# Coumarins from Phebalium tuberculosum ssp. megaphyllum and Phebalium filifolium

Mohammad A. Rashid, Alexander I. Gray, Peter G. Waterman, and James A. Armstrong

*J. Nat. Prod.*, **1992**, 55 (7), 851-858• DOI: 10.1021/np50085a003 • Publication Date (Web): 01 July 2004

*Journal of Natural Products*
*Vol. 55, No. 7, pp. 851-858, July 1992*



# COUMARINS FROM *PHEBALIUM TUBERCULOSUM* SSP. *MEGAPHYLLUM* AND *PHEBALIUM FILIFOLIUM*

MOHAMMAD A. RASHID, ALEXANDER I. GRAY, PETER G. WATERMAN,*

*Phytochemistry Research Laboratories, Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow G1 1XW, Scotland, UK*

and JAMES A. ARMSTRONG

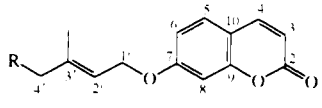
*Western Australian Herbarium, Department of Conservation and Land Management, PO Box 104, Como, Western Australia 6152*

ABSTRACT.—A total of 14 coumarins have been isolated from the aerial parts of *Phebalium tuberculosum* ssp. *megaphyllum* and 9 from *Phebalium filifolium* (Rutaceae). Three of the coumarins obtained from *P. tuberculosum* ssp. *megaphyllum* are novel and have been characterized, on the basis of spectroscopic analysis, as (*E*)-7-(6-hydroperoxy-3,7-dimethylocta-2,7-dienyloxy)coumarin [**3**], (*E*)-8-(6-hydroperoxy-3,7-dimethylocta-2,7-dienyloxy)psoralen [**16**] and (*E,E*)-8-(7-hydroxy-3,7-dimethylocta-2,5-dienyloxy)psoralen [**15**] In addition, both species yielded the simple acetophenone xanthoxylin, and *P. tuberculosum* ssp. *megaphyllum* gave (*E*)-betulin-3-*p*-coumarate [**20**] and (*Z*)-betulin-3-*p*-coumarate [**21**], both of which appear to be novel. The chemotaxonomic implications of coumarin distribution in the two species are discussed.

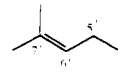
As part of our continuing investigations of the coumarins of *Phebalium* (Rutaceae) (1) we now report on two further species of West Australian origin, *Phebalium tuberculosum* ssp. *megaphyllum* P.G. Wilson and *Phebalium filifolium* Turcz. (2). A previous study (3) has recorded the presence of a single coumarin in each species; xanthotoxin [**11**] from *P. filifolium* and phebalosin [**7**] from *P. tuberculosum*.

## RESULTS AND DISCUSSION

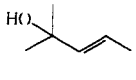
The concentrated petroleum ether and EtOAc extracts from the aerial parts of *P. tuberculosum* ssp. *megaphyllum* were subjected to vacuum liquid chromatography (vlc) with increasingly polar solvent mixtures. Each fraction was initially examined by tlc, and those containing mixtures of fluorescent compounds were further purified by preparative tlc. This resulted in the isolation of the simple acetophenone xanthoxylin [**19**], and a total of 14 coumarins. Of the coumarins, aurapten [**1**] (1), (*E,E*)-7-(7-hydroxy-3,7-dimethylocta-2,5-dienyloxy)coumarin [**2**] (1), osthol [**4**] (4), (+)-phebalosin [**7**]



