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Flavonoids from *Lonchocarpus muehlbergianus*

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ABSTRACT

The light petroleum extract from the roots of *Lonchocarpus muehlbergianus* Hassl contained nine flavonoids, including six new ones. These are 2,4-*cis*-2,4,5,8-tetramethoxy-(2´´,3´´:6,7)-furanoflavan; 2,4-*cis*-4-hydroxy-2,5,8-trimethoxy-(2´´,3´´:6,7)-furanoflavan; 2,4-*cis*-2-prenyloxy-4,5,8-trimethoxy-(2´´,3´´:6,7)-furanoflavan; 2,4-*cis*-2-prenyloxy-4-hydroxy-5,8-dimethoxy-(2´´,3´´:6,7)-furanoflavan; 2',5',6'-trimethoxy-9-(1,1-dimethylallyoxy)-[2´´,3´´:3´,4´]-furanochalcone; 5,6-dimethoxy-(2´´,3´´:7,8)-furanoflavone, identified by analysis of their spectral data (UV, IR, ^{1}H and ^{13}C NMR, 2D-NMR, NOE and MS). The natural occurrence of 2,4-dioxygenated flavan derivatives is being reported for the first time.

Quantitative analysis of the petrol extract, by using reversed-phase HPLC, showed that the most abundant flavonoid in the extract is 2,4-*cis*-2,4,5,8- tetramethoxy-(2´´,3´´:6,7)-furanoflavan.

Key words: *Lonchocarpus muehlbergianus*, Leguminosae, flavonoids, flavans.

1 INTRODUCTION

In continuation of our studies on the flavonoids of the *Lonchocarpus* species (*Leguminosae*), occurring in Brazil, we have examined *L. muehlbergianus* Hassl, which was allocated in *Lonchocarpus* subgenus *Punctati* together with *L. subglaucescens* (Magalhães et al. 1996).

Phytochemical data, obtained with several *Lonchocarpus* species previously investigated, allowed the characterization of many secondary metabolites mainly consisting of flavonoid structural types. Up to the present nothing is found in the literature about the natural occurrence of 2,4-dioxygenated flavans, while in *Lonchocarpus*, the occurrence of 4-oxygenated flavans, which are rarely found in nature, has only been observed in *L. subglaucescens* (Magalhães et al. 1996) and *L. orotinus* (Mahmoud and Waterman 1987). On the other side only three β-hydroxychalcones have been isolated from *Lonchocarpus* species (Magalhães et al. 1996, Waterman and Mahmoud 1985).

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2 MATERIALS AND METHODS

2.1 GENERAL

Melting points were determined on a Kofler block and are uncorrected. IR spectra were recorded in CH_2Cl_2 and as KBr discs, UV spectra in MeOH or $CHCl_3$. ^{1}H and ^{13}C NMR: run in $CDCl_3$ with TMS as internal standard; field strength given in text.

EIMS: direct probe insert at 70 eV. HREIMS: measurements were made on a VG Auto Spec-Fisions Instrument. HPLC: UV detector, reversed-phase column Varian C18, MCH, 10 μm (300 mm × 4.0 mm) and isocratic elution with CH_3CN:H_2O (70:30) as the mobile phase, at a flow rate of 0.8 ml/min. CC and TLC: silica gel 35-70 mesh, flash chromatography: silica gel 230-400 mesh.

2.2 Plant Material

Roots of *L. muehlbergianus* were collected in the Ecological Park – Unicamp, Campinas (SP) in February 1992. Voucher specimens have been deposited at the herbarium (A.M.G.A. Tozzi 95-30) of Campinas State University (UNICAMP), Campinas-SP, Brazil.

2.3 Extraction and Isolation

Dry roots (931.7 g) of *L. muehlbergianus* were successively extracted with petrol (30-60°C), chloroform and methanol in a Soxhlet apparatus. After solvent evaporation, the petrol extract gave a viscous yellow oil (9.9 g), while the chloroform extract gave a brown oil (4.0 g) and the methanol extract gave a brown gum (3.5 g). The petrol extract (9.9 g) was applied to a silica gel column eluted first with petrol. The eluent polarity was gradually increased by addition of ethyl acetate to furnish 230 fractions of 200 mL each which were reduced to 23 groups after TLC analysis. Most of the compounds were found in six groups ranging from fractions 47 to 199. A sample of each was further fractionated by successive preparative TLC (silica gel) respectively run with hexane:diethyl ether (95:05), petrol ether:ethyl acetate (90:10), dichloromethane:methanol (98:02) and dichloromethane:methanol (95:05). Flavonoids were visualized under UV light (λ = 254 and 366 nm) and recovered from TLC plates by extraction with mixtures of CH_2Cl_2 and MeOH to furnish compounds **1** (56.0 mg), **2** (12.0 mg), **3** (6.8 mg), **4** (16.0 mg), **5** (3.7 mg), **6** (5.9 mg), **7** (14.3 mg), **8** (11.0 mg) and **9** (7.0 mg). Analogously, a part of the chloroform extract (1 g) furnished compounds **1** (7.0 mg), **2** (1.7 mg), **3** (15.0 mg), **4** (5.0 mg), **5** (3.7 mg), **6** (3.0 mg), **7** (4.3 mg), 8 (11.0 mg) and **9** (3.4 mg).

