Phytochemistry, Vol. 34, No. 3, pp. 813–818, 1993
Printed in Great Britain.

0031–9422/93 $6.00 + 0.00
Pergamon Press Ltd

TWO CHROMENES AND A PRENYLATED BENZOIC ACID DERIVATIVE FROM *PIPER ADUNCUM*

JIMMY ORJALA, C. A. J. ERDELMEIER,* ANTHONY D. WRIGHT, TOPUL RALI† and OTTO STICHER

Department of Pharmacy, Swiss Federal Institute of Technology (ETH) Zurich, CH-8092 Zürich Switzerland; †Department of Chemistry, University of Papua New Guinea, Port Moresby, Papua New Guinea

(*Received* 18 *January* 1993)

Key Word Index—*Piper aduncum*; Piperaceae; leaves; chromene derivatives; prenylated benzoic acids; antimicrobial; molluscicidal.

Abstract—In addition to stigmasterol, piperiton, methyl 2,2-dimethyl-2H-chromene-6-carboxylate, methyl 3-(2-hydroxy-3-methyl-3-butenyl)-4-hydroxy-benzoate and methyl (6*S*)-2-*trans*-6-hydroxy-2,6-dimethyl-2,7-octadienoate, three new natural products were isolated from *Piper aduncum* and characterized as methyl 8-hydroxy-2,2-dimethyl-2H-chromene-6-carboxylate, 2,2-dimethyl-8-(3-methyl-2-butenyl)-2H-chromene-6-carboxylic acid and methyl 3-(6-hydroxy-3,7-dimethyl-2,7-octadienyl)-4-methoxy-benzoate. The structures of all isolates were elucidated by spectroscopic methods, mainly 1D and 2D NMR spectroscopy. The antibacterial, antifungal and molluscicidal activities of the isolates were also investigated.

INTRODUCTION

Piper aduncum L. is a small tree commonly found in Papua New Guinea (P.N.G.). Villagers from the coastal areas of the Morobe Province of P.N.G. use this plant to heal wounds [1]. In earlier investigations of *P. aduncum* phenylpropanoids, like myristicin and dillapiol, benzoic acid derivatives, flavonoids and terpenes were reported [2–4]. Some of these metabolites were also found to exhibit antibacterial activities [5]. In our study on biologically active metabolites derived from plants which are employed in the traditional medicine of P.N.G., we are currently investigating the leaves of *P. aduncum*.

The crude petrol extract of the leaves from *P. aduncum* showed, in *in vitro* biological screening, significant antibacterial activity against *Bacillus subtilis*, *Micrococcus luteus* and *Escherichia coli* as well as antifungal activity against *Penicillium oxalicum*. Molluscicidal potential against *Biomphalaria glabrata* was also detected.

We report on the isolation and structural elucidation of six bioactive molecules from *P. aduncum*, i.e. two new chromene derivatives (**1**, **2**) and a new benzoic acid derivative (**3**) as well as the known compounds **4**–**6**, together with an assessment of their antimicrobial and molluscicidal potential.

RESULTS AND DISCUSSION

Compound **1** showed a molecular ion in the EI mass spectrum at *m/z* 234, corresponding to the molecular formula of $C_{13}H_{14}O_4$. The IR spectrum showed the presence of ester (1703 cm^{-1}), hydroxyl (3380 cm^{-1}) and aromatic (1590, 1480 cm^{-1}) moieties, while the UV spectrum showed three absorption maxima at 254, 277 and 326 nm (log ε 4.38, 3.76 and 3.22) indicating the aromatic character of **1**.

