C-16

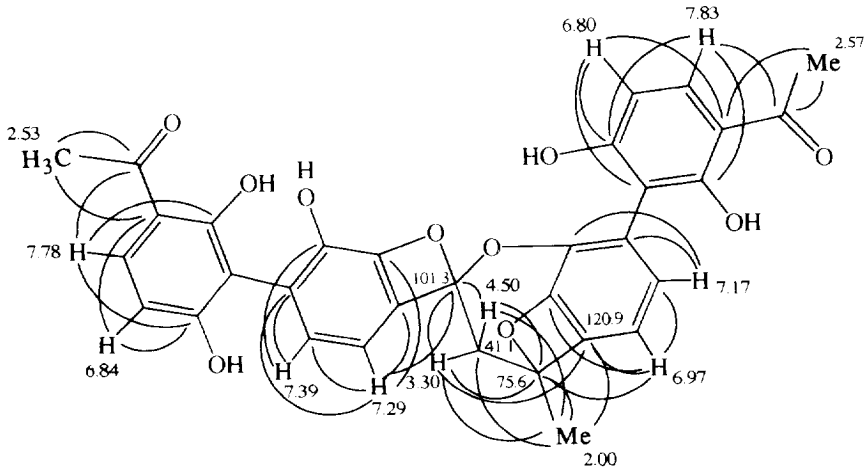
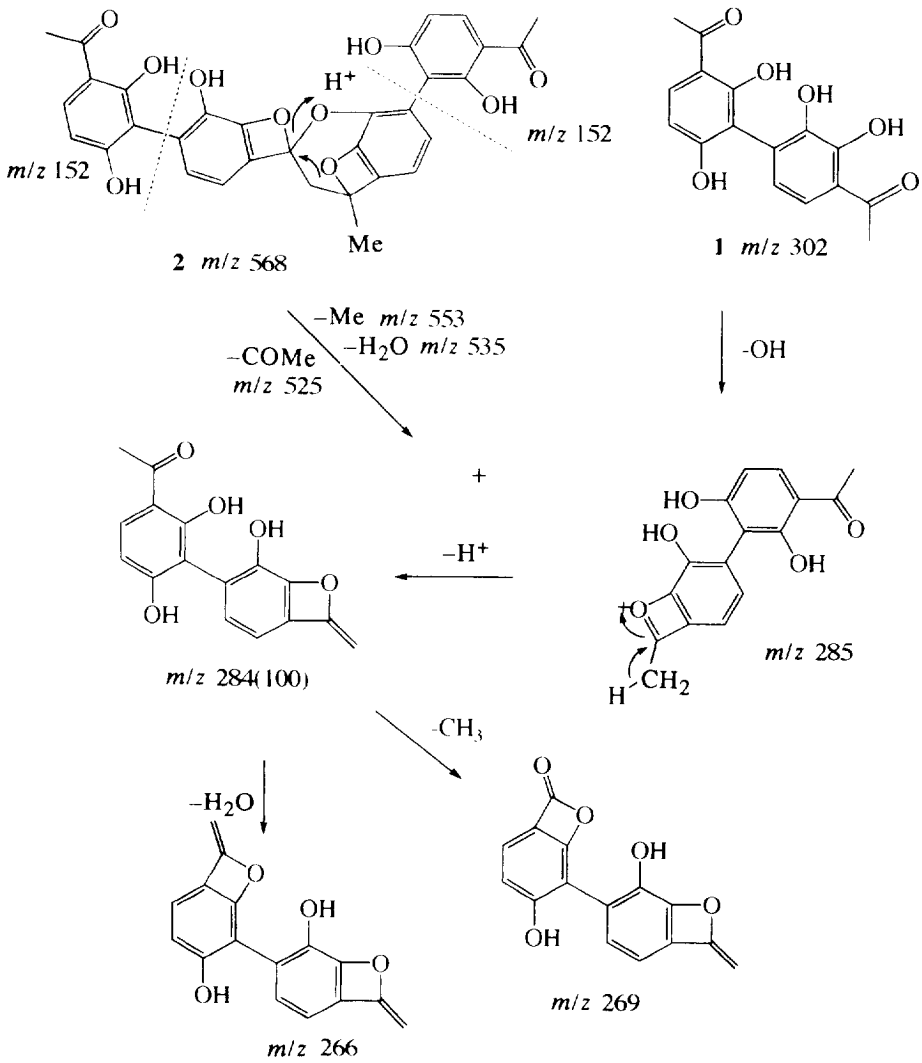


Fig. 2.



Scheme 1.

steric isomer of **2**. Unfortunately, the NOESY spectrum provided no useful information about the conformation of C-14 and C-16. The absolute configuration of C-14 and C-16 of both cynandione B (**2**) and C (**3**) remain to be defined.

EXPERIMENTAL

Extraction and isolation. Fresh rhizomes (5 kg) of *C. taiwanianum* were collected at Kaohsiung Hsiung, Taiwan, in July 1993. A voucher specimen is deposited in our laboratory. The fresh materials were chipped and extracted with MeOH at room temp. several times. The extract was chromatographed on a silica gel column. Elution with CH_2Cl_2 yielded germanicol-3-*O*-acetate, cycloartenol and β-sitosterol, elution with CH_2Cl_2–MeOH (9:1) yielded cynandione B (**2**) and C (**3**), and elution with CH_2Cl_2–MeOH(7:1) yielded cynandione A(**1**) and β-sitosterol-β-D-glucoside. The characterization of known compounds was achieved by spectral methods.