2.4 2, 4-*Cis*-2, 4, 5, 8-tetramethoxy-(2´´,3´´:6, 7)-furanoflavan (**1**)

Viscous oil; $[\alpha]^{20}_D$ + 100.59 ($CHCl_3$; c 21.95); UV (MeOH) λ_{max} (log ε): 257 (3.66) nm; IR (CH_2Cl_2) ν_{max} 2933, 2833, 1628, 1545, 1484, 1449, 1408, 1364, 1251, 1188, 1159, 1118, 1090, 1054, 1044, 990, 967, 921, 760, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$/TMS): see Table I; ^{13}C NMR (75.4 MHz, $CDCl_3$): see Table II; EIMS *m/z* 370 $[M^+]$ (7), 369 (25), 338 (32), 337 (93), 307 (76), 236 (100), 134 (4), 133 (9), 105 (18), 77 (19), 57 (38). HREIMS m/z: found 370.1333 $[M]^+$ ($C_{21}H_{22}O_6$ requires 370.1416).

2.5 2, 4-*Cis*-4-hydroxy-2, 5, 8-trimethoxy-(2´´,3´´:6, 7)-furanoflavan (**2**)

Viscous oil; $[\alpha]^{20}_D$ + 103.25 ($CHCl_3$; c 4.45); UV (MeOH) λ_{max} (log ε): 257 (3.51) nm; IR (CH_2Cl_2) ν_{max} 3508, 3055, 2932, 2850, 1629, 1545, 1485, 1437, 1407, 1364, 1265, 1189, 1155, 1116, 1063, 991, 736, 702 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$/TMS): see Table I; ^{13}C NMR (75.4 MHz, $CDCl_3$): see Table II; EIMS *m/z* 356 $[M^+]$ (absent), 307 (5), 222 (100), 207 (49), 179 (2), 134 (5), 105 (16), 77 (23). HR-EIMS m/z: found 356.1208 $[M]^+$ ($C_{20}H_{20}O_6$ requires 356.1260).

2.6 2, 4-*Cis*-2-prenyloxy-4, 5, 8-trimethoxy-(2´´,3´´:6, 7)-furanoflavan (**3**)

Viscous oil; $[\alpha]^{20}_D$ + 60.11 ($CHCl_3$; c 2.83); IR (CH_2Cl_2) ν_{max} 3044, 2922, 1635, 1484, 1364, 1265, 1117, 1065, 736, 703 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$/TMS): see Table I; ^{13}C NMR (75.4 MHz, $CDCl_3$): see Table II; EIMS *m/z* 424 $[M]^+$ (8), 338 (7), 324 (55), 292 (95), 236 (11), 221 (15), 105 (100), 77 (23), 69 (15).

2.7 2, 4-*Cis*-2-prenyloxy-4-hydroxy-5, 8-dimethoxy (2´´,3´´:6, 7) furanoflavan (**4**)

Viscous oil; $[\alpha]^{20}_D$ + 84.18 ($CHCl_3$; c 5.66); UV (MeOH) ν_{max} (log ε): 256 (3.76), 217 (4.31) nm; IR

TABLE I

^{1}H NMR assignments of 2,4-dioxygenated flavans 1-4*.

H	1	2	3	4
3	1.97 *dd* (5.4, 15.1 Hz)	2.12 *dd* (4.9, 15.0 Hz)	1.96 *dd* (5.4, 15.0 Hz)	2.08 *dd* (4.8, 14.9 Hz)
	2.76 *dd* (1.4, 15.1 Hz)	2.71 *dd* (1.8, 15.0 Hz)	2.76 *dd* (1.8, 15.0 Hz)	2.70 *dd* (1.8, 14.9 Hz)
4	4.55 *dd* (1.4, 5.4 Hz)	5.03 *ddd* (1.8, 4.9, 10.8 Hz)	4.56 *dd* (1.8, 5.4 Hz)	5.01 *ddd* (1.8, 4.8, 10.9 Hz)
2'	7.70-7.67 *m*	7.70-7.69 *m*	7.68-7.66 *m*	7.72-7.68 *m*
3'	7.47-7.35 *m*	7.50-7.40 *m*	7.45-7.35 *m*	7.49-7.37 *m*
4'	7.47-7.35 *m*	7.50-7.40 *m*	7.45-7.35 *m*	7.49-7.37 *m*
5'	7.47-7.35 *m*	7.50-7.40 *m*	7.45-7.35 *m*	7.49-7.37 *m*
6'	7.70-7.67 *m*	7.70-7.69 *m*	7.68-7.66 *m*	7.72-7.68 *m*
2''	7.53 *d* (2.2 Hz)	7.54 *d* (2.3 Hz)	7.52 *d* (2.3 Hz)	7.54 *d* (2.3 Hz)
3''	6.99 *d* (2.2 Hz)	6.93 *d* (2.3 Hz)	6.89 *d* (2.3 Hz)	6.93 *d* (2.3 Hz)
2'''	–	–	4.00 *m* 3.86 *m*	3.97-3.91 *m* 3.87-3.75 *m*
3'''	–	–	5.20 *m*	5.13 *m*
OMe	3.17 *s* 3.60 *s* 4.11 *s* 4.12 *s*	3.14 *s* 4.13 *s* 4.17 *s*	3.54 *s* 4.10 *s* 4.11 *s*	4.12 *s* 4.16 *s*
Me	–	–	1.58 *s* 1.58 *s*	1.58 *s* 1.58 *s*
OH	–	4.10 *d* (10.8 Hz)	–	4.34 *d* (10.9 Hz)

