Pergamon

0031-9422(94)00594-X

Phytochemistry, Vol. 38, No. 2, pp. 535–537, 1995

0031-9422/95 $9.50 + 0.00

ISOPRENYLATED FLAVONOLS OF FORMOSAN *BROUSSONETIA PAPYRIFERA*

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(*Received in revised form* 1 *July* 1994)

Key Word Index—*Broussonetia papyrifera*; Moraceae; isoprenylated flavonol; broussoflavonol E; broussoflavonol F.

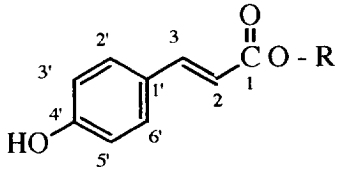
Abstract—Two new isoprenylated flavonols, broussoflavonol E [8,2′,6′-tri-(3,3-dimethylallyl)-5,7,3′,4′-tetrahydroxyflavonol], broussoflavonol F [8,3′-di-(3,3-dimethylallyl)-5,7,4′trihydroxyflavonol]; five known compounds, squalene, octacosan-1-ol, lignoceric acid, 4′-hydroxy-*cis*-cinnamic acid octacosyl ester, and (−)-marmesin; and a mixture of 4′-hydroxy-*trans*-cinnamates were further isolated and characterized from the root bark of Formosan *Broussonetia papyrifera*.

INTRODUCTION

In a previous paper [1], we reported the isolation of a new isoprenylated aurone and a novel isoprenylated flavan, and six known compounds from Formosan *Broussonetia papyrifera* (Moraceae). In continuous work on the same plant, two new isoprenylated flavonols, broussoflavonol E (**1**) [8,2′,6′-tri-(3,3-dimethylallyl)-5,7,3′,4′-tetrahydroxyflavonol], broussoflavonol F (**2**) [8,3′-di-(3,3-dimethylallyl)-5,7,4′-trihydroxyflavonol]; five known compounds, squalene, octacosan-1-ol, lignoceric acid, 4′-hydroxy-*cis*-cinnamaic acid octacosyl ester and (−)-marmesin; and a mixture of 4′-hydroxy-*trans*-cinnamic acid tetracosyl, hexacosyl, and octacosyl esters (**3**), were obtained from the root bark of this plant. In this paper, we report the isolation and structure elucidation of these two new compounds, **1** and **2**, and the mixture of 4′-hydroxy-*trans*-cinnamates (**3**).

RESULTS AND DISCUSSION

Compound **1** showed a positive Mg–HCl test. The IR spectrum of **1** showed the presence of a hydroxy group (3350 cm^{-1}), an aromatic ring (1595 and 1550 cm^{-1}) and a conjugated carbonyl group (1640 cm^{-1}). The UV spectrum showed characteristic absorption maxima at 218, 255, 290 (sh), 305 and 353 nm, resembling that of broussoflavonol C (**4**) [2]. The presence of bathochromic shifts with $AlCl_3$, $AlCl_3$–HCl, NaOAc, NaOAc–H_3BO_3 and NaOMe in the UV spectrum suggested that **1** is a 5,7,3′,4′-tetrahydroxylated flavonol. The EI mass spectrum of **1** showed a molecular ion peak at *m/z* 506 and significant peaks at *m/z* 286, 284 and 221. The EI mass spectrum indicated the presence of an isoprenyl group in the A-ring and two isoprenyl groups in the B-ring [2]. The ^{1}H NMR spectrum of **1** indicated the presence of three 3,3-dimethylallyl groups [δ1.39, 1.49, 1.56, 1.59, 1.68, 1.77 (each 3H, *br s* Me × 6), 3.32 (2H, *d*, *J* = 6.4 Hz), 3.38 (2H, *d*, *J* = 7.3 Hz), 3.48 (2H, *d*, *J* = 6.6 Hz), 5.01 (1H, *t*, *J* = 6.4 Hz) and 5.15 (2H, *m*)], two singlets of aromatic proton signals at δ6.35 and 6.90, and a hydrogen bonded hydroxy group at δ12.22 (1H, *br s*). The above results and a remarkable $AlCl_3$-induced shift in the UV spectrum of **1** clearly indicated that the prenyl groups in the A-ring of **1** were substituted at C-8 [3]. The ^{13}C NMR spectrum of **1** (Table 1) was assigned by ^{1}H-decoupled spectra, DEPT pulse sequence, HMQC and HMBC correlations (Fig. 1) and comparison of chemical shifts with those of corresponding data for broussoflavonol C (**4**) (Table 1). This established that **1** has the proposed structure. The characterization of **1** was also supported by HMBC correlations (Fig. 1). Thus, in the ^{1}H NMR of **1**, signals at δ1.39 (H-15′), 1.49 (H-16′), 1.56 (H-12), 1.59 (H-13), 1.68 (H-10′), 1.77 (H-11′), 3.32 (H-12′), 3.38 (H-9), 3.48 (H-7′), 5.01 (H-13′), 5.15 (H-10 and H-8′), 6.35 (H-6) and 6.90 (H-5′) were assigned.



