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COMPARISON BETWEEN METABOLITE PRODUCTIONS IN CELL CULTURE AND IN WHOLE PLANT OF *MACLURA POMIFERA**

GIULIANO DELLE MONACHE,† MARIA CRISTINA DE ROSA, ROSALBA SCURRIA, ALBERTO VITALI, ANGELA CUTERI,‡ BARBARA MONACELLI,‡ GABRIELLA PASQUA‡ and BRUNO BOTTA§

Centro Chimica dei Recettori del C.N.R., Università Cattolica S. Cuore, Largo F. Vito 1, 00168 Roma, Italy; ‡Dipartimento di Biologia Vegetale, Università La Sapienza, Piazzale A. Moro 5, 00185 Roma, Italy; §Dipartimento di Chimica e Tecnologia delle Sostanze Biologicamente Attive, Università La Sapienza, Piazzale A. Moro 5, 00185 Roma, Italy

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Key Word Index—*Maclura pomifera*; Moraceae; cell cultures; flavonoid production; biosynthesis.

Abstract—Plant tissue cultures of *Maclura pomifera* showed a metabolite accumulation pattern which was both quantitatively and qualitatively different from that of the parent plant. Triterpenes and flavonoids were isolated from callus and cell cultures, however, xanthones and stilbenes, which have been reported in the whole plant, were not found. Among the flavonoids, flavones and flavanones were produced preferentially by the suspended cells, but with the prenyl substituents exclusively on ring A, while the isoflavones did not show the 3′,4′-dihydroxyl substitution pattern found in the products isolated from fruits. A new prenylated 6′-deoxychalcone was also isolated from the callus and cell cultures.

INTRODUCTION

Maclura pomifera (Raf.) Schneid., known as Osage orange, is a hardwood tree native to the southwestern United States. Simple [1] and complex [2, 3] flavonoids have been isolated from the heartwood, the fruits and the root-bark. Triterpenes [4], xanthones [3] and stilbenes [1] were also reported to be present in the plant. Recently, we isolated from the fruit extract other unreported flavonoids [5], three of which were new. The antimicrobial activity of the fruit extract was attributed to the presence of the isoflavones osajin (**1a**) and pomiferin (**1b**) [6], while the wood gave an extract with antifungal properties [1]. No such activity was found in stem and leaves [7].

A tissue-culture system for the propagation of Osage orange has been developed: plantlet survival in the greenhouse after 2 months was more than 70% [8]. Calli and cell cultures of *M. pomifera* have now been initiated and stabilized for flavonoid accumulation. The hormonal requirements for establishment and optimization of the cultures have been previously reported [9]. This paper describes the metabolites accumulated in the tissue culture with special reference to flavonoids, and compares them with those of the differentiated plant tissue.

RESULTS AND DISCUSSION

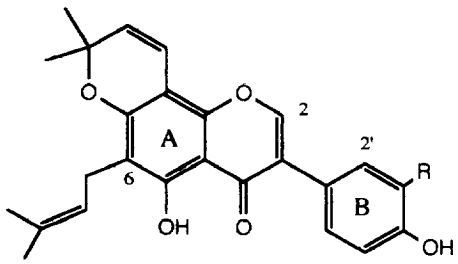
The *M. pomifera* cell suspension culture line selected for use in this study showed a greater level of metabolite accumulation (0.91%) than stem (0.26%), leaves (0.32%) and fruits (0.08%) of the parent plant [9]. Both calli and suspended cells produced triterpenes and flavonoids as the main components of the extract. By contrast, xanthones [3] and stilbenes [1], which are formed in intact plants from the same shikimate precursor as the flavonoids [10, 11], were not found. A number of flavonoids was isolated by a combination of column and preparative TLC (see Experimental). Most of the components of the extract had been previously isolated from various plants, mainly belonging to the family Leguminosae, and were identified by comparison of their spectral and physical data with those reported in the literature.

Among the isolated flavonoids only osajin (**1a**), warangalone (**2a**) and artocarpesin (**3**) were also present in the parent plant. The last compound was the most abundant component of the extracts from both calli and suspension cells.

