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Phenylpropanoid derivatives and biflavones at different stages of differentiation and development of *Araucaria angustifolia*

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Abstract

Chemical investigations carried out with tissues at different developmental stages of *Araucaria angustifolia* established the presence of *E* and *Z* isomers of octadecyl *p*-coumarate and octadecyl ferulate in undifferentiated callus; in the seedling stems, the source of explants, three biflavones of the amentoflavone-type were isolated, whereas the diterpene, *trans*-communic acid, was obtained from the seedling roots. Adult stems accumulated the benzaldehydes, vanillin, *p*-hydroxybenzaldehyde and coniferaldehyde; the lignans, pinoresinol, eudesmin and lariciresinol; and the isoflavones, cabreuvine and irisolidone.

Keywords: *Araucaria angustifolia*; Araucariaceae; Cell culture; Long chain cinnamate esters; Biflavonoids; Lignans; Isoflavonoids; Benzaldehydes

1. Introduction

Araucaria angustifolia (Araucariaceae) known as "Paraná pine" is an endemic conifer in southern Brazil (Handro, 1986). Its wood has economic importance as a raw material for paper and pulp production (Lorenzi, 1992). Due to the high content of its phenolics, its knot powder has been used as a partial substitute for phenolic resins (Anderegg and Rowe, 1974; Campello and Fonseca, 1975). The major chemical constituents in the wood and in the resin of *A. angustifolia* have been shown to be the lignans (+)-pinoresinol, (+)-lariciresinol, as well as (−)-secoisolariciresinol and its mono- and dimethyl ethers (Driselius and Lindberg, 1956; Campello and Fonseca, 1975; Fonseca et al., 1978, 1979). A biosynthetic sequence involving the oxidative dimerization of two molecules of coniferyl alcohol to produce (+)-pinoresinol, followed by two stepwise enantiospecific benzylic reductions to produce the last two lignans, has been demonstrated in *Forsythia intermedia* and is considered to be of widespread occurrence in vascular plants (Lewis et al., 1995). Another study carried out with *A. angustifolia* growing in Japan described two norlignans, cryptoresinol and 2,3-*bis*-(*p*-hydroxyphenyl)-2-cyclopentene-1-one, as well as 4-4′-dihydroxychalcone (Ohashi et al., 1992). Several biflavonoids have also been isolated from *Araucaria* species (Khan et al., 1971; Ilyas et al., 1978; Ilyas and Ilyas, 1994).

Since the "Paraná-pine" forest in the south of Brazil has been cleared due to extensive logging, several efforts have been made to propagate the conifers, including in vitro propagation of *A. angustifolia* (Handro, 1986). To date, all phytochemical investigations have addressed the adult plant. Since the accumulation of secondary compounds in seedlings or in cell tissue culture is potentialy important information for biosynthetic studies, herein we describe a phytochemical investigation carried out on callus developed from segments of seedling stems, in comparison to the stems and roots of seedlings, and to the adult wood of *A. angustifolia*.

2. Results

The callus culture of *A. angustifolia*, initiated from internodes of etiolated seedlings (Handro, 1986), was exhaustively extracted using EtOH. The resulting extract

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was submitted to partition and column chromatography to afford two pairs of isomers identified as *E* and *Z* isomers of octadecyl *p*-coumarate (**1a** and **1b**) (Bohlman et al., 1979; Snook et al., 1994) and of octadecyl ferulate (**2a** and **2b**) (Baldé et al., 1991; Bernards and Lewis, 1992). The structural identification, especially of the cinnamoyl moieties, was based on comparison of $^{1}H/^{13}C$ NMR and EIMS data to those described in the literature (Baldé et al., 1991). The relative composition of *E* versus *Z* isomers in both octadecyl *p*-coumarate and octadecyl ferulate were determined as 4:1 based on the integration of ^{1}H NMR of aromatic and olefinic proton signals. The chain length was determined in both cases as C18 based on the molecular ion peaks at *m/z* 416 and 446, respectively, in their EIMS spectra.