3 R = $C_{24}H_{49}$ or $C_{26}H_{53}$ or $C_{28}H_{57}$

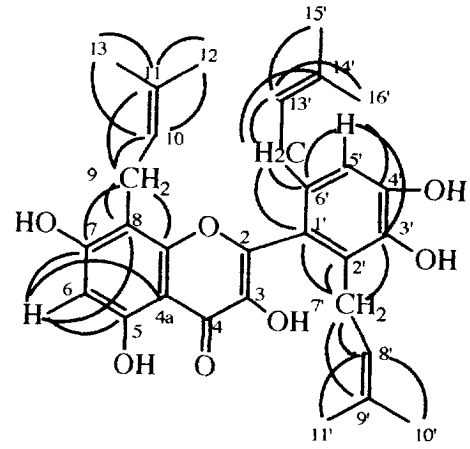
Table 1. ^{13}C NMR spectral data of **1***, **2***, **4** [2] and **5** [4]

Carbon	1†	2†	4‡	5†
C-2	151.4	147.5	147.8	147.0
C-3	137.9	136.8	135.6	136.6
C-4	177.6	177.1	174.9	176.6
C-4a	105.2	104.5	104.7	104.0
C-5	160.5	160.3	158.9	159.0
C-6	99.1	99.2	100.5	111.7
C-7	162.2	162.4	161.1	162.7
C-8	107.5	107.6	109.6	93.8
C-8a	156.2	155.3	155.1	155.6
C-9	22.4	22.6	29.2	21.9
C-10	123.5	123.8	122.9	123.3
C-11	131.8	132.5	132.1	131.7
C-12	17.8	18.5	17.7	17.9
C-13	25.9	26.2	25.4	25.9
C-1′	122.9	124.1	121.4	123.5
C-2′	128.7	130.3	126.4	129.1
C-3′	146.5	129.5	141.5	130.3
C-4′	143.3	158.1	144.0	159.0
C-5′	115.8	116.2	113.8	115.8
C-6′	131.4	128.6	130.8	128.0
C-7′	26.4	29.0	26.2	29.1
C-8′	124.6	123.8	121.1	123.2
C-9′	133.4	133.7	134.2	133.1
C-10′	25.9	18.2	17.9	17.9
C-11′	18.1	26.2	25.6	25.9
C-12′	29.4	—	40.5	—
C-13′	125.4	—	27.4	—
C-14′	132.2	—	28.0	—
C-15′	17.8	—	148.5	—
C-16′	25.6	—	113.1	—

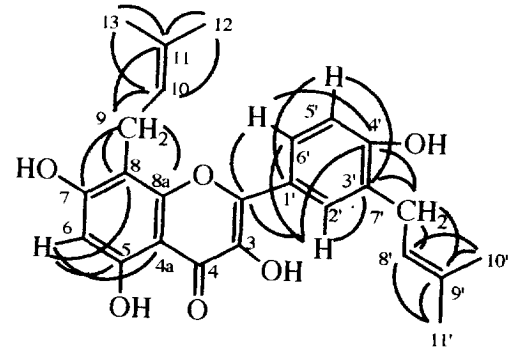
* The number of directly attached protons to each individual carbon was verified with DEPT pulse sequence.

† Measured in $(CD_3)_2CO$.

