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Flavanones and 3-hydroxyflavanones from *Lonchocarpus oaxacensis*[☆]

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Abstract

The roots of the tropical tree *Lonchocarpus oaxacensis* afforded the 3-hydroxyflavanones jayacanol and mundulinol, as well as two flavanones, mundulin and minimiflorin. Flavonoids bearing 6,7-(dimethylpyran) and 8-(γγ-dimethyl allyl) substituents are characteristic for species grouped in the *Minimiflori* subsection. Therefore this subsection seems to be chemically and morphologically homogeneous. The antifungal activity of the four isolated compounds was tested against the wood rotting fungus *Postia placenta*, but only jayacanol was active.

Keywords: *Lonchocarpus oaxacensis*; Leguminosae; Papilionoideae; Flavanones; 3-Hydroxyflavanones; Jayacanol; Mundulinol; Mundulin; Minimiflorin; Roots; Antifungal activity; *Postia placenta*; Wood rotting fungi; Chemotaxonomy

1. Introduction

As part of a chemotaxonomical study and survey of biologically active compounds from *Lonchocarpus* (Leguminosae, Papilionoideae, Millettieae) species growing in Mexico (Gómez-Garibay et al., 1990; Reyes-Chilpa et al., 1995), we have now investigated the flavonoids present in the roots of *Lonchocarpus oaxacensis*, a small tree endemic to the State of Oaxaca, Mexico. To the best of our knowledge, this plant has not been studied previously from the phytochemical point of view. According to a revised taxonomic classification, *L. oaxacensis* belongs to subgenus *Lonchocarpus*, section *Lonchocarpus*, subsection *Minimiflori* (M. Sousa-Sánchez, in preparation). This subsection comprises 15 species, only four of which have been chemically studied. At the present time, it is known that the seeds of *L. minimiflorus* (Mahmoud and Waterman, 1985), and *L. orotinus* (=*L. parviflorus*) (Waterman and Mahmoud, 1987) contain prenylated flavanones and 3-hydroxyflavanones. These types of flavonoids have also been obtained from the leaves of *L. minimiflorus* (Roussis et al., 1987), and the roots of *L. guatemalensis* (Ingham et al., 1988).

2. Results and discussion

The pooled petroleum ether and methylene chloride extracts from the roots of *L. oaxacensis* afforded, after column chromatography, a new 3-hydroxyflavanone, which was named jayacanol (**1**). Mundulinol (**2**), and two flavanones, mundulin (**3**) and minimiflorin (**4**) were also obtained. The molecular structure of **1** was established by spectroscopic methods, including HMQC, HMBC and NOESY NMR experiments.

Compound **1** was isolated as a yellow gum. The infrared spectrum displayed absorption bands for hydroxyl (3403 cm^{-1}) and conjugated carbonyl (1628 cm^{-1}) functional groups. The ^1H NMR spectrum (Table 1) showed an AB spin system located at δ 5.39 and δ 4.52 (both *d*, $J=12$ Hz, 1H) characteristic for H-2 and H-3, respectively, of a 3-hydroxyflavanone skeleton. A broad singlet (D_2O-exchangeable) at δ 4.65 was assigned to an hydroxyl on C-3. Signals for four coupled aromatic protons suggested that ring B was substituted by

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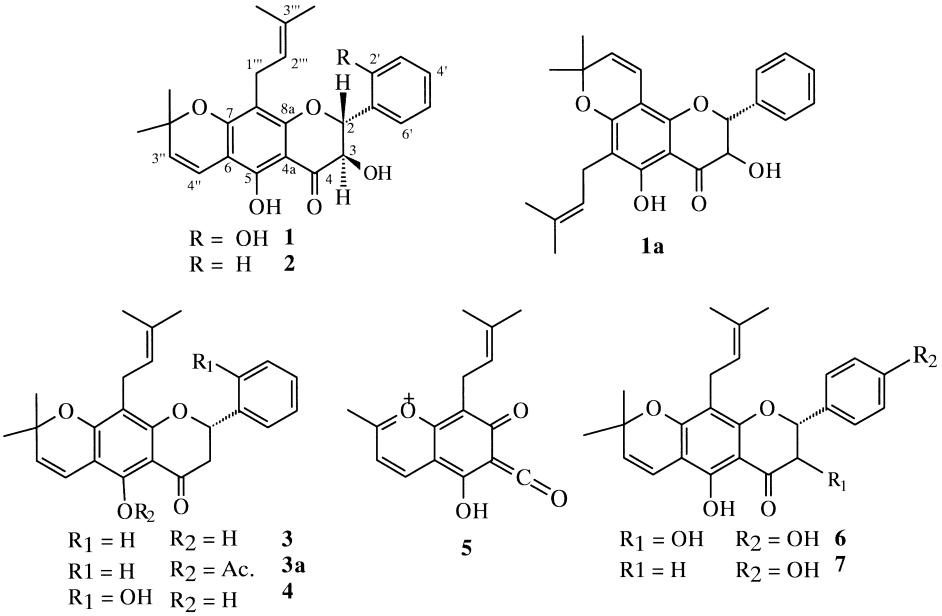

