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PRENYLATED FLAVONOIDS FROM *MACLURA POMIFERA*

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Key Word Index—*Maclura pomifera*; Moraceae; prenylated flavonoids; prenylated isoflavonoids.

Abstract—Eight prenylated flavonoids, including three new compounds, have been isolated from chloroform extracts of stems and leaves of *Maclura pomifera*. The new prenylated flavonoids have been characterized as 5,7,4′,2″-tetrahydoxy-6-[3″-methyl-but-3″-enyl]-flavone, 5,4′-dihydroxy-2″-(1-hydroxy-1-methylethyl)-3″-methoxyfurano (4″,5″ (6,7) isoflavone and 5,4′-dihydroxy-2″-(1-hydroxy-1-methylethyl)-3″-methoxyfurano (4″,5″ (6,7) flavone. The structures were elucidated using spectroscopic methods.

INTRODUCTION

Maclura pomifera is a dioecious tree known as the Osage Orange in its native habitat in the Southwestern United States. This plant is of interest because, in addition to ethanol extracts of various plant parts exhibiting antimicrobial and antifungal activity [1–3], a chloroform extract of stems and leaves has recently been shown to have activity against a highly specific oestrogen-regulated transcription system in transformed *Saccharomyces cerevisiae* cell cultures [4]. The oestrogenic activity of this extract results from phytooestrogens, a group which includes coumestan, isoflavones, flavones and lignans in other plants [5].

These reports led us to investigate in more detail the flavonoid chemistry of *M. pomifera*. Previous chemical studies led to the isolation and characterization of several flavonoids, including isoflavones from the fruits [6–8], the root bark [1] and the heartwood of the plant [9–11]. From the chloroform extract of the stems and leaves of this species, three new prenylated flavonoids (**6–8**), along with five known prenylated flavonoids, were isolated and identified.

RESULTS AND DISCUSSION

In this study, the isolation by partitioning and repeated column chromatography on silica gel and Sephadex LH-20 and the identification of the known compounds **1–5** [8, 13, 14] from the chloroform extracts of stems and leaves of *M. pomifera*, as well as three new prenylated flavonoids (**6–8**) are have been described.

The previously unreported flavone (**6**) exhibited the same M_r and fragmentation pattern as **3** with peaks at *m/z* 354, 336, 321 and 283. The ^{1}H NMR also exhibited signals similar to those for the side-chain in **3** at δ 4.48 and δ 4.71 for two allylic protons, δ 4.41 for H-2″, δ 3.06 and δ 2.90 for two H-1″. Moreover, the basic skeleton of this flavone resembles that of a C-6 substituted apigenin, with two aromatic proton signals at δ 7.9 (2H, d, J = 8.9 Hz) and δ 6.95 (2H, d, J = 8.9 Hz) and two singlet aromatic proton signals at δ 6.68 for H-3 and at δ 6.61 for H-8. The location at C-6 of the substituent was supported by a delay in the appearance of a bathochromic shift upon addition of $AlCl_3$ in the UV spectrum. These data established **6** to be 5,7,4′,2″-tetrahydroxy-6-[3″-methyl-3″ butenyl]-flavone.

The molecular formula of $C_{21}H_{20}O_7$ for **7** was provided by CI mass ($M^+ + 1$, *m/z* 385) and EI mass (M^+, *m/z* 384). The ^{13}C NMR spectrum confirmed the presence of 21 carbons and both the ^{1}H NMR and ^{13}C NMR spectra resembled those of a C-6 substituted genistein-type isoflavone with two aromatic protons signals at δ 7.37 (d, J = 8.7 Hz) and δ 6.84 (d, J = 8.7 Hz) and two singlet aromatic proton signals at δ 8.09 and δ 6.41. Furthermore, ^{1}H NMR signals at δ 1.31 and δ 1.41 indicated two

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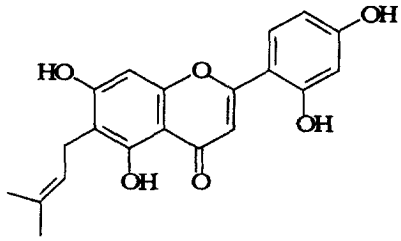

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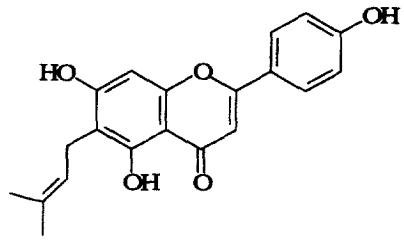