‡ Measured in $CDCl_3$.



1



2

Fig. 1. HMBC of **1** and **2**.

Compound **2** found as yellow prisms, $C_{25}H_{26}O_6$, has similar UV maxima to glyasperin A (**5**) [4]. The presence of bathochromic shift with $AlCl_3$, NaOAc and NaOMe, but disappearance of bathochromic shift with NaOAc on adding H_3BO_3 in the UV spectrum suggested that **2** has a flavonol skeleton with 4′,5,7-trihydroxyl groups. The IR spectrum of **2** showed the presence of a hydroxy group (3350 cm^{-1}), an aromatic ring (1605, 1550 cm^{-1}) and a conjugated carbonyl group (1655 cm^{-1}). The EI mass spectrum of **2** showed a molecular ion peak at *m/z* 422 (base peak) and a significant peak at *m/z* 367 [M $-C_4H_7]^+$, 165 ($C_8H_5O_4$), suggesting that a prenyl group and two hydroxy groups are located in the A-ring [2]. The ^1H NMR spectrum of **2** showed the presence of two 3,3-dimethylallyl groups [δ1.65, 1.81 (each 3H, *br s*, Me × 2), 1.75 (6H, *br s*, Me × 2), 3.42 (2H, *d*, J = 7.4 Hz), 3.57 (2H, *d*, J = 7.0 Hz), 5.32 (1H, *br t*, J = 7.4 Hz) and 5.40 (1H, *br t*, J = 7.0 Hz). The spectrum also indicated the presence of ABX type aromatic proton signals at δ7.02 (1H, *d*, J = 9.2 Hz), 8.05 (1H, *d*, J = 2.3 Hz) and 8.07 (1H, *dd*, J = 2.3, 9.2 Hz), a singlet of aromatic proton signal at δ6.35 (1H), and a hydrogen-bonded hydroxy group signal at δ12.1 (1H, *s*). The above results and a remarkable $AlCl_3$-induced shift in the UV spectrum of **2** [3] suggested that **2** is a 8,3′-di-(3,3-dimethylallyl)- or 8,5′-di-(3,3-dimethylallyl)-5,7,4′-trihydroxyflavonol. In the ^{13}C NMR spectrum of **2** (Table 1), the chemical shift values of C-2 to C-13 were almost identical to those of corresponding data for **1** and the chemical shift values of C-1′ to C-11′ were also almost identical to those of corresponding data for glyasperin A (**5**) (Table 1) [4]. This completes the characterization of **2**. The assignment of carbon signals of **2** was confirmed by ^1H-decoupled spectra, DEPT pulse sequence, HMQC and HMBC correlations (Fig. 1). The characterization of **2** was supported by HMBC corrections (Fig. 1). Therefore, in the ^1H NMR of **2**, signals at δ1.65 (H-12), 1.75 (H-10′ and H-11′), 1.81 (H-13), 3.42 (H-7′), 3.57 (H-9), 5.32 (H-10) and 5.40 (H-8′) were assigned.

Compound **3** was found as colorless prisms, which give a single spot on TLC plates, and was shown to be a mixture of straight chain *n*-alkyl (C_{24}, C_{26} and C_{28}), 4′-hydroxy-*trans*-cinnamates by NMR and mass spectroscopy (see Experimental) [5, 6].

EXPERIMENTAL

Extraction and separation. Root bark of *B. papyrifera* (1.45 kg) was collected at Kaohsiung, Taiwan during March, 1993. A voucher specimen was deposited in our laboratory. The air-dried root bark was chipped and extracted with acetone. The acetone extract was chromatographed on silica gel and elution with cyclohexane–EtOAc (10:1) yielded squalene and octacosan-1-ol; cyclohexane–EtOAc (2:1) yielded lignoceric acid, 4′-hydroxy-*cis*-cinnamic acid octacosyl ester, and a mixture of 4′-hydroxy-*trans*-cinnamates (**3**); and EtOAc–Me_2CO (10:1) yielded **1**, **2** and (−)-marmesin, respectively. The five known compounds were identified by UV, IR, MS and NMR [7–9].