The ethanolic extract from seedling stems was similarly submitted to organic partition and chromatographic procedures which resulted in the isolation of biflavonoids **3a–3c**. The establishment of these compounds as flavonoid dimers was based on the detection of molecular ions in the MS spectra of **3a** and **3b**. The definition of a common 3′-8″-interflavonoid linkage (amentoflavone-type) between the flavonoid unit was based on the analysis of ^{1}H NMR data (Table 1). In addition, signals corresponding to H3′ and H6″ protons were missing in the ^{1}H NMR sprectral spectra for all the biflavonoids. The position of the 3′-8″ interflavonoid linkage was further confirmed by ^{13}C NMR spectral data obtained for **3b** in which the signals δ 123.48 and 102.83 were assigned to C3′ and C8″, respectively (Markhan et al., 1987). Since no other type of dimer would match the obtained data, the amentoflavone-type emerged as the most likely dimer, and only the number and the location of hydroxyl and methoxyl groups in each compound remained to be defined. The presence of two chelated hydroxyls at C-5/C-5′, with the exception of **4**, could be observed in all ^{1}H NMR spectra.

The biflavonoids **3a** and **3b**, which have three methoxyls each, were respectively identified as 7,4′,7″-tri-*O*-methyl amentoflavone (Beckmann et al., 1971; Geiger and Quinn, 1982) and 7,4′,4‴-tri-*O*-methyl amentoflavone (sciadopitysin) (Konda et al., 1995; Ishratullah et al., 1978; Reddy and Krupadanam, 1996). The ^{13}C NMR spectra (see Experimental) were in agreement with the structures and with the reported data. Thus, the chemical shifts corresponding to the ring A, B, and C were similar to each other. The major differences observed were the signals of the other flavonoid moieties involving the C-6″/C-8″ carbons, in which the signals at δ 95.21/102.83 assigned to the compound **3** appeared deshielded at δ 99.5/103.49 for compound **4**, as expected when the methoxyl group at C-7″ is replaced by a hydroxyl. In contrast, the replacement of a hydroxyl by a methoxyl group at C-4 (ring B′) caused a deshielding and a shielding effect respectively at carbons C-4‴ (161.04 to 162.73 δ) and C-3‴/C-5‴ (116.08 to 114.60 δ). Additionally, the locations of the methoxyl groups for **3a** and **3b** were also assigned based on NOE effects with irradiation at the methoxyl frequencies (Table 2). The structure for the remaining biflavonoid was determined as 4′,4‴-di-*O*-methyl amento-flavone (**3c**) (isogingketin) (Baker et al., 1963; Geiger and Quinn, 1982).

The ethanolic extract from the seedling roots was submitted to partition and chromatographic procedures resulting in the isolation of *trans*-communic acid (**7**) as the major compound. Its structure was established based on comparison of ^{1}H and ^{13}C NMR spectral data to that of reference data (Zdero et al., 1991; Martins et al., 1999). This labdane diterpene was previously described from *Juniperus communis* (Arya et al., 1961), and from *Araucaria cunninghami* (Caputo et al., 1974) but this is the first report from *A. angustifolia*.

The ethanolic extracts from stems of the adult plant of *A. angustifolia*, when submitted to partition and

Table 1
^{1}H NMR spectral data of biflavonoids **3a**–**3c** isolated from *Araucaria angustifolia*[d]