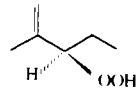
**1** R=

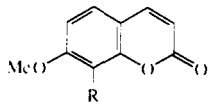


**2** R=

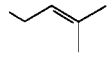


**3** R=

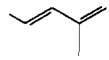




**4** R=

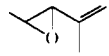


**5** R=

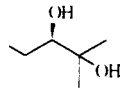


**6** R=

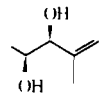
**7** R=



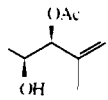
**8** R=



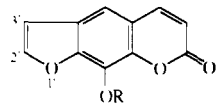
**9** R=



**10** R=

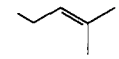


**17** R=

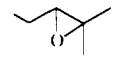


**11** R=

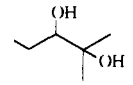
**12** R=



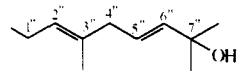
**13** R=



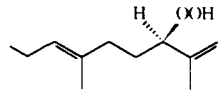
**14** R=



**15** R=



**16** R=



(1), (−)-(*R,R*)-murrangatin [**9**] (5,6), and imperatorin [**12**] (7) were identified by direct comparison with samples isolated previously in our laboratories. Five others, (*E*)-dehydroosthol [**5**] (5), (*Z*)-dehydroosthol [**6**] (5), (*S*)-meranzin hydrate [**8**] (5,8), (−)-(*R,R*)-murrangatin-2′-acetate [**10**] (1,5,6), and luvangetin [**18**] (9) gave spectral data in accord with those published in the literature.

Three further coumarins isolated from the petroleum ether extract appear to be novel. The most polar gave a highest fragment in the eims at *m/z* 314 $[C_{19}H_{22}O_4]^+$ with intense fragments at *m/z* 151 $[C_{10}H_{15}O]^+$ and 162 $[C_9H_6O_3]^+$ indicating an oxygenated geranyloxy-type and coumarin units, respectively. The $^1$H-nmr spectrum showed aromatic resonances for a 7-substituted coumarin. For the C-7 substituent, typical signals were observed for O-$CH_2$-CH=C(Me) and isopropenyl systems in the geranyloxy moiety. The remaining signals were attributable to two adjacent methylene groups and an oxymethine proton at δ 4.31. A NOESY spectrum revealed three strong interactions in the geranyloxy unit: (i) between the 1′-methylene protons and the 3′-Me, establishing a cis relationship between them; (ii) between one of the H-8′ protons (δ 5.03) and the 7′-Me, and (iii) between the other 8′-proton (δ 5.01) and the oxymethine proton, thereby establishing the position of oxygenation at C-6′.

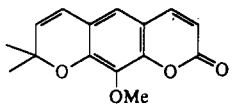
The $^{13}$C-nmr spectrum (Table 1) showed the anticipated resonances for a 7-oxygenated coumarin. However, when the chemical shifts for the geranyloxy substituent were compared with those for **22**, recently isolated from *Eriostemon tomentellus* (10), a 13.3 ppm downfield shift was observed for C-6′. This suggested that the C-6′ substituent must be a hydroperoxy moiety that underwent facile loss of oxygen in the eims to give a hydroxygeranyl fragment. The presence of the peroxide was confirmed by the reactivity of the coumarin with a ferrous thiocyanate solution (11) and by the occurrence of an $[M + 1]^+$ at 331 in the fabms. This allowed the assignment of structure **3** for the isolated compound.

The other two new coumarins were both readily characterized as furocoumarins with eims fragmentation revealing a major ion at *m/z* 202 $[C_{11}H_6O_4]^+$ for the furocoumarin nucleus. In the more polar of these, another fragment at *m/z* 151 could be attributed to the oxygenated geranyloxy substituent; in the second compound, this was observed at *m/z* 153. The linear annelation of both the furocoumarins was established by the uv spectrum (12) and by long range coupling between H-5 and H-2′. On this basis the two coumarins must carry a geranyloxy-type function on C-8.

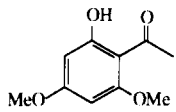
The $^1$H-nmr and $^{13}$C-nmr (Table 1) spectra of the more polar of the furocoumarins revealed the side chain to be identical to that of **3**, leading to the assignment of structure **16**. The side chain for the second furocoumarin was identical from C-1′ to C-3′, but thereafter the $^1$H-nmr spectrum revealed appreciable differences. Equivalent geminal Me groups resonating at δ 1.32 were indicative of an isopropanol terminal group. The remaining proton resonances indicated a trans-substituted double bond and a methylene. Placement of the methylene at C-4′ was established by an nOe interaction of the C-7′ methyls and the olefinic doublet, leading to structure **15** for the new

TABLE 1. $^{13}$C-nmr Chemical Shift Values for Coumarins **3**, **22**, and **16**.