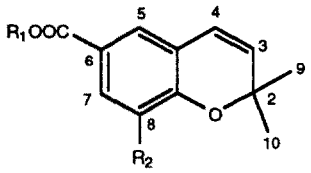
The ^1H NMR spectrum of **1** revealed a set of two *meta*-coupled protons (δ7.48 *d* and 7.32 *d*, $J = 1.8$ Hz), which implied the presence of a 1,3,4,5-substituted benzene ring, two olefinic protons (an AB system at δ5.66 and 6.35 with $J_{A,B} = 9.9$ Hz), a *gem*-dimethyl group attached to an oxygen-bearing carbon (6H, δ1.49 *s*), a sharp methyl singlet at δ3.88 belonging to a methyl ester and an exchangeable resonance (δ5.46, *s*), which implied a phenolic hydroxyl.

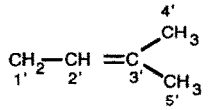
Methylation of **1** gave the monomethoxyl derivate **1b**, which lacked hydroxyl absorptions in the ^1H NMR spectrum, but gave an additional methoxyl resonance (δ3.90, *s*), hence confirming the nature of the hydroxyl function.

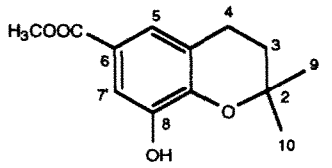
The five delineated molecular fragments were then associated from the results of a 2D NOESY measurement made with **1** and **1b**. Thus the NOE observed in **1** between H_3-9 and H-3, as well as between H_3-10 and H-3 implied the *gem*-dimethyl function to be allylic. Further, the C-4 to C-10 fragment could be positioned at C-3 on the basis of the NOE observed between H-4 and H-5. The final diagnostic NOE was between the methoxyl group and H-7 in **1b**, thus fixing the methoxyl function in **1b** at C-8 and consequently also the hydroxyl group in **1**. The ester group must then reside at C-6 and an ether bridge exists between C-2 and C-9, as consistent with the ^{13}C NMR data.

To conclusively prove these deductions, **1** was synthesized as outlined in the Experimental. The intermediates **1c** and **1d** in this synthesis were both fully

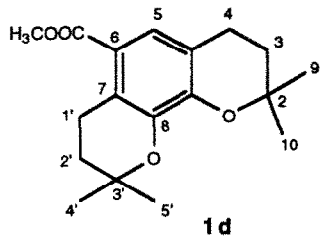
*Present address: Dr Willmar Schwabe Arzneimittel, Research and Development, D(W)-7500 Karlsruhe, F.R.G.



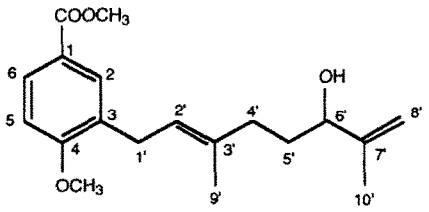
	R_1	R_2
1	CH_3	OH
1b	CH_3	OCH_3
2	H	
4	CH_3	H



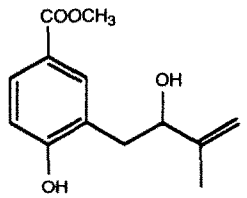
1c



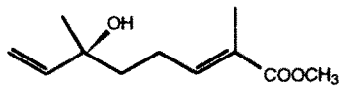
1d



3



5



6

characterized by ^1H, ^{13}CNMR and mass spectrometry. Compound **1** is methyl 8-hydroxy-2,2-dimethyl-2H-chromene-6-carboxylate.

Compound **2** had the molecular formula $C_{17}H_{20}O_3$ by mass spectrometry. The IR spectrum of **2** showed, as the only major difference to **1**, the presence of an aromatic acid carbonyl group (1680 cm^{-1}).

The ^1H and ^{13}CNMR spectra of **2** (Tables 1 and 2) exhibited enough characteristic features of **1** to suggest it was a C-8 substituted carboxylic acid derivative of **1**. The nature of the substituent at C-8 was deduced from a single ^1H–^1H double resonance experiment. Thus, the signal at δ3.28 (H_2-1′) collapsed to a singlet upon irradiation of the proton absorbing at δ5.27 (H-2′), the signals at δ1.73 (H_3-4′ and H_3-5′) thereby losing line-broadening.

