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Flavonoids of *Lonchocarpus montanus* A.M.G. Azevedo and biological activity

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ABSTRACT

The analysis of root extracts from *Lonchocarpus montanus* A.M.G. Azevedo resulted in the isolation of twenty three compounds chiefly flavonoids of which five (four flavonoids and one benzophenone) are described for the first time. The molecular structures of the new compounds (**1-5**) were determined through spectral analysis (UV, IR, MS and NMR) as being: 2′-hydroxy-8-(α,α-dimethylallyl)-2″, 2″-dimethylpyrano-(5″,6″:3′,4′)-dibenzoylmethane (**1**), 2′-methoxy-8-(α, α-dimethylallyl)-2″, 2″-dimethylpyrano-(5″,6″:3′,4′)-dibenzoylmethane (**2**), 4′-methoxy-2″,2″-dimethylpyrano-(5″,6″:8,7)-flavone (**3**), 2″-(1-hydroxy-1-methylethyl)-furano-(4″,5″:8,7)-flavone (**4**) and [2′-methoxy-furano-(4″,5″:3′,4′)-phenyl]-phenylmethanone (**5**). Additionally, fifteen fatty acids were detected through GC-MS analysis of the corresponding methyl esters [$(CH_3)_2CH(CH_2)_8COOH$ and $CH_3(CH_2)_nCOOH$ (n = 6, 12-24)]. Quantitative RP-HPLC showed that the most abundant flavonoids in the petroleum ether and dichloromethane extracts were pongamol (19%) and lanceolatine B (8.0%), respectively. In the bioautography assay, the extracts, pongamol (**9**), lanceolatine B (**10**), isolonchocarpin (**14**), derriobtusone A (**17**) and medicarpine (**18**) were active against *Staphilococus aureus* whereas **9** also against *Bacillus subtilis* and *Cladosporium cladosporioides*. Compound **1**, 2″,2″-dimethylpyrano-(5″,6″:8,7)-flavone (**11**) and furano-(12″,13″:7,8)-4′-methoxy flavone (**12**) were active against *Fusarium oxysporium* whereas **11** also against *Rhizopus orizae*. The extracts, compounds **9**, **10**, **17** and (*E*)-7-O-methoxypongamol (**23**) displayed high toxicity in the brine shrimp lethality assay.

Key words: *Lonchocarpus montanus* A.M.G. Azevedo, Leguminosae, dibenzoylmethanes, flavonoids, flavones, benzophenone, bioautography, *Artemia salina* lethality.

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INTRODUCTION

The genus *Lonchocarpus* encompasses 150 species, including 24 native to Brazil. *L. montanus* is a new native species (A.M.G.A. Tozzi, unpublished data). The botanical classification was based on morphology of the flowers and fruits and the inflorescence structure, which has been considered an outstanding character. Accordingly *L. montanus* and *L. obtusus* Benth revealed a strong similarity and were allocated to the Unguiflora section in *Lonchocarpus subgenus Lonchocarpus* (A.M.G.A. Tozzi, unpublished data). *L. obtusus* has furnished flavonoids, including the new auronol derriobtusone A, (Do Nascimento et al. 1976). The natural aurones and auronols comprise a very small group of flavonoids. *L. montanus* is an ornamental tree popularly known as "cabelouro" or "carrancudo". It is widely distributed through the states of Bahia, Goias, Minas Gerais and Tocantins.

Many flavonoids possess a remarkable spectrum of biological activities (Middleton et al. 2000). They have been reported to exhibit anti-cancer (Block 1992, Elangovan et al. 1994, Middleton et al. 2000), antiviral (Selway 1986) and anti-inflammatory (Middleton et al. 2000, Middleton 1998, Gabor 1986) effects as well as to reduce the risk of cardiovascular diseases (Middleton et al. 2000, Facino et al. 1999, Hertog et al. 1993, Mazur et al. 1999). These activities are broadly attributed to their antioxidant properties.

This paper describes the isolation and characterization of four new flavonoids (**1-4**) and one new benzophenone derivative (**5**) together with eighteen known compounds (**6-23**) and a mixture of fatty acids (**24-38**), from root extracts of *L. montanus*. The results obtained after submitting extracts (petroleum ether and dichloromethane) and compounds **1**, **6**, **9-12**, **14**, **17**, **18** to bioautography (against bacteria and fungi) as well as extracts (petroleum ether, dichloromethane and methanol) and compounds **9**, **10**, **17**, to brine shrimp lethality are also included. The analysis of the extracts was performed through chromatography (CC, TLC, GC-MS and RP-HPLC).

MATERIALS AND METHODS

GENERAL PROCEDURES

Melting points were determined using a Mettler FP5 apparatus. The optical rotations were measured on a Carl Zeiss Jena Polamat A polarimeter or a Jasco J-720 spectropolarimeter. IR spectra (film or KBr) were recorded using a Perkin-Elmer Model 1600 FT-IR 1600 instrument. UV spectra were recorded on a HP Diode Array Spectrophotometer 8452 A spectrometer using MeOH as solvent. NMR spectra were recorded on Varian INOVA-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C), Gemini 300 (300 MHz for ^{1}H and 75 MHz for ^{13}C) or Brucker AC 300 P (300 MHz for ^{1}H and 75 MHz for ^{13}C) spectrometers using $CDCl_3$ as solvent and TMS as internal standard. The DEPT experiments were performed using polarization transfer pulses of 90° and 135°. EIMS, direct probe, HREIMS and MS/MS experiments were performed on a VG Auto Spec-Fisions Instrument by using electron ionization at 70 eV. GC-MS was performed on a Hewlett Packard (Model HP 5890 B SERIES II) instrument. Column chromatography (CC) separations were on silica gel 60 (70-230 mesh, Merck). TLC was performed on commercial plates (silica gel G and GF_{254}, Merck) while preparative TLC used precoated 1000 m thick Merck silica gel 60 F_{254} glass plates. Compounds were detected by UV ($\lambda = 254$ and 366 nm) irradiation and/or with an ethanolic solution of anisaldehyde, sulfuric acid and acetic acid (90 mL:5mL:1mL), followed by heating. The solvent mixtures were prepared

TABLE I
Fatty acids identified by GC-MS as methyl ester derivatives in the petroleum ether extract from the roots of *Lonchocarpus montanus*.

Compound[a]	RI[b]	T_R (min)
Methyl caprylicate	1124	7.36
Methyl 10-methyl-undecanoate	1525	13.09
Methyl myristate	1726	15.55
Methyl pentadecanoate	1826	16.68
Methyl palmitate	1931	17.82
Methyl margaricate	2029	18.82
Methyl stereate	2133	19.82
Methyl nonadecanoate	2230	20.76
Methyl arachidate	2330	21.67
Methyl heneicosanoate	2431	22.53
Methyl docosanoate	2532	23.37
Methyl tricosanoate	2634	24.18
Methyl lignocericate	2733	24.97
Methyl pentacosanoate	2825	25.78
Methyl hexacosanoste	2935	26.68

[a]active compounds ($LD_{50} \leq 1000\ \mu g\ mL^{-1}$)[48].
[b]made using concentrations of 50, 5.0 and 0.5 $\mu g\ mL^{-1}$.

in volume ratios. Reversed-phase HPLC analyses were performed using a C-18 Nova Pak column, with diode-array detection. Identification of the peaks in the chromatogram of each extract (petroleum ether and dichloromethane) was carried out by co-injection with five flavonoids, **9**, **10**, **14**, **17**, **18** (one each time) isolated from the extracts of *L. montanus* roots and further purified by preparative HPLC.

