Pergamon

PII: S0031-9422(97)00599-2

Phytochemistry, Vol. 47, No. 6, pp. 1093–1099, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

A DITERPENOID SULPHATE AND FLAVONOIDS FROM *WEDELIA ASPERRIMA*

CLEOFE A. CALANASAN and JOHN K. MACLEOD*

Research School of Chemistry, The Australian National University, Canberra, ACT 0200, Australia

(*Received in revised form* 28 *April* 1997)

Key Word Index—*Wedelia asperrima*; Compositae; 4′-*O*-sulphowedeloside; flavanones; chalcones; chalcone glycosides; synthesis.

Abstract—A 4′-*O*-sulphated analogue of wedeloside, a new flavanone, 7,4′-dihydroxy-8,3′-dimethoxyflavanone and its related chalcone, together with the 4′-*O*-β-D-glucopyranoside of the latter, and a number of known compounds were isolated from the aerial parts of *Wedelia asperrima*. Their structures were established by spectroscopic techniques and by synthesis of the flavanone and chalcone. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The herb, *Wedelia asperrima* Benth. (Compositae) is distributed in parts of northern Australia where it has been shown to be responsible during drought conditions for severe loss of sheep [1]. The major toxic components of the plant have been identified as the kaurene aminoglycoside, wedeloside (**1**), and its two 4′-*O*-rhamnosyl analogues (**2**) and (**3**) [2, 3]. They act by inhibiting ADP/ATP transport across the mitochondrial membrane by binding to the carrier protein [4, 5]. These compounds were isolated from the plant using a toxicity-testing monitoring procedure which would not detect the presence of any minor or less toxic components.

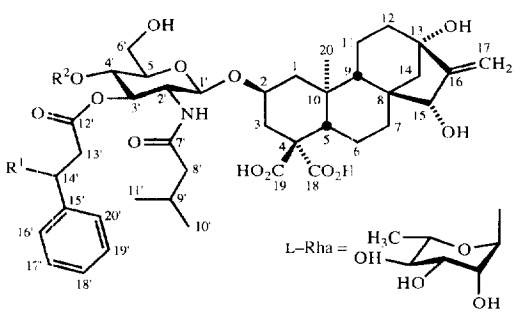
The present non-selective investigation of components of *W. asperrima* was undertaken mainly to look for additional analogues or precursors of wedeloside which might be present in the plant in small quantities or be of low toxicity and would not have been detected previously. These could help to define those structural features which are essential for the inhibitory properties of wedeloside, since chemical modification of wedeloside for this purpose posed difficulties. One new sulphated derivative (**4**) of wedeloside was isolated and identified, together with the previously unreported compound, 7,4′-dihydroxy-8,3′-dimethoxyflavanone (**5**), its related chalcone (**7**) and the 4′-*O*-glucopyranoside (**9**) of the chalcone. The proposed structures of **5** and **7** were confirmed by synthesis.

* Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

The milled, air-dried stems and leaves of *W. asperrima* were extracted first with ethyl acetate, then with methanol–water (1:1) and the extracts worked up as described in the Experimental. The less polar fraction of the ethyl acetate extract after chromatography provided the readily identifiable (NMR, MS) triterpenes, β-amyrin (12-oleanen-3β-ol), β-amyrin acetate and β-amyrone [6, 7], while the more polar fractions contained mixtures of phytosterols as evidenced from their ^{1}H NMR and mass spectra.

The methanol soluble fraction of a dichloromethane extract of the concentrated aqueous residue from the original methanol–water extract, after chromatography on a PVP column followed by reversed-phase HPLC of individual fractions from the column,



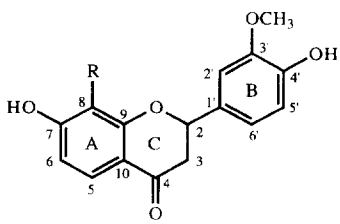
	R^1	R^2
1	H	H
2	H	L-Rha
3	OH	L-Rha
4	H	SO_3H

yielded flavanones (**5**) and (**6**) and their related chalcones (**7**) and (**8**). the methanol soluble fraction of a *n*-butanol extract of the same aqueous residue after LH-20 chromatography and reversed-phase HPLC, provided a mixture of the known 4′-*O*-rhamnosyl wedelosides (**2**) and (**3**) [3], 4′-*O*-sulphowedeloside (**4**) and the two chalcone glycosides (**9**) and (**10**).

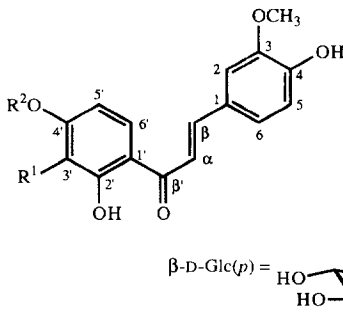
The negative FAB mass spectrum of **4** showed an $[M-H]^-$ ion at *m/z* 836.318 ($C_{40}H_{54}NO_{16}S$) which corresponded to wedeloside (**1**) plus one sulphate group. The presence of one sulphur atom in the molecule was confirmed by microanalysis. The EI mass spectrum of the per-TMSi derivative of **4** showed a strong fragment ion at *m/z* 651 corresponding to the tetra-TMSi aglycone fragment ion A^+ [2, 3] suggesting that the sulphate group was on the glycosidic portion of the molecule.