A 2D NOESY measurement made with **2** confirmed the position of the γ,γ-dimethyl allyl side chain at C-8. The key NOE being from H_2-1′ to H-7, thus **2** is 2,2-dimethyl-8-(3-methyl-2-butenyl)-2H-chromene-6-carboxylic acid.

The molecular formula of **3**, $C_{19}H_{26}O_4$, was established by means of EI-mass spectrometry and ^{13}CNMR spectroscopy. In its IR spectrum, absorptions for hydroxyl (3400 cm^{-1}), ester (1710 cm^{-1}) and aromatic ring (1600, 1490 cm^{-1}) functions were present, while the UV spectrum showed an absorption maximum at 255 nm (log ε 3.89) confirming the aromatic character of **3**.

The ^1HNMR spectrum of **3** contained a set of three coupled aromatic resonances (δ6.85, *d*, 1H; 7.83, *d*, 1H; 7.88, *dd*, 1H; J_{ortho} = 8.5 Hz, J_{meta} = 1.8 Hz), for a 1,3,4-substituted aryl ring, and two signals at δ3.88 (3H) and 3.89 (3H) for an aryl methoxyl group and an aryl methyl ester function, which were also evident in the ^{13}CNMR spectrum (55.5 and 51.8). The ^1HNMR spectrum of **3**

Table 1. ^{1}H NMR (300 MHz, $CDCl_3$) data of compounds **1**, **2**, **1b**, **1c** and **1d**

Proton (s) at carbon	1	2	1b	1c	1d
3	5.66 *d* (9.9)	5.63 *d* (9.8)	5.65 *d* (9.9)	1.82 *t* (6.7)	1.79 *t* (6.9)
4	6.35 *d* (9.9)	6.33 *d* (9.8)	6.33 *d* (9.9)	2.78 *t* (6.7)	2.75 *t* (6.9)
5	7.33 *d* (1.8)	7.59 *br s*	7.37 *d* (1.9)	7.38[a] *m*	7.34 *s*
7	7.48 *d* (1.8)	7.74 *br s*	7.45 *d* (1.9)	7.40[a] *m*	—
9+10	1.49 *s*	1.44 *s*	1.50 *s*	1.37 *s*	1.36 *s*
1′	—	3.28 *d* (7.3)	—	—	3.08 *t* (6.9)
2′	—	5.27 *t* (7.3)	—	—	1.77 *t* (6.9)
4′+5′	—	1.73 *s*	—	—	1.34 *s*
COOMe	3.88 *s*	—	3.88 *s*	3.85 *s*	3.83 *s*
OMe	—	—	3.90 *s*	—	—
OH	5.46 *s*	—	—	5.68	—

[a]Assignments interchangeable.

Table 2. ^{13}C NMR data (75.5 MHz, $CDCl_3$) of compounds **1**, **2**, **1b**, **1c** and **1d**