(CH_2Cl_2) ν_{max} 3526, 2924, 2852, 1627, 1485, 1353, 1247, 1150, 1113, 1061, 989, 761, 738, 700 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$/TMS): see Table I; ^{13}C NMR (75.4 MHz, $CDCl_3$): see Table II; EIMS *m/z* (410 [M$^+$] (2), 324 (20), 222 (57), 207 (17), 149 (17), 105 (100), 102 (4), 77 (16), 69 (25).

2.8 2',5',6'-Trimethoxy-9-(1, 1-dimethylallyoxy)- [2´´,3´´:3´,4´]-furanochalcone (**5**)

Viscous oil; UV (MeOH) λ_{max} (log ε): 341 (3.50), 272 (4.35), 214 (4.36) nm; ^{1}H NMR (300 MHz, $CDCl_3$/TMS): see Table V; EIMS *m/z* 422 [M$^+$] (absent), 322 (83), 307 (100), 279 (31), 221 (8), 107 (17), 77 (8).

2.9 5, 6-Dimethoxy-(2´´,3´´:7, 8)-furanoflavone (**6**)

Viscous oil; UV (MeOH) λ_{max} (log ε): 350 (3.87), 270 (4.72) nm; IR(CH_2Cl_2) ν_{max} 3450, 3044, 2930, 2855, 1641, 1479, 1450, 1370, 1265, 1195, 1132, 1067, 738, 704 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$/TMS): see Table V; ^{13}C NMR (75.4 MHz, $CDCl_3$): see Table II; EIMS *m/z* 322 [M$^+$] (98), 307 (100), 220 (4), 105 (20), 102 (15), 77 (17).

2.10 2, 4-*Cis*-4, 5, 6-trimethoxy-(2´´,3´´:7, 8)-furanoflavan (**7**)

White needles; mp 59-61°C; $[\alpha]^{20}_D$ – 28,98° ($CHCl_3$; *c* 22.0); UV (MeOH) λ_{max} (log ε): 292

TABLE II

^{13}C NMR assignments of flavonoids 1-4 and 6-9*.

C	1	2	3	4	6	7a	7b	9
1	–	–	–	–	–	–	–	–
2	100.4	101.9	100.5	101.7	161.6	73.3	73.6	147.6
3	38.8	41.9	39.0	42.1	107.6	34.5	38.1	142.0
4	68.9	60.1	68.6	60.9	178.9	68.6	60.3	174.6
5	147.6	143.8	147.4	147.6	?	148.0	148.5	104.6
6	113.8	113.8	135.3	113.6	116.0	132.7	132.5	147.2
7	149.1	147.2	149.1	148.9	147.0	143.7	147.7	154.8
8	130.1	130.0	113.4	140.7	119.8	114.5	114.8	110.4
9	142.0	141.0	142.5	147.2	149.5	142.0	143.8	146.8
10	111.2	113.2	111.1	113.2	114.0	110.2	112.3	115.6
1'	141.6	140.1	142.6	141.1	131.9	141.3	141.3	131.6
2'	126.5	126.4	126.5	128.7	129.3	126.4	126.5	128.5
3'	128.7	128.7	128.6	126.3	131.7	128.5	128.8	128.8
4'	128.4	128.7	128.3	126.3	131.7	128.0	128.3	130.6
5'	128.7	128.7	128.6	126.3	131.7	128.5	128.8	128.8
6'	126.5	126.4	126.5	128.7	129.3	126.4	126.5	128.5
2''	143.7	143.8	143.6	143.7	145.8	143.6	144.0	78.4
3''	105.0	105.2	105.1	105.2	105.5	104.5	104.7	130.7
4''	–	–	–	–	–	–	–	117.6
2'''	–	–	60.6	60.2	–	–	–	–
3'''	–	–	121.8	120.2	–	–	–	–
4'''	–	–	129.9	128.9	–	–	–	–
OMe	51.0 57.4 60.8 61.7	50.7 61.0 61.7	56.8 61.6 61.7	60.9 61.7	61.8 62.5	56.1 61.1 61.2	61.2 61.8	56.5 60.3
Me	–	–	17.9 25.6	– 25.6	–	–	28.1	 28.1

*Run at 75.4 MHz in $CDCl_3$ with TMS as internal standard.

(3.14), 284 (3.15), 254 (3.86) nm; IR (KBr) ν_{max} 2943, 1627, 1479, 1408, 1365, 1354, 1263, 1195, 1161, 1130, 1108, 1068, 1002, 964, 907, 877, 842, 764, 746, 698 cm^{-1}; COSY (Table VI); HETCOR (Table VI); COLOC (Table VI) and EIMS *m/z* 340 [M^+] (14), 339 (61), 308 (13), 236 (100), 221 (98), 105 (6), 104 (14), 91 (15), 77 (11).

