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2,3-Dihydrobenzofuran neolignans from *Aristolochia pubescens*

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Abstract

From a hexane extract of stems and roots of *Aristolochia pubescens*, the new neolignans (2*S*,3*S*,1′*R*,2′*R*)- and (2*S*,3*S*,1′*S*,2′*R*)-2,3-dihydro-5-(1′,2′-dihydroxypropyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-3-methylbenzofuran and (2*S*,3*S*,1′*R*,2′*R*)- and (2*S*,3*S*,1′*S*,2′*R*)-2,3-dihydro-5-(1′,2′-dihydroxypropyl)-2-(3,4-dimethoxyphenyl)-7-methoxy-3-methylbenzofuran were isolated, together with the known neolignan licarin A, and its *bis*nor-neolignan aldehyde and acid derivatives. In addition, sitosterol, 8*R*,9*R*-oxide-β-caryophyllene, kobusone, *ent*-kauran-16α,17-diol, vanillin, vanillic acid, (+)-sesamin, (+)-eudesmin, and (−)-cubebin were isolated. The structures of the new compounds have been elucidated by spectroscopic methods and by chemical transformation using Mosher's acid chloride.

Keywords: *Aristolochia pubescens*; Aristolochiaceae; Neolignans; *Bis*nor-neolignans; Lignans; Sesquiterpenes; Diterpenes; Vanillin; Vanillic acid

1. Introduction

In continuing chemical studies on medicinal plants belonging to the genus *Aristolochia* (Aristolochiaceae) (Lopes, Martins & Piasentin, 1991; Lopes & Humpfer, 1997), we report the isolation and structural elucidation of sixteen compounds from *A. pubescens* Willd. The compounds: sitosterol, (+)-sesamin, (+)-eudesmin and (−)-cubebin, whose occurrence is common in the Aristolochiaceae (Luiz, Bolzani, Trevisan & Lopes, 1990), were isolated together with the known compound *ent*-kauran-16α,17-diol, previously isolated from *A. triangularis* (Lopes, Bolzani, Trevisan & Grigolli, 1990). In addition, 8*R*,9*R*-oxide-β-caryophyllene, kobusone, vanillin and vanillic acid were isolated. Moreover, licarin A (**1**) and the corresponding *bis*nor-neolignan aldehyde (**2**) were also identified. Structural elucidations of the co-occurring *bis*nor-neolignan acid (**3**) as well as the new diols (**4**, **5**), structurally related to licarin A, are discussed.

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2. Results and discussion

The hexane extract from roots and stems of *A. pubescens* yielded 11 known compounds including lignans and terpenes which were purified by chromatographic methods and/or recrystallization. The compounds were identified by comparison of their physical and spectroscopic data with authentic samples, as well as with data described in the literature (Luiz et al., 1990; Lopes et al., 1990; Heymann, Tezuka, Kikuchi & Supriyatna, 1994; Pourchert, 1983; Abraham & Loftus, 1985; Achenbach et al., 1987; Wenkert et al., 1976; David, Yoshida & Gottlieb, 1994). The ^{13}C NMR data of **2** have not been reported in the literature. Thus, the chemical shift assignments of its carbons are presented in Table 1, which were made based on ^{1}H–^{13}C COSY and ^{1}H–^{13}C COSY long range experiments.

The main spectroscopic differences observed between **2** and **3** (Tables 1 and 2) were due to the substitution of the formyl group for a carboxyl group at C-5. This was evidenced by the mass spectrum of **3** which displayed a $[M]^+$ at m/z 330 (16 mass units more than **2**), and by the IR absorption bands for a carboxyl group (ν_{OH}: 3338, $\nu_{C=O}$: 1688 cm^{-1}). Besides, the ^{13}C NMR spectra showed the signals for C-5 and the carbonyl

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Table 1
^{13}C spectroscopic data of **2**–**5** ($CDCl_3$, δ)[b,d]