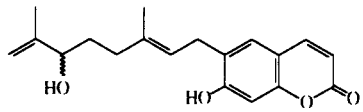
| Carbon | Compound | | |
|---|---|---|---|
| | **3** | **22** | **16** |
| C-2 | 162.5 | 162.2 | 160.8 |
| C-3 | 113.3 | 112.3 | 115.0 |
| C-4 | 143.7 | 144.1 | 144.6 |
| C-5 | 128.9 | 128.4 | 113.2 |
| C-6 | 113.5 | 125.6 | 126.1 |
| C-7 | 161.5 | 158.5 | 148.9 |
| C-8 | 101.8 | 103.1 | 131.6 |
| C-9 | 156.0 | 154.2 | 143.7 |
| C-10 | 112.7 | 112.2 | 116.7 |
| Furan ring | | | |
| C-2 | | | 146.9 |
| C-3 | | | 107.0 |
| Side chain | | | |
| C-1 | 65.6 | 28.6 | 70.3 |
| C-2 | 119.3 | 121.7 | 120.4 |
| C-3 | 141.8 | 137.9 | 142.4 |
| 3-Me | 16.9 | 16.2 | 16.7 |
| C-4 | 35.6 | 35.8 | 35.6 |
| C-5 | 28.9 | 32.9 | 28.8 |
| C-6 | 89.2 | 75.9 | 89.1 |
| C-7 | 143.6 | 147.1 | 144.1 |
| C-8 | 114.7 | 111.3 | 114.6 |
| 7-Me | 17.4 | 16.7 | 17.3 |



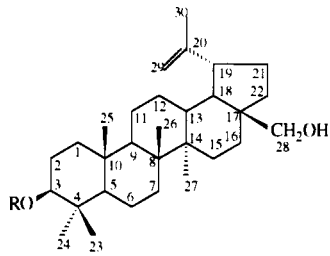
18



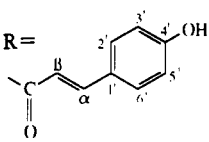
19



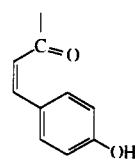
22



20 R=



21 R=



furocoumarin. Compound **15** failed to react with ferrous thiocyanate, ruling out the possibility that it was the corresponding hydroperoxide.

The 6′-hydroxygeranyl side chain corresponding to the 6′-hydroperoxygeranyl side chain identified in **3** and **16** has been synthesized with known *R* chirality (13) in a coumarin otherwise corresponding in structure to **3** and is reported to exhibit positive optical rotation. By contrast both **3** and **16** exhibit negative optical activity, indicating their occurrence in the *S* configuration.

In addition, the EtOAc extract yielded two triterpenes. These both analyzed, by hreims, for *m/z* 588 ($C_{39}H_{56}O_4$) and underwent hydrolysis with 2% KOH in MeOH to give a compound identical to an authentic sample of betulin. The distinction between the two compounds was, therefore, restricted to the esterifying group at C-3. This was readily resolved by $^1$H nmr, which revealed signals attributable to the *E* and *Z* forms of *p*-coumaric acid, leading to the formulation of structures **20** and **21**. These appear to be novel.

A comparable analysis of *P. filifolium* yielded **19** and seven coumarins from the petroleum ether extract. The latter were identified as **1–3**, **7**, and **18**, also obtained from *P. tuberculosum* ssp. *megaphyllum*, plus the furocoumarins xanthotoxin [**11**] (14) and heraclenin [**13**] (15), and the C-8 prenylated coumarin, murralongin [**17**] (14), all of which were identified by comparison with previously isolated material. From the EtOAc fraction, a further furocoumarin, heraclenol [**14**] (16), was isolated.

A recent paper (1) discussing the chemotaxonomic value of coumarins in *Phebalium* highlighted the ability of the genus to produce three groups of compounds: (a) 7-geranyloxycoumarins, (b) 8-prenylated coumarins, and (c) linear furocoumarins. Using a cladistic analysis of coumarin distribution (J.A. Armstrong and P.G. Waterman, unpublished) there was general agreement with the sub-generic classification of Wilson (2) but with some exceptions.