2.11 Hydrolysis of **7**

To a solution of **1** (17.4 mg) in $CHCl_3$ (2 ml) was added 1 $molL^{-1}$ HCl (0.5 ml). Work-up gave **7a** (8.4 mg) and **7b** (4.5 mg).

2.12 2, 4-*Trans*-4, 5, 6-trimethoxy-(2´´,3´´:7, 8)- furanoflavan (**7a**)

Viscous oil. ^1H NMR spectral data (300 MHz, $CDCl_3$/TMS): see Table V. ^{13}C NMR spectral data (75.4 MHz, $CDCl_3$): see Table II.

2.13 2, 4-*Trans*-4-Hydroxy-5, 6-dimethoxy-(2´´,3´´:7, 8)-furanoflavan (**7b**)

Light yellow needles; mp 126-128°C; UV ($CHCl_3$) λ_{max} (log ε): 289 (3.39), 283 (3.40), 257 (4.05), 254 (4.06), 216 (4.53) nm; IR (KBr) ν_{max} 3364, 2934, 1618, 1542, 1482, 1348, 1310, 1245, 1067, 954, 761, 699 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$/TMS): see Table V; ^{13}C NMR (75.4 MHz, $CDCl_3$): see Table II; EIMS *m/z* 326 [M^+] (12), 308 (8), 293 (6), 222 (95), 207 (100), 192 (14), 179 (5), 164 (8), 147 (12), 104 (13), 77 (25).

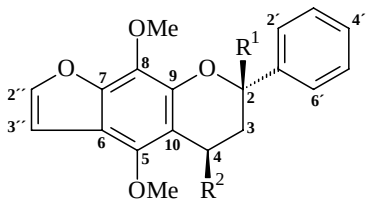
3 RESULTS AND DISCUSSION

The petrol extract from the roots of *L. muehlbergianus* was submitted to adsorption chromatographic separation analysis (column, chromatotron, TLC and preparative TLC) furnishing nine flavonoids (**1-9**, Fig. 1).

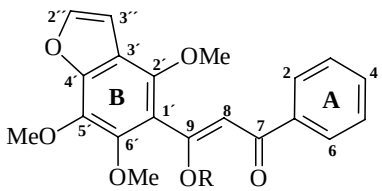
Flavans **1-4**, showed similar ^1H NMR spectra (Table I) as all of them have resonances for the hydrogens of a furan ring, two aromatic methoxyl groups and an unsubstituted B ring, where two of its hydrogens are deshielded. By comparison with flavan **7** ^1H NMR data (Magalhães et al. 1996), the lack of H-2 signal and a much simpler multiplet in the region expected for C-3 hydrogens, suggest that flavans **1-4** have an OR group (R = methoxyl or prenyl) at C-2 causing the paramagnetic shift of H-2´ and H-6´ hydrogens. The ^{13}C NMR spectrum (Table II) and DEPT (90° and 135°) allowed the assignment of all carbons; a peak around δ 100 (C_o) in place of a peak around δ 77 (CH) was taken as evidence of an OR group at C-2. In the NOE difference spectra irradiation of the methoxyl group hydrogens at C-4 enhanced the signal of one aromatic methoxyl group while irradiation of H-3´´ hydrogens caused enhancement of H-2´´ and of the same aromatic methoxyl group signal, indicating a linear fusion of furan with A ring (Fig. 2).

Flavan **1** was isolated as a yellow oil. Its HREIMS showed a molecular ion $[M]^+$ of m/z 370.1333, corresponding to $C_{21}H_{22}O_6$ (required M^+ 370.1416). The base peak at *m/z* 236 $[C_{12}H_{12}O_5]^+$ corresponds to the fragment bearing A ring from C-ring retro Diels-Alder (RDA) cleavage, while those at *m/z* 337 (93%) and *m/z* 307 (76%) can be represented by I and II fragments (Fig. 3). The UV spectrum indicated that the A and B rings are unconjugated. The ^1H NMR spectrum (Table I) also showed the signals of two aliphatic methoxyl groups which were located at C-2 and C-4, in accordance with the double doublets assigned to the H-4 and 2H-3 hydrogens. The respective chemical shifts were confirmed by COSY (Table III) and NOE data (Fig. 4).