C	2	3	4	5
2	94.9 *d*	94.8 *d*	93.8 *d*	93.7 *d*
3	44.8 *d*	45.0 *d*	45.5 *d*	45.5 *d*
3a	133.6 *s*	133.2 *s*	134.6 *s*	134.7 *s*
4	120.0 *d*	120.0 *d*	114.2 *d*	114.2 *d*
5	131.0 *s*	122.6 *s*	133.2 *s*	133.2 *s*
6	111.8 *d*	113.7 *d*	110.2 *d*	110.3 *d*
7	146.8 *s*	146.8 *s*	144.0 *s*	144.1 *s*
7a	153.2 *s*	152.5 *s*	147.1 *s*	[a]
8	190.6 *d*	171.3 *s*	2 × 79.6 *d*	2 × 79.7 *d*
9			72.3 *d*	72.3 *d*
10			18.8 *q*	18.9 *q*
1′	131.4 *s*	131.2 *s*	131.8 *s*	132.5 *s*
2′	108.9 *d*	108.9 *d*	109.0 *d*	109.5 *d*
3′	146.2 *s*	146.1 *s*	146.7 *s*	149.1 *s*
4′	144.9 *s*	144.0 *s*	145.8 *s*	149.1 *s*
5′	114.3 *d*	114.3 *d*	114.2 *d*	110.8 *d*
6′	119.9 *d*	119.1 *d*	119.8 *d*	119.2 *d*
CH_3-3	17.7 *q*	17.8 *q*	17.4 *q*	17.5 *q*
OCH_3-3′	56.1 *q*[c]	56.1 *q*[c]	56.0 *q*[c]	56.0 *q*[c]
OCH_3-4′				55.9 *q*[c]
OCH_3-7	56.0 *q*[c]	56.0 *q*[c]	55.9 *q*[c]	55.9 *q*[c]

[a] Signal not observed.
[b,c] Values bearing the same sign may be reversed.
[d] Multiplicity established by DEPT experiments.

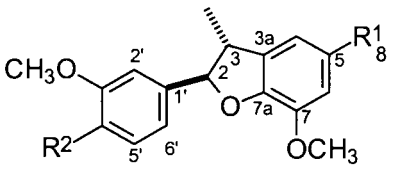
carbon were shifted upfield ($\Delta\delta_{C\text{-}5}$: 8.4, $\Delta\delta_{C=O}$: 19.3). These effects are in accordance with those observed comparing benzaldehyde with benzoic acid (Silverstein, Bassler & Morrill, 1991). Compound **3** had already been obtained by synthetic methods, but its NMR spectroscopic data had not been published (Hatakeyama, Nakano, Hatano & Migita, 1969; Hatakeyama & Nakano, 1970).

The acid **3**, the aldehyde **2** and the *O*-methyl derivative (**2a**) obtained from **2** should have the 2*R*,3*R* configuration, given that they have opposite optical rotations α_D (+14.7, +28.6 and +25.1°, respectively) from that displayed by (−)-kadsurenin M (**5b**, α_D −24.6°) earlier isolated from *Piper kadsura* (Ma, Han & Wang, 1993).

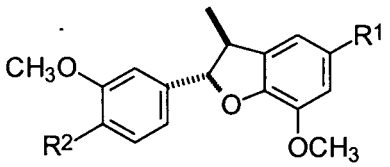
Table 2
^{1}H spectroscopic data of **2**–**5** [$CDCl_3$, δ, *J* (Hz)]

H	2	3	4[a]	5[a]
2	5.18 *d* (9.1)	5.16 *d* (9.3)	5.03 *d* (9.5)	5.06 *d* (9.5)
3	3.50 *m*	3.48 *m*	3.38 *m*	3.40 *m*
4, 6	7.30–7.27 *m*	7.54 *sl*	6.89–6.67 *m*	6.91–6.68 *m*
8	9.77 *s*		4.24 *d* (7.5)	4.26 *d* (7.5)
9			3.83 *m*	3.83 *m*
10			1.00 *d* (6.2)	1.02 *d* (6.2)
2′, 5′, 6′	6.87–6.84 *m*	6.89–6.86 *m*	6.89–6.67 *m*	6.91–6.68 *m*
CH_3-3	1.38 *d* (6.9)	1.37 *d* (6.5)	1.29 *d* (6.7)	1.31 *d* (6.8)
OCH_3-3′	3.88 *s*	3.88 *s*	3.81 *s*[b]	3.81 *s*[b]
OCH_3-4′				3.82 *s*[b]
OCH_3-7	3.81 *s*	3.83 *s*	3.79 *s*[b]	3.80 *s*[b]

[a,b] Values bearing the same sign may be reversed.