GC-MS Analysis of Methyl Esters

The methyl fatty acids esters were analyzed by GC-MS using a HP 5970 mass selective detector coupled to a HP-gas chromatograph having a HP5 fused silica capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μm film). The instrument was operated with injector and detector temperatures of 260°C and 280°C, respectively, in the splitless (2 μL injection) and constant flow mode. The temperature of the GC column was increased from 50 to 300°C at a rate of 10°C min^{-1} and held at 300°C for 5 min. The carrier gas was helium at a flow rate of 1.0 mL min^{-1}. Mass spectra were taken over the m/z 40-600 range with an ionizing voltage of 70 eV. Linear retention time indices (RI) for the methyl fatty acids esters **25-39** were determined by comparing their retention times with those of n-paraffin standards and mass spectral data (Adams 1995). All mass spectra were identified by using an on-line library (Wiley 275) or authentic compounds (Table I).

QUANTITATIVE HPLC ANALYSIS

Light petroleum and dichloromethane root extracts from *L. montanus* were dissolved in acetonitrile. An aliquot (2 μL) of each extract was analyzed on a Hewlett Packard model 1090, series II/M HPLC with a Waters Nova-Pak C-18, 3 μm, column, with mobile phase gradient of CH_3CN-H_2O (48-52) for 25 min and then to CH_3CN-H_2O (80-20) during 20 min at a flow rate of 0.8 mL min^{-1}, keeping the column at room temperature. Detection was with a HP photodiode array detector. The identification of the peaks in the chromatograms of each extract (petroleum ether and dichloromethane) was carried out by individual co-injections with each one of the most abundant flavonoids (**9**, **10**, **14**, **17** and **18**) isolated from the extracts of *L. montanus*, which were first purified by semi-preparative HPLC. Each compound was dissolved in acetonitrile and purified with a semi-preparative HPLC (Waters) system, consisting of an universal liquid chromatograph injector, a model 484 variable-wavelength detector (adjusted to 230 nm), a model 740 data module and a model 600E system controller and pump, with a Regis ODS, 5 μm, 250 $\times$ 10 mm i.d. column, and a CH_3CN-H_2O gradient of 20-100% CH_3CN in 45 min at a flow rate of 2.0 $mLmin^{-1}$. The corresponding retention times were: **9** (7.8 min), **10** (8.5 min), **14** (29 min), **17** (13 min) and **18** (4.2 min).

CALIBRATION CURVES

Standard solutions of each quantified flavonoid (**9**, **10**, **17**) were prepared by serial dilutions in acetonitrile. Calibration curves were obtained by plotting the integrated peak areas at the maximum UV absorption for each compound versus concentration by performing linear regression analysis with correlation coefficients of 0.9999 for each analyzed compound. Quantification was made by external calibration in the petroleum ether extract the concentrations were found to be **9** (19%), **10** (10.6%) and **17** (13.6%) while in the dichloromethane extract they were: **9** (6.7%), **10** (8.0%) and **17** (6.4%).

BIOLOGICAL ACTIVITY

The bioautography was performed according to Saxena (Saxena et al. 1995), with some modifications (Magalhães et al. 1998), against eight fungi [*Alternaria alternate* (CCT 1250), *Aspergillus fumigatus* (CCT 01277), *Aspergillus niger* (CCT 1435), *Candida albicans* (CCT 0776), *Cladosporium cladosporioides* (CCT 5039), *Fusarium oxysporium* (CCT 3244), *Penicilluim funiculosum* (CCT 0490), *Rhizopus orizae* (CCT 4964)] and seven bacteria [*Bacillus subtilis* (CCT 0089), *Escherichia colii* (CCT 5050), *Micrococcus luteus* (CCT 2720), *Rodococcus equi* (CCT 0541), *Salmonella typhimurium* (CCT 0528), *Staphylococcus aureus* (CCT 4295), *Streptococcus mutans* (CCT 3440)]. The standards used for comparison were, cyclopyrox olamine for fungi and chloramphenicol for bacteria. The extracts (petroleum ether, dichloromethane and methanol) and compounds **9**, **10**, **14**, **17** and **18** showed activity against *Staphylococcus aureus*, while **9** also against *Bacillus subtilis* and *Cladosporium cladosporioides*. Compounds **1**, **11** and **12** showed activity against *Fusarium oxysporium* while **11** also against *Rhizopus orizae*.

The Brine shrimp lethality assay was performed according to McLaughlin (McLaughlin 1995). The extracts (petroleum ether, dichloromethane and methanol) and compounds **9**, **10**, **17** displayed high toxicity as revealed by very low LC_{50} values (Table II).

TABLE II
Brine shrimp lethality test results for extracts and some flavonoids from Lonchocarpus montanus.[a]

Sample	LD_{50} ($\mu g \times mL^{-1}$)
Petroleum ether extract	2.5[b]
Dichloromethane extract	20.3[b]
Methanol extract	97.2[b]
9	10.7[c]
10	5.8[c]
17	1.6[c]
23	4.4[c]

[a]active compounds ($LD_{50} \leq 1000\ \mu g\ mL^{-1}$)[48].
[b]made using concentrations of 50, 5.0 and 0.5 $\mu g\ mL^{-1}$.
[c]made using concentrations of 1000, 100 and 10 $\mu g\ mL^{-1}$.

PLANT MATERIAL

Roots of *L. montanus* were collected in Taquatinga do Tocantins, Tocantins State, Brazil in March, 1998. The plant was identified by Dr. A.M.G. Azevedo from the Biology Institute of Campinas State University (UNICAMP), Campinas, SP, Brazil. A voucher specimen (B.A.S. Pereira and D. Alvarenga 1717 – UEC) is deposited at the herbarium of Campinas State University (UNICAMP).

EXTRACTION AND ISOLATION

Dried and pulverized roots (259 g) of *L. montanus* were successively extracted with petroleum ether (30-60°C), CH_2Cl_2 and MeOH in a Soxhlet apparatus. After solvent evaporation, the petrol ether extract gave a viscous yellow oil (5.1 g), while the dichloromethane (3.5 g) and the methanol (6.0 g) extracts gave brown oils. Part of the petroleum ether extract (4.32 g) was fractionated by silica gel CC eluted first with petroleum ether/$CHCl_3$ (1:1). The eluent polarity was gradually increased by addition of $CHCl_3$ and then MeOH to furnish 295 fractions (30 mL each) which were reduced to 28 groups after TLC analysis. Most of the compounds were found in ten groups ranging from fractions 34 to 240. A sample of each was further fractionated by successive preparative TLC (silica gel) as described below and recovered from TLC plates by extraction with mixtures of CH_2Cl_2 and MeOH.