Both of the species studied here exhibit the ability to produce all three of these classes of coumarins, a feature not currently shared by any other species examined. However, if the relative levels of production are considered (Table 2) then *P. tuberculosum* ssp. *megaphyllum* obviously emphasizes the production of prenylated coumarins (type b, about 95%), while *P. filifolium* emphasizes, to a lesser extent, furocoumarins (type c, about 60%). It is unclear if this variation in emphasis will necessitate modification of the way in which coumarin distribution is interpreted for chemotaxonomic purposes. If further studies suggest that all three pathways operate widely but to varying degrees, then it may be necessary to consider some weighting factor so that major metabolic pathways are emphasized in future cladistic analyses.

TABLE 2. Relative Quantities of Coumarins (expressed as percent of total) Isolated from Aerial Parts of *Phebalium tuberculosum* ssp. *megaphyllum* and *Phebalium filifolium*.

| Compound | Source | |
|---|---|---|
| | *P. tuberculosum* ssp. *megaphyllum* | *P. filifolium* |
| **1** | 1.9 | 12.7 |
| **2** | 0.5 | 2.0 |
| **3** | 0.5 | 4.7 |
| **4** | 0.5 | |
| **5** | 0.3 | |
| **6** | 1.7 | |
| **7** | 88.2 | 12.0 |
| **8** | 0.5 | |
| **9** | 3.1 | |
| **10** | 0.3 | |
| **11** | | 56.7 |
| **12** | 0.7 | |
| **13** | | 2.7 |
| **14** | | 1.3 |
| **15** | 0.7 | |
| **16** | 0.7 | |
| **17** | | 6.7 |
| **18** | 0.3 | 1.3 |

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All nmr experiments were run on a Bruker AMX400 instrument using $CDCl_3$ as solvent. Assignments of and homonuclear and heteronuclear correlation between nmr signals were achieved using standard Bruker programs: COSY-45, NOESY (0.8 sec mixing time), HMQC and HMBC (with delay set for $^2J$, $^3J$ of 7 Hz). Eims were obtained using an AEI-MS-902 double focussing spectrometer with direct probe insert, operating at 70 eV and elevated temperature. Fabms were recorded in the positive mode with glycerol as matrix. Petroleum ether was the bp 60–80° fraction. Preparative tlc was performed using Si gel (0.5 mm plates).

PLANT MATERIAL.—*P. tuberculosum* ssp. *megaphyllum* was collected 1 km southwest of Barbalin Rock, 13 km due southwest of Mukinbudin, Western Australia on 15 December 1989, and has been deposited at the Western Australia Herbarium, Perth under the accession number PERTH 01185373. *P. filifolium* was collected at Dragon Rock Nature Reserve, Buettners Road, 4 km south of Jilakin Flat Rock Road, Western Australia on 18 December 1989, and is deposited as PERTH 01185365.

EXTRACTION AND ISOLATION OF COMPOUNDS FROM *P. TUBERCULOSUM* SPP. *ME. GAPHYLLUM*.—Dried, ground, aerial parts (250 g) were extracted in a Soxhlet extractor sequentially with petroleum ether, EtOAc, and MeOH. The petroleum ether extract was concentrated, and pigments were removed by cc using Sephadex LH-20 eluting with $CHCl_3$-MeOH (9:1, then 4:1). The eluent was concentrated and subjected to vlc over Si gel eluting with solvent mixtures of increasing polarity. Elution with 10–15% EtOAc in petroleum ether gave **19** (675 mg), and 20% EtOAc in petroleum ether gave two bands, the less polar of which was purified by preparative tlc [toluene-EtOAc (8:1)] to give **1** (11 mg). Preparative tlc of the more polar [toluene-EtOAc (4:1), 3 developments] gave **4** (3 mg), **2** (3 mg), and **3** (3 mg). Elution with 25–30% EtOAc in petroleum ether gave a mixture of three compounds, which were separated by preparative tlc on Si gel [toluene-EtOAc (8:1)]. Final purification of each band required further preparative tlc and gave **5** [2 mg after preparative tlc with toluene-EtOAc (19:1), 2 developments], **6** [10 mg after preparative tlc with toluene-EtOAc (9:1), 3 developments], and **16** [4 mg after preparative tlc with toluene-EtOAc (4:1), 2 developments]. Elution with 35–40% EtOAc in petroleum ether yielded a mixture separated by preparative tlc [petroleum ether-EtOAc (9:1), 5 developments] to give **15** (4 mg), **12** (4 mg), and **10** (2 mg). Elution with 50% EtOAc gave a single compound purified by preparative tlc [petroleum ether-EtOAc (9:1), 5 developments] to give **18** (2 mg). Elution with 60% EtOAc gave **7** (515 mg).