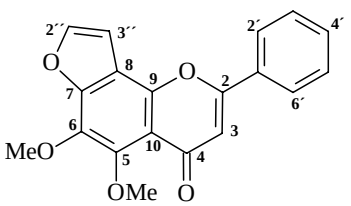
Flavan **2** was also isolated as a yellow oil and showed a similar UV spectrum. Its HREIMS showed a molecular ion $[M]^+$ of m/z 356.1208, corresponding to $C_{20}H_{20}O_6$ (required M^+ 356.1260), which is fourteen mass units lower than flavan **1**, suggesting that one methoxyl was replaced by one hydroxyl. The base peak at *m/z* 222 $[C_{11}H_{10}O_5]^+$, originating from RDA cleavage of the C-ring, indicates that the hydroxyl group is part of the fragment including A-ring. The ^1H NMR spectrum (Table I) showed all resonances of the A and B rings that were seen in flavan **1**, except for a signal corresponding to an aliphatic methoxyl group and the presence of a much more complex multiplet for H-4 as well as



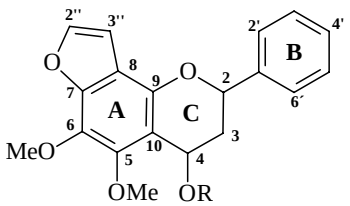
1 : R^1 = MeO-, R^2 = MeO- novel (most abundant)
2 : R^1 = MeO-, R^2 = OH novel
3 : R^1 = $OCH_2CH=C(CH_3)_2$, R^2 = MeO- novel
4 : R^1 = $OCH_2CH=C(CH_3)_2$, R^2 = OH novel



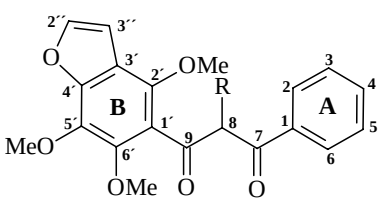
5 : R = $C(CH_3)_2CH=CH_2$ novel



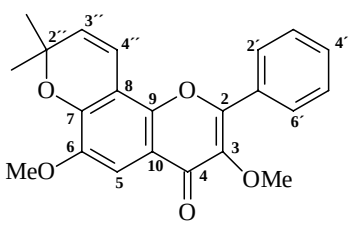
6 novel



7 : R = CH_3 2:4 - *cis* Magalhães et al. 1996
7a : R = CH_3 2:4 - *trans*
7b : R = H 2:4 - *trans*



8 : R = $C(CH_3)_2CH=CH_2$ Magalhães et al. 1997



9 Nascimento and Mors 1981

Fig. 1 – Flavonoids from *L. muehlbergianus*.

a doublet integrating for one hydrogen which was attributed to a hydroxyl group at C-4. The chemical shifts of C-ring hydrogens and carbons were confirmed by COSY (Table III) and HETCOR (Table III).

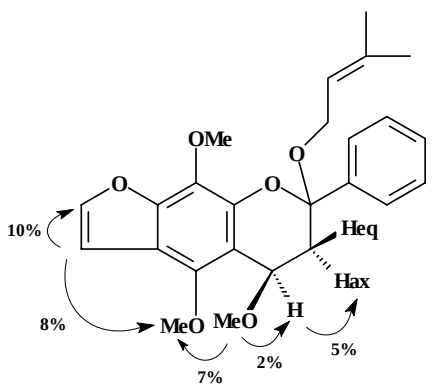
The ^{1}H NMR (Table I) and ^{13}C NMR (Table II) spectra of flavans **3** and **4** show the resonances for an O-prenyl group. In the case of flavan **3** there is also a resonance for an aliphatic methoxyl group while in flavan **4** there are the multiplets for a hydroxyl group at C-4 as in flavan **2**. The MS spectrum of flavan **3** showed $[M]^+$ at *m/z* 424 while in that of flavan **4** $[M^+]$ at *m/z* 410 is again fourteen mass units lower, confirming the replacement of a methoxyl group by a hydroxyl group. Both spectra showed a peak at *m/z* 324, which can be respectively rationalized by the simultaneous loss of methyl-1,1-dimethyl-1-vinyl ether $[CH_3OC(CH_3)_2CH = CH_2]$ and 1,1-dimethyl-1-vinyl alcohol $[HOC(CH_3)_2CH= CH_2]$ (Fig. 5). The molecular structure was also sup-

TABLE III

2D-NMR data of flavans 1 and 2.

Atom number	COSY **1**	COSY **2**	HETCOR **2**
H-3_{ax}	H-3_{eq}, H-4	H-3_{eq}, H-4	C-3
H-3_{eq}	H-3_{ax}, H-4	H-3_{ax}, H-4	
H-4	H-3_{ax}, H-3_{eq}	H-3_{ax}, H-3_{eq} C-4	
H-2´and H-6´	–	–	C-2´and C-6´
H-3´and H-5´	–	–	C-3´and C-5´
H-4´	–	–	C-4´
H-2´´	H-3´´	H-3´´	C-2´´
H-3´´	H-2´´	H-2´´	C-3´´

ported by COSY (Table IV) and NOE differential experiments (Fig. 2).



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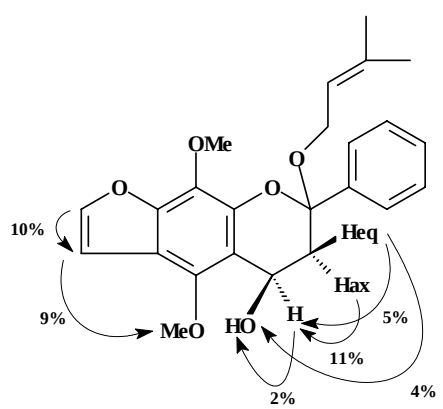


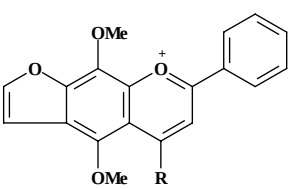
4

Fig. 2 – NOE effects observed for flavans **3** and **4**.