C	1	2	1b	1c	1d
2	78.5 *s*	77.2 *s*	77.6 *s*	76.6 *s*	75.0[a] *s*
3	130.8 *d*	130.7 *d*	131.0 *d*	21.9 *t*	21.8 *t*
4	122.8 *d*	122.1[a] *d*	121.9 *d*	32.6 *t*	32.5[b] *t*
4a	120.5[b] *s*	120.4[b] *s*	121.2[b] *s*	120.6[b] *s*	119.4[d] *s*
5	119.9 *d*	126.6 *d*	121.0 *d*	122.7 *d*	123.6 *d*
6	122.8[b] *s*	121.6[b] *s*	122.0[b] *s*	121.5[b] *s*	121.4[d] *s*
7	116.3 *d*	131.7 *d*	113.1 *d*	113.1 *d*	118.3[d] *s*
8	143.4[a] *s*	129.3 *s*	148.0[a] *q*	144.8[a] *s*	147.6[c] *s*
8a	144.1[a] *s*	155.4 *s*	146.3[a] *s*	145.2[a] *s*	143.7[c] *s*
9+10	28.3 *q*×2	28.3 *q*×2	28.2 *q*×2	26.8 *q*×2	26.7 *q*×2
1′	—	28.2 *t*	—	—	22.3 *t*
2′	—	122.0[a] *d*	—	—	32.7[b] *t*
3′	—	132.5 *s*	—	—	73.5[a] *s*
4′	—	25.8 *q*	—	—	26.5 *q*
5′	—	17.9 *q*	—	—	26.5 *q*
COOR	166.8 *s*	171.2 *s*	166.9 *s*	167.1 *s*	167.8 *s*
COOMe	51.9 *q*	—	51.9 *q*	51.8 *q*	51.4 *q*
OMe	—	—	56.3 *q*	—	—

[a–d]Assignments interchangeable.

contained a further set of coupled spins for a hydroxylated monoterpene side chain, whose structure could be determined from the ^{1}H–^{1}H and ^{1}H–^{13}C (one bond, $J = 136$ Hz) COSY spectra. Thus, the methylene protons at C-1′ (δ3.33) coupled to the olefinic proton at C-2′ (δ5.35) which in turn showed an allylic coupling to the protons of the C-4′ methylene group (δ2.11) and to the C-9′ methyl group (δ1.72). The two protons of the C-4′ methylene group further coupled to the methylene protons at C-5′ (δ1.67), which in turn coupled to the oxygen-bearing methine proton at C-6′ (δ4.06). The latter proton displayed allylic coupling to one of the C-8′ *exo*-methylene protons (δ4.93). Both C-8′ *exo*-methylene protons (δ4.82, 4.93) showed allylic coupling to the protons of the C-10′ methyl group (δ1.72), thus establishing the side chain to be 6-hydroxy-3,7-dimethyl-2,7-octadienyl.

The presence of a 6-hydroxy-3,7-dimethyl-2,7-octadienyl chain was also supported by the major ion at [M $-139]^+$ ($M-C_9H_{15}O$) in the EI mass spectrum of **3**. The stereochemistry of the $\Delta^{2',3'}$ double bond was established as (*E*) on the basis of the ^{13}C NMR shifts of the vinyl methyl group (C-9′) [6].

With the basic fragments of **3** established, the connectivity between them required solution. From the results of a 2D-NOESY experiment, it was evident that the 6-hydroxy-3,7-dimethyl-2,7-octadienyl, methoxyl and methyl ester functions had the regiochemical relationship as shown in **3**. The diagnostic NOEs being from H_2-1′ to H-2 and the methoxyl group, and from the methoxyl group to H-5, clearly indicating the methoxyl group to be adjacent to H-5 and the 6-hydroxy-3,7-dimethyl-2,7-octadienyl side chain. The methyl ester function must therefore be at C-1. Confirmation of the above structural deductions came from comparison of the ^{1}H and ^{13}C NMR data with those of compound **5** and with those published for methyl 3-(3,7-dimethyl-2,6-octadienyl)-

4-methoxy-benzoate [7]. Compound **3** is methyl 3-(6-hydroxy-3,7-dimethyl-2,7-octadienyl)-4-methoxy-benzoate.

Together with the new compounds the three known metabolites **4**, methyl 2,2-dimethyl-2H-chromene-6-carboxylate [8], **5**, methyl 3-(2-hydroxy-3-methyl-3-butenyl)-4-hydroxy-benzoate [8] and **6**, (6*S*)-2-*trans*-6-hydroxy-2,6-dimethyl-2,7-octadienoate [9, 10] were also isolated. Compounds **4** and **5** were reported from *Piper hostmannianum* (Piperaceae) [8], while **6** was reported from *Gymnocladus chinensis* (Leguminosae) and *Artemisia santolinifolia* (Compositae) [9, 10]. This is, however, the first report of compound **6** from Piperaceae.