Both the ^1H and ^{13}C NMR spectra of **4** were very similar to those of wedeloside (**1**) [2,3]. Its ^{13}C NMR spectrum contained the same number of carbon resonances as that of **1** and characteristic signals for the diterpene aglycone and the 3-phenylpropanoyl and 3-methyl-1-oxobutyl substituents on the aminosugar. There were, however, significant differences between the chemical shifts of the C-3′, C-4′ and C-5′ glycosidic carbons in **4** compared with those in **1**. C-4′ was deshielded by 5 ppm while both C-3′ and C-5′ were shielded by 1 ppm and 2.4 ppm, respectively. In keeping with the sulphate shift rules [8] this placed the sulphate group on C-4′.

Assignment of structures **5** and **6** to the flavanones and **7**–**10** to the corresponding chalcones and their glycosides was based initially on UV, mass and NMR spectrometric analysis. The EI mass spectrum of **5** showed an $[M]^+$ at *m/z* 316 which accurately mass measured for the composition $C_{17}H_{16}O_6$. Its mass spectrum contained significant fragment ions at *m/z* 167 (A_1+1, $C_8H_7O_4$) and 150 (B_3, $C710H_{10}O_2$) which placed one hydroxy and one methoxy substituent on each of the aromatic rings [9]. The ^1H NMR spectrum showed the two methoxy singlets at δ 3.81 and 3.87 and three doublet of doublets centred at δ 5.45, 3.10 and 2.75, corresponding to H-2 and the H-3 methylene protons. Two *ortho*-coupled aromatic protons at δ 6.56 and 7.51 were consistent with the presence of either a 5,6-, 7,8- or 5,8-disubstitution pattern on the A ring. The absence of a bathochromic shift of Band II in its UV spectrum in MeOH on addition of $AlCl_3$–HCl implied that the compound did not contain a 5-hydroxy group [10], while a shift of 54 nm (284 nm → 338 nm) on addition of sodium acetate confirmed the presence of a 7-hydroxy substituent. Thus, the ring A substituents could be placed at the 7,8-positions.



5 R = OCH_3
6 R = H



β-D-Glc(*p*) =

	R^1	R^2
7	OCH_3	H
8	H	H
9	OCH_3	β-D-Glc(*p*)
10	H	β-D-Glc(*p*)

In ring B, H-2′ appeared as a doublet at δ 7.13 in the ^1H NMR spectrum, *meta*-coupled to H-6′ at δ 6.96 which in turn was *ortho*-coupled to H-5′ at δ 6.82. Since H-2′ was deshielded compared with H-6′, this placed the methoxy substituent on C-3′ [11]. In the ^{13}C NMR spectrum of **5**, a signal at 56.5 ppm could be assigned to the 3′-OMe, being characteristic of a methoxy substituent with at least one unsubstituted *ortho*-position [12]. The other methoxy signal at 61.2 ppm placed this substituent at C-8 which has no *ortho*-protons. Since there had been no previous report of a compound possessing the proposed structure **5**, a synthesis was carried out to confirm its structure (see below).

The second flavanone (**6**) showed the characteristic ^1H NMR signals and coupling constants for the ring C protons and had one less methoxy signal compared with **5**. Its EI mass spectrum contained an $[M]^+$ ion at *m/z* 286 ($C_{16}H_{14}O_5$) and fragment ions at *m z* 137 (A_1+1, $C_7H_5O_3$) and 150 (B_3, $C_9H_{10}O_2$) which indicated that ring B contained a hydroxy and methoxy substituent while ring A had one hydroxy substituent only. Comparison of its UV, ^1H and ^{13}C NMR spectra with those of **5** showed that it lacked the methoxy group at C-8 and could be assigned structure **6**.

The structures of the chalcones (**7**) and (**8**) corresponding to the ring-opened flavanones (**5**) and (**6**), respectively, were readily determined from analysis of their ^1H and ^{13}C NMR and mass spectra. Compound **7** was isolated as a single compound from the *n*-butanol extract, while compound **8** was present as an inseparable mixture with (**7**) in the dichloromethane extract. The spectral data for **8** could be obtained by subtraction of that of **7** from the spectra of the mixture. Both the flavanone (**6**) and its chalcone (**8**) have been reported previously as synthetic com-

pounds [13, 14] while **8**, called homobutein, has been tentatively identified as a natural product from two plant sources [15, 16].

