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PRENYLATED FLAVONES AND PHENYLPROPANOID DERIVATIVES FROM ROOTS OF *DORSTENIA PSILURUS*

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Key Word Index—*Dorstenia psilurus*; Moraceae; roots; prenylated flavones; phenylpropanoid derivatives; psoralen; benzofuran; ferulate.

Abstract—Two new flavones, dorsilurins A and B, and a new benzofuran derivative have been isolated from *Dorstenia psilurus*, together with three known phenylpropanoid derivatives, stearyl-*p*-coumarate [octadecanyl 3-(4-hydroxyphenyl)prop-2-enoate], stearyl ferulate [octadecanyl 3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate] and psoralen. The structures of new compounds were determined from spectroscopic data. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Dorstenia psilurus is a small herb reaching 80 cm in height and 7 cm in girth; it grows in the tropical rain forest zone in west, east and central Africa [1]. It has many applications in traditional medicine; a decoction of leaves and roots is used to treat rheumatism, snakebites, headache and stomach disorders [2]. *Dorstenia* species are known to exhibit light-activated antimicrobial [3] and photobiocidal [4] activities. The methanol extract of *D. psilurus* is active against some mediators of inflammation (prostaglandins and platelet activating factor). However, the genus *Dorstenia* is relatively unstudied with only four out of the 170 taxa [5] cited in the chemical literature. Furocoumarins [3, 4, 6], styrenes [7], terpenoids [7, 8] and acetogenic acids [9] have been recorded from this genus. No previous phytochemical and pharmacological studies have been reported on *D. psilurus*. As part of our continuing studies on Cameroonian plants of medicinal interest, we have examined the *n*-hexane and ethyl acetate extracts of the roots of this species. In this report, we describe the isolation and characterization of two novel flavones, named dorsilurin A (**1a**) and B (**2**), a new benzofuran derivative (**4**), the known phenyl propanoid derivatives, psoralen (**5**) [10] stearyl-*p*-coumarate (**6**) [11], stearyl ferulate (**7**) [12], and the common sterol β-sitosterol and its glucoside.

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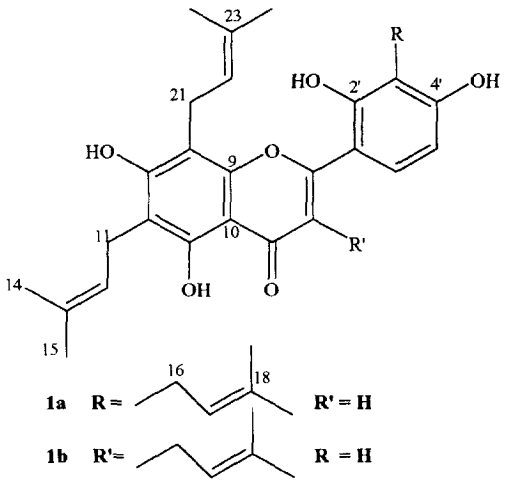
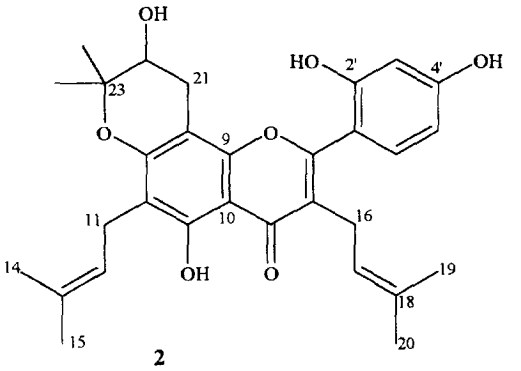
RESULTS AND DISCUSSION