Fractions 51-75 (63 mg): preparative TLC [*n*-hexane/CH_2Cl_2/EtOAc (30:10:5, 2×) and *n*-hexane/$CHCl_3$/EtOAc (35:5:2)] gave **1** (5.7 mg) and **9 + demethylpongamol** (30 mg).

Fractions 82-99 (100.3 mg): preparative TLC [$CHCl_3$ (100%, 2×), *n*-hexane/$CHCl_3$ (90:10, 4×), ether/*n*-hexane (90:10, 4×), petroleum ether/CH_2Cl_2/EtOAc (40:5:1) and *n*-hexane/$CHCl_3$/EtOAc (50:10:1, 3×)] gave **5** (0.9 mg).

Fractions 115-119 (93.1 mg): preparative TLC [$CHCl_3$ (100%) and petroleum ether/*n*-hexane (85:15, 7×)] gave **15** (1.9 mg) and **2** (1.7 mg).

Fractions 120-130 (96.4 mg): preparative TLC [(*n*-hexane/CH_2Cl_2/EtOAc (30:15:3, 6×)] gave **3 + demethylpongamol** (1.6 mg).

Fractions 189-215 (48 mg): preparative TLC [*n*-hexane/EtOAc (70:30)] gave **4** (2.4 mg).

Fractions 34-41 (16 mg): preparative TLC [*n*-hexane/$CHCl_3$/EtOAc (35:5:2)] gave **6** (1.1 mg).

Fractions 137-141 (98.7 mg): preparative TLC [*n*-hexane/CH_2Cl_2/MeOH (30:10:0.5, 2×)] gave **7** (2.1 mg).

Fractions 120-130 (98.6 mg): preparative TLC [petroleum ether/CH_2Cl_2/EtOAc (40:10:2) and *n*-hexane/$CHCl_3$/MeOH (35:5:1)] gave **8** (0.7 mg).

Fractions 120-130 (69.8 mg): preparative TLC [*n*-hexane/$CHCl_3$/EtOAc (35:5:5, 2×), *n*-hexane/$CHCl_3$ (94:6, 6×) and *n*-hexane/$CHCl_3$ (95:5, 5×)] gave **10** (8.1 mg).

Fractions 120-130 (100 mg): preparative TLC [*n*-hexane/$CHCl_3$/EtOAc (35:5:5, 2×) and CH_2Cl_2/petroleum ether (95:5, 5×)] gave **11** (2.7 mg).

Fractions 145-153 (71.6 mg): preparative TLC [$CHCl_3$/MeOH (99:1) and *n*-hexane/$CHCl_3$/MeOH (15:15:1)] gave **18** (5.2 mg) and **13** (1.6 mg).

Fractions 103-112 (100 mg): preparative TLC [*n*-hexane/$CHCl_3$/EtOAc (35:5:5) and *n*-hexane/EtOAc (97:3)] gave **14** (3.8 mg).

Fractions 115-119 (93.1 mg): preparative TLC [$CHCl_3$ (100%) and petroleum/*n*-hexane (85:15, 7×)] gave **15** (1.9 mg).

Fractions 103-112 (100 mg): preparative TLC [*n*-hexane/$CHCl_3$/EtOAc (35:5:5), Ag-TLC (5%) *n*-hexane/$CHCl_3$/EtOAc (35:5:0.3) and *n*-hexano/$CHCl_3$/EtOAc (35:5:5)] gave **17** (15.0 mg).

Fractions 137-141 (98.7 mg): preparative TLC [*n*-hexane/CH_2Cl_2/MeOH (30:10:0.5, 2×) and *n*-hexane/$CHCl_3$/EtOAc (35:5:5)] gave **19 + 20 + 12** (2.5 mg).

Fractions 137-141 (78.3 mg): preparative TLC [*n*-hexane/$CHCl_3$/MeOH (30:10:0.5, 2×) and *n*-hexane/$CHCl_3$/EtOAc (35:5:5)] gave **21 + 22** (3.0 mg).

Fractions 115-119 (116 mg) were flash chromatographed on silica gel, eluting with 2000 mL of petroleum ether/CH_2Cl_2 (70:30) and then washing with MeOH. After solvent evaporation, the methanolic fraction (28.1 mg) was submitted to preparative TLC [petroleum ether/$CHCl_3$/EtOAc (40:10:2)] to give **16** (2.7 mg).

The dichloromethane extract (2.4 g) was flash chromatographed on silica gel to give 501 fractions (30 mL each) which were reduced to 49 groups after TLC analysis. A sample of each was further fractionated by successive preparative TLC (silica gel) as described below and recovered from TLC plates by extraction with mixtures of CH_2Cl_2 and MeOH.

Fractions 154-161 (45.3 mg): preparative TLC ($CHCl_3$ 100%, 4×) gave **10** (7.8 mg) and **23** (4.6 mg).

Fractions 194-295 (116.4 mg): preparative TLC [*n*-hexane/EtOAc (35:5:5)] gave **18** (20.1 mg).

Fractions 126-135 (268.4 mg) were flash chromatographed on silica gel, eluting with *n*-hexane/$CHCl_3$ (6:4). The eluent polarity was gradually increased by addition of $CHCl_3$ and then MeOH to furnish 200 fractions (30 mL each) which were monitored by TLC. A sample of each was further fractionated by successive preparative TLC (silica gel) and recovered from TLC plates by extraction with mixtures of CH_2Cl_2 and MeOH.

Fractions 14-19 (32.8 mg): preparative TLC [$CHCl_3$/MeOH (98:2, 3×)] gave **17** (15.7 mg).

Fractions 60-74 (51 mg): preparative TLC [$CHCl_3$/*n*-hexane/MeOH (17:3:0.1, 3×)] gave **10** (20.7 mg) and **23** (13.0 mg).

New Compounds

2′-hydroxy-8-(α,α-dimethylallyl)-2″, 2″-dimethylpyrano-(5″,6″:3′,4′)-dibenzoylmethane (1): yellow needles, $[\alpha]_D^{25}$: –8.68 (MeOH, c 0.008 gmL^{-1}). HREIMS, m/z (%): 390.1831 (13) $[M]^+$, 375.1609 (11) $[M\text{-}Me]^+$, 307.0944 (100) $[M\text{-}Me\text{-}68]^+$, 203.0641 (39) $[M\text{-}187]$+, 187.0363 (65) $[M\text{-}Me\text{-}188]^+$, 105.0267 (51) $[M\text{-}285]^+$, 77.0311 (28) $[M\text{-}313]^+$.UV (MeOH): λ_{max} (log ε) = 253 (4.40), 319 (4.03), nm. IR (KBr): ν_{max} = 3438, 2923, 2850, 1690, 1644, 1598, 1384, 1118 cm^{-1}. ^{1}H NMR (300 MHz): δ and ^{13}C NMR (75.4 MHz): δ, see Table III.