TABLE IV

COSY (^{1}H-^{1}H) data of flavans 3 and 4.

Hydrogen (δ ppm)	Hydrogen correlated
H-3_{ax} (1.96)	H-3_{eq}, H-4
H-3_{eq} (2.76)	H-3_{ax}, H-4
H-4 (4.56)	H-3_{ax}, H-3_{eq}
H-2" (7.52)	H-3"
H-3" (6.89)	H-2"
H-2' and H-6' (7.68-7.66)	H-3', H-4' and H-5'

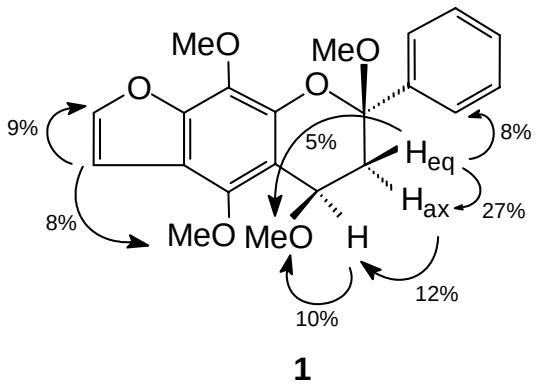


I , R = OMe (m/z = 337)

II, R = H (m/z = 307)

Fig. 3 – Fragments corresponding to the most abundant peaks in the mass spectrum of **1**.

The buds of *Populus nigra* excudate (Wollenweber and Egger 1971) furnished 2,5-dihydroxy-7-methoxyflavanone, but the natural occurrence of 2,4-dioxygenated flavans is now being registered for the first time. A recent publication (Knaggs 2000) about the biosynthesis of shikimate metabolites mentions that a (2*S*)-flavanone 2-hydroxylase is involved in biosynthetic pathways to flavones and dibenzoylmethanes. Based on these findings it seems reasonable to suggest a biogenetic route



1

Fig. 4 – NOE effects observed for flavan **1**.

where a 2-hydroxyflavanone derivative is also reduced to a 4-hydroxy derivative which, in turn, could be further alkylated.

The ^{1}H NMR spectrum of flavonoid **5** (Table V) is very similar to that of dibenzoylmethane **8** (Magalhães et al. 1997) because both have resonances for the hydrogens of an unsubstituted aromatic ring, three aromatic methoxyl groups and one dimethylallyl group. The singlet of a methinic hydrogen on **5**, however, is deshielded suggesting a corresponding enol ether. Through a NOE experiment it was observed that irradiation of the H-8 enhanced (21%) H-2 and H-6 signals, while irradiation of H-2 and H-6 has not caused an enhancement of dimethylallyl resonances, suggesting that flavonoid **5** is the Z regioisomer of the corresponding 9-OR enol ether of **8**. In the ^{13}C NMR spectrum two signals of low intensity at δ 191.6 and δ 169.1 were attributed to C-7 and C-9, respectively while a signal at δ 107,4 was attributed to C-8. Many weak signals in the ^{13}C-NMR spectrum may be due to the presence of several tautomeric forms. In the UV spectrum, an additional band at λ_{max} 341 nm was attributed to the cinamoyl system, now present in the enol ether. The main peaks in the MS spectrum can be explained by the simultaneous loss of methoxyl and 1,1-dimethyl-1-vinyl radicals to give a flavone type fragment *m/z* 322 (83%), followed by the loss of a methyl radical to give the fragment *m/z* 307 (100%) (Fig. 6).

The UV spectrum of compound **6** suggested a flavone structure where the bands at λ_{max} 270 and 350 nm indicate the presence of benzoyl and cinamoyl systems, respectively. Its IR spectrum exhibited the carbonyl group at 1641 cm^{-1}. The MS spectrum displayed $[M]^+$ at *m/z* 322 [98%] and the peaks at *m/z* 307 [100%, M – 15]$^+$, 105 [20%, $C_7H_5O]^+$ and 77 [17%, $C_6H_5]^+$. The base peak can be taken as evidence of a methoxyl group on C-6. The ^{1}H NMR (Table V) and ^{13}C NMR (Table II) spectral data are compatible with a flavone analogous of flavan **7**.

Flavan **7** was isolated in much lower amounts from *L. subglaucescens* (Magalhães et al. 1996), when its structure was established by ^{1}H NMR, ^{13}C NMR and NOE data. We now report additional spectral data (UV, IR and MS) including the use of 2D-NMR techniques such as COSY, HETCOR and COLOC (Table VI) which support its molecular constitution. The UV spectrum is in accordance with the lack of conjugation between the aromatic rings while the MS spectrum displayed a molecular ion at *m/z* 340 $[M]^+$, together with peaks at *m/z* 236 $[C_{12}H_{12}O_5]^+$ and 104 $[C_8H_8]^+$ corresponding to fragments derived from C ring RDA cleavage. Flavan **7** was submitted to a hydrolysis reaction. After reaction work up two major products were obtained: **7a** and **7b** while some of the starting material was recovered.