The isolates **1**–**6** were tested for their biological activity against the bacteria *Bacillus subtilis*, *Micrococcus luteus* and *Escherichia coli*, and the fungus *Penicillium oxalicum* using a TLC bioassay [11]. Further the molluscicidal effect against *Biomphalaria glabrata* was evaluated. Minimum growth inhibition concentrations on TLC as well as the lethal concentration are given in Table 3.

The biological activities reported here suggest that the topical application of *P. aduncum* leaves will have a beneficial effect on infected wounds and the antimicrobial activity of these metabolites also support the traditional use as a remedy for wounds.

EXPERIMENTAL

General. Mps: uncorr; UV: MeOH; IR: KBr or film; optical rotations: MeOH; EIMS 70 eV; ^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz): $CDCl_3$ using TMS or solvent (δ 7.26 resp. 77.0) as int. standard.

Separation. All solvents were of analyt. quality. Silica gel (Merck) and RP-18-material (Baker) for VLC had a particle size of 40–63 μm resp. average particle size of 40 μm. MPLC sepn was carried out using Büchi MPLC columns 80 cm × 4.9 cm (column A), 46 × 3.6 cm (column B), and 46 cm × 2.6 cm (column C). The columns were drypacked with TLC silica gel HF 254 (Merck), particle size 15 μm. HPLC sepns were performed on a Spherisorb S5 ODS II, 5 μm, 250 × 16 mm column with UV detection at 254 nm and Lichrosorb Si 60, 5 μm, 250 × 8 mm with UV detection at 340 nm.

Plant material. The plant material was collected near Gawam village, Morobe Province of P.N.G., during September, 1988 [12]. Herbarium specimens are deposited at Herbarium ZT, ETH, Zurich, Switzerland, as well as at UPNG Herbarium, Port Moresby, P.N.G. and at the National Herbarium in Lae, P.N.G.

Extraction and isolation. Air-dried and powdered leaves (1.55 kg) were percolated with petrol at room temp. Removal of the solvent under red. pres. furnished a resinous mass (91.0 g, 5.9%). The extract was divided into 5 parts and each part was subjected to VLC (RP-18 material, 40 g) using a step gradient of MeOH–H_2O (3:2, 4:1, 10:0) to give three frs, A (1.5 g), B (48.3 g), and C (15.2 g). Frs A and B were worked up as follows: Fr. A was subjected to VLC (silica gel, 50 g) using a hexane–EtOAc step gradient to afford 4 frs (A1–A4). From fr. A3, **5** (7.2 mg) was isolated by RP 18 HPLC (MeOH–H_2O, 3:2). Fr. B was subjected to VLC (silica gel, 150 g) using a hexane–EtOAc step gradient to yield 5 frs (B1–B5). Fr. B2 was further fractionated by MPLC (silica gel, column A) giving 8 frs (B2.1–B2.8), the mobile phase being EtOAc–hexane (1:9). Fr. B2.2 was further purified by HPLC on RP18 material using a mixt. of MeOH–H_2O (9:1) as eluent, to give **4** (8.5 mg). Fr. B2.4 yielded piperiton. Fr. B3 was further fractionated into 10 frs (B3.1–B3.10) by MPLC (silica gel, column B), the mobile phase was Me_2CO–hexane (1:9). Fr. B3.3 was further purified by HPLC on RP18 material using MeOH–H_2O (4:1) as eluent, to give **1** (20.4 mg). Fr. 3.4 gave stigmasterol upon addition of Me_2CO. Fr. B3.7 was further purified by HPLC on RP18 material using a mixt. of MeOH–H_2O (7:3) as eluent, to afford **2** (10.7 mg). Fr. B4 was further fractionated by MPLC (silica gel, column B) yielding 9 frs (B4.1–B4.9). The mobile phase being Me_2CO–hexane (1:9). Fr. B4.3 gave **6** (15.3 mg) and fr. B4.5 yielded **3** (6.3 mg), both using prep. TLC with toluene–EtOAc (9:1) as an eluent.