2′-methoxy-8-(α, α-dimethylallyl)-2″, 2″-dimethylpyrano-(5″, 6″:3′,4′)-dibenzoylmethane (2): orange needles. HREIMS, m/z (%): 404.1988 (4) $[M]^+$, 389.1768 (20) $[M\text{-}Me]^+$, 217.0852 (66) $[M\text{-}187]^+$. GC-MS, m/z (%): 404 (4) $[M]^+$, 389 (19) $[M\text{-}Me]^+$, 321 (13) $[M\text{-}Me\text{-}68]^+$, 217 (100) $[M\text{-}187]^+$, 105 (28) $[M\text{-}299]^+$, 77 (30) $[M\text{-}327]^+$. UV (MeOH): λ_{max} (log ε) = 248 (4.0). ^{1}H NMR (300 MHz): δ and ^{13}C NMR (75.4 MHz): δ, see Table III.

2′-hydroxy-furano-(4″, 5″:3′,4′)-dibenzoylmethane (demethylpongamol): GC-MS, m/z (%): 280 (23) $[M]^+$, 161 (16) $[M\text{-}119]^+$, 160 (23) $[M\text{-}120]^+$, 105 (100) $[M\text{-}175]^+$, 77 (45) $[M\text{-}175\text{-}CO]^+$, 51 (19) $[M\text{-}175\text{-}CO\text{-}C_2H_2]^+$.

4′-methoxy-2″, 2″-dimethylpyrano-(5″, 6″:8,7)-flavone (3): colorless amorphous solid. GC-MS, m/z (%): 334 (13) $[M]^+$, 319 (100) $[M\text{-}Me]^+$, 187 (72) $[M\text{-}Me\text{-}132]^+$, 159 (8) $[M\text{-}Me\text{-}132\text{-}CO]^+$, 132 (9) $[M\text{-}Me\text{-}187]^+$. ^{1}H NMR (500 MHz): δ, see Table III.

2″-(1-hydroxy-1-methylethyl)-furano- (4″,5″:8,7)-flavone (4): colorless needles, HREIMS, m/z (%): 320.1050 (38) $[M]^+$, 305.0816 (100) $[M\text{-}Me]^+$, 203.0368 (17) $[M\text{-}Me\text{-}102]^+$, 68.9970 (52)$[M\text{-}252]^+$. MS, m/z (%): 320 (26) $[M]^+$, 305 (100) $[M\text{-}Me]^+$, 203 (25) $[M\text{-}Me\text{-}102]^+$, 102 (33) $[M\text{-}Me\text{-}203]^+$, 77 (22) $[M\text{-}Me\text{-}228]^+$, 43 (99) $[M\text{-}Me\ C_{17}H_{10}O_3]^+$. ^{1}H NMR (500 MHz): δ, see Table III.

2′-methoxyfurano(4″,5″: 3′,4′)-phenyl-phenylmethanone (5): colorless amorphous solid. MS, m/z (%): 252 $[M]^+$ (absent), 175 (100) $[M\text{-}77]^+$, 160 (24) $[M\text{-}77\text{-}Me]^+$, 132 (5) $[M\text{-}77\text{-}Me\text{-}CO]^+$, 105 (8) $[M\text{-}147]^+$, 77 (28) $[M\text{-}147\text{-}CO]^+$. UV (MeOH): λ_{max} (log ε) = 248 (3.77), 276 (3.36), 322 (2.79), nm. IR (film): ν_{max} = 2924, 2853, 1651, 1585, 1472, 1257, 733 cm^{-1}. ^{1}H NMR (500 MHz): δ, see Table III.

TABLE III
The NMR data of compounds *1*, *2*, *4*, *5* and *6*.

Position	1**		2		3	4	5
	δ_H^*	δ_C^*	δ_H^*	δ_C^*	δ_H^*	δ_H^*	δ_H^*
1	–	138.2	–	138	–	–	–
2	7.91 *d* (9.0)	128.6	7.99 *d* (8.5)	128	–	–	–
3	7.42 *m*	129.0	7.42-7.55 *m*	129	6.69 *s*	6.90 *s*	–
4	7.50 *m*	133.4	7.42-7.55 *m*	131	–	–	–
5	7.42 *m*	129.0	7.42-7.55 *m*	129	7.99 *d* (8.5)	8.15 *d* (9.0)	7.37 *dd* (8.5,1.0)
6	7.91 *d* (9.0)	128.6	7.99 *d* (8.5)	128	6.86 *d* (8.5)	7.53 *dd* (9.0, 1.0)	8.04 *d* (9.0)
7	–	194.2	–	195.8	–	–	–
8	5.42 *s*	61.3	5.65-5.68 *m*	62	–	–	–
9	–	198.6	–	196.4	–	–	7.97 *d* (8.0)
10		–		–	–	–	7.52 *t* (8.0)
11	–	–	–	–	–	–	7.65 *m*
12		–		–	–	–	7.52 *t* (8.0)
13	–	–	–	–	–	–	7.97 *d* (8.0)
1′	–	114.4		113		–	–
2′	–	161.0	–	161	7.86 *d* (8.5)	7.96-8.0 *m*	7.65 *m*
3′	–	109.7	–	110	7.04 *d* (8.5)	7.54-7.58 *m*	6.92 *d* (2.5)
4′	–	160.3	–	160	–	7.54-7.58 *m*	–
5′	6.35 *d* (9.0)	108.8	6.56 *d* (9.0)	109	7.04 *d* (8.5)	7.54-7.58 *m*	–
6′	7.70 *d* (9.0)	131.7	7.42-7.55 *m*	130	7.86 *d* (8.5)	7.96-8.0 *m*	–
1″	–	78.3		78	–	–	–
2″	–	–	–	–	–	–	–
3″	5.56 *d* (9.9)	128.5	5.65-5.68 *m*	127	5.77 *d* (10.0)	7.04 *d* (1.0)	–
4″	6.68 *d* (9.9)	115.8	6.53 *d* (10.0)	116	6.94 *d* (10.0)	–	–
1‴	–	41.8		42	–	–	–
2‴	6.17 *dd* (17.4,10.8)	145.7	6.09 *dd* (17.4,11.0)	145	–	–	–
3a‴	5.00 *d* (17.4)	112.3	4.91 *d* (17.4)	112	–	–	–
3b‴	4.97 *d* (10.8)	112.3	4.89 *d* (11.0)	112	–	–	–
4‴	–	–	–	–	–	–	–
OCH_3	–	–	3.51 *s*	66.0	3.91 *s*	–	3.81 *s*
2″-CH_3	1.45 *s*	28.5	1.45 *s*	28	–	–	–
2″-CH_3	1.43 *s*	28.5	1.40 *s*	28	–	–	–
1‴-CH_3	1.31 *s*	26.4	1.21 *s*	27	–	–	–
1‴-CH_3	1.29 *s*	25.9	1.20 *s*	26	–	–	–
2″2″$(CH_3)_2$C-	–	–	–	–	–	1.76 *s*	–
OH	13.03 *s*	–	–	–	–	4.78 *sl*	–

*δ, $CDCl_3$, J values in Hz.