	R1	R2
1	$CH=CHCH_3$	OH
1a	$CH=CHCH_3$	OCH_3
2	CHO	OH
2a	CHO	OCH_3
3	COOH	OH



	R1	R2
4	$CH(OH)CH(OH)CH_3$	OH
5	$CH(OH)CH(OH)CH_3$	OCH_3

Scheme 1. Chemical transformations of **4** and **5**.

The spectroscopic data of compound **4** were somewhat similar to the known co-occurring licarin A (**1**). The mass spectrum of **4** displayed a $[M]^+$ at *m*/*z* 360, corresponding to the addition of two hydroxyl groups to the unsaturated carbons in the side chain of **1**. The ^{1}H, ^{13}C and DEPT NMR spectra allowed the characterisation of this chain by signals of a glycol (δ_H: 4.24, 3.83, and δ_C: 79.6, 72.3) and of a methyl group at a saturated carbon (δ_H: 1.00 and δ_C: 18.8). The correlation between the hydrogens of this unit was observed by a ^{1}H–^{1}H COSY experiment while the correlation between these hydrogens with carbons was observed with the ^{1}H–^{13}C COSY experiment.

Compound **4** was submitted to acetylation. The ^{1}H NMR spectrum of its product showed two aliphatic and one aromatic acetyl groups and double signals corresponding to CH_3-3 and CH-8, suggesting the pre-

sence of diastereoisomeric compounds in a mixture (**4a**:**4b** 1:1). These compounds could not be successfully separated using usual methods due to their close retention time values by HPLC under the conditions employed.

The IR, UV and NMR spectroscopic data of **5** were very similar to those of **4**, although the former differed by the presence of a methoxyl group at C-4′ instead of an hydroxyl group. This difference was supported by a $[M]^+$ at *m/z* 374, methoxyl signals (δ_H: 3.82 and δ_C: 55.9), deshielding observed for C-4′ ($\Delta\delta = 3.3$) and C-3′ ($\Delta\delta = 2.4$), and shielding for C-5′ ($\Delta\delta = 3.4$).

Compounds **1**–**5** displayed ^{1}H NMR spectroscopic data characteristic of *trans*-2-aryl-3-methyl-2,3-dihydro-benzofuranoid-type neolignans (Achenbach et al., 1987; David et al., 1994; Li, Iliefski, Lundquist & Wallis, 1997) [$\delta_{H\text{-}2}$: 5.0 (*d*, $J \sim 9$ Hz), $\delta_{Me\text{-}3}$: 1.3 to 1.4 (*d*, $J \sim 6.7$ Hz)]. In order to separate the diastereoisomers and establish the absolute configuration of the new compounds **4** was transformed into **5**. This in turn was submitted to esterification using (*R*)-(−)-α- and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) (Scheme 1). The products **5a** and **5b** from (*R*)-MTPA-Cl were isolated by preparative TLC, and the products from (*S*)-MTPA-Cl were analyzed as a mixture (**5c** + **5d**). When the transformation of **5** was attempted using the (*R*)-MTPA-Cl reagent, the ester derivatives were not produced, probably due to steric hindrance between the methoxyl group linked to (*R*)-MTPA at C-9 and the benzofuran ring. Instead of esters it afforded one ketone (**5a**) and one aldehyde (**5b**). The former could be produced via 1,2-rearrangement (Atkinson, 1995). The NMR spectral and α_D data of the aldehyde **5b** allowed us to identify it as (−)-kadsurenin M (Ma et al., 1993).