Protons	**3a**[a]	**3b**[b]	**3c**[c]
H-3	6.46, *s*	6.57, *s*	6.53, *s*
H-6	6.23, *d* (2.2),	6.27, *d* (2.0)	6.20, *d* (2.1)
H-8	6.39 *d* (2.2)	6.41 *d* (2.0)	6.38 *d* (2.1)
H-2′	7.81 *d* (2.3)	7.93 *d* (2.3)	7.68 *d* (2.4)
H-5′	7.10, *d* (8.8)	7.19, *d* (8.8)	7.12, *d* (8.8)
H-6′	7.92 *dd* (8.7, 2.3)	8.01 *dd* (8.8, 2.4)	7.94 *dd* (8.8, 2.4)
H-3″	6.50, *s*	6.59, *s*	6.54, *s*
H-6″	6.57, *s*	6.46, *s*	6.38, *s*
H-2‴/H-6‴	7.32, *d* (8.8)	7.48, *d* (8.8)	7.42, *d* (8.7)
H-3‴/H-5‴	6.71, *d* (8.8)	6.84, *d* (8.8)	6.77, *d* (8.8)
C-5″ OH	13.11, *s*	–	12.89, *s*,
C-5′OH	12.73, *s*	–	12.71, *s*,
C-7 OMe	3.78, *s*	3.81, *s*	–
C-4′OMe	3.74, *s*	–	3.81, *s*
C-7″ OMe	3.80, *s*	3.80, *s*	–
C-4‴ OMe	–	3.82, *s*	3.74, *s*

[a] 200 MHz, $CDCl_3$+MeOD.
[b] 300 MHz, MeOD.
[c] 500 MHz, MeOD.
[d] Coupling constants in Hz.

Table 2
NOE-diff spectra observed for biflavonoids of *Araucaria angustifolia*

Biflavonoid	Methoxyl irradiated (δ)	Correlations observed
3a	3.78 (C-7)	6.39, *d* (H-8), 6.23, *d* (H-6)
	3.80 (C-4′)	7.10, *d* (H-5′)
	3.74 (C-7″)	6.57, *s* (H-6″)
3b	3.81 (C-7)	6.27, *s* (H-6)
	3.80 (C-7″)	6.46, *s* (H-6″)
	3.82 (C-4‴)	6.48, *d* (H-3‴/ H-5‴)
3c	3.81 (C-4″)	7.12, *d* (H-5″)
	3.74 (C-4‴)	6.77, *d* (H-3‴/ H-5‴)

chromatographic procedures, resulted in the isolation of the known isoflavonoids, cabreuvine (**4a**) (Villain and Jadot, 1976) and irisolidone (**4b**) (Carman et al., 1985), and the lignans, (+)-pinoresinol (**5a**) (Ludwig et al., 1964), (+)-eudesmin (**5b**) (Kato et al., 1990), (+)-lariciresinol (**6a**) and *O*-acetyl-lariciresinol (**6b**). The lignan secoisolariciresinol, previously detected in high concentration in both *A. angustifolia* resin and knots (Ohashi et al., 1992), was not detected in the tissues examined. Other phenolics isolated and characterized included the benzaldehydes, vanillin, *p*-hydroxybenzaldehyde (Beistel and Edwards, 1976), and coniferaldehyde (Etse et al., 1988; Farah and Samuelsson, 1992).

3. Discussion

A. angustifolia at the undifferentiated stage accumulated octadecyl *p*-coumarate (**1**) and octadecyl ferulate (**2**) as detectable secondary metabolites; similar compounds have already been described for the Pinaceae species (Chatterjee et al., 1977). Since such compounds have also been detected as constituents of suberin in injured potato tubers (Bernards and Lewis, 1992), it is expected that they may play similar protective roles in cell cultures of *A. angustifolia*. Alternatively, the compounds **1a–2b** might represent the non-toxic forms of *p*-coumaric and ferulic acid as previously demonstrated (Nair et al., 1988). There is no previous report for the occurrence of these compounds in *A. angustifolia*.