Broussoflavonol E (**1**). Yellow prisms (Me_2CO–*n*-hexane), mp 195–196°, IR ν_{max}^{KBr} cm^{-1}: 3350, 1650, 1640, 1595, 1550, 1425. UV λ_{max}^{MeOH} nm (log ε): 218 (4.76), 255 (4.45), 290 (3.85), 305 (3.87), 353 (4.04); MeOH–$AlCl_3$ nm: 220, 261, 318, 420; MeOH–$AlCl_3$–HCl nm: 215, 265, 322, 410; MeOH–NaOAc nm: 220, 277, 318, 392; MeOH–NaOAc–H_3BO_3 nm: 222, 252, 270, 330, 375; MeOH–NaOMe nm: 273, 325, 384. ^{1}H NMR [$(CD_3)_2CO$]: see text. ^{13}C NMR [$(CD_3)_2CO$]: see Table 1. EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 506 $[M]^+$ (42), 489 (9), 450 (16), 435 (9), 407 (6), 284 (11), 257 (9), 221 (26), 201 (18), 165 (82), 43 (100). Anal. calcd for $C_{30}H_{34}O_7$: 506.2303. Found (MS): 506.2279.

Broussoflavonol F (**2**). Yellow prisms (Me_2CO–*n*-hexane), mp 155–157°, IR ν_{max}^{KBr} cm^{-1}: 3350, 1655, 1625, 1605, 1550, 1505, 1425. UV λ_{max}^{MeOH} nm (log ε): 250 (4.24), 270 (4.26), 325 (4.06), 374 (4.23); MeOH–$AlCl_3$ nm: 270, 310, 356, 433; MeOH–NaOAc nm: 276, 318, 392; MeOH–NaOAc–H_3BO_3 nm: 250, 270, 325, 375; MeOH–NaOMe nm: 278, 328, 425. ^{1}H NMR [$(CD_3)_2CO$]: see text. ^{13}C NMR [$(CD_3)_2CO$]: see Table 1. EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 422 $[M]^+$ (100), 407 (39), 367 (25), 351 (16), 311 (13), 205 (8), 189 (16), 422.1736.

4′-*Hydroxy*-trans-*cinnamates* (**3**). Colourless prisms ($CHCl_3$–MeOH), mp 97°, Gibbs test (−), IR ν_{max}^{KBr} cm^{-1}: 3390 (*br* OH), 1670 (C=O), 1600, 1580. UV λ_{max}^{MeOH} nm (log ε): 290 (4.09), 308 (4.06). ^{1}H NMR ($CDCl_3$): 0.88 (3H, *t*, *J* = 6.3 Hz, terminal Me), 1.25 (*br s*, methylene chain), 1.63 (4H, *m*, $-CO_2CH_2\underline{CH_2}-$ and $-CH_2\underline{CH_2}Me$), 4.19 (2H, *t*, *J* = 6.6 Hz, $CO_2\underline{CH_2}$), 5.45 (1H, *br s*, OH, exchangeable with D_2O), 6.30 (1H, *d*, *J* = 15.9 Hz, H-2), 6.84 (2H, *d*, *J* = 8.7 Hz, H-3′ and H-5′), 7.43 (2H, *d*, *J* = 8.7 Hz, H-2′ and H-6′), 7.63 (1H, *d*, *J* = 15.9 Hz, H-3). ^{13}C NMR ($CDCl_3$): 14.1 (terminal Me), 22.7, 29.3, 29.4 ($CH_2 \times 3$), 26.0 ($-\underline{CH_2}Me$), 28.8 ($-CO_2CH_2\underline{CH_2}-$), 29.5 ($CH_2$), 31.9 ($-CH_2\underline{CH_2}CH_2Me$), 61.7 ($-CO_2\underline{CH_2}-$), 115.8 (C-2, C-3′ and C-5′), 127.4 (C-1′), 129.9 (C-2′ and C-6′), 144.2 (C-3), 157.6 (C-4′), 167.5 (C=O). EIMS (direct inlet) 70 eV, *m/z* (rel. int.): 556 (2), 528 (19), 500 (3), 165 (42), 164 (100), 147 (65), 120 (17).

Acknowledgements—This work was supported, in part, by grants from the National Science Council of R.O.C. (NSC-84-2331-B037-075).

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