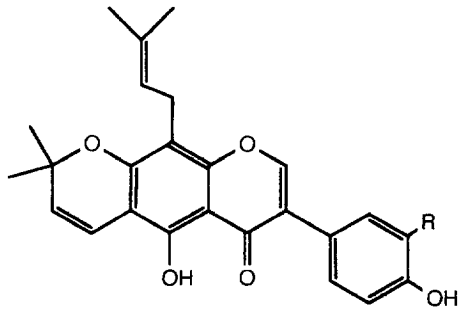
Four A-ring prenylated flavanones (**4**–**7**) were isolated. The regioisomeric **4** and **5** exhibited the same relationship as the isoflavones **1a** and **2a**. Cajaflavanone (**4**) had been isolated previously from *Cajanus cajan* [12]. The structure **4** had also been assigned to a flavanone isolated from *Erythrina senegalensis* [13] and named erythrisenegalone. Lupininifolin (**5**) had been reported previously from *Tephrosia lupinifolia* [14], *Mundulea sericea* [15] and *Lonchocarpus minimiflorus* [16].

*Part 3 in the series 'Cell Suspension Cultures of *Maclura pomifera*'. For part 2 see ref. [5].

†Author to whom correspondence should be addressed.



	R
1a	H
1b	OH



	R
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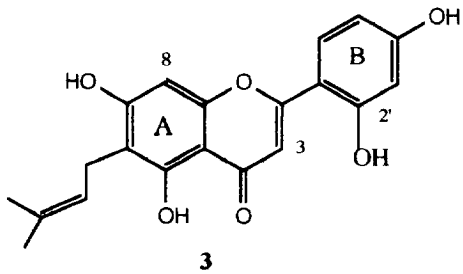
2b	OH



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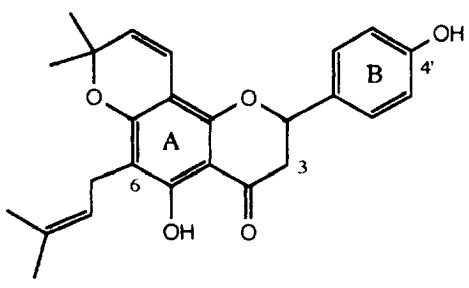

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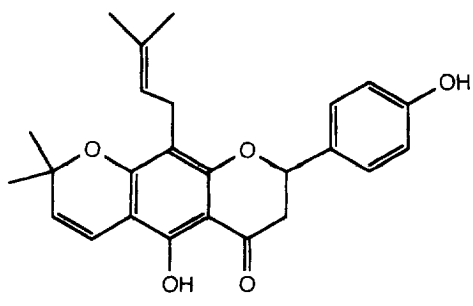

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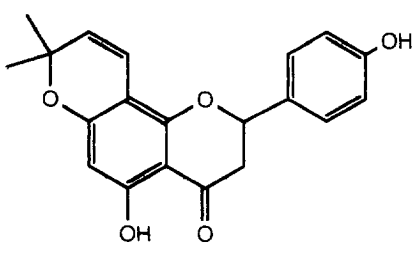

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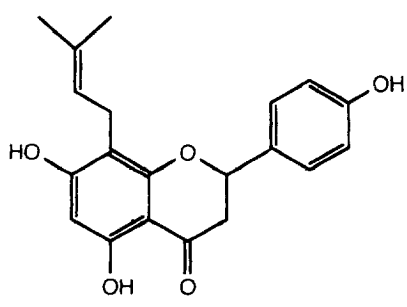

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The structure of the new flavanone **6** was assigned on the basis of ^{1}H NMR data and the fragmentation ions in the mass spectrum, where the first fragment ion was formed by loss of 15 amu, typical of chromene derivatives, and underwent RDA fragmentation at ring C to give the base peak at *m/z* 203. The angular arrangement of the pyran ring was supported by the immediate bathochromic shift in the UV spectrum after addition of $AlCl_3$ [17]. Moreover, the signal of a chelated hydroxyl proton (OH-5) appeared at δ 12.10, as required for 8-isopentenylated flavanones [18].