Table 1
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data (δ, ppm) of jayacanol (**1**)

Position	^{1}H NMR	^{13}C NMR
2	5.39 (*d*, *J*=12.0 Hz, 1H)	78.50
3	4.52 (*d*, *J*=12.0 Hz, 1H)	73.22
4	–	195.42
4a	–	100.29
5	–	160.99
6	–	103.45
7	–	156.13
8	–	109.56
8a	–	159.07
1′	–	124.19
2′	–	154.00
3′	6.97 (*dd*, *J*= 1.0 and 8.0 Hz, 1H)	118.01
4′	7.27 (*td*, *J*= 1.5 and 7.5 Hz, 1H)	129.93
5′	7.02 (*td*, *J*= 1.5 and 7.5 Hz, 1H)	121.19
6′	7.56 (*dd*, *J*= 1.5 and 7.5 Hz, 1H)	126.93
2″	–	78.64
3″	5.53 (*d*, *J*= 10 Hz,1H)	126.46
4″	6.63 (*d*, *J*= 10 Hz,1H)	115.33
1‴	3.19 (*dd*, *J*= 7.25 and 14.4, 1H) and 3.25 (*dd*, *J*= 7.5 and 14.5, 2H)	21.29
2‴	5.13 (*tt*, *J*= 1.2 and 7.5 Hz, 1H)	122.12
3‴	–	131.60
CH_3-3‴	1.60 and 1.65 (*s*, 6H)	17.81 25.69
CH_3-2″	1.45 (*s*, Me, 6H)	28.38
OH-5	11.31 (*s*, D_2O ex, 1H)	
OH-3	4.65 (*s*, D_2O ex, 1H)	
OH-2′	~7.05 (obscure)	

one hydroxyl group (δ 7.05) located at C-2′ as shown by analysis of the multiplicity and coupling constants of the aromatic protons (2 *dd* with *J*=*ortho, meta*-2H- and 2 *td* with *J*=*ortho, meta*-2H-). Since no further aromatic protons were evident, ring A should be fully substituted, presumably with a chelated hydroxyl on C-5 as indicated by a D_2O-exchangeable singlet at δ 11.31, a dimethylpyran ring, and a $\gamma\gamma$-dimethyl allyl group. The presence of the ring substituent was deduced from the characteristic signals for two vinylic protons at δ 5.53 and 6.63 (both *d*, *J*=10 Hz, 1H), and two gem-methyls at δ 1.45 (*s*, 6H). The latter substituent could be deduced from signals for two benzylic methylene protons at δ 3.19 and 3.25 (each *dd*, *J*= 7.5 and 14.5 Hz, 1H, H-1‴), one vinylic proton at δ 5.13 (*tt*, *J*= 1.2 and 7.5 Hz, 1H, H-2‴) and two methyls at δ 1.60 and 1.65 (each *s*, 3H). The observed multiplicity for H-1‴ protons indicated they were not magnetically equivalent, probably due to hampered rotation of the prenyl substituent caused by H bonding between OH-2′ and H-2‴. Therefore, irradiation of the vinylic proton signal caused a simplification of both H-1‴ signals, each now appearing as a doublet with *J*= 14.4 Hz (geminal coupling). The mass spectrum showed $[M]^+ = m/z$ 422, in agreement with the molecular formula $C_{25}H_{26}O_6$. A prominent diagnostic ion (**5**) was observed at m/z 271, clearly indicating that ring A supported both the dimethylpyran ring and the $\gamma\gamma$-dimethyl allyl group (Ingham et al., 1988). The ^{13}C NMR spectrum with the aid of DEPT analysis showed signals for 25 carbons, comprising four methyls, one methylene, nine methynes and 11 non-protonated carbons (Table 1). Signals at δ 73.22 (HC–OH) and 195.42 (C=O) were assigned to C-3 and C-4, respectively. The above data can fit either structure **1** or **1a**. In the former, the dimethylpyran ring is fused to C-6 and C-7 (linear arrangement), while in the latter, the attachment is to C-7 and C-8 (angular arrangement), which would place the $\gamma\gamma$-dimethyl allyl group at C-8 or C-6, respectively. Structure **1** was selected based on analysis of HMBC and NOESY data (Fig. 1). The HMBC spectrum clearly