The ketone **5a** seems to be a new compound. Its side chain was established based on NMR spectral data, which showed signals corresponding to a methylene group (δ_H: 3.59 *s*, δ_C: 50.9), two methyl groups [δ_H: 2.12 *s*, δ_C: 29.7 and δ_H: 1.31 *d* (J = 6.8 Hz), δ_C: 17.5], and two methine groups [δ_H: 3.40 *m*, δ_C: 45.6 and δ_H: 5.05 *d* (J = 9.6 Hz), δ_C: 93.7]. Moreover, the IR spectrum displayed a carbonyl absorption band at 1720 cm^{-1}.

The NMR spectra of the products obtained from (*S*)-MTPA-Cl treatment revealed the presence of two compounds (**5c** + **5d**). Comparing the ^{1}H NMR spectrum of the mixture with that of **5**, significant chemical shift differences for H-4, 6, 8, 9, 10, and for CH_3-3 were observed. The resonances for H-4, 6 and CH_3-3 were shifted upfield, whereas those of H-8, 9, 10 were shifted downfield. It is interesting to notice that the magnitude of the coupling constant between H-8,9 decreased (**5**: $J_{H\text{-}8,9} = 7.5$ Hz, **5c**: $J_{H\text{-}8,9} = 5.9$ Hz, **5 d**: $J_{H\text{-}8,9} = 5.2$ Hz). It was also observed for the acetyl derivatives (**4a**, **4b**) obtained from **4** (**4**: $J_{H\text{-}8,9} = 7.5$ Hz, **4a**, **4b**: $J_{H\text{-}8,9} = 6.5$ Hz). It was probably due to an increased electron-withdrawing effect of the —O-MTPA or —O-Ac, in comparison to —OH as well as due to conformation changes. The deshielding observed for CH_3-10 ($\Delta\delta = 0.20$) suggests that this group was *anti*-periplanar to the benzofuran ring. The most stable conformation, in solution, for the MTPA moiety should be that established by Mosher (Ohtani, Kusumi, Kashman & Kakisawa, 1991) (the carbinyl proton, the ester carbonyl, and trifluoromethyl groups lie in the same plane). Taking into account this conformation and that CH_3-3 was shielded by the MTPA phenyl group linked to C-9, we could select two conformational isomers (shown by the Newman projections **5e** and **5f**) among the six theoretically possible. Based on the magnitude of coupling constants between H-8,9, we could infer that the hydrogens with J = 5.9 Hz should be in an *anti* periplanar relationship (**5e**). On the other hand, those with J = 5.2 Hz should be in a *syn* periplanar relationship (**5f**) in which the dihedral angle between them should be ca 45°. Thus, **5c** must adopt the conformation **5e**, that is 2*S*,3*S*,8*S*,9*R* in its absolute configuration, and **5d** the conformation **5f** being 2*S*,3*S*,8*R*,9*R* in its absolute configuration. Hence, **4** must contain the same mixture of (2*S*,3*S*,8*S*,9*R*) with (2*S*,3*S*,8*R*,9*R*) neolignans which differ from **5** by substitution at C-4′. By analogy with licarin A, we named the neolignans **4** as licarinediol A and B, and the neolignans **5** as *O*-methyl-licarinediol A and B in which A represents the (2*S*,3*S*,8*S*,9*R*) isomer and B the (2*S*,3*S*,8*R*,9*R*) isomer.

The absolute configuration of licarin A (**1**) was established as being 2*R*,3*R* by comparison of its CD curve and α_D measurement with those reported for (+)-licarin A isolated from *Piper kadsura* (Ma et al., 1993). The optical activity of its *O*-methyl derivative [**1a**, (+)-acuminatin] provides further corroboration with the proposed 2*R*,3*R* configuration.

Considering that the configurations of C-2 and C-3 do not change during the transformation we could suggest that the enantiomer (not isolated) of **4** might be the key biosynthetic intermediate from **1** to **2**. Thus, this later one by oxidative processes could yield **3**.

The occurrence of lignans, such as those described in this paper, is widespread in *Aristolochia* species (Lopes, Bolzani & Trevisan, 1988). Yet, the reported occurrence of neolignans has been restricted to *A. arcuata* (Watanabe & Lopes, 1995), *A. birostris* (Conserva, Silva & Filho, 1990) and *A. taliscana* (Ionescu, Jolad & Cole, 1977). From *A. pubescens*, however, we obtained seven benzofuranoid neolignans, but only licarin A had already been isolated from species belonging to this genus.