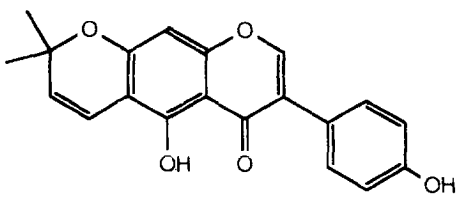
In **7**, which is the isopentenyl derivative corresponding to **6**, the substituent was located at C-8 for the same reasons as described above (see Experimental). 8-*C*-Prenylnaringenin (**7**) had been reported from various *Wyethia* species [19]. The compound isolated from cell cultures of *Maclura pomifera* was contaminated by traces of the 6-isomer, as shown by the presence of ^{1}H NMR signals at δ 12.36 (1H, *s*, OH-5), 6.40 (1H, *s*, H-8), 1.85 and 1.78 (3H each, *s*, 2 × Me).

The isoflavones **8** and **9**, two other components of the *in vitro* extract, represent a pair of chromene- and prenyl-derivative, respectively. The former is alpinumisoflavone (**8**), which was found in *Laburnum alpinum* [20] and, more recently, in the seeds of *Milletia thonningii* [21]. The latter is wighteone (**9**), which had been isolated as a phytoalexin from fungus-inoculated stems of *Glycine wightii* [22]. The compound had also been reported from the while lupin, *Lupinus albus* [23].

Whereas **8** and **9** are both oxygenated at position 4′ of the B-ring as **1a** and **2a**, a further isoflavone exhibited the unique 2′,4′-dihydroxy substitution pattern and was identified as parvisoflavone A (**10**), a metabolite present in *Poecilanthe parviflora* [24] and *Lupinus albus* [23].

Cycloartocarpesin (**11**), the chromene derivative corresponding to **3**, was the second flavone present in the extract. The compound has been previously reported among the colouring matters of the wood of *Artocarpus heterophyllus* [25].

A further component of the extract was a new chalcone, which was assigned as 2,4,2′,4′-tetrahydroxy-3′-(3-methyl-2-butenyl)-chalcone (**12**). The presence in the ^{1}H NMR spectrum of **12** of the signals for two *ortho*-coupled aromatic protons (one of which is clearly *peri* to



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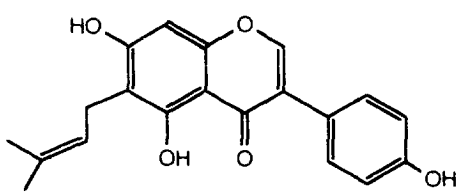

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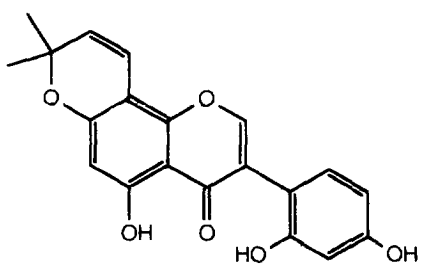

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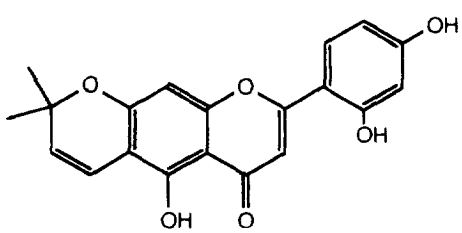

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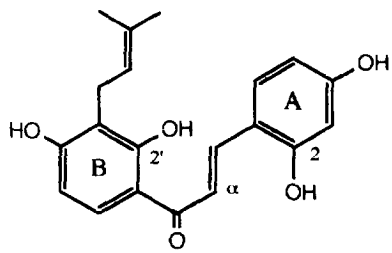