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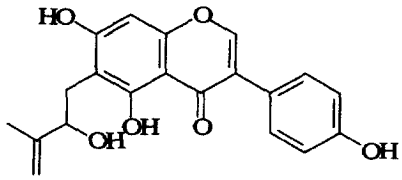

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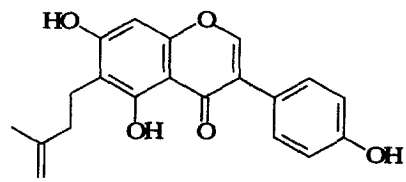

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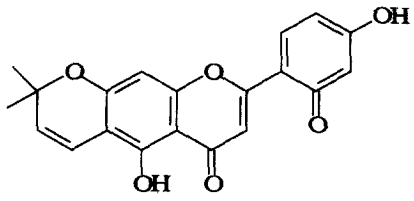

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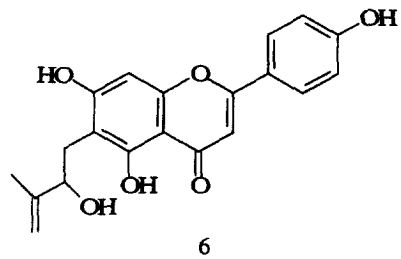

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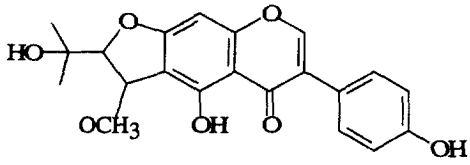

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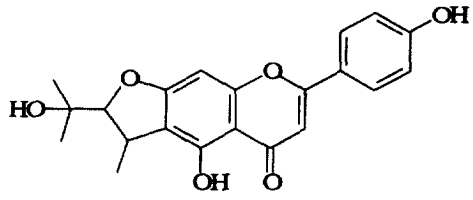

8

methyl groups typical for a 1-hydroxy-1-methylethyl side-attachment, the ^{1}H NMR signal for one methoxyl group at δ 3.52 indicated this group was on the 1-hydroxy-1-methylethyl side-chain. A *trans*-orientation for H-2″ and H-3″ is supported by a 2.6 Hz coupling between these two protons, with signals at δ 5.15 and δ 4.45. Moreover, the EI mass spectrum with peaks at *m/z* 353 $[M-CH_3O]^+$ (55), 335 $[M-CH_3O + H_2O]^+$ (20) and 295 $[M-C_4H_{10}O_2]^+$ (100) suggested a [1″-methoxy-2″-(2-hydroxyisopropyl)-dihydrofurano]-type substituent. HMQC and HMBC experiments allowed the assignment of all ^{1}H and ^{13}C NMR signals. Correlation from C-3″ proton to C-5, C-6 and C-7 and correlation from the C-8 proton to C-6, C-7, C-9 AND C-10 suggested a dihydrofurano substituent at position 6 and a proton at C-2″, which correlated with C-7, indicating that the dihydrofurano moiety was at the C-6 and C-7 position. The position of the methoxyl group was established by a correlation between the resonance of the methoxyl protons and C-3″. These spectral data established the structure of (**7**) as 5,4′-dihydroxy-2″-(1-hydroxy-1-methylethyl)-3″-methoxyfurano(4″,5″(6,7)isoflavone.

Compound **8** appeared as a purple fluorescent spot on a paper chromatogram in UV light, changing to yellow with ammonia in accordance with the presence of free 5- and 4′-hydroxyl groups. The UV maxima in MeOH at 267 and 334 nm and no shifts with NaOAc suggested that the oxygen function at C-7 was a substituted hydroxyl. The CI and EI mass spectra indicated a formula of $C_{21}H_{20}O_7$ the same as **7**, viz: ($[M^+ + 1]$, 385) and ($[M]^+$, *m/z* 384), respectively. Furthermore, the ^{1}H NMR indicated that **8** had the same side-chain as in **7**: δ 5.16 and δ 4.46 (1H each, J = 2.6 Hz) for two protons, δ 1.31 and δ 1.41 for a γ,γ-dimethyl group and δ 3.52 for the 3″-methoxyl group (Table 1). These