The analysis of seedling stems, which was the source of explants for callus initiation, revealed a variety of dimers of apigenin (**3a–3c**), produced by oxidative coupling followed by methylation at different positions of amentoflavone. Such dimers of flavonoids have been isolated from several sources, including adult ***A. angustifolia*** **(Ilyas and Ilyas, 1994), but not from the seedlings. The benzaldehydes, *p*-hydroxybenzaldehyde, and vanillin observed in the adult stems were not previously described in *A. angustifolia*. Coniferaldehyde, also detected in the adult stems, is an intermediate in the general phenylpropanoid metabolism and is seldom detectable as a natural product in woody plants.**

This study of tissues of *A. angustifolia* at different growth stages, including callus cultures, revealed a broad range of secondary compounds that can be linked by a biosynthetic scheme (Fig. 1). The aspects involved in the regulation of phenylpropanoid metabolism that

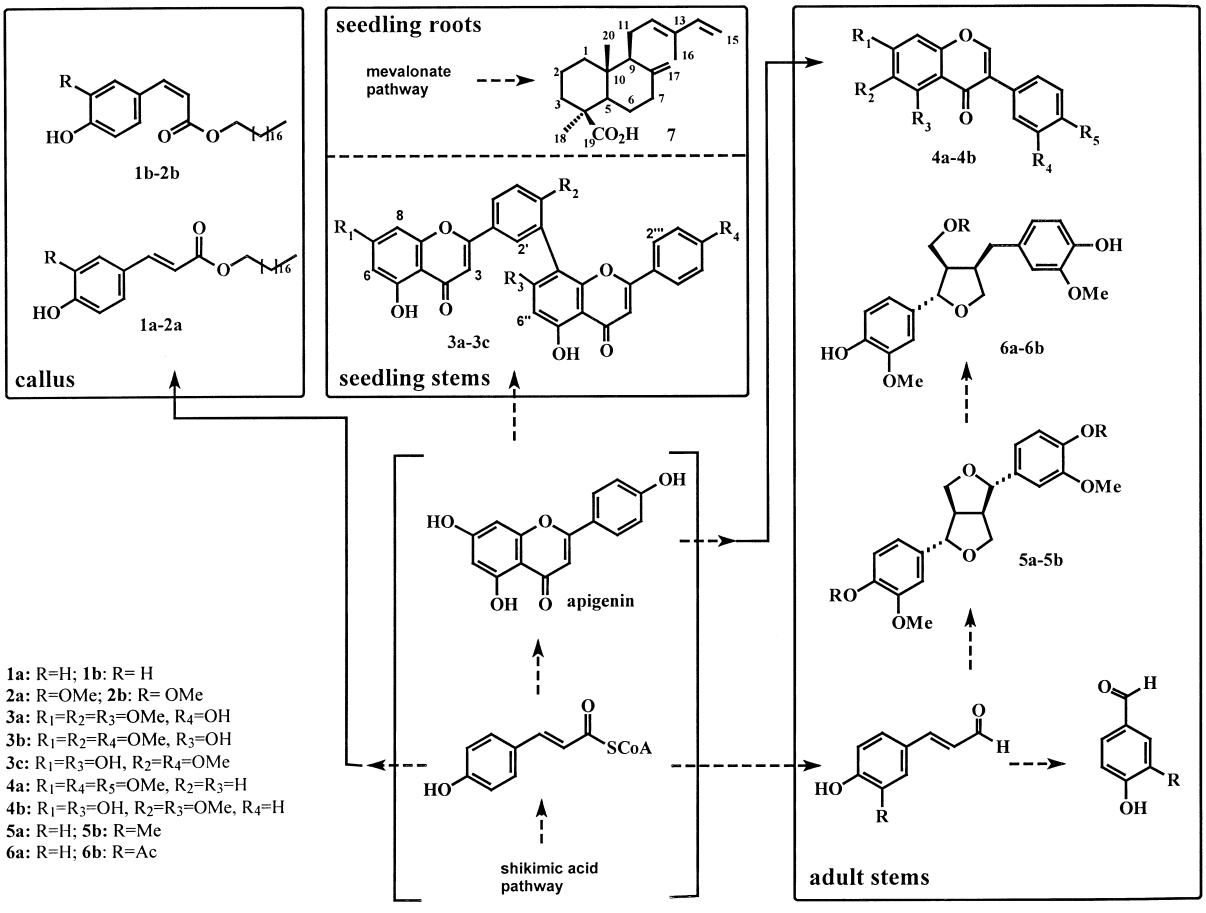

Fig. 1. Secondary metabolites isolated from *Araucaria angustifolia* and their biosynthetic relationship.

account for these differences observed during the differentiation and development of *A. angustifolia* requires further investigation.