Dorsilurin A was isolated as an oil and its molecular formula determined as $C_{30}H_{34}O_6$ from NMR and mass spectral data. Colour tests with magnesium and concentrated hydrochloric acid (pink), together with the NMR and UV spectral data (see Experimental), indicated that it was a 5-hydroxyflavone [13]. The bathochromic shift in the UV spectrum of dorsilurin A induced by sodium acetate and aluminum chloride are consistent with its formulation as a 5,7-dihydroxyflavone [13]. The chelated 5-hydroxyl proton appeared at δ 13.03. Since the aromatic region of the ^{1}H NMR spectrum displayed only three proton resonance signals, dorsilurin A was a highly substituted flavone. It was possible to conclude at this stage that there were four hydroxyl and three prenyl (see below) groups. One of the signals in the aromatic region appeared as a sharp singlet at δ 6.53, which was appropriate for a proton located at H-3. It was, therefore, assumed that ring A was fully substituted with two hydroxyls at positions 5 and 7 and two prenyl groups at 6 and 8. The two aryl protons observed in the ^{1}H NMR spectrum were *ortho* coupled forming an AB-system at δ 7.16 and 6.51 (J = 8.2 Hz) and these had to be in ring B, together with the remaining substituents (one prenyl and two hydroxyl groups). The signals that could be assigned to the three prenyl groups were as follows: three overlapping olefinic proton signals at δ 5.16 (*m*, 3H), three methylenes protons at δ 3.40 (*brd*, J = 6.9 Hz, 4H) and at δ 3.14 (*brd*, J = 6.5 Hz, 2H) and six olefinic methyl protons at δ 1.83, 1.75, 1.69, 1.68, 1.60 and 1.41. The proposed





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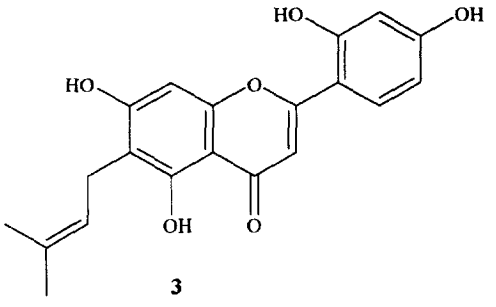

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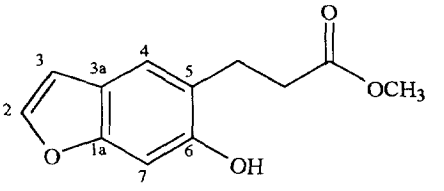

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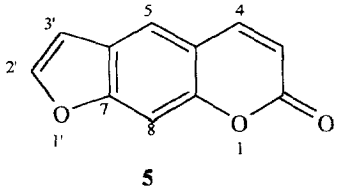

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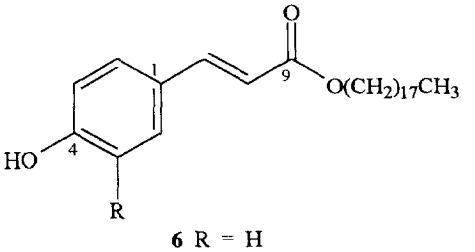

6 R = H
7 R = OMe

structure of dorsilurin A as **1a** was further confirmed by ^{13}C NMR data (see Experimetal), which was fully assigned using DEPT spectra and by comparison of measured values with those reported for similar compounds [14]. Dorsilurin A (**1a**) is reported here for the first time, while its isomer, artelasticin (**1b**) has recently been isolated from *Artocarpus elasticus* [14]. Not surprisingly, dorsilurin A is a labile compound. A chloroform solution of **1a**, when examined by NMR after a few days, showed at least four compounds, presumed to be cyclization products.