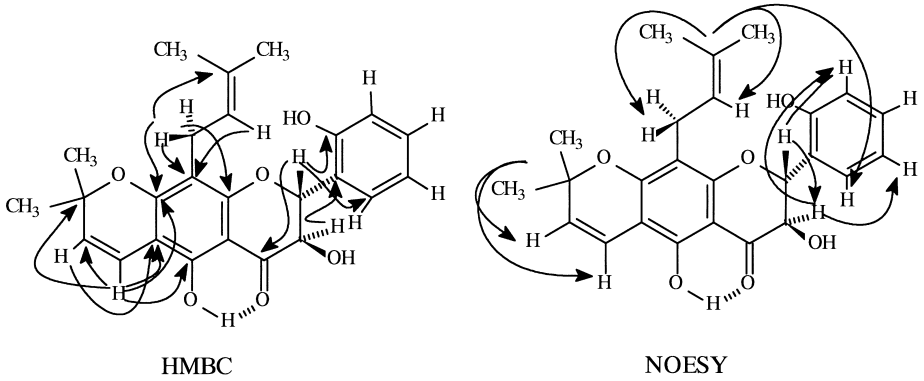

Fig. 1. HMBC and NOESY interactions in jayacanol **1**.

showed dimethylpyran H-3″ and H-4″ long range coupling with C-6. In addition, H-4″ was coupled with C-7, C-5, and C-2″, while prenyl H-1‴ was coupled with C-8, C-8a, C-2‴ and C-3‴. The NOESY spectrum showed interaction of both isoprenyl methyls with H-6′ of ring B (Fig. 1). The absolute configuration of jayacanol (**1**) was deduced mainly from analysis of the CD spectrum and comparison to published data for lupinifolinol (**6**) and lupinifolin (**7**) compounds of known stereochemistry (Ingham et al., 1988; Smalberger et al., 1974). Negative and positive Cotton effects were observed at 302 and 323 nm, respectively. In addition, the large $J_{2,3}$ value (12 Hz) between H-2 and H-3 was indicative of equatorial substituents on C-2 and C-3. The above data suggested the absolute configuration of jayacanol to be 2*R*, 3*R*.

The known compounds mundulinol (**2**), mundulin (**3**) and minimiflorin (**4**) were also isolated. Their spectroscopic data (IR, UV, EIMS, ^{1}H and ^{13}C NMR) were very similar to those previously described; however some differences were observed on comparison of the optical rotation data with reported values. Compounds **2** and **3** have been previously isolated only from *Mundulea sericea* (Van Zyl et al., 1979), while **4** was obtained from *L. minimiflorus* (Mahmoud and Waterman, 1985; Roussis et al., 1987). Linear dimethylpyran arrangement previously proposed for compounds **2** and **4** was confirmed here by HMBC and NOESY evidence, as described for compound **1**. HMBC also allowed correct assignment of the chemical shifts for quaternary carbons C-6, C-7, C-8 and C-8a of compounds **2** and **4** (see Experimental). In addition, mundulin acetate (**3a**) was prepared, showing the expected downfield shift for H-4″ (−0.25 ppm), as well as the upfield shift for H-3″ (+0.13 ppm) as compared to the parent compound **3**. This behavior is characteristic of flavanones with linear dimethylpyran arrangement (Mahmoud and Waterman, 1985; Ingham et al., 1988).

Flavonoids **1**–**4** are closely related to those described previously for other species included in the *Minimiflori* subsection of *Lonchocarpus* genus. Thus *L. minimiflorus* (Mahmoud and Waterman, 1985; Waterman and Mahmoud, 1987), *L. orotinus* (= *L. parviflorus*) (Roussis et al., 1987), and *L. guatemalensis* (Ingham et al., 1988), and now *L. oaxacensis* seem to be chemically and morphologically homogenous. All synthesize 6,8-prenylated flavanones and 3-hydroxyflavanones. Cyclization of 6-prenyl substituents is rather common, especially to yield a 6,7-dimethylpyran ring. In addition, the chalcone orotinichalcone with the described substitution pattern has been reported for *L. orotinus* (Smalberger et al., 1974).

Jayacanol (**1**) treatment (0.25 mg/ml) caused 48% inhibition of the growth of wood rotting fungus *Postia placenta* mycelia. Mundulinol, mundulin and minimiflorin at the same concentration inhibited fungal growth less than 10% (Table 2).

3. Experimental

3.1. Plant material

L. oaxacensis was collected near Jayacatlán, District of Etla, State of Oaxaca, Mexico. The specimen was identified by one of us (M. Sousa-Sánchez). A voucher specimen (No. 739,650) is deposited in the Mexican National Herbarium (MEXU).