4. Experimental

4.1. General

^{1}H NMR (200 and 500 MHz), ^{13}C NMR (75 and 125 MHz), COSY and HMBC spectra ($^3J_{C\text{-}H}$ optimized to 8.0 Hz) were recorded on VARIAN DPX-300 and DPX-500 spectrometers using $CDCl_3$ and MeOD as solvents and TMS as internal standard. IR spectra were measured in KBr. EIMS were measured at 70 eV on a HP 5990/5988 A spectrometer. CC: silica gel 60 (Merck) 230–400 mesh, TLC were performed on silica gel GF $_{254}$ (Merck) plates.

4.2. Plant material

A. angustifolia (Bert.) O. Kuntz, plants and seeds were collected in Salesópolis (State of São Paulo, Brazil) and identified by Professor Walter Handro (Instituto de Biociências da USP).

4.3. Tissue culture

Callus cultures were initiated from stems explants (~1 cm) derived from one month old etiolated seedlings. The inocula were maintained in MS media complemented with 2,4-D (1 mg/ml) and incubated at 26°±C, 45 μM $s^{-1}m^{-2}$, 16 h light day^{-1}. Calli were developed after 10 days and were routinely transferred at 8 week intervals under the same conditions.

4.4. Extraction and isolation of compounds

Fresh cells (182 g) were extracted with ethanol (4×500 ml)at room temperature with the aid of a sonicator and filtered. The crude extract (3.68 g) was suspended in methanol/water (4:1, 300 ml) partitioned with hexane (3×100 ml) and dichloromethane (3×300 ml). The dichloromethane fraction (239 mg) was subjected to silica gel column chromatography this being eluted with CH_2Cl_2:MeOH at increasing polarities, followed by prep-TLC to afford the isomers octadecyl *E*/*Z*-*p*-coumarate (**1a**/**1b**) (6.4 mg) and of octadecyl *E*/*Z*-ferulate (**2a**/**2b**) (10.3 mg). Fresh stems of seedlings (335 g) were extracted with EtOH (4×300 ml) at in an ultrasonic bath, yielding a crude extract (3.2 g) which was submitted to column chromatography and eluted with CH_2Cl_2–MeOH at increasing polarities, yielding 7,4′,7″-tri-*O*-methyl amentoflavone (3a) (7 mg), 7,4′,4‴-tri-*O*-methyl amentoflavone (**3b**) (8 mg), and 4′,4‴-di-*O*-methyl amentoflavone (3c) (3.2 mg). Fresh roots from seedlings (600 g) were extracted with ethanol in an ultrasonic bath (5×100 ml) at room temprature. The ethanol extract was saturated with water (100 ml) and extracted with hexane (3×300 ml) and then with dichloromethane (3×300 ml). The hexane fraction (3.2 g) was submitted to column chromatography over silica gel to yield sixteen fractions. Fraction twelve (76 mg) was submitted to prep TLC (CH_2Cl_2–MeOH, 5%) to give the diterpene, *trans*-communic acid (**7**) (29.0 mg). Dried pieces of adult wood (1.68 kg) were exhaustively extracted with ethanol in an ultrasonic bath at room temperature. The ethanolic extract (10 g) was suspended in EtOH (300 ml) and partitioned with hexane (3×200 ml) and dichloromethane (5×200 ml). The dichloromethane fraction was concentrated in vacuo, and then applied to a silica gel column, eluted with CH_2Cl_2–MeOH at increasing polarities to yield, *p*-hydroxybenzaldehyde (2.0 mg), vanillin (4.7 mg), coniferaldehyde (2.8 mg), cabreuvine (**4a**) (2.5 mg), irisolidone (**4b**) (1.0 mg), pinoresinol (**5a**) (13.8 mg), eudesmin (**5b**) (2.6 mg) and lariciresinol (**6**) (27.7 mg), respectively.