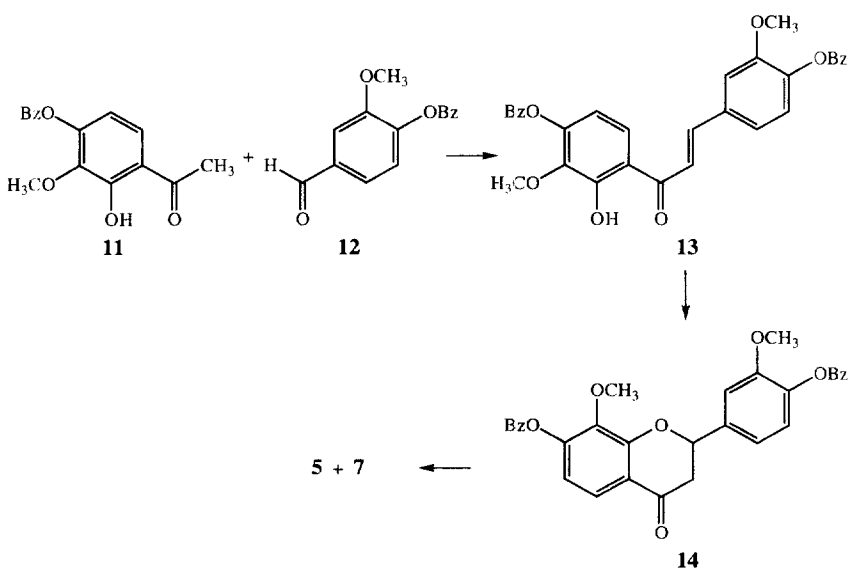
The two chalcone glycosides, isolated in small amounts from the *n*-butanol fraction, were assigned structures **9** and **10** based on a comparison of their ^1H and ^{13}C NMR and mass spectra with those of the corresponding aglycones (**7**) and (**8**). The negative FAB mass spectrum of **9** showed an $[M-H]^-$ ion at *m/z* 477, while in the positive FAB mass spectrum there was a prominent $[MH]^+$ ion at *m/z* 479 which mass measured for $C_{23}H_{27}O_{11}$, corresponding to chalcone (**7**) plus a hexose unit. Comparison of the ^1H NMR spectrum of **9** with that of the chalcone (**7**) showed that the aromatic rings in both compounds had the same substitution patterns. The identity of the glycoside as a β-glucopyranoside was determined by comparison with reported ^{13}C NMR data for different hexosides [17], while the 4′-site of glycosylation was inferred from the diagnostic upfield shift of C-4′ (−6.9 ppm) and downfield of C-3′ (+2.1 ppm), C-5′ (+1.1 ppm) and C-1′ (+2.0 ppm) compared with the corresponding carbon resonances in **7** [18] (Table 1).

From the FAB mass spectral data on the second chalcone glycoside and similar comparisons of its NMR data with that of **8** (Table 1), its structure could be assigned as **10**. A synthesis of **10** has been reported but it has not been previously identified as a natural product.

An unambiguous synthesis of the flavanone (**5**) and its related chalcone (**7**) was carried out to confirm their proposed structures (Scheme 1). Aldol condensation of 4-benzyloxy-2-hydroxy-3-methoxyacetophenone (**11**), prepared from gallacetophenone using modifications of reported procedures [20], with 4-benzyloxy-3-methoxybenzaldehyde (**12**) gave the protected chalcone (**13**). On heating in ethanol containing a trace of H_3PO_4, **13** cyclized to the protected flavanone (**14**). Removal of the benzyl protecting groups from **13** and **14** proved more difficult than expected. Hydrogenation ($Pd/C/H_2$) of the flavanone (**14**) resulted in debenzylation but was accompanied by reduction of the ketone to a methylene group. However, treatment of **14** with TMSiI gave a low yield of the didebenzylated product together with some monodebenzylated compound as determined by ^1H NMR. Work-up of the reaction mixture resulted in the isolation of the flavanone (**5**) and its chalcone

Table 1. ^{13}C NMR spectral date of the chalcones (**7**) and (**8**) and their 4′-*O*-glucosides (**9**) and (**10**) (75.5 Hz, δ, CD_3OD)

C	7	9	8	10
1	127.9	127.6	128.4	128.2
2	112.2	112.4	112.1	112.3
3	151.1	151.7	151.0	149.6
4	149.5	149.7	149.4	148.0
5	116.9	117.8	116.5	116.7
6	125.1	125.5	125.1	125.3
1′	114.7	116.7	114.7	116.7
2′	159.4	157.5	166.6	166.9
3′	136.2	138.3	103.8	105.1
4′	158.6	151.7	167.4	165.1
5′	109.0	107.9	109.2	109.3
6′	131.9	136.3	133.5	133.1
α	118.5	118.5	118.6	118.3
β	145.8	147.2	146.0	147.6
β'	194.0	194.6	193.5	194.0
3-OMe	56.5	56.6	56.5	56.5
3′-OMe	60.8	61.4	—	—
1″	—	101.8	—	101.4
2″	—	74.8	—	74.8
3″	—	78.5	—	78.3
4″	—	71.2	—	71.2
5″	—	78.0	—	77.9
6″	—	62.5	—	62.4



Scheme 1.

isomer (**7**) as a 4:3 mixture which were separated by reversed-phase HPLC. The spectral data (UV, MS, ^{1}H NMR, ^{13}C NMR) for the synthetic compounds **5** and **7** matched that obtained for the natural products from *W. asperrima*, confirming their structures.

The facility with which the flavanone (**5**) ring-opens to the chalcone (**7**) casts some doubt, however, on whether the latter occurs naturally or is formed during the isolation procedure.

EXPERIMENTAL

General. Mps: uncorr. ^{1}H and ^{13}C NMR: 300 and 75.5 MHz, respectively, TMS int. standard. MS: 7070F(EI) and ZAB-2SEQ(±FAB) mass spectrometers. TLC: Merck silica gel 60; Merck RP-18. CC: silica gel 230–400 mesh; PVP; Sephadex LH-20. HPLC: Waters 510 pump with UV detection (column Nova-Pak HR C18, 60 Å, 6 μm, 7.8 mm × 30 cm).

Plant material. Details have been reported previously [21].

Extraction and isolation. Milled, air-dried stems and leaves of *W. asperrima* (170 g) were extracted with EtOAc (1.2 l) at room temp. by mechanical shaking. After filtration, the concd extract was adsorbed on silica gel, and flash chromatography, using hexane with increasing concs of EtOAc, providing eight frs. The first fr. was subjected to centrifugal TLC (CTLC) with 10% EtOAc–hexane, yielding β-amyrin acetate (45.7 mg) and β-amyrone (18 mg). CTLC of the second fr. using 5–20% EtOAc–hexane gave β-amyrin (15 mg). Purification of the other frs was attempted by a combination of CC and CTLC, and gave only mixts of phytosterols, based on ^{1}H NMR and mass spectral data.