Bioassay procedures. The bioautographic assays were carried out as previously described [11]. Test organisms were *B. subtilis* (ATCC 6633), *M. luteus* (ATCC 9341), *E. coli* (ATCC 25922) and *P. oxalicum* (Table 3). The screening for molluscicidal potential was carried out as previously described [13], with the modification that the

Table 3. Biological activity of isolates **1**–**6**

	Compound							
Organism	**1**	**2**	**3**	**4**	**5**	**6**	CA	M.NO_3
*E. coli**	8.5	—	—	—	—	—	0.15	NT
*M. luteus**	8.5	3.2	10.8	—	6.6	—	0.10	NT
*B. subtilis**	8.5	2.0	10.8	—	13.0	—	0.10	NT
*P. oxalicum**	17.0	15.5	—	—	—	—	NT	1.66
B. glabrata†	30	30	NT	NT	‡	‡	NT	NT

*Minimum growth inhibition concentration in nmol on TLC.

†100% lethal concentration in ppm.

‡ = No activity at 35 ppm.

CA = Chloramphenicol; M.NO_3 = miconazole NO_3; — = no inhibition at 20 nmol; NT = not tested.

samples were first dissolved in 100 μl of EtOH and then diluted to 100 ml with distilled H_2O. The test organism was *Biomphalaria glabrata.*

Methyl 8-hydroxy-2,2-dimethyl-2H-chromene-6-carboxylate (**1**) (20.4 mg, 0.0013%). Amorphous solid; mp 94°; UV λ_{max}^{MeOH} nm (log ε): 254 nm (4.38), 277 nm sh (3.76), 326 nm (3.22); IR ν_{max}^{KBr} cm^{-1}: 3380, 1708, 1590, 1480, 1438, 1322, 1204; EIMS *m/z* (rel.int.): 234 $[M]^+$ (14), 219 (100), 174 (8), 160 (19), 129 (17), 115 (7), 103 (9), 91 (10), 77 (15); ^1H NMR: Table 1, ^{13}C NMR: Table 2.

Methylation of **1** (4.2 mg) with CH_2N_2 afforded **1b** (4.3 mg, 97%).

Methyl 8-methoxy-2,2-dimethyl-2H-chromene-6-carboxylate (**1b**). Clear oil; UV λ_{max}^{MeOH} nm (log ε): 290 nm (3.48), 249 nm (4.18), 243 nm (4.16); IR ν_{max}^{KBr} cm^{-1}: 2940, 1710, 1365, 1305, 1200, 1090; EI-MS *m/z* (rel.int.): 248 $[M]^+$ (29), 233 $[M-Me]^+$ (100), 218 (7), 174 (6), 129 (8), 77 (8); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Total synthesis of **1**. Methyl 3,4-dihydroxybenzoate was obtained by dissolving 4 g 3,4-dihydroxybenzoic acid (Fluka, purum) in 10 ml MeOH (Fluka p.a), adding conc. H_2SO_4 (1 ml) and heating the resultant soln under reflux for 5 hr. Aq. work-up followed by VLC (silica gel, EtOAc–hexane step-gradient), gave 3.5 g of the methyl ester. The ester (1.5 g) was dissolved in toluene and condensed with isoprene (Fluka purum) as described elsewhere [14]. The condensation with isoprene gave a mixt. of 2 products in a ratio of 2:1, which were sepd by MPLC (silica gel, column C) using a EtOAc–hexane gradient. The more polar product **1c** (600 mg), methyl 8-hydroxy-2,2-dimethylchroman-6-carboxylate, showed the introduction and condensation of one isoprene moiety, while the least polar compound **1d** (350 mg), methyl 2,2,9,9-tetramethyltetrahydropyrano[3,2-h] chroman-6-carboxylate, showed the introduction of two isoprene units. Compound **1c** (100 mg) was dehydrogenated with DDQ in C_6H_6, as described [8]. The resulting product (15 mg) was purified by HPLC (silica gel) using Me_2CO–hexane (1:4) as eluent. The synthetic product showed identical chemical and spectroscopic data as the isolated compound **1**.