Compound **1**. Yellow needles (CH_2Cl_2), mp 203 ~ 206°, $[\alpha]_D^{25}$ 0° (0.11, MeOH). UV λ_{max}^{MeOH} nm (log ε): 217 (4.36), 232 (4.22) (sh), 280 (4.03), 317 (3.90), 400 (3.23) (sh); IR ν_{max}^{KBr} cm^{-1}: 3550 (chelated OH), 3395 (OH), 3125 (chelated OH), 1675 (C=O), 1638 (C=O); EIMS (direct inlet) 70 eV, *m/z* (rel. int.): 302 $[M]^+$ (17), 285 (18), 284 $[M - H_2O]^+$ (100), 269 (26), 266 (32), 245 (18), 227 (8), 213 (5), 137 (10), 115 (10), 95 (17), 77 (18), 43 (86); ^1H NMR (CD_3OD): Table 1; ^{13}C NMR (CD_3OD): Table 1; HRMS Calc. for $C_{16}H_{14}O_6$: 302.0790; found: 302.0786.

Compound **1** was acetylated by the usual method to yield a powder ($CHCl_3$) of **1a**, mp 37 ~ 40°. IR ν_{max}^{KBr} cm^{-1}: 1770 (ester C=O), 1690 (C=O), 1595; ^1H NMR ($CDCl_3$): Table 1; ^{13}C NMR ($CDCl_3$): Table 1; EIMS (direct inlet) 70 eV, *m/z* (rel. int.): 470 $[M]^+$ (0.04), 428 $[M - 42]^+$ (1), 386 $[M - 42 \times 2]^+$ (8), 344 $[M - 42 \times 3]^+$ (56), 302 $[M - 42 \times 4]^+$ (8), 285 (29), 284 (95), 283 (23), 269 (9), 243 (8), 137 (7), 115 (2), 95 (6), 43 (100).

Compound **2**. Yellow–orange powders, mp > 300°, $[\alpha]_D^{25}$ + 30° (0.01, MeOH). UV λ_{max}^{MeOH} nm (log ε): 208 (4.55), 222 (4.45) (sh), 268 (4.40), 300 (4.20) and 400 (3.61) (sh); EIMS (direct inlet) 70 eV, *m/z* (rel. int.): 568 $[M]^+$ (18), 554 $[M - CH_2]^+$ (30), 553 $[M - Me]^+$ (87), 551 $[M - 17]^+$ (19), 535 (6), 309 (5), 286 (12), 285 (69), 284 (100), 269 (11), 267 (10), 266 (21), 265 (6), 251 (4), 237 (12), 227 (4), 213 (6), 152 (6), 128 (12), 127 (10), 115 (11), 95 (16), 77 (13), 43 (91); ^1H NMR (pyridine-d_5): Table 1; ^{13}C NMR (pyridine-d_5): Table 1; HRMS Calc. for $C_{32}H_{24}O_{10}$: 568.1369; found $[M]^+$: 568.1378.

Compound **3**. Yellow needles, mp 255 ~ 257°, $[\alpha]_D^{25}$ −18.2° (0.01, MeOH). UV λ_{max}^{MeOH} nm (log ε): 208 (4.64), 220 (4.66) (sh), 261 (4.57), 308 (4.37) and 405 (3.21) (sh); EIMS (direct inlet) 70 eV, *m/z* (rel. int.): 568 $[M]^+$ (12), 554 $[M - CH_2]^+$ (17), 553 $[M - Me]^+$ (49), 551 $[M - 17]^+$ (11), 535 (3), 309 (2), 286 (11), 285 (63), 284 (77), 269 (7), 267 (7), 266 (12), 265 (3), 251 (2), 237 (6), 227 (2), 213 (3), 152 (3), 128 (5), 127 (3), 115 (4), 95 (9), 77 (7), 43 (100); ^1H NMR (pyridine-d_5): Table 1; ^{13}C NMR (pyridine-d_5): Table 1; HRMS Calc. for $C_{32}H_{24}O_{10}$: 568.1369; found $[M]^+$: 568.1370.

Acknowledgements—This work was supported by grants from the National Science Council of R.O.C. (NSC-83-0412-B037-078 and 84-2331-B037-019) and the National Research Institute of Chinese Medicine, Taipei, Taiwan, R.O.C.

REFERENCES

1. Chen, Z. S., Lai, J. S. and Kuo, Y. H. (1991) *Chem. Pharm. Bull.* **39**, 3034.
2. Li, J., Kadota, S., Kawata, Y., Hattori, M., Xu, G. J. and Namba, T. (1992) *Chem. Pharm. Bull.* **40**, 3133.
3. Chen, Z. S., Lai, J. S. and Kuo, Y. H. (1991) *J. Chin. Chem. Soc.* **38**, 393.
4. Lin, C. N. and Gan, K. H. (1989) *Planta Med.* **55**, 48.
5. Lin, C. N., Lu, C. M., Cheng, M. K., Gan, K. H. and Won, S. J. (1990) *J. Nat. Prod.* **53**, 513.
6. Scott, A. I. (1964) in *Interpretation of the Ultraviolet Spectra of Natural Products*, pp. 103–110. Pergamon Press, Oxford.
7. Steglich, W. and Losel, W. (1969) *Tetrahedron* **25**, 4301.