Dorsilurin B, the second novel flavone (**2**) was assumed to have the molecular formula $C_{30}H_{34}O_7$ on the basis of ^{13}C DEPT editing exercises. Its EI and CI mass spectra did not show a $[M]^+$. However, under electrospray ionization (ESI) conditions it was possible to observe ions at *m/z* 529 $[M+Na]^+$ and 545 $[M+K]^+$. The UV spectral pattern, including applications of shift reagents (see Experimental), and the characteristic IR absorption at 1640 cm^{-1}, indicated that dorsilurin B was also a 5-hydroxyflavone [13]. The ^1H NMR of this compound showed the presence of two prenyl groups (see below) and only three aryl proton resonances which form an ABX-system, a doublet at δ 7.03 ($J = 8.5$ Hz), an *ortho* and *meta*-coupled double doublet at δ 6.39 ($J = 8.5, 2.2$ Hz) and a *meta*-coupled signal at δ 6.35 ($J = 2.2$ Hz). It was also observed that the 3-position should be substituted by a prenyl group, since a singlet signal was not observed. Irradiation of one of the two CH_2 protons

Table 1. HETCOR spectrum of dorsilurin B (**2**)

Proton	Position	^{1}J-correlated carbon
7.03	6′	132.5
6.39	5′	107.7
6.35	3′	103.7
5.12	12/17	123.8/123.3
3.79	22	69.8
3.39	11	25.9
3.08	16	27.8
2.93	21	22.9
2.58	21	22.9
1.58	14	17.8
1.51	19	17.7

of the prenyl groups at δ 3.08 led to a NOE (3%) enhancement of the doublet at δ 7.03. The above observations are consistent with the location of the ABX-protons on ring B and the doublet signal at δ 7.03 must be due to a proton located at the 6′ position of ring B. It follows from the foregoing, that ring B is disubstituted at positions 2′ and 4′. The proton at 3′ is considerably shielded by the two adjacent hydroxyl groups and gives rise to a signal at δ 6.35. The ^{13}C NMR of dorsilurin B gave additional support to the attachment of a carbon residue and not an oxygen function at C-3, since the chemical shift of the latter was observed at δ 123.5. An oxygen substituent at this position would have led to a signal at ca 139 ± 1, which is not observed [15]. A chelated OH signal was not observed probably because of deuterium exchange with the CD_3OD solvent, but the chemical shift of the carbonyl group at δ 180.3 was consistent with a 5-hydroxyflavone. The ^{1}H NMR spectrum also showed an AMX-sytem [δ 2.58 (*dd*, J = 16.8, 7.6 Hz) 2.93 (*dd*, J = 16.8, 5.8 Hz) and 3.79 (*dd*, J = 7.6, 5.8 Hz)] and two methyl groups that could be assigned to a hydroxydimethylpyran group. Regarding the position of the second prenyl group, two possibilities were considered, one with an angular pyran ring (**2**) or an alternative structure with a linear pyran ring and a prenyl substituent at C-8. The ^{13}C chemical shift of δ 113.8 strongly favours the attachment of the prenyl group at C-6 [15]. The ^{13}C signals were assigned (see Experimental) using the HETCOR data (Table 1) and by comparison of measured values with those reported for **3** isolated from *Moclura pomifera* [16]. Structure **2** is, therefore, assigned to dorsilurin B. Like dorsilurin A, **2** is also quite labile and a chloroform solution of it when freed of solvent gave an orange-red resinous material.

Compound **4** was a viscous, pale brown oil. EI mass spectrometry indicated the $[M]^+$ at m/z 220. The IR spectrum showed bands at 3350 and 1720 cm^{-1}. ^{13}C NMR, DEPT analysis and mass spectrometry led to the formulation of this compound as $C_{12}H_{12}O_4$. Its ^{1}H NMR spectrum showed the signals for a methoxyl group (δ 3.69), a benzylic proton at δ 2.76 coupled to a deshielded methylene at δ 3.00 (t, J = 6.8 Hz), two isolated aromatic protons at δ 7.29 and 7.05 and two furan protons at δ 6.64 (*dd*, J = 2.3, 0.8 Hz) and 7.49 (*d*, J = 2.3 Hz). The above data and comparison with reported ^{13}C data in the literature [7, 17] led to the proposal of a phenolic benzofuran with a side-chain propanoyl ester. The position of the substituents was established by consideration of the inter-ring coupling between the H-3 furan proton [δ 6.64 (*dd*, J = 2.3, 0.8 Hz)] and the upfield proton H-7 at δ 7.05 (*brs*) [7, 18]. The corresponding ethyl ester has been reported by Lin and Kuo from *Psoralea corylifolia* [19]. The spectral data obtained for **4** are almost identical to those reported for the ethyl ester, except for differences due to the change in the esterifying alcohol. It is interesting to speculate whether these esters are true natural products or artefacts resulting from transesterification reactions. Although methanol was not used for extraction of *D. psilurus* in this work, we note that the above workers isolated the ethyl ester after using 60% ethanol as the extracting solvent. Methyl ester **4** and the corresponding ethyl ester have been synthesized from psoralen [19] in two steps by partial hydrogenation and subsequent treatment with a 1:1 mixture of ethanol and methanol. Ester **4** is reported here as a natural product for the first time.