The structure of **7a** was deduced through the comparison of its ^{1}H NMR spectrum with that of flavan **7** (Table V). The summation of the coupling constant values obtained for H-2, H-3 and H-4 signals [$J_{2,3ax} + J_{2,3eq} = 14.1$ Hz and $J_{4,3ax}$ (3.0) + $J_{4,3eq}$ (2.0) = 5.0 Hz], now are nearly those attributed to trans relative configuration. The H-4 signal also appeared as a triplet with a coupling constant of J=3.0 Hz (Clark-Lewis 1968, Bolger et al. 1966). So in flavan **7**, the ''triplet'' corresponding to H-4 signal is in fact, a superimposed double doublet. Finally, the downfield chemical shift value of H-2 is strong evidence that it is being deshielded by the 4-OMe group which is on the same side of the C-ring in **7a**. These findings suggest that the relative configuration of 4,5,7-trimethoxy-8-prenyl flavan, previously iso-

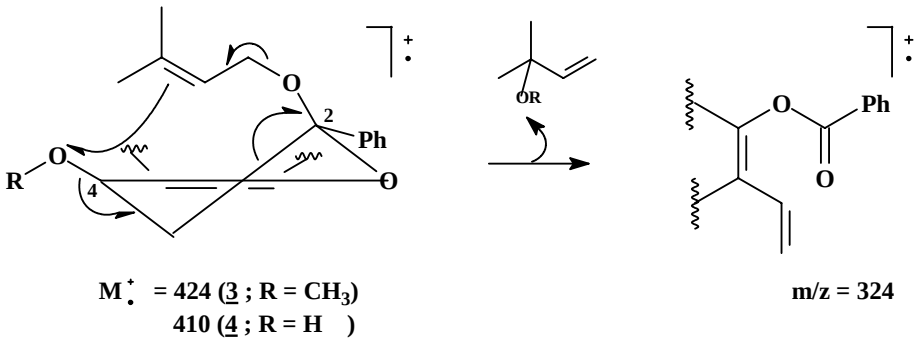


Fig. 5 – Rationalization of a pathway leading to a common fragment in the mass spectra of **3** and **4**.

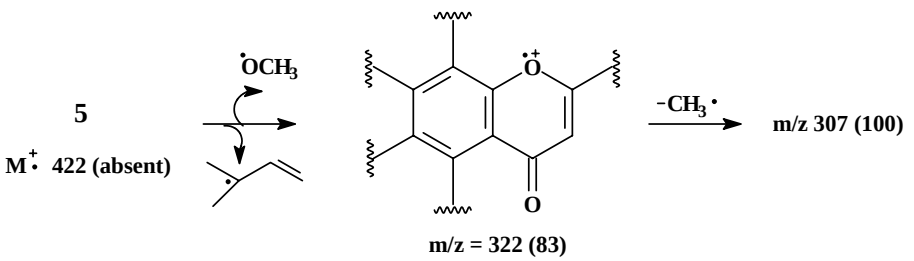


Fig. 6 – Mass spectrum rationalization of compound **5**.

lated from *Tephrosia quercetorum* (Gómez-Garibay et al. 1988) is also 2:4-*trans*, since in its ^{1}H NMR spectrum the signal at δ 5.29 (1H, *dd*, *J*= 12 and 4 Hz) is very close to that of H-2 in **7a** (Table V).

The ^{1}H NMR spectrum of **7b** (Table V) closely resembled that of **7a**, except for the lack of a singlet corresponding to the methoxyl group at C-4 and the presence of an absorption corresponding to hydroxyl groups (δ 2.63). Significant differences caused by the presence of the hydroxyl group, however, were observed in the ^{13}C NMR spectrum (Table II); the chemical shifts that were assigned to C-3 and C-4 appeared deshielded (+3.5 ppm) and shielded (−8.3 ppm), respectively. The NOE spectrum exhibited the expected interactions (Fig. 7). The IR spectrum showed the presence of a hydroxyl group (ν = 3364 cm^{-1}). The MS spectrum displayed the molecular ion peak at *m/z* 326 $[M]^+$, other significant peaks at *m/z* 308 $[M - H_2O]^+$ and those originating from RDA cleavage of the C-ring at *m/z* 222 $[C_{11}H_{10}O_5]^+$ and *m/z* 104 $[C_8H_8]^+$.

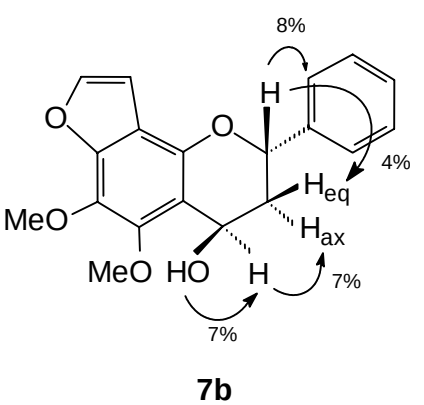


Fig. 7 – NOE effects observed for flavan **7b**.