Methyl 8-hydroxy-2,2-dimethylchroman-6-carboxylate (**1c**). Crystalline solid; mp 95°; UV λ_{max}^{MeOH} nm (log ε): 298 nm (3.77), 268 nm (4.05); IR ν_{max}^{KBr} cm^{-1}: 3400 br, 2970, 1700, 1590, 1490, 1370, 1330, 1200, 1120; EI-MS *m/z* (rel.int.): 236 $[M]^+$ (14), 221 (7), 205 (24), 189 (11), 181 (100), 149 (17); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Methyl 2,2,9,9-tetramethyltetrahydropyrano[3,2-h] chroman-6-carboxylate (**1d**). Amorphous solid; mp 125°; UV λ_{max}^{MeOH} nm (log ε): 301 nm (3.90), 274 nm (3.96), 232 nm (4.21); IR ν_{max}^{film} cm^{-1}: 2960, 1705, 1560, 1210, 1155, 1115, 990; EI-MS *m/z* (rel.int.): 304 $[M]^+$ (100), 272 (33), 261 (21), 249 (100), 216 (26), 205 (39), 193 (35); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

2,2-Dimethyl-8-(3-methyl-2-butenyl)-2H-chromene-6-carboxylic acid (**2**) (10.7 mg, 0.0007%). Clear oil; UV λ_{max}^{MeOH} nm (log ε): 315 nm (3.40), 277 nm (3.48), 239 nm (4.26); IR ν_{max}^{film} cm^{-1}: 3600–2600, 2920, 1680, 1600, 1410, 1280, 1250, 1200, 1120; EI-MS *m/z* (rel.int.): 272 $[M]^+$ (14), 257 $[M-15]^+$ (100), 227 (2), 197 (3), 128 (4), 115 (4); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Methyl 3-(6-hydroxy-3,7-dimethyl-2,7-octadienyl)-4-methoxy-benzoate (**3**) (6.3 mg, 0.0004%). Clear oil; $[\alpha]_D^{20}$: −10.0° (MeOH; *c* 0.23); UV λ_{max}^{MeOH} nm (log ε): 285 nm sh (3.37), 257 nm (3.89); IR ν_{max}^{film} cm^{-1}: 3400, 2920, 1710, 1600, 1490, 1435, 1295, 1265, 1250, 1120; EI-MS *m/z* (rel.int.): 318 $[M]^+$ (3), 300 (6), 286 (21), 232 (16), 179 (100), 161 (17), 149 (15), 121 (24); ^1H NMR ($CDCl_3$, 300 MHz): δ 1.67 (2H, *m*, H-5′), 1.72 (6H, *s*, H-9′ and H-10′), 2.11 (2H, *t*, *J* = 6.9 Hz, H-4′), 3.33 (2H, *d*, *J* = 7.3 Hz, H-1′), 3.87 (3H, *s*, COOMe), 3.88 (3H, *s*, OMe), 4.06 (1H, *t*, *J* = 6.2 Hz, H-6′), 4.82 (1H, *s*, Ha-8′), 4.93 (1H, *s*, Hb-8′), 5.35 (1H, *t*, *J* = 7.4 Hz, H-2′), 6.85 (1H, *d*, *J* = 8.5 Hz, H-5), 7.83 (1H, *d*, *J* = 1.8 Hz, H-2), 7.88 (1H, *dd*, *J* = 1.8, 8.5 Hz, H-6); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 16.0* (*q*, C-9′), 17.7* (*q*, C-10′), 28.3 (*t*, C-1′), 33.0 (*t*, C-5′), 35.8 (*t*, C-4′), 51.8 (*q*, COOMe), 55.5(*q*, OMe), 75.5 (*d*, C-6′), 109.5 (*d*, C-5), 110.9 (*t*, C-8′), 122.2 (*s*, C-1), 122.3 (*d*, C-2′), 129.4 (*d*, C-6), 129.9 (*s*, C-3), 130.8 (*d*, C-5), 136.3 (*s*, C-3′), 147.6 (*s*, C-7′), 161.1 (*t*, C-4), 167.2 (*s*, COOMe). *Assignments may be interchanged.