Psoralen (**5**), the cinnamate (**6**) and the ferulate (**7**) were identifed on the basis of physical and spectroscopic data (m.p., IR, UV, NMR and mass spectrometry) and by comparison of acquired data with those reported for the respective compounds in the literature [10, 11, 12]. The ferulic acid ester (**7**) was first reported in 1991 as an oil from *Pavetta owariensis* [12].

It is possible to characterize the plant under study as a rich source of psoralen (ca 0.1%) and β-sitosterol glucoside (ca 0.025%). Kurster *et al.* have observed variations in the relative amounts of psoralen and bergapten in [20, 21] rhizomes of *D. brasiliensis* collected from different regions in Brazil. They also claim that sitosterol and stigmasterol present in the latter may account for the traditional use of the plant for the treatment of snake-bites [20, 22]. The roots of this species are used by Cameroonian women as an antibiotic substitute to avoid post-delivery infections. Although psoralen is described as a potent antibacterial and antifungal agent [10], evidence for attributing such claims to the presence of this compound in *D. psilurus* is however, lacking.

EXPERIMENTAL

General

M.p.s: uncorr. UV–vis: MeOH soln. CIMS and EIMS: direct inlet 70 eV. ESIMS flow injection of an MeCN–MeOH (1:1) soln in 6.25 M TFA buffer, 4.5 kV, needle at 200°. IR: KBr disk. ^{1}H and ^{13}C NMR ($CDCl_3$ or CD_3OD): 300 MHz and 75 MHz, respectively, with residual solvent peak as int. reference.

Plant material

Roots of *D. psilurus* Welwistsch were bought from Mbouda (West Province of Cameroon) market in 1996. A voucher specimen (no. 2109) is deposited at the National Herbarium in Yaounde.

Extraction, isolation and characterization

Finely ground, sun-dried roots (4 kg) were exhaustively extracted with *n*-hexane (8 l) and EtOAc (7 l) in a Soxhlet extractor. Removal of solvents under red. pres. gave 25 g and 50 g, respectively, as two dark brown residues. The *n*-hexane extract (23 g) was applied on 200 g of silica gel column (Merck Kiselgel 60) and eluted with *n*-hexane followed by a *n*-hexane-EtOAc gradient. Frs of 250 ml were collected and these were monitored by TLC. Frs 1–15 (2.4 g) contained mixts of hydrocarbons, which was mainly β-sitosterol. Recrystallization of the combined residues gave (1.5 g) of β-sitosterol. Frs 16–30 (2 g) eluted with *n*-hexane–EtOAc (9:1) was rechromatographed as described above to give octadecanyl ferulate (180 mg) and octadecanyl-4-hydroxycinnamate (80 mg). Recrystallization of combined frs 31–45 (5.3 g, *n*-hexane–EtOAc , 17:3) afforded psoralen (4.2 g). The EtOAc extract (40 g) was chromatographed on a silica gel 60 (300 g) column. Elution was started with $CHCl_3$ and continued stepwise with $CHCl_3$-MeOH mixts and MeOH. Frs were combined on the basis of ^1H NMR or TLC. From the above chromatographic separation and, in some cases, with the aid of successive prep. TLC, β-sitosterol glucoside (600 mg), dorsilurin A (2.8 g), compound **4** (15 mg) and dorsilurin B (20 mg) were obtained. Known compounds were identified by comparison (mp, ^1H and ^{13}C NMR) with authentic samples or published information. β-sitosterol glucoside (100 mg) which was insol. in usual organic solvents was acetylated using boiling Ac_2O (10 ml) for 2 h. The reaction mixt. was evapd in a Petri dish to leave a residue which was chromatographed by CC (*n*-hexane–EtOAc, 3:2) to give white platelets of the tetraacetate of β-sitosterol-3-β-D-glucopyranoside. (80 mg), m.p. 166–7°.