4.5. Octadecyl (E)-p-coumarate (*1a*)

ν_{max}^{film} cm^{-1}: 1716, 1675, 1588, 1465, 980. MS *m*/*e*: 416 [M^+] (13), 207 (1), 177 (2), 164 (100), 147 (56), 119 (12), 107 (17). ^{1}H NMR 300 MHz ($CDCl_3$) δ: 0.81 (*t*, 6.9 Hz, 3H, H-20′), 1.18 (*bs*, 30H, -$(CH_2)_{17}$-), 1.61 (*m*, 2H, H-2′), 4.11 (*t*, 6.7 Hz, 2H, H-1′), 5.46 (*bs*, 1H, Ar-OH), 6.23 (*d*, 15.9 Hz, 1H, H-8), 6.79 (*d*, 8.6 Hz, 2H, H-2/H-6), 7.36 (*d*, 8.6 Hz, 2H, H-3/H-5), 7.55 (*d*, 15.9 Hz, 1H, H-7). ^{13}C NMR 75 MHz, $CDCl_3$) δ: 14.07 (C-20′), 25.98 (C-3′), 29.68 (C-4′-C-19′), 31.92 (C-2′), 64.63 (C-1′), 115.75 (C-8), 115.85 (C-3/C-5), 129.92 (C-1), 132.00 (C-2/C-6), 144.34 (C-24), 157.78 (C-4), 167.53 (C-9).

4.6. Octadecyl (Z)-p-coumarate (*1b*)

ν_{max}^{film} cm^{-1}: 1716, 1675, 1588, 1465, 720. MS *m*/*e*: 416 [M^+] (3), 207 (1), 177 (2), 164 (100), 147 (56), 119 (12), 107 (17). ^{1}H NMR 300 MHz ($CDCl_3$) δ: 0.81 (*t*, 6.9 Hz, 3H, H-20′), 1.18 (*bs*, 30H,-$(CH_2)_{17}$-), 1.61 (*m*, 2H, H-2′), 4.05 (*t*, 6.7 Hz, 2H, H-1′), 5.46 (*bs*, 1H, Ar-OH), 5.76 (*d*, 12.7 Hz, 1H, H-8), 6.74 (*d*, 12.7 Hz, 1H, H-7), 6.79 (*d*, 8.6 Hz, 2H, H-2/H-6), 7.36 (*d*, 8.6 Hz, 2H, H-3/H-5).

4.7. Octadecyl (E)-ferulate (*2a*)

ν_{max}^{film} cm^{-1}: 3422, 2851, 1711, 1634, 1161, 982. MS *m*/*e*: 446 [M^+] (14), 207 (1), 194 (100), 177 (55), 149 (16), 17 (23). ^{1}H NMR 300 MHz ($CDCl_3$) δ: 0.77 (*t*, 6.8 Hz, 3H, H-20′), 1.18 (*bs*, 30H, -$(CH_2)_{17}$-), 1.61 (*m*, 2H, H-2′), 4.12 (*t*, 6.8 Hz, 1H, H-1′), 6.22 (*d*, 15.9 Hz, 1H, H-8), 6.84 (*d*, 8.1 Hz, 1H, H-5), 6.97 (*d*, 1.8 Hz, 1H, H-2), 6.84 (*d*, 8.1 Hz, 1H, H-5), 7.00 (*dd*, 8.1 and 1.8 Hz, 1H, H-6), 7.54 (*d*, 15.9 Hz, 1H, H-7). ^{13}C NMR 75 MHz ($CDCl_3$) δ: 14.11 (C-20′), 25.98 (C-3′), 29.30 (C-4′-C-19′), 31.91

(C-2′), 55.92 (Ar-OCH_3), 64.44 (C-1′), 109.26 (C-2), 114.67 (C-5), 115.68 (C-8), 123.03 (C-6), 128.83 (C-1), 144.60 (C-7), 146.72 (C-3), 147.86 (C-4), 167.38 (C-9).