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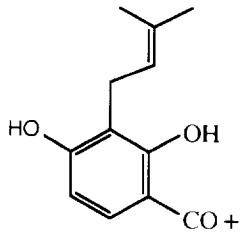
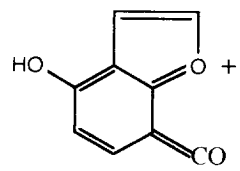
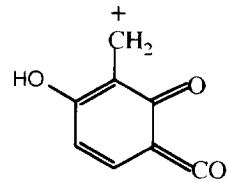


m/z 205



m/z 161



m/z 149

the carbonyl group owing to the lowfield chemical shift) required the placement of the prenyl group at C-3′, thus revealing the substitution pattern of the B-ring. Conversely, the 2,4-dihydroxy substitution of the A-ring was supported by the multiplicities of the proton signals, which showed an *ortho–ortho–meta* coupling system. Notably, in the ^{13}C NMR spectrum (Table 1) the chemical shifts of B-ring carbons were very similar to those of synthetic 2,4,2′,4′-tetrahydroxychalcone, while the signals of the A-ring were comparable with those of the natural compound isocordoin, i.e. 2,4-dihydroxy-3′-(3-methyl-2-butenyl)-chalcone [26]. The structure of **12** was confirmed by its fragmentation in the mass spectrum, which was characterized by the loss of H_2O from the molecular ion, followed by the mass losses typical for a prenyl group (43 and 55 amu). The ions formed, in turn, underwent an RDA-type reaction to give peaks at *m/z* 161 and 149. These B-ring fragment ions are rationalized in the structures shown together with the ion at *m/z* 205, which is formed by RDA directly from the molecular ion.

Simple flavonoids were also present in the extract from calli. Aromadendrin (3,5,7,4′-tetrahydroxyflavanone) was the major metabolite and steppogenin (5,7,2′,4′-tetrahydroxyflavanone) as well as morin (5,7,2′,4′-tetrahydroxyflavonol) were also isolated, but dihydromorin (3,5,7,2′,4′-pentahydroxyflavanone) was absent. Moreover, naringenin (5,7,4′-trihydroxyflavanone), apigenin (5,7,4′-trihydroxyflavone) and the dimer 8,8′-bis-naringenin were found.

In conclusion, the comparison between *in vitro* and *in vivo* flavonoid production revealed the following: (1) with respect to the class of flavonoids, flavones were the major constituents in the extracts from callus cultures, whereas isoflavones were the most abundant class in the parent plant. (2) All of the simple flavonoids obtained from the callus culture were biosynthetically related to naringenin [11], e.g. naringenin *per se*, apigenin, steppogenin, 8,8′-bis-naringenin and aromadendrin. (3) The complex flavonoids produced by the cell cultures were prenyl substituted exclusively in ring A. Moreover, these flavonoids showed a marked selectivity at the level of the B-ring hydroxylation. (4) The 3′,4′-dihydroxyl substitution, typical of pomiferin (**1b**), the most abundant component of the fruit extract, and auriculasin (**2b**) [5], was not observed in components of callus and cell cultures. Surprisingly, only 4′-hydroxy substituted isoflavones **1a** and **2a** were present. This finding indicates that flavonoid 3′-hydroxylase [27] is apparently not active in suspended cells. (5) The 2′,4′-dihydroxyl substitution pattern of the isoflavone **10** present in suspension but not in callus cells, must have originated from the action of a specific hydroxylase at the C_{15}-stage, possibly the flavanone **6**. (6) Notably, the flavanones **4** and **5**, which may give isoflavones **1a** and **2a**, respectively, by 2,3-migration of the aryl side chain, were also found in cell cultures. (7) Finally, the flavones present in cell cultures showed the same B-ring substitution pattern as in whole plant.

Table 1. ^{13}C NMR data of compound **12** and model chalcones*

C	**12**	Isocordoin	2,4,2′,4′-Tetrahydroxychalcone
α	145.3	144.4	141.0
β	117.6	121.8	117.3
CO	193.2	192.8	193.1
1′	114.1	114.2	114.5
2′	162.7	163.0	—
3′	115.9	116.0	—
4′	164.5	164.7	—
5′	107.8	108.1	108.5
6′	131.7	131.3	131.7
1	115.6	—	115.1
2	159.7	—	159.9
3	103.6	—	103.6
4	162.5	—	162.2
5	109.1	—	109.1
6	132.8	—	132.8
CH_2	22.3	22.2	—
CH =	123.4	123.1	—
C =	131.8	131.5	—
E-Me	25.8	25.8	—
Z-Me	17.9	17.9	—

*Me_2CO-d_6, 75 MHz, TMS as internal standard. Only the signals significant for the comparison are reported.