5,7,2′,4′-Tetrahydroxy-6,8,3′-*tri*(3-*methylbut*-2-*enyl*)*flavone-dorsilurin A* (**1a**). Dark orange oil. CI-MS *m/z* (rel. int.): 490 ([M^+] $C_{30}H_{34}O_6$, 100), 446 (56), 378 (32), 334 (34), 188 (21). IR ν_{max}^{KBr} cm^{-1}: 3430–3410 (—OH), 2925, 1645, 1550, 1440, 1360. UV λ_{max}^{MeOH} nm (log ε): 210.0 (5.13), 269.0 (4.83), 301.0 (4.60), 333.5 (4.62); UV $\lambda_{max}^{MeOH+AlCl3}$ nm (log ε): 210.0 (5.14), 274.0 (3.81), 300.6 (4.61), 351.4 (4.58); UV $\lambda_{max}^{MeOH-ALCl3+HCl}$ nm (log ε): 209.4 (5.13), 277.4 (4.81), 300.4 (4.63), 351.6 (4.58); UV $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 219.4 (5.33), 269.6 (4.87), 300.2 (4.62), 350.2 (4.60). ^1H NMR (300 MHz, $CDCl_3$): δ 13.03 (1H, *brs*, OH), 7.16 (1H, *d*, $J = 8.2$ Hz, H-2′), 6.53 (1H, *s*, H-3), 6.51 (1H, *d*, $J = 8.2$ Hz, H-3′), 6.38 (1H, *brs*, —OH), 5.16 (3H, *m*, olefinic protons), 3.40 (4H, *d*, $J = 6.9$ Hz, Ar—CH_2), 3.14 (2H, *d*, $J = 6.5$ Hz, Ar—CH_2), 1.83, 1.75, 1.69, 1.60, 1.68, 1.41 (3H each, *s*, olefinic CH_3).^{13}C NMR (75 MHz, $CDCl_3$): δ 182.8 (*s*, C-4), 160.2, 159.5, 159.3 (*s* each, C-2, 4′ and 7), 156.7, 155.4 (*s* each, C-2′ and 5), 153.5 (*s*, C-9), 135.4, 134.2, 133.1 (*s* each C-13, 18, and 23), 131.6 (*d*, C-6′), 121.5, 121.4, 121.0 (*d* each, C-12, 17 and 22), 120.8 (*s*, C-1′), 112.7 (*s*, C-6), 109.9 (*s*, C-3′), 108.4 (*d*, C-5′), 105.3, 104.8 (*s* each, C-8 and 10), 104.0 (*d*, C-3), 25.8, 25.7, 25.6 (*q* each, $3 \times CH_3$), 24.3 (*t*, C-21), 21.8, 21.7 (*t* each, C-11 and 16), 17.9, 17.8, 17.6 (*q* each $3 \times CH_3$).