4.8. Octadecyl (Z)-ferulate (**2b**)

ν_{max}^{film} cm^{-1}: 3422, 2851, 1711, 1634, 1161, 982 cm^{-1}. MS *m/e*: 446 [M^+] (14), 207 (1), 194 (100), 177 (55), 149 (16), 17 (23). ^1H NMR 300 MHz ($CDCl_3$) δ: 0.77 (*t*, 6.8 Hz, 3H, H-20′), 1.18 (*bs*, 30H, -$(CH_2)_{17}$-), 1.61 (*m*, 2H, H-2′), 4.05 (*t*, 6.8 Hz, 1H, H-1′), 5.74 (*d*, 12.9 Hz, 1H, H-8), 6.72 (*d*, 12.9 Hz, 1H, H-7), 6.84 (*d*, 8.1 Hz, 1H, H-5), 6.97 (*d*, 1.8 Hz, 1H, H-2), 7.00 (*dd*, 8.1 and 1.8 Hz, 1H, H-6).

4.9. 7,4′,7″-tri-O-Methyl amentoflavone (**3a**)

MS *m/e*: 580 ($C_{32}H_{10}O_{22}$) [M^+] (3), 446 (5), 326 (21), 290 (12), 281 (9), 256 (13), 207 (27), 165 (10), 149 (100), 73 (21). ^1H NMR see Table 1. ^{13}C NMR 75 MHz (MeOD) δ: 55.65, 55.82, 56.18 (3 MeO), 92.41 (C-8), 95.21 (C-6″), 98.17 (C-6), 102.83 (C-8″), 103.49 (C-3″), 104.40 (C-3), 104.60 (C-10″), 104.94 (C-8′), 105.50 (C-10), 111.17 (C-5′), 116.08 (C-3‴, C-5‴), 121.80 (C-1″)[a], 122.00 (C-1‴)[a], 123.19 (C-3′), 127.77 (C-2‴/C-6‴), 127.85 (C-2′), 130.93 (C-6′), 154.17 (C-9″), 157.66 (C-9), 160.63 (C-4′), 161.04 (C-4‴), 161.96 (C-7″)[b], 162.26 (C-5″)[b], 162.49 (C-5), 164.05 (C-2″)[c], 164.29 (C-2)[c], 165.38 (C-7), 182.28 (C-4)[d], 182.75 (C-4″)[d].

[a, b, c, d] Assignments which can be reversed.

4.10. 7, 4′,4‴-tri-O-Methyl amentoflavone (**3b**)

^{13}C NMR 75 MHz (MeOD) δ: 55.65, 55.82, 56.18 (3 MeO), 95.57 (C-8), 99.51 (C-6″), 99.37 (C-6), 103.49 (C-8″), 102.81 (C-3″), 104.21 (C-3), 103.41 (C-10″), 104.94 (C-8′), 104.21 (C-10), 111.44 (C-5′), 114.60 (C-3″, C-5‴), 121.80 (C-1″)[a], 122.10 (C-1‴)[a], 123.58 (C-3′), 127.90 (C-2″/C-6‴), 128.25 (C-2′), 131.34 (C-6′), 155.81 (C-9″), 158.17 (C-9), 160.94 (C-4′), 162.72 (C-4‴), 161.77 (C-7″)[b], 162.28 (C-5″)[b], 161.16 (C-5), 163.56 (C-2″)[c], 164.38 (C-2)[c], 164.95 (C-7), 183.36 (C-4)[d], 182.63 (C-4″)[d].

[a, b, c, d] Assignments which can be reversed.

4.11. 4′,4‴-di-O-Methyl amentoflavone (**3c**)

MS *m/e*: 566 ($C_{32}H_{10}O_{22}$) [M^+] (100), 538(6), 537(15), 535(8), 296 (7) 268 (7), 185 (12), 169 (11), 153(21), 135(72), 109 (24), 73 (21). For ^1H NMR spectrum, see Table 1.