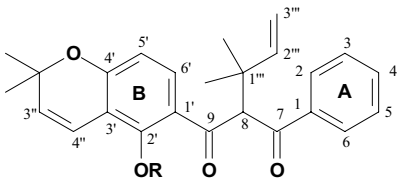
**The complete assignments of ^{1}H NMR, ^{13}C NMR and DEPT data of this compound were confirmed by correlations observed in 2D-NMR spectra (HSQC and HMBC).

RESULTS AND DISCUSSION

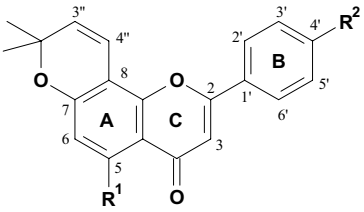
The petroleum ether extract of *L. montanus* roots was submitted to successive chromatographic analysis (CC, TLC and preparative TLC), allowing the isolation of nineteen flavonoids (**1-4**, **6-20**), one benzophenone derivative (**5**) (Fig. 1) and two steroids (**21** and **22**). A mixture of fatty acids was first methylated with diazomethane followed by GC-MS analysis, allowing the identification of fifteen methyl esters: **24** [$CH_3(CH_2)_6COOCH_3$], **25** [$(CH_3)_2CH\ (CH_2)_7COOCH_3$] and **26-38** [$CH_3(CH_2)_nCOOCH_3$ (n = 12-24)]. The dichloromethane extract gave the flavonoids **10**, **17**, **18** and **23**.

Compound 1 gave a molecular ion $[M]^+$ at m/z 390.18314 in HREIMS which corresponds to $C_{25}H_{26}O_4$ (calc. 390.18311). The NMR data (Table III) were very similar to those of the dibenzoylmethane derivative described earlier (Magalhães et al. 1997). In the ^{1}H NMR spectrum, signals for a monosubstituted aromatic ring [δ 7.91 (2H, *d*, J = 9.0 Hz, H-2, H-6), δ 7.42 (2H, *m*, H-3,H-5) and δ 7.50 (1H, *m*, H-4)], one α,α-dimethylallyl group [δ 6.17 (1H, *dd*, J = 17.4 and 10.8 Hz, H-2″'), δ 5.0 (1H, *d*, J = 17.4 Hz, H-3a″'), δ 4.97 (1H, *d*, J = 10.8 Hz, H-3b″'), δ 1.31 (3H, *s*) and δ 1.29 (3H, *s*)], one uncoupled methynic hydrogen [δ 5.42 (1H, *s*, H-8)], two *ortho* coupled aromatic hydrogens [δ 7.70 (1H, *d*, J = 9.0 Hz, H-6′) and δ 6.35 (1H, *d*, J = 9.0 Hz, H-5′)] and one hydrogen bonded hydroxy group [δ 13.03 (1H, *s*)] were also observed. However instead of a furano ring, compound **1** has a 2,2-dimethylpyrano ring shown in the ^{1}H NMR spectrum by the signals at δ 5.57 (1H, *d*, J = 9.9 Hz, H-3″), δ 6.68 (1H, *d*, J = 9.9 Hz, H-4″), δ 1.45 (3H, *s*, 2″-CH_3) and δ 1.43 (3H, *s*, 2″-CH_3). This was confirmed in the ^{13}C NMR spectrum (Table III) by the signals at δ 109.7 (C-3′), δ 160.3 (C-4′), δ 128.5 (C-3″), δ 115.8 (C-4″), δ 28.5 (2″-CH_3) and δ 28.5 (2″-CH_3). The signals of two carbonyl groups (δ 198.6 and δ 194.2) in the ^{13}C NMR is in accordance with a non-enolizable dibenzoylmethane skeleton (Parmar et al. 1989, Zeng et al. 1994, Fukai et al. 1994). The ^{13}C NMR spectrum and DEPT (90° and 135°) indicated the presence of nine quaternary carbons, eleven CH, one CH_2 and four CH_3. Based on these data, four structural possibilities were suggested concerning the alternative oxygenation pattern of the B ring (2′,4′; 2′,5′; 2′,3′; 2′,6′). In the nOe difference experiment, irradiation of H-4″ (δ 6.68) caused an enhancement of the signals at δ 5.56 (H-3″) and δ 13.03 (chelated 2′-OH). These findings are in agreement with structure **1**. Irradiation of H-8 (δ 5.42) caused a higher enhancement of the signal at δ 7.70 (H-6′, 9.66%) than of the signal at δ 7.91 (H-2 and H-6, 5.04%), suggesting that the preferential conformation is the one where the B ring benzoyl moiety and C-8/H-8 lie in the same plane (Parmar et al. 1989). Thus, C-8 is a stereogenic center, which was further confirmed by optical rotation measurement. Based on MS/MS experiments selecting the ions m/z 375 [307 (12%), 187 (100%)] and m/z 307 [187(100%)], it was possible to suggest a fragmentation pathway (Fig. 2).

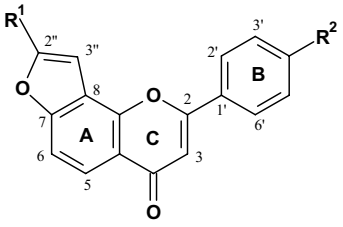
Compound **2** exhibited a molecular ion $[M]^+$ at m/z 404.198792 in HREIMS, which is fourteen mass units higher than **1**, suggesting that the hydroxyl group was replaced by a methoxy group, for a molecular formula of $C_{26}H_{28}O_4$ (calc. 404.198760). In the low resolution mass spectrum the base peak at m/z 217 $[C_{13}H_{13}O_3]^+$ originates from C-8/C-9 bond cleavage proving that the methoxy group is on B ring. The fragmentaion pathway (Fig. 2) is analogous to that of **1**. NMR data (Table III) were also very similar those of **1** except for the lack of a signal corresponding to a hydrogen bonded hydroxy group at C-2′ in the ^{1}H NMR spectrum and the presence of a methoxy group (δ 3.51, 3H, *s*, 2′-OCH_3) that was confirmed in the ^{13}C NMR spectrum (δ 66.0, 2′-OCH_3). The unusual chemical shift value observed for the methoxy



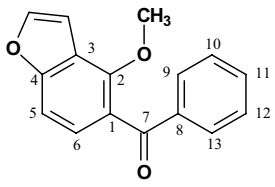
1: R = H **new**
2: R = CH_3 **new**



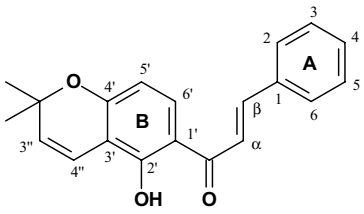
3: R^1 = H ; R^2 = OCH_3 **new**
11: R^1 = R^2 = H
13: R^1 = OCH_3 ; R^2 = H