Methyl 2,2-dimethyl-2H-chromene-6-carboxylate (**4**) (8.5 mg, 0.0005%). Spectroscopic and chemical data are identical with those previously reported [8].

Methyl 3-(2-hydroxy-3-methyl-3-butenyl)-4-hydroxybenzoate (**5**) (7.2 mg, 0.0005%). $[\alpha]_D^{20}$: +4.8° (MeOH; *c* 0.29); spectroscopic and chemical data are identical with those previously reported [8].

Methyl (6S)-2-trans-6-*hydroxy-2,6-dimethyl-2,7-octadienoate* (**6**) (15.3 mg, 0.0010%). $[\alpha]_D^{20}$: +1.4° (MeOH; *c* 2.01); Spectroscopic and chemical data are identical with those previously reported [9, 10].

Acknowledgements—This research was supported by the Swiss National Science Foundation. We are indebted to Dr M. Baltisberger (ETH Zurich) for the preparation of the herbarium specimens and for his assistance with collection of plant materials. Our thanks are also due to Mr P. Katik (National Herbarium, Lae) for his valuable help concerning botanical matters. We are also grateful to Mr R. Häfliger and Mr O. Greter (ETH Zurich) for recording the mass spectra and to Dr E. Zass (ETH Zurich) for performing literature searches.

REFERENCES

1. Holdsworth, D. and Damasc, F. (1986) *Int. J. Crude Drug Res.* **24**, 217.
2. Dias, P., Maldonado, E. and Ospina, E. (1984) *Rev. Latinoam. Quim.* **15**, 136.
3. Jairo, C. A. (1983) *Rev. Colomb. Cienc. Quim. Farm.* **1**, 47.
4. Burke, B. and Nair, M. (1986) *Phytochemistry* **25**, 1427.
5. Nair, M. and Burke, B. (1990) *J. Agric. Food Chem.* **38**, 1093.
6. De Haan, J. W. and van de Ven, L. J. M. (1973) *Org. Magn. Reson.* **5**, 147.

7. Orjala, J., Erdelmeier, C. A. J., Wright, A. D., Rali, T. and Sticher, O. (1993) *Planta Med.* (in press).
8. Diaz, P. P., Arias, T. and Nathan, P. J. (1987) *Phytochemistry* **26**, 809.
9. Konoshima, T. and Sawada, T. (1985) *Chem. Pharm. Bull.* **32**, 2617.
10. Banjerjee, S., Grenz, M., Jakupovic, J. and Bohlmann, F. (1985) *Planta Med.* **39**, 177.
11. Baumgartner, B., Erdelmeier, C. A. J., Wright, A. D., Rali, T. and Sticher, O. (1990) *Phytochemistry* **29**, 3327.
12. Baltisberger, M., Erdelmeier, C. A. J. and Rali, T. (1989) *Ber. Geobot. Inst. ETH, Stiftung Rübel, Zürich* **55**, 252.
13. Hostettmann, K., Kizu, H. and Tomimori, T. (1982) *Planta Med.* **44**, 34.
14. Ahluwalia, V. K., Jolly, R. S. and Tehim, A. K. (1982) *Tetrahedron* **38**, 3673.