4.12. Cabreuvin (**4a**)

MS *m/e*: 312 ($C_{18}H_{16}O_6$) [M^+] (2), 162 (15), 156 (3), 297 (3), 150 (6), 122 (11), 119 (15). ^1H NMR 300 MHz ($CDCl_3$) δ: 3.84 (*s*, 3H; Ar-OMe), 3.85 (*s*, 3H; Ar-OMe), 3.86 (*s*, 3H; Ar-OMe), 6.80 (*d*, 2.4 Hz, 1H; H-8), 6.86 (*d*, 8.3 Hz, 1H; H-5′), 6.94 (*dd*, 8.9 and 2.4 Hz; H-6), 7.00 (*dd*, 8.3 and 1.9 Hz, 1H; H-6′), 7.15 (*d*, 1.9 Hz, 1H; H-2′), 7.90 (*s*, 1H; H-2), 8.84 (*d*, 8.9 Hz, 1H; H-5).

4.13. Irisolidone (**4b**)

MS *m/e*: 314 ($C_{17}H_{14}O_6$) [M^+] (66), 299 (100), 271 (40), 182 (1), 167 (4), 139 (33), 132 (4). ^1H NMR 500 MHz ($CDCl_3$) δ: 3.78 (*s*, 3H; Ar-OMe), 3.91 (*s*, 3H; Ar-OMe), 6.20 (*ls*, 1H; C7-OH), 6.38 (*bs*, 1H; H-8), 6.86 (*d*, 8.8 Hz, 2H; H-3′/H-5′), 7.40 (*d*, 8.8 Hz, 2H; H-2′/H-6′), 7.86 (*s*, 1H; H-2), 12.55 (*s*, 1H; C5-OH).

4.14. (+)-Pinoresinol (**5a**)

$[\alpha]_D^{25} + 84.4°$ (*c* 1.0; MeOH), MS *m/e*: 386 ($C_{22}H_{26}O_6$) [M^+] (29), 220 (4), 219 (1), 194 (9), 193 (4), 180 (3), 177 (67), 165 (100), 151 (65), 137 (7).

4.15. (+)-Eudesmin (**5b**)

$[\alpha]_D^{25} + 60.5°$ (*c* 1.0; MeOH), MS *m/e*: 386 ($C_{22}H_{26}O_6$) [M^+] (29), 220 (4), 219 (1), 194 (9), 193 (4), 180 (3), 177 (67), 165 (100), 151 (65), 137 (7).

4.16. (+)-Lariciresinol (**6**)

$[\alpha]_D^{25} + 23°$ (*c* 1.0; Me_2CO), MS *m/e*: 360 ($C_{20}H_{24}O_6$) [M^+] (27), 342 (2), 219 (10), 194 (30), 152 (16), 151 (51), 137 (100), 123 (13), 122 (27).

4.17. trans-Communic acid (7)

^1H NMR 200 MHz ($CDCl_3$) δ: 0.58 (*s*, 3H-20), 1.18 (*s*, 3H-18), 1.68 (*s*, 3H-16), 4.38 (*bs*, 1H; H-17a), 4.78 (*bs*, 1H; H-17b), 4.81 (*d*, 11.2, H-15a), 4.98 (*d*, 17.0, H-15b), 5.34 (*t*, 6.6, H-12), 6.26 (*dd*, 17.0, 11.2, H-14). ^{13}C NMR 50 MHz ($CDCl_3$) δ: 11.8 (C-20), 12.8 (C-16), 19.9 (C-2), 23.2 (C-6), 25.8 (C-11), 29.0 (C-18), 37.9 (C-10), 38.4 (C-1), 39.2 (C-3), 40.3 (C-7), 44.2 (C-4), 56.2 (C-9), 56.3 (C-5), 107.8 (C-17), 109.9 (C-15), 133.4 (C-12), 133.8 (C-14), 141.6 (C-13), 147.9 (C-8), 184.2 (C-19).

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