Two new compounds were isolated from the callus culture, notably the rare prenylated chalcone **12**. This is the first example of the *in vitro* formation of 6′-deoxychalcones.

EXPERIMENTAL

General. Mps: uncorr.; EI-MS: direct inlet, 70 eV; FAB-MS: glycerol; ^{1}H NMR ($CDCl_3$ for **4–9**, Me_2CO-d_6 for **10–12**): 300 MHz, TMS as int. standard.

Isolation. Stabilization and optimization of *in vitro* cultures of *Maclura pomifera* (Raf.) Schneid. are reported

in a previous paper [9]. Calli and cells were extracted exhaustively by percolation with cold MeOH, the solvent evapd and the residue partitioned between H_2O and EtOAc (x3). For example, the pooled organic layers from dark calli (6 g, fr. wt) gave a residue (1.2 g), which on silica gel with $CHCl_3$–MeOH mixture (5–20%) gave fractions MC-I to MC-IX. These fractions were further purified by CC or prep. TLC to give the following products: MC-I (55 mg), CC ($CHCl_3$): butyrospermol (8 mg) and β-sitosterol (45 mg); MC-II (40 mg), CC ($CHCl_3$–MeOH, 19:1) and TLC (hexane–EtOAc, 4:1): cajaflavanone (**4**, 6 mg), lupinifolin (**5**, 4 mg), warangalone (**2a**, 4 mg) and osajin (**1a**, 8 mg); MC-III (24 mg), TLC (hexane–EtOAc, 3:2 × 2): alpinumisoflavone (**8**, 8 mg), 4′,5-dihydroxy-2″, 2″-dimethylpyrano-[5″,6″; 7,8]-flavanone (**6**, 4 mg) and an unidentified flavonoid (2 mg); MC-IV (9 mg) TLC (hexane–EtOAc, 3:2 × 2): 8-*C*-prenylnaringenin (**7**, 4 mg); MC-V (39 mg), CC ($CHCl_3$–MeOH, 19:1), and TLC ($CHCl_3$–MeOH, 97:3 × 2): wighteone (**9**, 4 mg) and parvisoflavone A (**10**, 3 mg); MC-VI (35 mg), CC and TLC ($CHCl_3$–MeOH, 19:1): naringenin (3 mg) and 8,8′-bisnaringenin (4 mg); MC-VII (59 mg),CC ($CHCl_3$–MeOH, 19:1) and TLC (CH_2Cl_2–EtOAc–MeOH, 90:7:3): cycloartocarpesin (**11**, 15 mg), apigenin (5 mg) and aromadendrin (21 mg); MC-VIII (46 mg), CC ($CHCl_3$–MeOH, 19:1) and TLC (CH_2Cl_2–EtOAc–MeOH, 90:7:3): 2,4,2′,4′-tetrahydroxy-3-(3-methyl-2-butenyl)-chalcone (**12**, 5 mg) steppogenin (9 mg) and morin (4 mg); MC-IX (104 mg), crystallization from $CHCl_3$–MeOH: artocarpesin (**3**, 46 mg). Osajin (**1a**), warangalone (**2a**) and artocarpesin (**3**) were identified by comparison with authentic specimens, isolated from the fruits [5]. Simple flavonoids were identified by ^{1}H NMR spectra and by comparison with authentic samples.

8, *8′-bis-Naringenin*. UV λ_{max}^{MeOH} nm (log ε): 229 (4.16), 273 (4.12), 284sh (4.10); (+ NaOAc): 271, 322; (+ $AlCl_3$): 225, 273, 310, 364 (immediate); ^{1}H NMR: δ7.40 (4H, *d*, *J* = 8.5 Hz, H-2′, H-2‴, H-6′, H-6‴), 6.90 (4H, *d*, H-3′, H-3‴), 5.96 (2H, *s*, H-6, H-6″), 5.46 (2H, *dd*, *J* = 13 and 3 Hz, H-2′, H-2″), 3.19 (2H, *dd*, *J* = 17 and 13 Hz, H-3_{ax}), H-$3''_{ax}$), 2.73 (2H, *dd*, *J* = 17 and 3 Hz, H-3_{eq}, H-$3''_{eq}$); FAB-MS: $[MH]^+$ *m/z* 543.