3. Experimental

3.1. General

^{1}H NMR 1- and 2-D spectra were obtained at 200 MHz; ^{13}C NMR and DEPT spectra were taken at 50 MHz; ^{1}H–^{13}C COSY were optimized for $J = 7$ and 145 Hz. The mass spectra were obtained on an HP 5985 spectrometer. TLC: Silica gel 60 PF_{254}.

3.2. Plant material

The plant was collected in Ituiutaba, state of Minas Gerais, Brazil. The botanical material was identified as *A. pubescens* Willd, by Dr Condorcet Aranha, and a voucher specimen was deposited at the herbarium of the Instituto Agronômico de Campinas, Campinas, SP, Brazil. The material was separated by plant parts, dried (~40°) and ground.

3.3. Reagents

Commercially available (*R*)- and (*S*)-MTPA-Cl (Aldrich, Nos 42,339-4 and 42,340-8, respectively) were used without purification.

3.4. Isolation

Ground roots and stems (648.37 g) were extracted exhaustively at room temp. with hexane, Me_2CO and EtOH successively and then individually conc. The crude hexane extract (4.0 g) was fractionated by CC (silica gel, 90 g, hexane–EtOAc gradient) leading to 12 frs (120 ml). After prep. TLC [hexane–EtOAc (95:5)], Fr. 2 (432.8 mg) yielded 8*R*,9*R*-oxide-β-caryophyllene (14.5 mg). Fr. 4 (98.4 mg) yielded kobusone (76.3 mg) as an oil on precipitation from CH_3OH. Fr. 5 (138.3 mg) led to sitosterol (62.7 mg) after recrystallization from CH_3OH. Fr. 6 (115.0 mg) after prep. TLC [hexane–EtOAc (7:3)] afforded **1** (14.3 mg) and (+)-sesamin (33.4 mg). Fr. 8 (222.0 mg) by prep. TLC [$CHCl_3$–CH_3OH–NH_4OH (95:5:0.5)] yielded vanillin (8.5 mg). Fr. 9 (365.6 mg) was also submitted to prep. TLC [$CHCl_3$–CH_3OH (97:3)] leading to (−)-cubebin (55.9 mg) and **2** (10.6 mg). Fr. 10 (239.4 mg) by CC (silica gel 6 g, $CHCl_3$–CH_3OH + 0.5% NH_4OH) afforded (+)-eudesmin (15.6 mg) and *ent*-kauran-16α, 17-diol (3.2 mg) after recrystallization from CCl_4. Fr. 11 by prep. TLC [$CHCl_3$–CH_3OH–NH_4OH (93:7:0.5)] afforded **4** (42.3 mg), **5** (26.3 mg) and a sub-fr. that was also submitted to prep. TLC [$CHCl_3$–CH_3OH–NH_4OH (60:40:0.5)] leading to vanillic acid (2.5 mg) and **3** (7.5 mg).

3.5. Methylation of *1*, *2* and *4*

Compounds **1** (5.0 mg), **2** (2.3 mg) and **4** (20.0 mg) were individually reacted with CH_2N_2 (standard conditions) to yield (+)-acuminatin (**1a**, 5.2 mg), (+)-kadsurenin M (**2a**, 2.4 mg) and **5** (20.7 mg), respectively. **1a**: yellow oil, $[\alpha]_D^{25}$ +8.5° ($CHCl_3$; *c* 1.184) (lit., Ma et al., 1993, $[\alpha]_D^{25}$ −9.5°, $CHCl_3$). **2a**: yellow oil, $[\alpha]_D^{25}$ +25.1° ($CHCl_3$; *c* 0.380). The IR, UV, ^{1}H and ^{13}C NMR data of **1a** and **2a** were identical to those of acuminatin (Ma et al., 1993) and **5b**, respectively.