4: R^1 = $C(CH_3)_2OH$; R^2 = H **new**
10: R^1 = R^2 = H
12: R^1 = H ; R^2 = OCH_3



5 **new**



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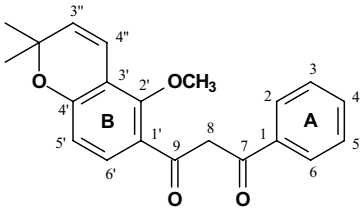

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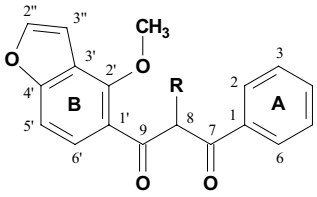
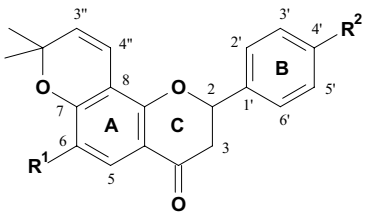
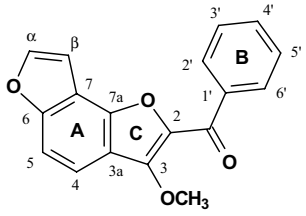


8: R = α-α-dimethylallyl
9: R = H



14: R^1 = R^2 = H
15: R^1 = H ; R^2 = OCH_3
16: R^1 = OCH_3 ; R^2 = H



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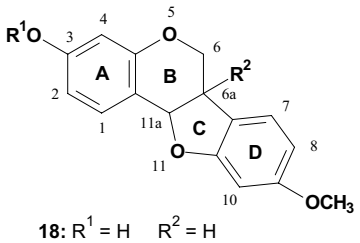
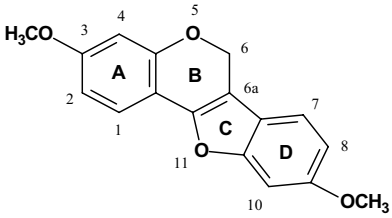


18: R^1 = H R^2 = H
19: R^1 = CH_3 R^2 = OH



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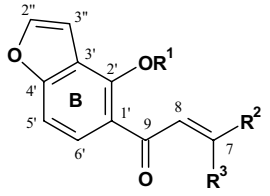

23: R^1 = CH_3 ; R^2 = OCH_3 ; R^3 = Ph

Fig. 1 – Flavonoids isolated from roots of *Lonchocarpus montanus* A.M.G. Azevedo.

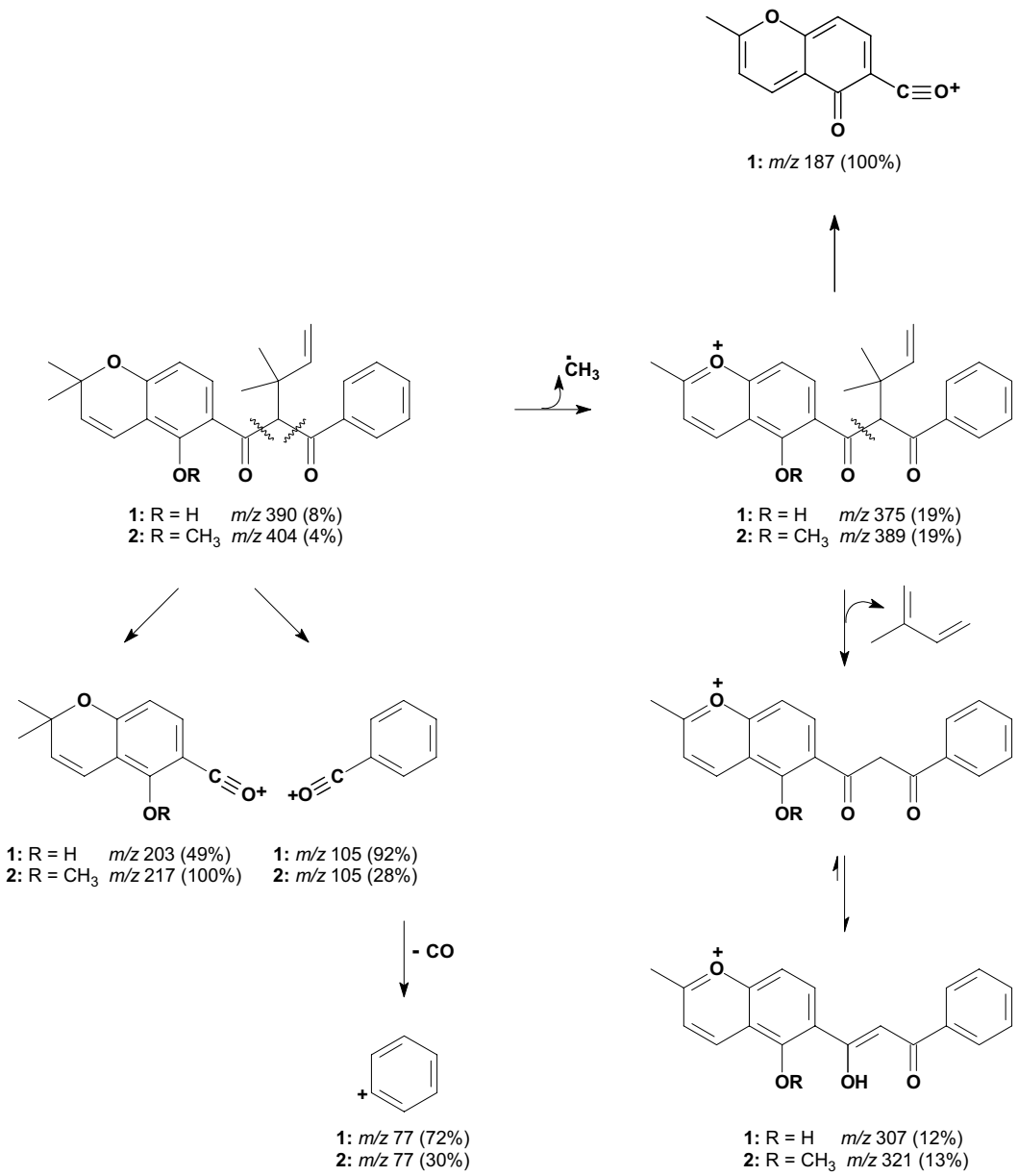

Fig. 2 – Rationalization of a pathway leading to a common fragment in the mass spectra of **1** and **2**.

group in the ^{1}H NMR spectrum evidenced that the methyl group stays out of the aromatic ring plane in order to relieve sterical hindrance with the α,α-dimethylallyl group at C-8, as can be demonstrated through the corresponding molecular model.

A minor compound together with **9** was detected through GC-MS. The corresponding mass spectrum (t_R = 23.3 min) displayed a molecular ion $[M]^+$ of m/z 280 (23%) which is fourteen mass units lower than that of **9**, according to the molecular formula $C_{17}H_{12}O_4$. The peaks at m/z 161 (16%) and m/z 105 (100%)

correspond to the fragments from the cleavage of C8-C9 and C7-C8 bonds, respectively, leading to the suggestion that this compound can be demethylpongamol, a new compound that is probably the biogenetic precursor of **1**. Alternative modes of furano fusion with ring A however cannot be discarded.