5,4′-*Dihydroxy*-6-(3-*methyl*-2-*butenyl*)-2″,2″-*dimethylpyrano*-[5″,6″;7,8]-*flavanone* (**4**, *cajaflavanone*. Mp 125–127°, lit. [12] Mp 129–130°, $[\alpha]_D$ − 5, lit [13]. [α] − 5 ($CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 267 (4.0), 273 (4.04), 298 (3.85), 311 (3.64), 360sh (3.48); (+ $AlCl_3$): 236, 273, 318, 361 (after 20 min); ^{1}H NMR and mass spectral data were in agreement with those reported in the literature [12, 13].

5,4′-*Dihydroxy*-8-(3-*methyl*-2-*butenyl*)-2″,2″-*dimethylpyrano*-[5″,6″;6,7]-*flavanone* (**5**, *lupinifolin*). Mp 115–116°, lit. [14] mp 117–119°, $[\alpha]_D$ − 6.5, lit. [α] − 8.7; UV λ_{max}^{MeOH} nm (log ε): 267 (4.08), 275 (4.12), 298 (3.71), 312 (3.70), 355 (3.58); (+ $ALCl_3$): 231, 273, 320, 361 (after 20 min); ^{1}H and ^{13}C NMR and mass spectral data were in agreement with those published in the literature [14–16].

5,4′-*Dihydroxy*-2″,2″-*dimethylpyrano*-{5″,6″;7,8]-*flavanone* (**6**). Vitreous solid, [α] 0; UV λ_{max}^{MeOH} nm (log ε): 230 (4.05), 262sh (4.25), 270 (4.34), 294sh (3.99), 308sh (3.88), 358sh (3.70); (+ $AlCl_3$): 231, 238sh, 270sh, 280, 323, 358sh (immediate); ^{1}H NMR ($CDCl_3$): 12.10 (1H, *br s*, ex. D_2O, OH-5), 7.33 (2H, *d*, *J* = 8.5 Hz, H-2′, H-6′), 6.90 (2H, *d*, H-3′, H-5′), 6.53 (1H, *d*, *J* = 10 Hz, H-4″), 6.00 (1H, *s*, H-6), 5.46 (1H, *d*, *J* = 10 Hz, H-3″), 5.36 (1H, *dd*, *J* = 13 + 3 Hz, H-2), 3.07 (1H, *dd*, *J* = 17 + 13 Hz, H-3ax), 2.79 (1H, *dd*, *J* = 17 + 3 Hz, H-3eq), 1.45, 1.42 (3H each, *s*, 2 × Me); EI-MS *m/z* (rel. int.): 338 $[M]^+$ (50), 323 $[M - Me]^+$ (65), 203 $[C_{11}H_7O_4$, ring A$]^+$ (100), 120 $[C_8H_8O$, ring B$]^+$ (10).

5,7,4′-*Trihydroxy*-8-(3-*methyl*-2-*butenyl*)-*flavanone* (7, 8-c-*prenylnaringenin*): Vitreous solid; UV λ_{max}^{MeOH} nm (log ε): 227 (4.21), 264sh (3.98), 288 (4.08); (+ NaOAc): 230, 281, 330; (+ $AlCl_3$): 230, 312, 362 (immediate); ^{1}H NMR ($CDCl_3$): δ12.15 1H, *s*, ex. D_2O, 5-OH), 7.42 (2H, *d*, *J* = 8.5 Hz, H-2′, H-6′), 6.92 (2H, *d*, *J* = 8.5 Hz, H-3′, H-5′), 6.03 (1H, *s*, H-6), 5.45 (1H, *dd*, *J* = 13 and 3 Hz, H-2), 5.20 (1H, *br t*, *J* = 7 Hz, =CH), 3.22 (2H, *d*, *J* = 7 Hz, CH_2), 3.05 (1H, *dd*, *J* = 17 and 13 Hz, H-3_{ax}), 2.80 (1H, *dd*, *J* = 17 and 3 Hz, H-3_{eq}), 1.76 (6H, *br s*, 2 × Me).