Identical treatment of the EtOAc extract gave, from 50% EtOAc in petroleum ether, a mixture that was separated by preparative tlc [$CHCl_3$-EtOAc-HOAc (75:25:1), 2 developments] to give **21** (6 mg) and **20** (25 mg). From 20% MeOH in EtOAc with subsequent preparative tlc [toluene-EtOAc-HOAc (7:3:1)], **9** (18 mg) and **8** (3 mg) were obtained.

EXTRACTION AND ISOLATION OF COMPOUNDS FROM *P. FILIFOLIUM*.—Dried, ground, aerial parts (200 g) were treated as described for *P. tuberculosum* ssp. *megaphyllum*. From vlc of the petroleum ether extract, **19** (2 mg) was obtained by elution with 10% EtOAc in petroleum ether. Elution with 15–20% EtOAc followed by preparative tlc [toluene-EtOAc (9:1)] gave **1** (19 mg), **3** (7 mg), and **2** (3 mg). The 30–40% EtOAc eluate, on concentration, gave **11** (76 mg). Preparative tlc of the supernatant [toluene-EtOAc (9:1), 2 developments] gave **11** (9 mg) and two further bands. The less polar of these after preparative tlc [petroleum ether-EtOAc (19:1), 7 developments] gave **18** (2 mg). The more polar, after preparative tlc [petroleum ether-EtOAc (8:3), 3 developments] gave **13** (4 mg) and **7** (6 mg). Elution with 45% EtOAc gave **7** (12 mg), and elution with 60–75% EtOAc gave a mixture from which **17** (10 mg) was obtained by preparative tlc with toluene-EtOAc (8:3) (2 developments) followed by $CHCl_3$-MeOH (98:2).

Identical treatment of the EtOAc extract yielded only **14** (2 mg) by elution from vlc with 50% EtOAc in petroleum ether followed by preparative tlc [toluene-EtOAc (8:2)].

KNOWN COMPOUNDS.—Aurapten [**1**] (1), (*E,E*)-7-(7-hydroxy-3,7-dimethylocta-2,5-dienyloxy) coumarin [**2**] (1), osthol [**4**] (4), (+)-phebalosin [**7**] (1), (−)-(*R,R*)-murrangatin [**9**] (6), (−)-(*R,R*)-murrangatin-2′-acetate [**10**] (5,6), xanthotoxin [**11**] (14), imperatorin [**12**] (7), heraclenin [**13**] (15), murralongin [**17**] (14), and xanthoxylin [**19**] (17) were characterized by direct comparison with authentic samples available at the Strathclyde laboratory.

*(E)-Dehydroosthol* [**5**].—Gum: *m/z* 242.0949 (calcd for $C_{15}H_{14}O_3$ 242.0943). Uv, ir, $^1$H nmr, and eims in agreement with literature (5).

*(Z)-Dehydroosthol* [**6**].—Gum: *m/z* 242.0919 (calcd for $C_{15}H_{14}O_3$ 242.0943). Uv, ir, $^1$H nmr, and eims in agreement with literature (5).

*Meranzin hydrate* [**8**].—Gum: *m/z* 278.1172 (calcd for $C_{15}H_{18}O_5$ 278.1154); $[\alpha]_D$ −25° ($c$ = 0.3, $CHCl_3$). Uv, ir, $^1$H nmr, eims, and optical rotation in agreement with literature (5,8).

*Heraclenol* [**14**].—Gum: $[\alpha]_D$ +11° ($c$ = 0.08 $CHCl_3$). Uv, ir, $^1$H nmr, eims, and optical rotation in agreement with literature (16).