Compound **3** also was isolated as a minor compound together with **12**. The ^{1}H NMR spectrum of the mixture showed a ratio of 17:83 (**4:13**). Among the signals corresponding to **3** there are those of a *p*-disubstituted aromatic ring [δ 7.86 (2H, *d*, J = 8.5 Hz, H-2′ and H-6′) and δ 7.04 (2H, *d*, J = 8.5 Hz, H-3′ and H-5′)] and one methoxy group [δ 3.91 (3H, *s*, 4′-OCH_3)]. A typical flavone H-3 signal at δ 6.69 (1H, *s*) was observed. GC-MS analysis furnished a chromatogram with two peaks [t_R = 28.7 min (**12**); t_R = 32.3 min (**3**)]. In the mass spectrum of **3**, the molecular ion $[M]^+$ at m/z 334 (10%) and the fragments at m/z 399 (100%), m/z 187 (80%), can be rationalized by the loss of one methyl radical from a 2,2-dimethylpyrano group followed by C ring RDA rearrangement. Based on the biosynthetic pathway of flavonoids (Cooper-Driver and Bhattacharya 1998), it could be suggested a close biosynthetic relation between **15** and **3**.

Compound **4** gave a molecular ion $[M]^+$ at m/z 320.1052 in HREIMS according to $C_{20}H_{16}0_4$ (calc. 320.1049). The ^{1}H NMR spectrum (Table III) showed signals of one monosubstituted aromatic ring [δ 7.96-8.0 (2H, *m*, H-2′, H-6′) and δ 7.54-7.58 (3H, *m*, H-3′, H-4′, H-5′)], two *ortho* coupled hydrogens [δ 8.15 (1H, *d*, J = 9.0 Hz) and δ 7.53 (1H, *dd*, J = 9.0 and 1.0 Hz)], a flavone H-3 (δ 6.90, *s*, 1H) and a long range coupled hydrogen [δ 7.04 (1H, *d*, J = 1.0 Hz)]. In addition, the signals at δ 1.76 [6H, *s*, 2″2″$(CH_3)_2$C-] and δ 4.78 (1H, *bs*, 2″-C-OH) are similar to those corresponding to the isopropyl group in oroselol (Lee 1995). In the low resolution mass spectrum the most intense peaks at m/z 305 [100% (M^+.-15)] and m/z 43 [99%, CH_3CO^+ (M^+.-15-$C_{17}H_{10}O_3$)] can be rationalized by the loss of a methyl group followed by the loss of (4,5:8,7)-furanoflavone while the peak at m/z 203 (25%) corresponds to C ring RDA cleavage after the molecule loses a methyl group [(M^+.-CH_3)-C_8H_6].

Compound **5** did not give the molecular ion $[M]^+$ in HREIMS even after lowering the energy of the ionizing electron bean. The ^{1}H NMR spectrum (Table III) showed signals corresponding to one monosubstituted aromatic ring [δ 7.97 (2H, *d*, J = 8 Hz, H-9 and H-13), δ 7.65 (1H, *m*, H-11) and δ 7.52 (2H, *t*, J = 8 Hz, H-10 and H-12)], one furan [δ 6.92 (1H, *d*, J = 2.5 Hz, H-3″) and δ 7.65 (1H, *m*, H-2″], two *ortho*-coupled hydrogens [δ 8.04 (1H, *d*, J = 8.5 Hz, H-6) and δ 7.37 (1H, *dd*, J = 8.5 and 1 Hz, H-5)] and one aromatic methoxy group [δ 3.81 (3H, *s*, 2-OCH_3)]. In the low resolution mass spectrum the base peak at m/z 175 (100%) corresponds to the fragment ion containing the B-ring, which results from the α carbonyl bond cleavage.

The known flavonoids (**6-20**, **23**; see Table IV), β-sitosterol (**21**) and stigmasterol (**22**) were characterized by comparison of the respective spectral data with those found in the literature.

HPLC analyses of the petroleum ether and dichloromethane extracts allowed the identification of **9**, **10**, **14**, **17** and **18**. The most abundant flavonoids in the petroleum ether were found to be **9** (19%), **10** (10%) and **17** (13%) while in dichloromethane they were **9** (6.7%), **10** (8.0%) and **17** (6.4%).

The root extracts and the flavonoids **1**, **6**, **9-12**, **14**, **17** and **18** were submitted to bioautography assay (Saxena et al. 1995, Magalhães et al. 1998) against seven bacteria and eight fungi. Root extracts (petroleum ether and dichloromethane) and the flavonoids **9**, **10**, **14**, **17** and **18** showed antibacterial activity against *Staphilococcus aureus*, whereas **9** was also active against *Bacillus subtilis* and *Cladosporium cladospo-*

TABLE IV
Known compounds isolated from root extract of *Lonchocarpus montanus* A.M.G. Azevedo.

Compound	Botanical source*	References
Lonchocarpin (**6**)	*L. sericeu*	Do Nascimento and Mors 1972
	Cordoa piaca	Delle Monache et al. 1974
	Pongamia glabra	Subrahmanyam et al. 1977
Purpurenone (**7**)	*L. subglaucescens*	Magalhães et al. 1996
	Tephrosia purpurea	Rao and Raju 1984
2′-methoxy-8-(α-α-dimethylallyl)-furano-(4″,5″:3′,4′)-dibenzoylmethane (**8**)	*L. latifolius*	Magalhães et al. 1998
Pongamol (**9**)	*Tephrosia purpúrea*	Sinha et al. 1982
	Milletia sanagana	Parmar et al. 1989
		Mahli et al. 1989
		Mbafor et al. 1995
Lanceolatin B (**10**)	*Pongamia glabra*	Talapatra et al. 1980
	Milletia sanagana	Mbafor et al. 1995
	L. montanus	C.M.D.P. Soriano, unpublished data
	L. latifolius	Magalhães et al. 2000
2″,2″-dimethylpyrano-(5″,6″:8,7)-flavone (**11**)	*Dahlstedtia pentaphylla*	Garcez et al. 1988
	L. subglaucescens	Magalhães et al. 1996
Furano-(4″,5″:7,8)-4′-methoxyflavone (**12**)	*Pongamia glabra*	Garg 1979
	Derris mollis	Lyra et al. 1979
Isopongaflavone (**13**)	*Tephrosia bracteolate*	Khalid and Waterman 1981
	L. costaricensis	Waterman and Mahmoud 1985
		Bentley et al. 1987
Isolonchocarpin (**14**)	*Tephrosia purpurea*	Rao and Raju 1979
	L. xuul	Delle Monache et al. 1978
	L. campestri	C.A. Firmino, unpublished data
4′-methoxyisolonchocarpin (**15**)	*L. campestri*	C.A. Firmino, unpublished data
6-methoxyisolonchocarpin (**16**)	*L. subglaucescens*	Magalhães et al. 1996
Derriobtusone A (**17**)	*L.obtusus*	Do Nascimento et al. 1976
	L.montanus	C.M.D.P. Soriano, unpublished data
Medicarpin (**18**)	*L. campestri*	Vanetten et al. 1983
	L.montanus	C.A. Firmino, unpublished data
	L. latifolius	C.M.D.P. Soriano, unpublished data
		Magalhães et al. 2000
Variabilin (**19**)	*Trifolium pretense*	Bilton et al. 1976
	Dalbergia variabilis	Kurosawa et al. 1978
	Rhynchosia acuminatifolia	Ingham 1990
Anhydrovariabilin (**20**)	*Lespedeza cyrtobotrya*	Miyase et al. 1980
(*E*)-7-O-methoxy-pongamol (**23**)	*Tephrosia purpurea*	Parmar et al. 1989
	L. montanus	C.M.D.P. Soriano, unpublished data