Table 1. NMR data of compounds **7** and **8** in CD_3OD

C	7			8
	^{13}C	^1H	HMBC	^1H
2	154.9	8.09(s)	C = 3, 4, 9, 1′	
3	127.0			6.68(s)
4	180.8			
5	160.5			
6	111.1			
7	168.8			
8	90.23	6.41(s)	C = 6, 7, 9, 10	6.61(s)
9	161.0			
10	107.1			
1′	123.0			
2′	131.4	7.37(d,8.7)	C = 3, 2′, 4′, 6′	7.90(d,8.9)
3′	116.2	6.84(d,8.7)	C = 1′, 3′, 5′	6.95(d,8.9)
4′	158.9			
5′	116.2	6.84(d,8.7)	C = 1′, 3′, 5′	6.95(d,8.9)
6′	131.4	7.37(d,8.7)	C = 3, 2′, 4′, 6′	7.90(d,8.9)
1″	79.8	5.15(d,2.6)	C = 5, 6, 7, 3″, OCH_3	5,16(d,2.6)
2″	98.1	4.45(d,2.6)	C = 1″, 7	4.46(d,2.6)
3″	71.6			
4″	24.6	1.16(s)	C = 2″, 3″, 5″	1.16(s)
5″	26.0	1.26(s)	C = 2″, 3″, 4″	1.27(s)
OCH_3	57.3	3.52(s)	C = 1″	3.52(s)

NMR findings were in accord with the EI mass spectrum, which showed fragments at *m/z* 353, 335 and 295. A C-6 substituted apigenin-type flavone was indicated by the ^1H NMR with two aromatic proton signals at δ 7.9 (2H, d, J = 8.9 Hz) and δ 6.95 (2H, d, J = 8.9 Hz) and two singlet aromatic proton signals at δ 6.68 and δ 6.61. Additional HMBC and HMQC experiments established this flavonoid to be 5,4′-dihydroxy-2″-(1-hydroxy-1-methylethyl)-3″-methoxyfurano(4″,5″-(6,7)flavone.

EXPERIMENTAL

General

One and two dimensional NMR were recorded on a Bruker AM500 and chemical shifts reported as δ values using the solvent as a reference. CC employed Sephadex LH 20 (Pharmacia) and silica gel 60–120 mesh (Aldrich). Precoated cellulose plates (Merck), polyamide (Macherey-Nagel) and silica gel 60 GF-254 (Merck) were used for TLC. The solvent systems were TBA (*t*-BuOH–HOAc–H_2O, 3:1:1) and *n*-BAW, upper layer (*n*-BuOH–HOAc–H_2O, 4:1:5). All flavonoids were purified over Sephadex LH-20 using MeOH prior to spectral analysis by standard procedures [12]. Visualization of the flavonoids on TLC plates was realized either by UV light + NH_3 or by spraying with NA (Naturstofferagenz-A in MeOH).

Plant material

Leaves and stems of *M. pomifera* (Raf.) Schnider were collected in December 1995 in Pease Park at 24th and Lamar streets in Austin, Texas. A voucher specimen (Wood 97-1; TEX) was deposited in the Plant Resources Center at the University of Texas at Austin.

Extraction and isolation

Dried stems and leaves (1.5 kg) were cut into small pieces and extracted with $CHCl_3$ (5 l) at 25°C. Extracts were concentrated under reduced pressure to yield a dark brown resin (35 g). A silica gel column (15 × 45 cm) for prep. separation was used with a series of mixtures of hexane, $CHCl_3$, EtOAc and MeOH starting with hexane–$CHCl_3$ (9:1) continuing with increasing polarity up to MeOH–EtOAc (1:9). In all, 35 frs (500 ml each) were collected and all frs were examined by 1D TLC on silica gel (benzene–Et_2O–EtOAc–HOAc, 80:10:10:0.2) and 2D paper chromatography on 3 MM Whatman paper for flavonoids. Some of the flavonoids were detected in fr. 24 (EtOAc–$CHCl_3$, 2:1). Fr. 24 from the first column was further chromatographed on another silica gel column (5 × 45 cm) which was eluted with a series of solvent mixtures yielding another 26 frs; 1–5 (hexane–$CHCl_3$, 4:1), 6–8 ($CHCl_3$–hexane, 4:1), 9–11 (hexane–$CHCl_3$–EtOAc), 12–14 ($CHCl_3$–EtOAc, 4:1), 15–18 ($CHCl_3$–EtOAc) (1:1), 19–21 (EtOAc) and 22–26 (EtOAc–MeOH) (4:1). Frs 17 and 18 contained most of the flavonoids. Further Sephadex LH-20 column chromatography of fr. 17 eluted with MeOH yielded 5 mg of **2**, 70 mg of **4** and 10 mg of **5**. Concentrated fr. 18 was applied to a Sephadex LH-20 column and **1** (12 mg), **3** (4 mg), **6** (3 mg), **7** (15 mg) and **8** (3 mg) were obtained.