*Luvangetin* [**18**].—Gum: *m/z* 258.0891 (calcd for $C_{15}H_{14}O_4$ 258.0892). Uv, ir, $^1$H nmr, eims, and optical rotation in agreement with literature (9).

(*E*)-7-(6-HYDROPEROXY-3,7-DIMETHYL-2,7-DIENYLOXY)COUMARIN [**3**].—Gum: $[\alpha]_D$ −11° ($c$ = 0.15, $CHCl_3$); uv λ max (log ε) (EtOH) 250 (3.45), 294 (3.97), 322 (4.18); ir ν max (film) 3380, 1725, 1610, 1550, 1500, 1400, 1350, 1280, 1230, 1125, 1000, 900, 830; $^1$H nmr δ 1.65 (2H, m, H-5′), 1.74 (3H, br s, 7′-Me), 1.77 (3H, d, $J$ = 1.4 Hz, 3′-Me), 2.14 (2H, m, H-4′), 4.31 (1H, t, $J$ = 6.7

Hz, H-6′), 4.61 (2H, d, $J$ = 6.6 Hz, H-1′), 5.01 (1H, m, H-8′), 5.03 (1H, m, H-8′), 5.50 (1H, tq, $J$ = 6.6, 1.4 Hz, H-2′), 6.81 (1H, d, $J$ = 2.4 Hz, H-8), 6.84 (1H, dd, $J$ = 8.5, 2.4 Hz, H-6), 7.37 (1H, d, $J$ = 8.5 Hz, H-5), 6.25, 7.64 (2H, ABq, $J$ = 9.5 Hz, H-3, H-4), 7.92 (1H, br s, 6′-OOH); $^{13}$C nmr see Table 1; fabms $[M + 1]^+$ 331; eims *m/z* (rel. int.) $[M - O]^+$ 314 (3), 162 (100), 151 (48), 134 (46), 69 (75).

(*E*)-8-(6-HYDROPEROXY-3,7-DIMETHYLOCTA-2,7-DIENYLOXY)PSORALEN [**16**].—Gum: $[\alpha]_D$ −13° ($c$ = 0.3, $CHCl_3$); uv λ max (log ε) (EtOH) 241 (4.20), 249 (4.27), 260 (4.05), 299 (3.98); ir ν max (film) 3390, 3140, 3120, 3065, 1725, 1620, 1585, 1540, 1465, 1440, 1290, 1215, 1180, 1150, 1100, 1030, 990, 905, 875, 825, 750; $^1$H nmr δ 1.53 (1H, m, H-5″), 1.65 (1H, ddd, $J$ = 16.0, 7.8, 6.7 Hz, H-5″), 1.70 (3H, d, $J$ = 1.3 Hz, 3″-Me), 1.72 (3H, br s, 7″-Me), 2.07 (2H, br t, $J$ = 7.8 Hz, H-4″), 4.24 (1H, t, $J$ = 6.7 Hz, H-6″), 4.95 (1H, m, H-8″), 5.00 (1H, m, H-8″), 5.04 (2H, d, $J$ = 7.1 Hz, H-1″), 5.65 (1H, tq, $J$ = 7.1, 1.3 Hz, H-2″), 6.37, 7.78 (2H, ABq, $J$ = 9.6 Hz, H-3, H-4), 6.83, 7.70 (2H, ABq, $J$ = 2.2 Hz, H-3′, H-2′), 7.37 (1H, s, H-5), 7.98 (1H, s, 6″-OOH); $^{13}$C nmr see Table 1; fabms $[M + Na]^+$ 393, $[M + H]^+$ 371; eims *m/z* (rel. int.) 202 (100), 174 (34), 151 (56), 69 (33).