5,4′-*Dihydroxy*-2″,2″-*dimethylpyrano*-[5″,6″; 6,7]-*isoflavone* (**8**, *alpinumisoflavone*). Mp 210–212° lit. [20] Mp 213–214°; identical with an authentic specimen [21] (mmp 210–212°).

5,7,4′-*Trihydroxy*-6-(3-*methyl*-2-*butenyl*)-*isoflavone* (**9**, *wighteone*). Vitreous solid; UV λ_{max}^{MeOH} nm (log ε): 227 (4.25), 265 (4.12); (+ NaOAc): 230, 270, 331; (+ $AlCl_3$): 235, 277, 313, 363 (after 20 min); ^{1}H NMR ($CDCl_3$): δ12.56 (1H, *s*, ex. D_2O, OH-5), 7.84 (1H, *s*, H-2), 7.41 (2H, *d*, *J* = 8.5 Hz, H-2′, H-6′), 6.92 (2H, *d*, *J* = 8.5 Hz, H-3′, H-5′), 6.40 (1H, *s*, H-8), 5.29 (1H, *br t*, *J* = 7 Hz, =CH), 3.47 (2H, *d*, *J* = 7 Hz, CH_2), 1.85, 1.79 (3H each, *br s*, 2 × Me). Mass spectral data were coincident with those reported in the literature [22].

5, 2′, 4′-*Trihydroxy*-2″, 2″-*dimethylpyrano*-[5″, 6″: 6,7]-*isoflavone* (**10**, *parvisoflavone A*): Vitreous solid; ^{1}H NMR: δ12.36 (1H, *s*, ex. D_2O, OH-5), 7.19 (1H, *d*, *J* = 8.5 Hz, H-6′), 6.67 (1H, *d*, *J* = 10 Hz, H-4″), 6.57 (1H, *d*, *J* = 2 Hz, H-3′), 6.50 (1H, *dd*, *J* = 8.5 and 2 Hz, H-5′), 6.26 (1H, *s*, H-8), 5.74 (1H, *d*, *J* = 10 Hz, H-3″), 1.44 (6H, *s*, 2 × Me); UV and MS were coincident with those of parvisoflavone A [24].

5, 2′, 4′-*Trihydroxy*-2″, 2″-*dimethylpyrano*-[5″, 6″: 6,7]-*flavone* (**11**, *cycloartocarpesin*). Mp 275–276°, lit. [25] mp 277–278°; UV λ_{max}^{MeOH} nm (log ε): 286 (4.16), 308sh (4.02), 354 (4.12); ^{1}H NMR (MeCO-d_6): δ12.12 (1H, *s*, ex. D_2O, OH-5), 7.84 (1H, *d*, *J* = 8.5 Hz, H-6′), 7.10 (1H, *s*, H-3), 6.65 (1H, *d*, *J* = 10 Hz, H-4″), 6.59 (1H, *d*, *J* = 2 Hz, H-3′), 5.56 (1H, *dd*, *J* = 8.5 and 2 Hz, H-5′), 6.44 (1H, *s*, H-6), 5.14 (1H, *d*, *J* = 10 Hz, H-3″), 1.45 (6H, *s*, 2 × Me); ^{13}C NMR (Me_2CO-d_6, 75 MHz); δ183.4 (*s*, CO), 162.9, 162.6 (*s* each, C-2, C-7), 159.9, 159.3 (*s* each, C-5, C-8a), 157.9 (*s*, C-4′), 156.8 (*s*, C-2′), 130.9 (*d*, C-6′), 129.1 (*d*, C-3″),115.8 (*d*, C-4″), 110.5 (*s*, C-1′), 109.0, 108.51 (*d* each, C-3, C-5′), 108.4 (*s*, C-6), 105.7 (*s*, C-4a), 104.2 (*d*, C-3′), 95.5 (*d*, C-8), 78.6 (*s*, C-2″), 28.3 (*q* 2 × Me); EI-MS *m/z* (rel. int.): 352 $[M]^+$ (32), 337 $[M - Me]^+$ (100), 203 [ring A$]^+$ (25), 168.5 $[M - Me/2]^{2+}$, 137 [ring B$]^+$ (6).