3.6. (2*R*,3*R*)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-3-methylbenzofuran-5-carboxylic acid (*3*)

Yellow oil. $[\alpha]_D^{25}$ +14.7° ($CHCl_3$; *c* 0.574). (Found: C, 65.3; H, 5.5 $C_{18}H_{18}O_6$ requires: C, 65.4; H, 5.5; O, 29.1%). UV $\lambda_{max}^{CHCl_3}$ nm (log ϵ): 244 (3.88), 278 (3.74), 304 *sh* (3.42). CD (CH_3OH, *c* 0.1) $[\theta]_{235}$ +56,100, $[\theta]_{250}$ +9900, $[\theta]_{280}$ +39,600. IR ν_{max}^{KBr} cm^{-1}: 3338, 2929, 2859, 1688, 1604, 1513, 1457, 1277, 1200. EIMS 70 eV *m*/*z* (rel. int.): 330 $[M]^+$ (100), 150 $[C_6H_2(OCH_3)COOH]^+$ (10), 137 $[C_7H_5(OCH_3)OH]^+$ (31). ^{1}H and ^{13}C NMR spectra see Tables 1 and 2.

3.7. (2*S*,3*S*,1′*S*,2′*R*)- and (2*S*,3*S*,1′*R*,2′*R*)-2,3-dihydro-5-(1′,2′-dihydroxypropyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-3-methylbenzofuran (licarinediol *A* + *B*, *4*)

Yellow oil. $[\alpha]_D^{25}$ +3.6° ($CHCl_3$; *c* 1.024). (Found: C, 66.5; H, 6.7. $C_{20}H_{24}O_6$ requires: C, 66.6; H, 6.7; O 26.6%). UV $\lambda_{max}^{CHCl_3}$ nm (log ϵ): 242 (3.91), 282 (3.80), 310 *sh* (3.57) IR ν_{max}^{KBr} cm^{-1}: 3444, 2925, 1603, 1515, 1458, 1381, 1271, 1125, 1033. EIMS 70 eV *m*/*z* (rel. int.): 360 $[M]^+$ (17), 315 $[M\text{-}CH_3CHOH]^+$ (100), 163 (27), 151 $[C_6H_3(OCH_3)(OH)CO]^+$ (21), 137 $[C_7H_5(OCH_3)OH]^+$ (23). ^{1}H and ^{13}C NMR spectra see Tables 1 and 2.

3.8. Acetylation of *4*

Compound **4** (5.2 mg) was submitted to acetylation (Ac_2O, pyridine) yielding a mixture of isomeric compounds (**4a** + **4b**, 6.5 mg) in a 1:1 ratio as evidenced by ^{1}H NMR. Yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.04 (6H, *d*, $J = 6.5$ Hz, 2 × C$\underline{H}_3$-10), 1.34, 1.35 (6H, *d*, $J = 6.6$ Hz 2 × C$\underline{H}_3$-3), 2.00 (6H, *s*, 2 × OAc), 2.02 (6H, *s*, 2 × OAc), 2.25 (6H, *s*, 2 × OAc-4′), 3.40 (2H, *m*, H-3), 3.77 (6H, *s*, 2 × OC$\underline{H}_3$-3′), 3.84 (6H, *s*, 2 × OC$\underline{H}_3$-7), 5.11 (2H, *d*, $J = 9.3$ Hz, 2 × H-2), 5.23 (2H, *m*, 2 × H-9), 5.62, 5.63 (2H, *d*, $J = 6.5$ Hz, 2 × H-8), 6.72 (4H, *sl*, 2 × H-4, 6), 6.99 to 6.88 (6H, *m*, 2 × H-2′, 5′, 6′).