The plant residue was then soaked in 50% MeOH–H_2O (800 ml) and kept at 5° for 2 months. After filtration, the concd extract was partitioned between hexane and H_2O. The aq. phase was then extracted successively with CH_2Cl_2 and *n*-BuOH. The less polar components of the combined CH_2Cl_2 and hexane extracts were discarded, while the MeOH-soluble ones were passed through a PVP column using EtOAc–MeOH (4:1) as eluent. Frs containing compounds **5**–**8** were further purified by reversed-phase HPLC using a C18 column. A gradient of MeOH–H_2O (9:11 → 3:2) over 25 min at 2.0 ml min^{-1} yielded **5** (5mg) after 16.5 min and **6** (7.4 mg) after 17.9 min. The mixt. of the chalcones (**7**) and (**8**) (9.4 mg) eluted after 21.7 min using a gradient of MeOH–H_2O (17:3 → 9:1) over 25 min at 3.0 ml min^{-1}.

The MeOH-soluble portion of the *n*-BuOH extract was chromatographed on a Sephadex LH-20 column (MeOH) and four major frs were collected. Reversed-phase HPLC of the first fr. using a H_2O–MeOH (3:7 → 1:9) gradient over 15 min resulted in the mixt. of (**2**) and (**3**) (17 mg) eluting after 5.6 min, and the pure compound (**4**) (16 mg) after 6.5 min.

The last fr. obtained from gel chromatography was purified by reversed-phase HPLC using H_2O–MeOH (3:17) as solvent. The chalcone (**7**) (4.8 mg) eluted after 10.7 min while the glycosides (**9**) (0.4 mg) and (**10**) (0.7 mg) eluted as a mixt. after 8.07 min.

4′-O-*Sulphowedeloside* (**4**). Off-white solid. Negative HRFAB MS $[M-H]^-$, found 836.3182 $[M-H]^-$. $C_{40}H_{54}NO_{16}S$ requires 836.3163. Found: S, 3.0. $C_{40}H_{54}NO_{16}S$ requires S, 3.8. ^{1}H NMR (75.5 MHz, CD_3OD): δ 0.84 (3H, *bs*, H-10′), 0.89 (3H, *bs*, H-11′), 1.00 (3H, *s*, H-20), 3.76 (1H, *s*, H-15), 4.32 (1H, *d*, J = 7.2 Hz, H-1′), 5.20 (1H, *s*, H-17), 5.23 (1H, *s*, H-17), 7.1–7.4 (5H, *m*, H-16′–H-20′). ^{13}C NMR (75.5 MHz, D_2O): δ 17.2 (C-20), 19.8 (C-11), 22.2 (C-11′), 22.4 (C-10′), 23.3 (C-6), 26.8 (C-9′), 30.3 (C-13′), 35.1 (C-7), 35.6 (C-14′), 38.8 (C-12), 40.2 (C-3), 40.4 (C-10), 42.8 (C-14), 45.8 (C-8′), 46.2 (C-8), 47.0 (C-1), 51.8 (C-5), 52.1 (C-9), 54.2 (C-2′), 61.0 (C-4), 61.1 (C-6′), 73.7 (C-2), 74.8 (C-4′), 75.0 (C-5′), 75.2 (C-3′), 79.8 (C-13), 81.5 (C-15), 100.0 (C-1′), 109.3 (C-17), 127.2 (C-18′), 129.0 (C-16′, C-20′), 129.5 (C-17′, C-19′), 141.4 (C-15′), 158.9 (C-16), 172.7 (C-12′), 175.5 (C-7′), 177.3 (C-18, C-19).

Silylated derivative of **4**. Compound **4** (1.0 mg) was dissolved in dry pyridine (10 μl) and treated with BSTFA–TMCS (9:1) 50 μl) at 70° for 20 min. EIMS (probe) 70 eV, *m/z* above 600 (rel. int.) 1114(34), 998(18), 958(22), 842(12), 728(25), 651[A]$^+$ (100).

4′-O-*Rhamnosyl wedelosides* (**2**) *and* (**3**). Identified by comparison of their FAB MS and ^{13}C NMR with those previously reported [3].

7,4′-*Dihydroxy*-8,3′-*dimethoxyflavanone* (**5**). White solid. Mp: 149–150° $[\alpha]_D$ −6.7° (*c* 0.15, MeOH); HREIMS $[M]^+$, found 316.0947. $C_{17}H_{16}O_6$ requires 316.0945. Found 167.0344. $C_8H_7O_4$ requires 167.0345. Found 150.0681. $C_9H_{10}O_2$ requires 150.0681. UV λ_{max} nm: 284 (MeOH), 253, 338 (MeOH + NaOMe), 256, 338 (MeOH + NaOAc), 284 (MeOH + $AlCl_3$ + HCl). EIMS (probe) 70 eV *m/z* (rel. int.): 316 $[M]^+$ (17), 167 (86), 150 (99), 138 (84). ^{1}H NMR (300 MHz, CD_3OD): δ 2.75, (1H, *dd*, J = 3, 17 Hz, H-3*eq*), 3.16 (1H, *dd*, J = 13, 17 Hz, H-3*ax*), 3.81 (3H, *s*, 3′-OCH_3), 3.87 (3H, *s*, 8-OCH_3), 5.45 (1H, *dd*, J = 3, 13 Hz, H-2*ax*), 6.56 (1H, *d*, J = 9 Hz, H-6), 6.82 (1H, *d*, J = 8 Hz, H-5′), 6.96 (1H, *dd*, J = 2, 8 Hz, H-6′), 7.13 (1H, *d*, J = 2 Hz, H-2′), 7.51 (1H, *d*, J = 9 Hz, H-5). ^{13}C NMR (75.5 MHz, CD_3OD): δ 44.9 (C-3), 56.5 (3′-OCH_3), 60.8 (8-OCH_3), 81.5 (C-2), 111.3 (C-6), 112.1 (C-10), 115.7 (C-2′), 116.2 (C-6′), 120.5 (C-5′), 124.0 (C-5), 132.1 (C-1′), 136.9 (C-8), 148.2 (C-4′), 149.2 (C-3′), 157.8 (C-9), 159.8 (C-7), 193.6 (C-4).