Dorsilurin B (**2**). Yellow oil. $[\alpha]_D$ −20° (MeOH, *c* 0.02). ESIMS *m/z* (rel. int.): 529 $[M+Na]^+$ (16), 545 $[M+K]^+$ (12), 491 (100); CIMS *m/z* (rel. int.): 386 (40), 220 (20), 177 (40), 165 (100). UV λ_{max}^{MeOH} nm (log ε): 207.2 (4.95), 262.4 (4.78), 298.8 (4.72), 325.0 (4.65); UV $\lambda_{max}^{MeOH+AlCl3}$ nm (log ε): 207.2 (4.97), 266.8 (4.46), 303.8 (4.52), 345.0 (4.58), UV $\lambda_{max}^{MeOH+AlCl3+HCl}$ nm (log ε): 207.1 (4.96), 267.2 (4.70), 303.6 (4.62), 345.0 (4.57). IR ν_{max}^{KBr} cm^{-1}: 3450–3400, 2900, 1640, 1550, 1255. ^1H NMR (300 MHz, CD_3OD): δ 7.03 (*d*, $J = 8.5$ Hz, H-6′), 6.39 (*dd*, $J = 8.5$, 2.2 Hz, H-5′), 6.35 (*d*, $J = 2.2$ Hz, H-3′), 5.12 (*brt*, $J = 7.0$ Hz, H-12, H-17), 3.79 (*dd*, $J = 7.6$, 5.8 Hz, H-22), 3.39 (*d*, $J = 7.1$ Hz, 2H-11), 3.08 (*d*, $J = 6.6$ Hz, 2H-16), 2.93 (*dd*, $J = 16.8$, 5.8 Hz, H-21), 2.58 (*dd*, $J = 16.8$, 7.6 Hz, H-21), 1.58, 1.51, 1.46, 1.44, 1.37, 1.31 (6 × Me). ^{13}C NMR (75 MHz, CD_3OD): δ 180.3 (*s*, C-4), 161.5, 160.9, 159.0 (*s*, each, C-2, 4′ and 7), 157.8, 157.3 (*s*, each, C-2′ and 5), 152.9 (*s*, C-9), 132.5 (*d*, C-6′), 132.3, 131.9 (*s*, each C-13,18), 123.8, 123.3 (*d*, each C-12, 17), 123.5 (*s*, C-3), 113.8 (*s*, C-6), 108.7, 108.7 (*s*, each, C-1′, 10), 107.7 (*d*, C-5′), 106.3 (*s*, C-8), 103.7 (*d*, C-3′), 79.0 (*s*, C-23), 69.8 (*d*, C-22), 30.7, 20.4 (*q*, each, C-24 and 25), 27.8 (*t*, C-16), 26.0, 25.9 (*q*, each, C-15 and 20), 25.9 (*t*, C-11), 22.9 (*t*, C-21), 17.8, 17.7 (*q*, each, C-14 and 19).

Compound (**4**). Oil. EIMS *m/z* (rel. int.): 220 $[M]^+$ (80), 189 $[M\text{-}OMe]^+$ (16), 188 $[M\text{-}MeOH]^+$ (100), 160 $[M\text{-}MeOH\text{-}CO]^+$ (76), 147 $[M\text{-}CH_2CO_2Me]^+$ (47), 146 $[M\text{-}CH_2CO_2Me\text{-}H]^+$ (55). IR ν_{max}^{KBr} cm^{-1}: 3350, 1720, 1660, 1640, 1600, 1500. ^1H NMR (300 MHz, $CDCl_3$): δ 2.76 (2H, *t*, $J = 6.8$ Hz, Ar-CH_2), 3.00 (2H, *t*, $J = 6.7$ Hz, CO—CH_2), 3.69 (3H, *s*, OCH_3), 6.64 (1H, *dd*, $J = 2.3$, 0.8 Hz, H-3), 7.05 (1H, *brs*, H-7), 7.29 (1H, *s*, H-4), 7.49 (1H, *d*, $J = 2.3$ Hz, H-2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.2 (*s*, C = O), 154.8, 152.2 (*s* each, C-6 and 1a), 144.2 (*d*, C-2), 123.9 (*s*, C-3a), 121.7 (*d*, C-4), 121.1 (*s*, C-5), 106.1 (*d*, C-3), 99.9 (*d*, C-7), 52.4 (*q*, O-CH_3), 35.6 (*t*, CH_2-CO), 24.9 (*t*, ArCH_2).