3.9. (2S,3S,1′S,2′R)- and (2S,3S,1′R,2′R)-2,3-dihydro-5-(1′,2′-dihydroxypropyl)-2-(3,4-dimethoxyphenyl)-7-methoxy-3-methylbenzofuran (O-methyl-licarinediol A + O-methyl-licarinediol B, **5**)

Yellow oil. $[\alpha]_D^{25}$ +3.5° ($CHCl_3$; *c* 1.036). (Found: C, 67.4; H, 6.9. $C_{21}H_{26}O_6$ requires: C, 67.4, H, 7.0; O, 25.6%). UV $\lambda^{CHCl_3}_{max}$ nm (log ϵ). 242 (3.83), 282 (3.68), 310 *sh* (3.47). IR ν^{KBr}_{max} cm^{-1}: 3444, 2923, 1599, 1514, 1459, 1264, 1136, 1025. EIMS 70 eV *m/z* (rel. int.): 374 $[M]^+$ (17), 329 $[M\text{-}CH_3CHOH]^+$ (100), 165 $[C_6H_3(OCH_3)(OCH_3)CO]^+$ (26), 151 $[C_7H_5(OCH_3)(OCH_3)]^+$ (34). ^1H and ^{13}C NMR spectra see Tables 1 and 2.

3.10. (2S,3S)-2,3-dihydro-2-(3,4-dimethoxyphenyl)-7-methoxy-3-methyl-5-(2-oxopropyl)benzofuran (licarinone, **5a**)

Yellow oil. $[\alpha]_D^{25}$ −36.3° ($CHCl_3$; *c* 0.880). CD (CH_3OH, *c* 0.1) $[\theta]_{228}$ −10,324, $[\theta]_{242}$ 0, $[\theta]_{255}$ +3204. IR ν cm^{-1}: 2930, 2849, 1720, 1604, 1508, 1264, 1154. ^1H NMR ($CDCl_3$): δ 1.31 (3H, *d*, J = 6.8 Hz, C<u>H</u>$_3$-3), 2.12 (3H, *s*, H-10), 3.40 (1H, *m*, H-3), 3.59 (2H, *s*, H-8), 3.81 (9H, *s*, 3 × OC<u>H</u>$_3$), 5.05 (1H, *d*, J = 9.6 Hz, H-2), 6.55 (2H, *brs*, H-4 and H-6), 6.77 (1H, *d*, J = 8.1 Hz, H-5′), 6.92 to 6.88 (2H, *m*, H-2′ and H-6′).

3.11. (−)-Kadsurenin M (**5b**)

Yellow oil. $[\alpha]_D^{25}$ −25.3° ($CHCl_3$; *c* 0.501) (lit., Ma et al., 1993, $[\alpha]_D^{25}$ −24.6°, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 1.39 (3H, *d*, J = 6.8 Hz, C<u>H</u>$_3$-3), 3.50 (1H, *m*, H-3), 3.82 (3H, *s*, OC<u>H</u>$_3$-7), 3.83 (3H, *s*, OC<u>H</u>$_3$-4′), 3.89 (3H, *s*, OC<u>H</u>$_3$-3′), 5.21 (1H, *d*, J = 9.3 Hz, H-2), 6.92 to 6.78 (3H, *m*, H-2′, H-5′ and H-6′), 7.29 (1H, *brs*, H-4), 7.32 (1H, *brs*, H-6), 9.79 (1H, *s*, H-8). The IR, UV and ^{13}C NMR data were identical to those of (−)-kadsurenin M (Ma et al., 1993).

3.12. (R)- and (S)-MTPA-Cl derivatives

The derivatives were obtained by using Mosher's procedure (Dale & Mosher, 1973). A soln of (*R*)-MTPA-Cl (20 µl, 107 µmol) in pyridine (250 µl) was added to a soln of **5** (9 mg, 24 µmol) in CCl_4 (600 µl). The reaction mixture was then shaken for 15 min and allowed to stand at room temp. for 24 h. The solvent was evaporated and the residue after prep. TLC [hexane–EtOAc (1:1)] yielded **5a** (5.0 mg) and **5b** (3.6 mg). Using the same procedure earlier described for obtaining **5a** and **5b**, a mixture of **5c** and **5 d** (8.3 mg) in a 1:1 ratio was obtained from **5** and (*S*)-MTPA-Cl. The NMR spectra were taken of this mixture. ^{13}C NMR ($CDCl_3$): δ 16.3, 16.5 (C<u>H</u>$_3$-3A and B); 17.3, 17.5 (C-10A and B); 45.3, 45.5 (C-3A and B); 56.0 (6 × OC<u>H</u>$_3$), 74.2, 74.5 (C-9A and B); 78.8, 79.4 (C-8A and B), 93.8 (C-2A and B). ^1H NMR ($CDCl_3$): δ 1.24 (12H, *m*, H-10A and B, C<u>H</u>$_3$-3A and B), 3.82 (18H, *s*, 6 × OC<u>H</u>$_3$), 5.08 (2H, *d*, J = 9.5 Hz, H-2A and B), 5.40 (2H, *m*, H-9A and B), 5.90 (1H, *d*, J = 5.9 Hz, H-8A), 5.95 (1H, *d*, J = 5.2 Hz, H-8B), 6.65 to 6.53 (4H, *m*, H-4A and B, H-6A and B), 6.80 (2H, *d*, J = 8.8 Hz, H-5′A and B), 6.92 to 6.88 (4H, *m*, H-2′A and B, H-6′A and B).