7,4′-*Dihydroxy*-3′-*methoxyflavanone* (**6**). White solid. $[\alpha]_D$ −6.5° (*c* 0.19, MeOH); HREIMS $[M]^+$, found 286.0841. $C_{16}H_{14}O_5$ requires 286.0840. Found 137.0239. $C_7H_5O_3$ requires 137.0239. Found 150.0681. $C_9H_{10}O_2$ requires 150.0681. UV λ_{max} nm: 277, 312 (MeOH), 253, 335 (MeOH + NaOMe), 253, 335 (MeOH + NaOAc), 277, 311 (MeOH + $AlCl_3$ + HCl). EIMS (probe) 70 eV, *m/z* (rel. int.): 286 $[M]^+$ (26), 163 (10), 150 (65), 137 (100). ^{1}H NMR (300 MHz, CD_3OD): δ 2.79 (1H, *dd*, J = 3, 17 Hz, H-3*eq*), 3.18

(1H, *dd*, *J* = 13, 17 Hz, H-3*ax*), 3.44 (3H, *s*, 3′-OCH_3), 5.47 (1H, *dd*, *J* = 3, 13 Hz, H-2*ax*), 6.44 (1H, *d*, *J* = 2 Hz, H-8), 6.58 (1H, *dd*, *J* = 2, 9 Hz, H-6), 6.91 (1H, *d*, *J* = 8 Hz, H-5′), 7.02 (1H, *dd*, *J* = 2, 8 Hz, H-6′), 7.18 (1H, *d*, *J* = Hz, H-2′), 7.82 (1H, *d*, *J* = 8 Hz, H-5). ^{13}C NMR (75.5 MHz, CD_3OD): δ 45.1 (C-3), 56.4 (3′-OCH_3), 81.3 (C-2), 103.9 (C-8), 111.2 (C-2′), 112.1 (C-6), 114.7 (C-10), 116.1 (C-6′), 120.5 (C-5′), 129.8 (C-5), 132.0 (C-1′), 148.0 (C-4′), 149.1 (C-3), 165.6 (C-9), 167.7 (C-7), 193.5 (C-4).

2′,4′,4-*Trihydroxy*-3′,3-*dimethoxychalcone* (**7**). Orange-yellow solid. HREIMS $[M]^+$, found 316.0947. $C_{17}H_{16}O_6$ requires 316.0961. Found 167.0344. $C_8H_7O_4$ requires 167.0332. Found 150.0681. $C_9H_{10}O_2$ requires 150.0681. UV λ_{max} nm: 271, 341 (MeOH) 272, 398 (MeOH + NaOMe), 271, 353 (MeOH + NaOAc), 279, 365 (MeOH + $AlCl_3$), 281, 361 (MeOH + $AlCl_3$ + HCl), EIMS (probe) 70 eV, *m/z* (rel. int.): 316 $[M]^+$ (26),167 (85), 150 (100), 138 (71). ^{1}H NMR (300 MHz, CD_3OD): δ 3.83 (3H, *s*, 3-OCH_3), 3.97 (3H, *s*, 3′-OCH_3), 6.47 (1H, *d*, *J* = 9 Hz, H-5′), 6.84 (1H, *d*, *J* = 9 Hz, H-5), 7.22 (1H, *dd*, *J* = 2, 9 Hz H-6), 7.37 (1H, *d*, *J* = 2 Hz, H-2), 7.64 (1H, *d*, *J* = 15 Hz, H-α), 7.80 (1H, *d*, *J* = 15 Hz, H-β), 7.83 (1H, *d*, *J* = 9 Hz, H-6′). ^{13}C NMR (75.5 MHz, CD_3OD): see Table 1.

2′,4′,4-*Trihydroxy*-3-*methoxychalcone* (**8**). Orange-yellow solid. Mixt. with (**7**). Spectral data obtained after subtraction of that of **7**. HREIMS $[M]^+$, found 286.0841. $C_{16}H_{14}O_5$ requires 286.0840. Found 137.0239. $C_7H_5O_3$ requires 137.0239. Found 150.0681. $C_9H_{10}O_2$ requires 150.0681. EIMS (probe) 70 eV, *m/z* (rel. int.): 286 $[M]^+$ (12), 150 (36), 137 (49). ^{1}H NMR (300 MHz, CD_3OD): δ 3.93 (3H, *s*, 3-OCH_3), 6.28 (1H, *d*, *J* = 2 Hz, H-3′), 6.40 (1H, *dd*, *J* = 2, 9 Hz, H-5′), 6.84 (1H, *d*, *J* = 8 Hz, H-5), 7.21 (1H, *dd*, *J* = 2, 8 Hz, H-6), 7.36 (1H, *d*, *J* = 2 Hz, H-2), 7.63 (1H, *d*, *J* = 15 Hz, H-α), 7.77 (1H, *d*, *J* = 15 Hz, H-β), 8.01 (1H, *d*, *J* = 9 Hz, H-6′). ^{13}C NMR (75.5 MHz, CD_3OD): See Table 1.