(*E,E*)-8-(7-HYDROXY-3,7-DIMETHYLOCTA-2,5-DIENYLOXY)PSORALEN [**15**].—Gum: uv λ max (log ε) (EtOH) 241 (4.29), 247 (4.31), 262 (4.10), 298 (4.01); ir ν max (film) 3400, 3150, 3100, 1725, 1620, 1585, 1465, 1400, 1325, 1290, 1215, 1130, 1095, 1030, 990, 875, 825, 750; $^1$H nmr δ 1.32 (6H, s, 7″-$Me_2$), 1.67 (3H, d, $J$ = 1.4 Hz, 3″-Me), 2.74 (2H, br d, $J$ = 6.0 Hz, H-4″), 5.03 (2H, d, $J$ = 7.1 Hz, H-1″), 5.52 (1H, d, $J$ = 15.9 Hz, H-6″), 5.57 (1H, dt, $J$ = 15.9, 6.0 Hz, H-5″), 5.65 (1H, tq, $J$ = 7.1, 1.4 Hz, H-2″), 6.39, 7.78 (2H, ABq, $J$ = 9.6 Hz, H-3, H-4), 6.83, 7.71 (2H, ABq, $J$ = 2.2 Hz, H-3′, H-2′), 7.39 (1H, s, H-5), 7.56 (1H, s, 7″-OH); fabms $[M + Na]^+$ 377; eims *m/z* (rel. int.) 202 (100), 174 (41), 135 (21).

(*E*)-BETULIN-3β-*p*-COUMARATE [**20**].—Gum: $[\alpha]_D$ +29° ($c$ = 0.8, $CHCl_3$); uv λ max (log ε) (EtOH) 256 (3.75), 302 (4.39), 314 (4.44) (+NaOH) 312, 368; ir ν max (KBr) 3350, 2940, 1680, 1605, 1585, 1510, 1450, 1375, 1280, 1170, 1100, 1015, 980, 910, 885, 830, 760; $^1$H nmr (250 MHz, $C_5D_5N$) δ 0.76, 0.96, 0.99, 1.00, 1.08 (5 × 3H, 5 × s, 5 × Me), 1.83 (3H, br s, 30-Me), 2.65 (1H, dt, $J$ = 10.9, 6.3 Hz, H-19), 3.70, 4.12 (2H, ABq, $J$ = 10.5 Hz, H-28), 4.77 (1H, br s, H-29), 4.90 (1H, br s, H-29), 4.91 (1H, dd, $J$ = 9.6, 6.5 Hz, H-3), 6.74, 8.08 (2H, ABq, $J$ = 15.9 Hz, H-β, H-α), 7.21, 7.69 (4H, AA′BB′, $J$ = 8.6 Hz, H-3′/5′, H-2′/6′); (400 MHz, $CDCl_3$) δ 0.88 (3H, s, Me-25), 0.89 (3H, s, Me-23), 0.92 (3H, s, Me-24), 0.99 (3H, s, Me-26), 1.04 (3H, s, Me-27), 1.71 (3H, br s, Me-30), 2.39 (1H, dt, $J$ = 10.9, 6.4 Hz, H-19), 3.36, 3.82 (2H, ABq, $J$ = 10.5 Hz, H-28), 4.58 (1H, br s, H-29), 4.59 (1H, dd, $J$ = 9.5, 6.5 Hz, H-3), 4.69 (1H, br s, H-29), 6.28, 7.61 (2H, ABq, $J$ = 15.9 Hz, H-β, H-α), 6.87, 7.42 (4H, AA′BB′, $J$ = 8.6 Hz, H-3′/5′, H-2′/6′); $^{13}$C nmr (100.6 MHz) ppm s at 167.7 (C=O), 159.3 (C-4′), 150.7 (C-20), 126.7 (C-1′), 48.0 (C-17), 42.9 (C-14), 41.2 (C-8), 38.3 (C-4), 37.3 (C-10), d at 144.5 (C-α), 130.1 (C-2′/6′), 116.3 (C-β), 115.8 (C-3′/5′), 81.0 (C-3), 55.6 (C-5), 50.5 (C-9), 49.0 (C-18), 48.0 (C-19), 37.3 (C-13), t at 109.9 (C-29), 60.8 (C-28), 38.6 (C-1), 34.4 (C-22), 34.2 (C-7), 30.0 (C-21), 29.3 (C-16), 27.3, (C-15), 25.4 (C-12), 24.0 (C-2), 21.1 (C-11), 18.4 (C-6), q at 28.2 (C-23), 19.3 (C-30), 16.9 (C-24), 16.4 (C-25), 16.2 (C-26), 15.0 (C-27); eims *m/z* (rel. int.) 588.4209 (calcd for $C_{39}H_{56}O_4$ 588.4178) (19), 557 (13), 424 (19), 234 (6), 203 (29), 189 (62), 147 (100), 119 (22), 107 (23).