5,7,2′,4′-Tetrahydroxy-6-[3″-methylbut-3″-enyl]-flavone (**1**) *[8]*

UV λ_{max} nm, (MeOH): 250, 271, 355; (+NaOMe): 273, 326, 402; (+$AlCl_3$): 258, 280, 365; (+NaOAc): 269, 358. ^{1}H NMR (CD_3OD): δ 7.80 (1H, d, J = 8.5 Hz, H-6′), 7.80 (1H, s, H-8), 6.62 (1H, d, J = 2.5 Hz, H-3′), 6.69 (1H, s, H-3), 6.56 (1H, dd, J = 8.5 and 2.5 Hz, H-5′), 5.28 (1H, br t, J = 7 Hz, CH=), 3.35 (2H, br d, J = 7 Hz, CH_2), 1.78, 1.65 (3H each, br s, 2 × Me). EIMS *m/z* (rel. int.): 354 $[M]^+$ (50), 339 $[M-Me]^+$ (17), 337 $[M-OH]^+$ (15), 311 $[M-C_3H_7]^+$ (100).

5,7,4′-Trihydroxy-6-[3″-methylbut-3″-enyl]-flavone, (**2**) *[8]*

UV λ_{max} nm, (MeOH): 273, 333; (+$AlCl_3$): 281, 358; (+NaOAc): 273, 343. ^{1}H NMR (CD_3OD): δ 7.90 (2H, d, J = 9 Hz, H-2′, 6′), 7.00 (2H, d, J = 9 Hz, H-3′, H-5′), 6.62 (1H, s, H-8), 6.61 (1H, s, H-3), 5.27 (1H, br t, J = 7 Hz, CH=), 3.34 (2H, d, J = 7 Hz, CH_2), 1.76, 1.63 (3H each, br s, Me_2). EIMS *m/z* (rel. int.): 338 $[M]^+$ (51), 323 $[M-Me]^+$ (16), 321 $[M-OH]^+$ (18), 309 $[M-CHO]^+$ (7).

5,7,4′,2″-Tetrahydroxy-6-[3″-methyl-3″-butenyl]-isoflavone, (**3**) *[13]*

UV λ_{max} nm, (MeOH): 266, 342; (MeOH + $AlCl_3$): 277, 344; (+NaOAc): 273, 332. ^{1}H NMR (CD_3OD): δ 8.15 (1H, s, H-2), 7.45 (2H, d, J = 8.5 Hz, H-2′, H-6′), 6.91 (2H, d, J = 8.5 Hz, H-3′, H-5′), 6.51 (1H, s, H-8), 4.93, 4.75 (1H, br s, $=CH_2$), 4.43 (1H, dd, J = 7.5 and 3.5 Hz, CH–O), 3.06 (1H, dd, J = 14 and 3.5 Hz, CH_aH_b), 2.93 (1H, dd, 14 and 7.5 Hz, CH_aH_b), 1.83 (3H, br, s, Me). EIMS *m/z* (rel. int.): 354 $[M]^+$ (8), 336 $[M-H_2O]^+$ (22), 321 $[M-C_3H_7]^+$ (27), 283 $[M-C_4H_7O]^+$ (100).

5,7,4′-Trihydroxy-6-[3,3-DMA]-isoflavone, (**4**) *[13]*

UV λ_{max} nm, (MeOH): 215, 268; (MeOH + NaOMe): 277; (+$AlCl_3$): 277; (+NaOAc): 270. ^{1}H NMR (CD_3OD): δ 8.02 (1H, s, H-2), 7.39 (2H, d, J = 8.5 Hz, H-2′, H-6′), 6.82 (2H, d, J = 8.5, H-3′, H-5′), 6.39 (1H, s, H-8), 5.23 (1H, br t, J = 7 Hz, CH=), 3.35 (2H, br d, J = 7 Hz, H-1″), 1.78, 1.65 (3H, each, br s, 2 × Me). ^{13}C NMR: 180.4 (H-4), 162.1 (H-7), 159.0 (H-5), 157.5 (H-4′), 155.5 (H-9), 153.8 (H-2), 130.8 (H-3″), 130.3 (H-2′, H-6′), 122.3 (H-3, H-2′), 121.5 (H-1′), 115.2 (H-3′, H-5′), 111.2 (H-6), 104.4 (H-10), 93.0 (H-8), 25.6 (H-4″), 21.1 (H-1″), 17.8 (H-5″). EIMS *m/z* (rel. int.): 339 $[M+1]^+$ (11), 338 $[M-C_{20}H_{18}O_5]^+$ (100), 337 (6), 323 $[C_{19}H_{15}O_5]^+$ (15), 321 (16), 296 $[C_{16}H_{11}O_3]^+$ (16).