4′-O-β-D-*Glucopyranosyl*-2′,4-*dihydroxy*-3′,3-*dimethoxychalcone* (**9**). Orange-yellow solid. $[\alpha]_D$ −48.9° (*c* 0.045, MeOH); HR FAB-MS $[MH]^+$, found 479.1553. $C_{23}H_{27}O_{11}$ requires 479.1554. Negative FAB-MS *m/z* 477 $[M-H]^-$, 315 $[A-H]^-$ EIMS (probe) 70 eV, *m/z* (rel. int.): 316 $[A]^+$ (19), 167 (91), 150 (81). UV λ_{max} nm: 265, 379 (MeOH), 291, 453 (MeOH + NaOMe), 291, 465 (MeOH + NaOAc), 271, 434 (MeOH + $AlCl_3$), 273, 408 (MeOH + $AlCl_3$ + HCl). ^{1}H NMR (300 MHz, CD_3OD): δ 3.5–4.0 (6H, *m*, H-2″-H-6″), 3.98 (3H, *s*, OCH_3), 4.04 (3H, *s*, OCH_3), 5.20 (1H, *d*, *J* = 7 Hz, H-1″), 6.93 (1H, *d*, *J* = 9 Hz, H-5′), 6.95 (1H, *d*, *J* = 8 Hz, H-5), 7.35 (1H, *dd*, *J* = 2, 8Hz, H-6), 7.49 (1H, *d*, *J* = 2 Hz, H-2), 7.78 (1H, *d*, *J* = 15 Hz, H-α), 7.96 (1H, *d*, *J* = 15 Hz, H-β), 8.06 (1H, *d*, *J* = 9 Hz, H-6′). ^{13}C NMR (75.5 MHz, CD_3OD): see Table 1.

4′-O-*Glucopyranosyl*-2′,4-*dihydroxy*-3-*methoxychalcone* (**10**). Orange-yellow solid. $[\alpha]_D$ −40.0 (*c* 0.06, MeOH); EIMS (probe) 70 eV, *m/z* (rel. int.): 286 $[A]^+$ (8), 150 (65), 137 (97). Negative FAB-MS *m/z* 447.0 $[M-H]^-$. UV λ_{max} nm: 265, 379 (MeOH), 284, 453 (MeOH + NaOMe), 282. 461 (MeOH + NaOAc), 273, 443 (MeOH + $AlCl_3$), 273, 434 (MeOH + $AlCl_3$ + HCl). ^{1}H NMR (300 MHz, CD_3OD): δ 3.5–4.0 (6H, *m*, H-2″-H-6″), 4.04 (3H, *s*, OCH_3), 5.13 (1H, *d*, *J* = Hz, H-1′), 6.72 (1H, *d*, *J* = 2 Hz, H-3′), 6.79 (1H, *dd*, *J* = 2, 9 Hz, H-5′), 6.94 (1H, *d*, *J* = 8 Hz, H-5), 7.34 (1H, *dd*, *J* = 2, 8 Hz, H-6), 7.49 (1H, *d*, *J* = 2 Hz, H-2), 7.79 (1H, *d*, *J* = 15 Hz, H-α), 7.94 (1H, *d*, *J* = 15 Hz, H-β), 8.24 (1H, *d*, *J* = 9 Hz, H-6). ^{13}C NMR (75.5 MHz, CD_3OD): see Table 1.

4-*Benzyloxy*-2,3-*dihydroxyacetophenone*. To a soln of gallacetophenone (0.007 mol, 1.20 g) in DMF (18 ml) were added Li_2CO_3 (0.007 mol, 0.59 g) and benzyl bromide (0.1 mol, 1.17 ml) dropwise. The resulting suspension was stirred under N_2 at 60° for 24 hr. The reaction mixt. was poured into cold, dilute aq. HCl and extracted with $CHCl_3$. The organic phase was washed with H_2O and satd brine, dried ($MgSO_4$) and concd under red. pres. The reaction product was purified by repeated CC using PVP and eluting with MeOH. The required compound was obtained as a yellow solid (0.90 g, 49%). Mp 136–137°, lit. [20] 137–138°. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.53 (3H, *s*, $COCH_3$), 5.20 (2H, *s*, OCH_2Ph), 6.51 (1H, *d*, *J* = 9 Hz, H-5′), 7.24 (1H, *d*, *J* = 9 Hz, H-6′), 7.34–7.43 (5H, *m*, ArH), 12.52 (1H, *s*, 2′-OH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 26.7, 71.3, 104.7, 115.2, 122.8, 127.8, 128.7, 129.1, 134.1, 136.3, 150.9, 151.4, 208.9.