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References

Abraham, R. J., & Loftus, P. (1985). In *Proton and Carbon-13 NMR Spectroscopy—An Integrated Approach* (p. 202). Chichester: John Wiley and Sons.

Achenbach, H., Groß, J., Dominguez, X. A., Cano, G., Star, J. V., Brussolo del, L. C., Muñoz, G., Salgado, F., & López, L. (1987). *Phytochemistry*, *26*, 1159.

Atkinson, R. S. (1995). In *Stereoselective Synthesis* (pp. 92–97). UK: John Wiley and Sons Ltd.

Conserva, L. M., da Silva, M. S., & Filho, R. B. (1990). *Phytochemistry*, *29*, 257.

Dale, J. A., & Mosher, H. S. (1973). *Journal of the American Chemical Society*, *95*, 512.

David, J. M., Yoshida, M., & Gottlieb, O. R. (1994). *Phytochemistry*, *36*, 491.

Hatakeyama, H., & Nakano, J. (1970). *Cellulose Chemistry and Technology*, *4*, 281.

Hatakeyama, H., Nakano, J., Hatano, A., & Migita, N. (1969). *Tappi*, *52*, 1724.

Heymann, H., Tezuka, Y., Kikuchi, T., & Supriyatna, S. (1994). *Chemical Pharmaceutical Bulletin*, *42*, 138.

Ionescu, F., Jolad, S. D., & Cole, J. R. (1977). *Journal of Pharmaceutical Science*, *66*, 1489.

Li, S., Iliefski, T., Lundquist, K., & Wallis, A. F. A. (1997). *Phytochemistry*, *46*, 929.

Lopes, L. M. X., & Humpfer, E. (1997). *Phytochemistry*, *45*, 431.

Lopes, L. M. X., Bolzani da S, V., & Trevisan, L. M. V. (1988). *Revista Latinoamericana de Química*, *19*, 113.

Lopes, L. M. X., Martins, J. A., & Piasentin, R. M. (1991). *Eclética Química*, *16*, 63.

Lopes, L. M. X., Bolzani da S, V., Trevisan, L. M. V., & Grigolli, T. M. (1990). *Phytochemistry*, *29*, 660.

Luiz, V., Bolzani da S, V., Trevisan, L. M. V., & Lopes, L. M. X. (1990). *Química Nova*, *13*, 250.

Ma, Y., Han, G. Q., & Wang, Y. Y. (1993). *Acta Pharmaceutica Sinica*, *28*, 370.

Ohtani, I., Kusumi, T., Kashman, Y., & Kakisawa, H. (1991). *Journal of the American Chemical Society*, *113*, 4092.

Pourchert, C. J. (1983). *The Aldrich Library of NMR Spectra, 2nd ed., vol. 2* (p. 212A). Aldrich Chemical Company.

Silverstein, R. M., Bassler, G. C., & Morrill, T. C. (1991). In *Spectrometric Identification of Organic Compounds* (5th ed.) (pp. 245–246). New York: John Wiley and Sons.

Watanabe, L. Y., & Lopes, L. M. X. (1995). *Phytochemistry*, *40*, 991.

Wenkert, E., Gottlieb, H. E., Gottlieb, O. R., Pereira da S, M. O., & Formiga, M. D. (1976). *Phytochemistry*, *15*, 1547.