Psoralen (**5**). White needles, m.p. 163°, lit. 164° [10]. EIMS *m/z* (rel. int.): 186 (100). UV λ_{max}^{MeOH} nm (log ε): 240 (4.18), 290 (3.80), 320 (3.50). IR ν_{max}^{KBr} cm^{-1} 1700, 1620, 1580, 1500. ^1H NMR (300 MHz, $CDCl_3$): δ 6.39 (1H, *d*, $J = 9.8$ Hz, H-3), 6.84 (1H, *dd*, $J = 2.2$, 0.8 Hz, H-3′), 7.49 (1H, *brs*, H-8), 7.70 (1H, *s*, H-5), 7.70 (1H, *d*, $J = 2.3$ Hz, H-2′), 7.81 (1H, *d*, $J = 9.8$ Hz, H-4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.5 (*s*, C-2), 157.1 (*s*, C-7), 152.5 (*s*, C-8a), 147.0 (*d*, C-2′), 144.5 (*d*, C-4), 125.1 (*s*, C-6), 120.0 (*d*, C-5), 115.1 (*s*, C-4a), 114.8 (*d*, C-3), 107.1 (*d*, C3′), 99.6 (*d*, C-8).

Octadecanyl-3[4-*hydroxyphenyl*]-*prop*-2-*enoate*

(**6**). White powder, m.p. 97–9°, lit. 99–100° [11]. CIMS *m/z* (rel int): 416 [M$^+$]. UV λ_{max}^{MeOH} nm (log ε): 203.5 (3.97), 228.0 (3.85), 311.5 (4.12), 424.5 (2.68). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.82 (3H, *t*, J = 6.8 Hz, CH_3), 4.15 (2H, *t*, J = 7.0 Hz, OCH_2), 6.23 (1H, *d*, J = 15.9 Hz, H-α), 6.78 (2H, *brd*, J = 8.6 Hz, H-3, H-5), 7.38 (2H, *brd*, J = 8.6 Hz, H-2, H-6), 7.41 (1H, *brs*, —OH), 7.56 (1H, *d*, J = 15.9 Hz, H-β).

Octadecanyl-3[(4-hydroxy-3-methoxyphenyl)-prop-2-enoate (**7**). White needles, m.p. 110–1°. CIMS *m/z* (rel. int): 446 ([M]$^+$, $C_{28}H_{46}O_4$, 100), 338 (8). UV λ_{max}^{MeOH} nm (log ε): 204 (4.24), 237 (3.95), 324 (4.25). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (3H, *t*, J = 7.0 Hz, CH_3), 1.25 (*brs*, CH_2), 3.93 (3H, *s*, OCH_3), 4.19 (2H, *t*, J = 6.8 Hz, —OCH_2, 5.86 (1H, *brs*, OH), 6.30 (1H, *d*, J = 15.9 Hz, -α), 6.92 (1H, *d*, J = 8.1 Hz, H-5), 7.04 (1H, *d*, J = 2 Hz, H-2), 7.08 (1H, *dd*, J = 8.1, 2.0 Hz, H-6), 7.61 (1H, *d*, J = 15.9 Hz, Hβ). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.9 (*s*, C-9), 145.8, 144.8 (*s,s*, C-3, C-4), 144.6 (*d*, C-7), 128.0 (*s*, C-1), 123.1 (*d*, C-6), 116.8 (*d*, C-8), 114.7 (*d*, C-5), 109.3 (*d*, C-2), 64.7 (*t*, C-1′), 55.0 (*q*, -OMe), 31.0 (*t*, C-2′), 29.8 (*t*, C-3′), 29.4 (*t*, C-4′–16′), 14.2 (*q*, Me).

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