The spectral data obtained for compound **9** agree with those previously reported (Nascimento et al. 1976, Nascimento and Mors 1981). Additionally a NOE experiment was carried out in order to confirm the angular closure of the dimethylchromene ring in the A-ring and the location of the methoxyl group on C-6.

TABLE V

^{1}H NMR assignments of flavonoids 5, 6*, 7a and 7b.

H	5	6	7	7a	7b
2	8.00–7.97 *m*	–	5.17 *dd* (9.9, 3.2 Hz)	5.28 *dd* (12.1, 2.0 Hz)	5.24 *dd* (12.3, 2.0 Hz)
3	7.57–7.53 *m*	6.74 *s*	2.40 *ddd* (13.9, 9.9, 7.2 Hz)	1.94 *ddd* (14.4, 12.1, 3.0 Hz)	2.10 *ddd* (14.5, 12.3, 4.0 Hz)
			2.62 *ddd* (13.9, 7.2, 3.2 Hz)	2.42 *dt* (14.4, 2.0 Hz)	2.36 *dt* (14.5, 2.0 Hz)
4	7.57–7.53 *m*	–	4.84 *t* (7.2 Hz)	4.60 *t* (3.0 Hz)	5.06 *m*
5	7.57–7.53 *m*	–	–	–	–
6	8.00–7.97 *m*	–	–	–	–
8	6.74 *s*	–	–	–	–
2'	–	8.01–7.99 *m*	7.47–7.32 *m*	7.67–7.35 *m*	7.55–7.52 *m*
3'	–	7.56–7.54 *m*	7.47–7.32 *m*	7.67–7.35 *m*	7.47–7.35 *m*
4'	–	7.56–7.54 *m*	7.47–7.32 *m*	7.67–7.35 *m*	7.47–7.35 *m*
5'	–	7.56–7.54 *m*	7.47–7.32 *m*	7.67–7.35 *m*	7.47–7.35 *m*
6'	–	8.01–7.99 *m*	7.47–7.32 *m*	7.67–7.35 *m*	7.55–7.52 *m*
2''	7.66 *d* (2.2 Hz)	7.66 *d* (2.2 Hz)	7.51 *d* (2.2 Hz)	7.51 *d* (2.2 Hz)	7.54 *d* (2.2 Hz)
3''	7.05 *d* (2.2 Hz)	7.06 *d* (2.2 Hz)	6.82 *d* (2.2 Hz)	6.82 *d* (2.2 Hz)	6.84 *d* (2.2 Hz)
3'''	6.00 *dd* (10.7, 17.4)	–	–	–	–
4'''	5.21 *dd* (1.1, 17.4 Hz)	–	–	–	–
	4.99 *dd* (1.1, 10.7 Hz)				
OME	3.71 *s* 4.11 *s* 4.27 *s*	4.11 *s* 4.27 *s*	3.36 *s* 3.99 *s* 4.07 *s*	3.55 *s* 4.03 *s* 4.08 *s*	4.08 *s* 4.08 *s*
Me	1.25 *s* 1.32 *s*	– –	– –	– –	– –
OH	–	–	–	–	2.63 *s*

*Run at 300 MHz in $CDCl_3$ with TMS as external standard.

HP-HPLC showed that **1** is the most abundant constituent in the roots (26.3 mg/g of dried roots).

Analogously, compounds **1, 2, 3, 4, 6, 7, 8** and **9** were isolated from the chloroform extract.

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RESUMO

O extrato éter de petróleo das raízes de *Lonchocarpus muehlbergianus* foi submetido a sucessivas análises cromatográficas (CC, CCD e CDC preparativa) levando ao isolamento de nove flavonóides (**1-9**) dos quais seis são

TABLE VI

2D-NMR data of flavan 7.

Atom number	COSY	HETCOR	COLOC
H-2	H-3_{ax}, H-3_{eq}	C-2	C-2*
H-3_{ax}	H-2, H-3_{eq}, H-4	C-3	–
H-3_{eq}	H-2, H-3_{ax}, H-4		–
H-4	H-3_{ax}, H-3_{eq}	C-4	–
H-2´and H-6´	–	C-2´and C-6´	–
H-3´and H-5´	–	C-3´and C-5´	–
H-4´	–	C-4´	–
H-2´´	–	C-2´´	C-3†, C-7‡, C-8‡, C-2´´*
H-3´´	H-2´	C-3´´	C-2´´†, C-7‡
OMe	–	–	C-4‡
OMe	–	–	C-5‡
OMe	–	–	C-6‡

*one bond correlation / † two bonds correlation / ‡ three bonds correlation.

inéditos na literatura (**1-6**) destacando-se as quatro flavanas 2,4-dioxigenadas (**1-4**) que representam uma nova classe de flavonóides.

As estruturas moleculares foram determinadas através da análise dos respectivos espectros de RMN ^{1}H, RMN ^{13}C e DEPT, RMN-2D (COSY, HETCOR e COLOC), NOE, IV, UV e EM.

A análise quantitativa por CLAE, mostrou que a nova flavana **1** é o flavonóide que ocorre em maior abundância no extrato.

Palavras-chave: *Lonchocarpus muehlbergianus*, Leguminosae, flavonóides, flavanas.

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