(*Z*)-BETULIN-3-*p*-COUMARATE [**21**].—Gum: uv and ir identical to those of **20**; $^1$H nmr (250 MHz, $CDCl_3$) δ 0.80, 0.85, 0.86, 0.99, 1.03 (5 × 3H, 5 × s, 5 × Me), 1.69 (3H, br s, 30-Me), 2.39 (1H, dt, $J$ = 10.9, 6.3 Hz, H-19), 3.35, 3.81 (2H, ABq, $J$ = 10.5 Hz, H-28), 4.53 (1H, dd, $J$ = 10.8, 5.3 Hz, H-3), 4.59 (1H, br s, H-29), 4.68 (1H, br s, H-29), 5.84, 6.84 (2H, ABq, $J$ = 12.7 Hz, H-β, H-α), 6.80, 7.68 (4H, AA′BB′, $J$ = 8.8 Hz, H-3′/5′, H-2′/6′); eims *m/z* (rel. int.) 588.4169 (calcd for $C_{39}H_{56}O_4$ 588.4178) (12), 557 (8), 424 (16), 203 (17), 189 (43), 147 (100), 119 (22), 107 (14).

## ACKNOWLEDGMENTS

One of us (M.A.R.) thanks the Association of Commonwealth Universities for the award of a scholarship. Nmr studies were performed at the Strathclyde University NMR Laboratory. Fabms were obtained through the S.E.R.C. mass spectroscopy service at University College, Swansea. Dr. B.R. Maslin, Western Australia Herbarium, Perth, is thanked for making the collections of plant materials used in this study.

## LITERATURE CITED

1. A.M. Quader, J.A. El-Turbi, J.A. Armstrong, A.I. Gray, and P.G. Waterman, *Phytochemistry*, in press.
2. P.G. Wilson, *Nuytsia*, **1**, 4 (1970).
3. P.W. Chow, A.M. Duffield, and P.R. Jefferies, *Aust. J. Chem.*, **19**, 483 (1966).

4. M.A. Quader, A.I. Gray, T.G. Hartley, and P.G. Waterman, *Biochem. Syst. Ecol.*, **19**, 91 (1991).
5. C. Ito and H. Furukawa, *Chem. Pharm. Bull.*, **35**, 4277 (1987).
6. C. Ito, H. Furukawa, H. Ishii, T. Ishikawa, and J. Haginawa, *J. Chem. Soc., Perkin Trans. 1*, 2047 (1990).
7. P.G. Waterman, *Phytochemistry*, **15**, 578 (1976).
8. P. Ceccherelli, M. Curini, M.C. Marcotullio, G. Madruzza, and A. Menghini, *J. Nat. Prod.*, **52**, 888 (1989).
9. J. Hlubucek, E. Ritchie, and W.C. Taylor, *Aust. J. Chem.*, **24**, 2347 (1971).
10. M.A. Rashid, J.A. Armstrong, A.I. Gray, and P.G. Waterman, *Z. Naturforsch.*, **476**, 284 (1992).
11. E. Merck, "Dyeing Reagents for Thin Layer and Paper Chromatography," Darmstadt, Germany, 1974, p. 55.
12. B.E. Nielsen and J. Lemnich, *Acta Chem. Scand.*, **23**, 962 (1969).
13. M. Aziz and F. Rouessac, *Tetrahedron*, **44**, 101 (1988).
14. F. Bevalot, J.A. Armstrong, A.I. Gray, and P.G. Waterman, *Biochem. Syst. Ecol.*, **16**, 631 (1988).
15. M.A. Rashid, J.A. Armstrong, A.I. Gray, and P.G. Waterman, *J. Nat. Prod.*, **54**, 667 (1992).
16. S. Harkarc, T.K. Razdan, and E.S. Waight, *Phytochemistry*, **23**, 426 (1984).
17. M.A. Quader, J.A. Armstrong, A.I. Gray, T.G. Hartley, and P.G. Waterman, *Biochem. Syst. Ecol.*, **19**, 171 (1990).

*Received 12 August 1991*