4-*Benzyloxy*-2-*hydroxy*-3-*methoxyacetophenone* (**11**). A soln of the above monobenzylated gallacetophenone (0.0024 mol, 0.631 g) in dry Me_2CO (20 ml) was added to K_2CO_3 (2.2 g) under N_2. Dimethyl sulphate (0.23 ml) was added in batches and the mixt. stirred for 20 hr at 5°. The mixt. was filtered and the residue washed with Me_2CO. The filtrate on evapn gave a pale yellow solid which was purified by CC (PVP) using MeOH as eluent. The monomethylated product (**11**) was obtained as a yellow solid (0.5976 g, 90%). Mp 143–145°, lit. [20] 146°. TLC R_f 0.52 (30% EtOAc–petrol). EIMS (probe) 70 eV, *m/z* (rel. int): 272 $[M]^+$ (2), 230 (1), 153 (1), 135 (1), 91 (100). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.56 (3H, *s*, $COCH_3$), 3.90 (3H, *s*, OCH_3), 5.22 (2H, *s*, OCH_2Ph), 6.51 (1H, *d*, *J* = 9 Hz, H-5′), 7.39–7.40 (5H, *m*, ArH), 7.44 (1H, *d*, *J* = 9 Hz, H-6′), 12.59 (1H, *s*, 2′-OH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 26.4, 60.7, 70.7, 104.6, 115.4, 126.8, 127.1, 128.2, 128.7, 136.2, 137.0, 157.3, 157.6, 203.2.

4′,4-*Dibenzyloxy*-2′-*hydroxy*-3′,3-*dimethoxychalcone* (**13**). To a soln of the protected acetophenone (**11**) (0.0019 mol, 0.51 g) in EtOH (30 ml) and 40% aq. NaOH (18 ml) was added 4-benzyloxy-3-methoxybenzaldehyde (**12**), prepd by benzylation of vanillin, (0.0019 mol, 0.45 g) while keeping the reaction temp. between 0–5°. The soln was stirred at this temp. for 2 hr, then at room temp. for 7 days. The mixt. was neutralized using cold 10% aq. HCl, and then extracted with CH_2Cl_2. The organic extract was

washed with satd $NaHCO_3$, then dried ($MgSO_4$) and reduced under vacuum. The residue was passed through a silica gel column using CH_2Cl_2–EtOAc–petrol (4:1:5) as eluting solvent. The first compound eluted was the recovered starting acetophenone (**11**), then the desired condensation product (**13**) (0.723 g, 79% yield). Compound (**13**. Mp 133–135°. Found: C, 74.7; H, 5.9. $C_{31}H_{28}O_6$ requires: C, 75.0; H, 5.7. EIMs (probe) 70 eV, *m/z* (rel. int.): 496 $[M]^+$ (25), 177 (12), 91 (100). ^1H NMR (300 MHz, $CDCl_3$): δ 3.96 (6H, *s*, 3′-OCH_3, 3-OCH_3), 5.23 (2H, *s*, OCH_2Ph), 5.26 (2H, *s*, OCH_2Ph), 6.55 (1H, *d*, $J = 9$ Hz, H-5′), 6.92 (1H, *d*, $J = 9$ Hz, H-5), 7.17 (1H, *dd*, $J = 2$, 9 Hz, H-6), 7.35–7.47 (12H, *m*, 2 × 5 ArH, H-2, H-α), 7.64 (1H, *d*, $J = 9$ Hz, H-6′), 7.84 (1H, *d*, $J = 15$ Hz, H-β). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 56.1 (3-OCH_3), 60.1 (3′-OCH_3), 70.7 (OCH_2Ph), 70.8 (OCH_2Ph), 104.5 (C-5′), 110.7 (C-2), 113.3 (C-1′), 115.7 (C-5), 117.9 (C-α), 123.2 (C-6), 125.7 (C-1), 127.1 (C-2″, C-6″), 127.2 (C-2″, C-6″), 128.0 (C-4″), 128.2 (C-4″), 128.7 (C-3″, C-5″), 128.8 (C-3″, C-5″), 136.3 (C-1″), 136.4 (C-1″), 137.1 (C-3′), 144.9 (C-4),. 150.8 (C-3), 157.5 (C-4′), 158.5 (C-2′), 192.3 (C-β′).

7,4′-Dibenzyloxy-8,3′-dimethoxyflavanone (**14**). To a soln of the chalcone (**13**) (0.114 mmol, 0.0566 g) in EtOH (20 ml), was added 5 drops of 85% aq. H_3PO_4, and the mixt. refluxed for 24 hr. The soln was concd under red. pres. and the residue dissolved in CH_2Cl_2. The organic extract was washed with satd $NaHCO_3$, H_2O, dried ($MgSO_4$) and concd under vacuum. The residue was chromatographed on silica gel (20% EtOAc–petrol) to yield the recovered starting material (**13**) in the first fr., while the second fr. yielded the flavanone (**14**) (0.337 g, 60% yield), TLC, R_f 0.34 (30% EtOAc–petrol). Compound (**14**). Mp 40–42°. Found: C, 75.0; H, 5.4. $C_{31}H_{28}O_6$ requires: C, 75.0; H, 5.7. EIMS (probe) 70 eV, *m/z* (rel. int.): 496.3 $[M]^+$ (20), 269 (49), 177 (8), 91 (100). ^1H NMR (300 MHz, $CDCl_3$): δ 2.85 (1H, *dd*, $J = 3$, 17 Hz, H-3*eq*), 3.02 (1H, *dd*, $J = 12$, 17 Hz, H-3*ax*), 3.87 (3H, *s*, 3′-OCH_3), 3.90 (3H, *s*, 8-OCH_3), 5.16 (2H, *s*, OCH_2Ph), 5.20 (2H, *s*, OCH_2Ph), 5.44 (1H, *dd*, $J = 3$, 12 Hz, H-2*ax*), 6.66 (1H, *d*, $J = 9$ Hz, H-6), 6.88 (1H, *d*, $J = 8$ Hz, H-5′), 6.92 (1H, *dd*, $J = 2$, 8 Hz, H-6′), 7.04 (1H, *d*, $J = 2$ Hz, H-2′), 7.29–7.44 (10H, *m*, 2 × 5 ArH), 7.62 (1H, *d*, $J = 9$ Hz, H-5). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 44.2 (C-3), 56.2 (3′-OCH_3), 60.5 (8-OCH_3), 70.7 (OCH_2Ph), 70.8 (OCH_2Ph), 79.5 (C-2), 107.4 (C-10), 109.8 (C-6), 110.7 (C-2′), 113.5 (C-5′), 116.3 (C-6′), 118.4 (C-5), 122.6 (C-8), 127.0 (C-2″, C-6″), 127.1 (C-2″, C-6″), 127.8 (C-4″), 128.0 (C-4″), 128.5 (C-3″, C-5″), 128.6 (C-3″, C-5″), 131.7 (C-1′), 136.1 (C-1″), 136.8 (C-1″), 148.3 (C-3′), 149.6 (C-4′), 155.5 (C-9), 157.8 (C-7), 190.9 (C-4).