5,7,4′-Trihydroxy-6,7-[2,2-dimethylchromeno]-flavone, (**5**) *[14]*

UV λ_{max} nm, (MeOH): 234, 308, 344; (+NaOMe): 230, 289, 317, 391; (+$AlCl_3$): 350, 402. ^{1}H NMR (CD_3OD): δ 7.78 (2H, d, J = 9 Hz, H-2″, H-6′), 6.93 (2H, d, J = 9 Hz, H-3′, 5′), 6.71 (1H, d, J = 10 Hz, H-4″), 6.52 (1H, s, H-8), 6.38 (1H, s, H-3), 5.61 (1H, d, J = 10, 3″), 1.42, 1.60 (3H each, br s, 2 × Me). EIMS *m/z* (rel. int.): 336 $[M]^+$ (39), 323 (13), 321 $[M-CH_3]^+$ (100), 307 $[M-CHO]^+$ (5), 203 (18), 161 (5), 160 (11), 69 (7).

5,7,4′,2″-Tetrahydroxy-6-[3″-methyl-but-3″-enyl]-flavone, (**6**)

UV λ_{max} nm, (MeOH): 271, 336; (+NaOMe): 275, 327, 395; (+$AlCl_3$): 276, 303, 350, 383; (+NaOAc): 277, 302, 382. ^{1}H NMR (CD_3OD): δ 7.82 (2H, d, J = 9 Hz, H-2″, 6″), 6.95 (2H, d, J = 9 H, H-3″, 5″), 6.6 (1H, s, H-8), 6.51 (1H, s, H-3), 4.84, 4.71 (1H each, br s, $=CH_2$), 4.41 (1H, dd, J = 7.5 and 3.5, C*H*–O), 3.06 (1H, dd, J = 14 and 3.5 Hz, C*H*aHb), 2.90 (1H, dd, J = 14 and 7.5 Hz, CHa*H*b), 1.83 (3H, br s, Me). EIMS *m/z* (rel. int.): 354 $[M]^+$ (11), 336 $[M-H_2O]^+$ (31), 321 $[M-C_3H_7]^+$ (21), 283 $[M-C_4H_7O]^+$ (100).

5,4′-Dihydroxy-2″-(1-hydroxy-1-methylethyl)-3″-methoxyfurano (4″,5″ (6,7) *isoflavone* (**7**)

UV λ_{max} nm: (MeOH): 211, 262, 331 sh.; (+$AlCl_3$): 211, 265, 371: (+$AlCl_3$/HCl): 211, 267, 371; (+NaOAc): 207, 263: (+NaOAc/H_3BO_3): 207, 263. CIMS *m/z* (rel. int.): 413 $[M + C_2H_4]^+$ (8), 385 $[M + 1]^-$ (34), 354 $[M + 1\text{-}CH_3O]^+$ (16), 353 $[M + 1\text{-}CH_3OH]^+$ (62), 335 $[M + 1\text{-}CH_3OH + H_2O]^+$ (16), 295 $[M + 1\text{-}C_4H_{10}O_2]^-$ (100). EIMS *m/z* (rel. int.): 384 $[M]^+$ (9), 352 $[M-CH_3OH]^+$ (21), 334 $[M-CH_3OH + H_2O]^+$ (31), 294 $[M-C_4H_{10}O_2]^+$ (100), 256 (32), 207 (6), 176, 147.

5,4′-Dihydroxy-2″-(1-hydroxy-1-methylethyl)-3″-methoxyfurano (4″,5″ (6,7) *flavone* (**8**)

UV λ_{max} nm: (MeOH): 216, 267, 334 sh; (+NaOMe): 213, 267, 387; (+$AlCl_3$): 275, 303, 348 sh, 374; (+NaOAc): 216, 265. CIMS *m/z* (rel. int.): 413 $[M + 28]^+$ (6), 385 $[M + 1]^+$ (36), 354 $[M-CH_3O]^+$ (17), 353 $[M-CH_3OH]^+$ (55), 335 $[M-CH_3OH + H_2O]^+$ (20), 295 $[M-C_4H_{10}O_2]^-$ (100); EIMS *m/z* (rel. int.): 384 $[M]^+$ (5), 352 $[M-CH_3OH]^+$ (11), 334 $[M-CH_3OH-H_2O]^+$ (12), 294 $[M-C_4H_{10}O_2]^+$ (100).

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