*all species belong to family Leguminosae, sub-family Papilonoideae.

rioides. The flavonoids **1**, **11** and **12** showed antifungal activity against *Fusarium oxysporium* whereas **11** was also active against *Rhizopus oryzae*.

The petroleum ether, dichloromethane and methanol extracts and the flavonoids **9**, **10**, **17** and **23** were also submitted to the brine shrimp lethality bioassay (McLaughlin 1995) and displayed high toxicity as revealed by very low LC_{50} values (Table II).

Derriobtusone A (**17**) is a very rare auronol which was isolated only from *L. obtusus*. The occurrence of Derriobtusone A (**17**), among the most abundant compounds in *L. montanus* reinforces its allocation together with *L. obtusus* at Unguiflora section in *Lonchocarpus* subgenus *Lonchocarpus*. The structures of the most abundant flavonoids (**9**, **10**, **17**) furnished by *L. montanus* are closely related. These findings suggest a biosynthetic pathway consisted by alternative oxidative steps starting from the same chalcone (Fig. 3). However no aurone was found in *L. montanus*.

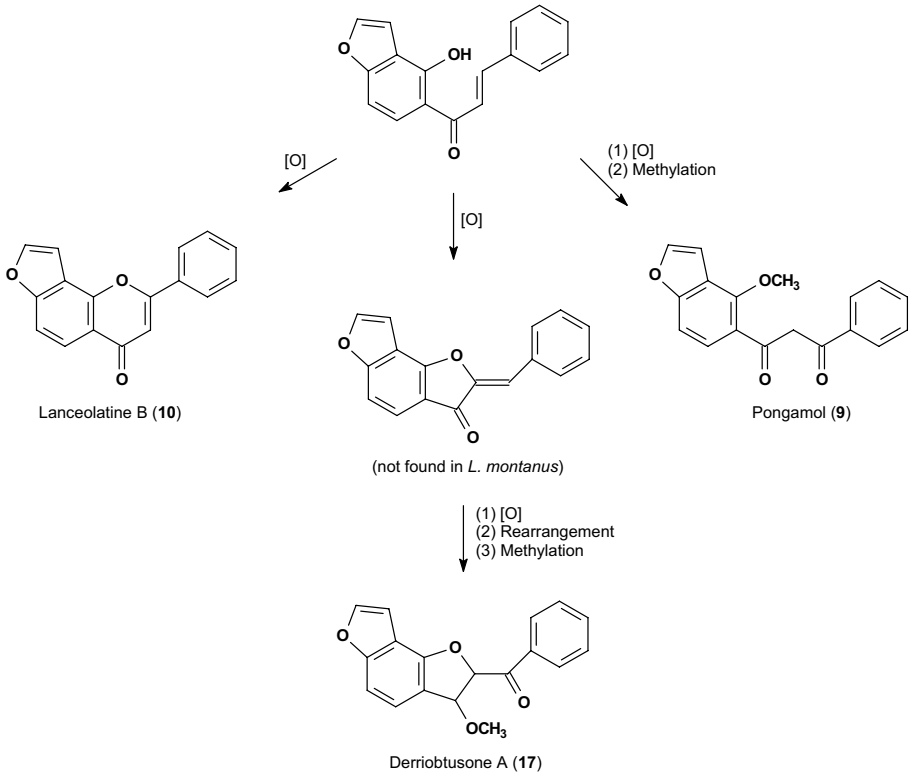

Fig. 3 – Rationalization of a biosynthetic pathway leading to the most abundant flavonoids isolated from *Lonchocarpus montanus* A.M.G. Azevedo.

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RESUMO

A análise dos extratos das raízes de *L. montanus* A.M.G. Azevedo resultou no isolamento de vinte e três compostos principalmente flavonóides dos quais cinco são descritos pela primeira vez. As estruturas moleculares dos novos compostos (**1-5**) foram propostas através da análise dos espectros de UV, IV, EM e RMN como sendo: 2′-hidroxi-8-(α, α-dimetilalil)-2″, 2″-dimetilpirano-(5″, 6″:3′,4′)-dibenzoilmetano (**1**), 2′-metoxi-8-(α,α-dimetilalil)-2″, 2″-dimetilpirano-(5″, 6″:3′,4′)-dibenzoilmetano (**2**), 4′-metoxi-2″, 2″-dimetilpirano-(5″, 6″:8,7)-flavona (**3**), 2″-(1-hidroxi-1-metiletil)-furano-(4″, 5″:8,7)-flavona (**4**) e [2′-metoxi-furano(4″, 5″:3′,4′)-fenil]-fenilmetanona (**5**). Adicionalmente quinze ácidos graxos foram detectados através da análise de CG-EM dos ésteres metílicos correspondentes [$(CH_3)_2CH(CH_2)_8COOH$ e $CH_3(CH_2)_nCOOH$ (n = 6, 12-24)]. A análise quantitativa por CLAE mostrou que os flavonóides mais abundantes nos extratos éter de petróleo e diclorometânico foram pongamol (19%) e lanceolatina B (8.0%), respectivamente. Nos ensaios de bioautografia, os extratos, pongamol (**9**), lanceolatina B (**10**), isolonchocarpina (**14**), derriobtusona A (**17**) e medicarpina (**18**) foram ativos contra *Staphilococcus aureus* enquanto **9**, também contra *Bacillus subtilis* e *Cladosporium cladosporióides*. O composto **1**, 2″, 2″-dimetilpirano-(5″, 6″:8,7)-flavona (**11**) e furano-(2″, 3″:7,8)-4′-metoxiflavona (**12**) foram ativos contra *Fusarium oxysporium*, enquanto **11**, também contra *Rhizopus oryzae*. Os extratos assim como os compostos **9**, **10**, **17** e (E)-7-O-metoxipongamol (**23**) apresentaram alta toxicidade no ensaio de letalidade com *Artemia salina*.

Palavras-chave: *Lonchocarpus montanus* A.M.G. Azevedo, Leguminosae, dibenzoilmetano, flavonoides, flavonas, benzofenona, bioautografia, letalidade com *Artemia salina*.

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