Debenzylation of protected flavanone (**14**). Hexamethyldisilane (100 μl) was added to I_2 (0.21 mmol, 0.053 g) in a flame-dried flask fitted with a condenser under N_2. The mixt. was heated at about 55° for 2 hr until a colourless soln was formed [22]. The protected flavanone (**14**) (0.05 mmole, 26.2 mg) in dry CCl_4 was then added at room temp. and the mixt. stirred for 24 hr while maintaining the temp. of the reaction between 30–35°. The reaction was quenched with MeOH and then concd under red pres. The residue was dissolved in EtOAc and washed with $NaHSO_3$ and H_2O, then dried. Evapn of the solvent and initial HPLC purification (RP C-18 column/10% H_2O–MeOH) resulted in partially purified frs. Re-chromatography (silica gel/70% EtOAc–petrol) yielded first the monodebenzylated product (2 mg, 10%) and then the fully deprotected products (6 mg, 38%) as a mixt. of the flavanone (**5**) and its chalcone isomer (**7**). Compounds **5** and **7** were sepd by reversed-phase HPLC using MeOH–H_2O (11:9) as eluent. The UV, MS, ^1H NMR and ^{13}C NMR spectra of the synthetic **5** and **7** were identical to those of the natural products.

Acknowledgements—We thank Dr Peter Oelrichs for supplying the milled plant material and IDP Education Australia for providing a Ph.D. fellowship award to C.A.C.

REFERENCES

1. Everist, S. L., *Poisonous Plants of Australia*, 2nd ed. Angus and Robertson, Sydney, 1981, p. 166.
2. Lewis, I. A. S., MacLeod, J. K. and Oelrichs. P. B., *Tetrahedron*, 1981, **37**, 4305.
3. MacLeod, J. K., Lewis, I. A. S., Moeller, P. D. R. and Oelrichs, P. B., *Journal of Natural Products*, 1990, **53**, 1256.
4. Klingenberg, M., Appel, M. and Oelrichs, P. B., *FEBS Letters*, 1985, **189**, 245.
5. Calanasan, C. A., Capon, R. J., Gaul, K. L., Klingenberg, M., MacLeod, J. K., Moeller, P. D. R. and Oelrichs, P. B., *Plant-associated Toxins*, ed. S. M. Colegate and P. R., Dorling. CAB International, Wallingford, 1994, p. 238.
6. Seo, S., Tomita, Y. and Tori, K., *Tetrahedron Letters*, 1975, 7.
7. Knight, S. A., *Organic Magnetic Resonance*, 1974, **6**, 603.
8. Archbald, J. P., Fen, M. D., and Roy, A. B., *Carbohydrate Research*, 1981, **93**, 177.
9. Hedin, P. A. and Phillips, V. A., *Journal of Agriculture and Food Chemistry*, 1992, **40**, 607.
10. Mabry, T. J., Markham, K. R. and Thomas, M. B., *The Systematic Identification of Flavanoids*. Springer, New York, 1970, p. 165.
11. Ref. 10, p. 266.
12. Panichpol, K. and Waterman, P. G., *Phytochemistry*, 1978, **17**, 1363.
13. Nabaei-Bidehendi, G. and Bannerjee, N. R., *Journal of the Indian Chemical Society*, 1990, **67**, 43.
14. Begum, S. D. and Parthasarathi, J., *Indian Journal of Chemistry*, 1988, **27B**, 464.
15. Clark-Lewis, J. W. and Porter, L. J., *Australian Journal of Chemistry*, 1972, **25**, 1943.
16. DeAlmeida, M. E. L., Braz Filho, R., Von Bülow, M. V., Corrêa, J. J. L., Gottlieb, O. R. Maia, J.

G. S. and DaSilva, M. S., *Phytochemistry*, 1979, **18**, 1015.
17. Agrawal, P. K. and Bansal, M. C., *Carbon*-13 *NMR of Flavanoids*, ed. P. K. Agrawal. Elsevier, New York, 1989, p. 287.
18. Reference 17 p. 418.
19. Gupta S. R., Varindranath, B. and Seshadri, T. R., *Phytochemistry*, 1971, **10**, 877.
20. Khanna, R. N. and Seshadri, T. R., *Indian Journal of Chemistry*, 1963, **1**, 385.
21. Oelrichs, P. B., Vallely, P. J., MacLeod, J. K. and Lewis, I. A. S., *Journal of Natural Products*, 1980, **43**, 414.
22. Salurai, H., Shirahata, A., Sasaki, K. and Hosomi, A., *Synthesis*, 1979, 740.