2,4,2′,4′-Tetrahydroxy-3-(3-methyl-2-butenyl)-chalcone (**12**). Mp 204–205° (Et_2O); UV λ_{max}^{MeOH} nm (log ε): 218 (4.08), 262sh (3.78), 312sh (3.80), 385 (4.17); (+ NaOAc): 222, 262sh, 320sh, 396; (+ CH_3ONa): 219, 276sh, 340sh, 452; (+ $AlCl_3$, after 10 min): 219, 272sh, 322, 420; ^{1}H NMR: δ12.25 (1H, *s*, ex. D_2O, 5-OH), 8.19 (1H, *d*, *J* = 15.5 Hz, H-β), 7.86 (1H, *d*, *J* = 9 Hz; H-6′), 7.78 (1H, *d*, *J* = 15.5 Hz; H-α), 7.66 (1H, *d*, *J* = 8.5 Hz; H-6), 6.51 (1H, *d*, *J* = 9 Hz, H-5′), 6.50 (1H, *d*, *J* = 2.5 Hz, H-3), 6.43 (1H, *dd*, *J* = 8.5 + 2.5 Hz, H-5), 5.25 (1H, *br t*, *J* = 7 Hz, CH =), 3.35 (2H, *d*, *J* = 7 Hz, CH_2), 1.76, 1.62 (3H each, *br s*, 2 × Me); ^{13}C NMR Table 1; EI-MS *m/z* (rel. int.): 340 $[M]^+$ (23), 322 $[M - H_2O]^+$ (89), 311 $[M - CHO]^+$ (17), 307 $[M - Me]^+$ (12), 305 (12), 279 $[322 - C_3H_7]^+$ (50), 267 $[322 - C_4H_7]^+$ (100), 205 [RDA of 340]$^+$ (19), 176 (19), 161 [RDA of 279]$^+$ (30), 149 [RDA of 267]$^+$ (35), 134 $[C_8H_6O_2]^+$ (23), 123 (23).

Synthesis of 2,4,2′,4′-tetrahydroxychalcone. 2,4-Dibenzyloxybenzaldehyde (1.9 g, 6 mM) and 2,4-dibenzyloxyresacetophenone (1 g 3 mM) in MeOH (20 ml) and 50% KOH (10 ml) were held at reflux for 3 hr. The reaction mixture was poured into ice-water, acidified and extracted with EtOAc. The residue on silica gel CC eluted with hexane–EtOAc (7:3) gave 2,4,2′,4′-tetrabenzyloxychalcone (1.9 g, 95% yield). To a soln of the benzylated chalcone (0.9 g) in CH_2Cl_2 (18 ml) was added 1 M BCl_3 (9 ml) at 0° and the mixture stirred for 25 min. The reaction mixture was poured into ice-water and stirred for 2 hr. The aq. soln was washed with $CHCl_3$ and extracted with EtOAc. The residue of the latter extract on a silica gel CC eluted with CH_2Cl_2–EtOAc–MeOH (8:2:1) gave 2,4,2′,4′-tetrahydroxychalcone (260 mg, 67% yield): mp > 320°; ^{1}H NMR (Me_2CO-d_6): δ12.2 (1H, ex. D_2O, OH-5), 8.10 (1H, *d*, *J* = 15.5 Hz, H-α), 7.89 (1H, *d*, *J* = 9 Hz, H-6′), 7.66 (1H, *d*, *J* = 15.5 Hz, H-β), 7.56 (1H, *d*, *J* = 9 Hz, H-6), 6.40 (1H, *J* = 2 Hz, H-3), 6.34 (2H, *dd*, *J* = 9 and 2 Hz, H-5, H-5′), 6.24 (1H, *d*, *J* = 2 Hz, H-3′); ^{13}C NMR Table 1: 167.4 (*s*, C-4′), 166.2 (*s*, C-2′), 103.7 (*s*, C-3′); EI-MS *m/z* (rel. int.): 272 (12), 153 (23), 137 (100), 123 (26).

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