3.2. Extraction and isolation

The roots (939.2 g) were dried at room temperature and cut in small pieces. Extraction was carried out at room temperature with petroleum ether, CH_2Cl_2, ethyl acetate, and methanol. The petroleum ether and CH_2Cl_2 extracts were similar as judged by TLC and were pooled. The combined extracts (10.3 g) were subjected to column chromatography (silica gel 60, 330 g) eluted with petroleum ether and CH_2Cl_2 and mixtures of these solvents. All compounds were obtained as oily substances and purified by TLC. Mundulin (**3**) was isolated from fractions 35–41 eluted with a petroleum ether: CH_2Cl_2 mixture (7:3), mundulinol (**2**) was isolated from

fractions 50–52 eluted with a petroleum ether:CH_2Cl_2 mixture (1:1). Minimiflorin (**4**) and jayacanol (**1**) were isolated from fractions 118 and 130–140, respectively, eluted with CH_2Cl_2.

3.3. Jayacanol (*1*) (3,5,2′-trihydroxy-6,7-(2″,2″-dimethyl cromene)-8-(3‴,3‴-dimethylallyl)-dihydroflavonol)

Yellow oil, purified by TLC (petroleum ether–ethyl acetate, 8:2), $[\alpha]_D^{23}$ −147.5° ($CHCl_3$; *c* 1.2); UV λ_{max}^{MeOH} nm (log ε): 265 (3.44), 276 (4.5), 315 (4.02), 365 (3.44), 276 (4.5), and 203 (4.48). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3403 (OH), 2930.7 (C–H aliph.), 2857.8 (C–H aliph.), 1628 (C=O), 1465 (C–C aliph.), 1709 (C=O); HREIMS *m*/*z* 422.1742 for $C_{25}H_{26}O_6$ (calcd 422.1729); EIMS 70 eV, *m*/*z* (rel. int): 422 (100), $[M]^+$ (C_{25} H_{26} O_6), 407 $[M-CH_3]^+$ (89.7), 287 (27.5), 271 (30), 215 (41.4); ^1H NMR spectral data (500 MHz, $CDCl_3$/TMS): see Table 1; ^{13}C NMR spectral data (125 MHz, $CDCl_3$/TMS): see Table 1; CD (MeOH: *c* 0.0123): $[\theta]_{213}$ −4.470, $[\theta]_{225}$ 0, $[\theta]_{245}$ +1.950, $[\theta]_{256}$ 0, $[\theta]_{267}$ −2.780, $[\theta]_{274}$ 0, $[\theta]_{284}$ +6.4, $[\theta]_{293}$ 0, $[\theta]_{302}$ −2.500, $[\theta]_{313}$ 0, $[\theta]_{323}$ +1.780, $[\theta]_{359}$ +1.170, $[\theta]_{425}$ 0.

3.4. Mundulinol (*2*) (3,5-dihydroxy-6,7-(2″,2″-dimethyl cromene)-8-(3‴,3‴-dimethylallyl)-dihydroflavonol)

Yellow oil, purified by TLC (petrol ether–CH_2Cl_2, 1:1); $[\alpha]_D^{23}$ = + 34.82°; ($CHCl_3$; *c* 1.78), reported $[\alpha]_D^{23}$ = +94.9° ($CHCl_3$; *c* 0.01) (Van Zyl et al., 1979). ^{13}C NMR spectral data 125 MHz, ($CDCl_3$/TMS): C-6 (103.17), C-7 (160.73) C-8 (109.30), C-8a (159.25), other data as reported (Van Zyl et al., 1979); CD (MeOH: *c* 0.0123): $[\theta]_{216}$ 0, $[\theta]_{232}$ +4.0, $[\theta]_{285}$ 0, $[\theta]_{299}$ −3.209, $[\theta]_{318}$ 0, $[\theta]_{323}$ +0.857, $[\theta]_{353}$ +1.150, $[\theta]_{414}$ 0.

3.5. Mundulin (*3*) (5,2′-dihydroxy-6,7-(2″,2″-dimethyl cromene)-8-(3‴,3‴-dimethylallyl)-flavanone)

Yellow oil, purified by TLC (petrol ether–CH_2Cl_2, 2:1); $[\alpha]_D^{23}$ = −0.029° ($CHCl_3$; *c* 2.92), reported $[\alpha]_D^{23}$ = −221.0° ($CHCl_3$; *c* 0.01) (Van Zyl et al., 1979); CD (MeOH: *c* 0.019): $[\theta]_{208}$ −0.099, $[\theta]_{209}$ 0, $[\theta]_{224}$ +2.380, $[\theta]_{237}$ 0, $[\theta]_{276}$ −2.650, $[\theta]_{297}$ −3.070, $[\theta]_{302}$ 0, $[\theta]_{322}$ +0.652, $[\theta]_{363}$ +0.653, $[\theta]_{411}$ 0.

3.5.1. Mundulin acetate (*3a*)

Compound **3** was converted into **3a** (5,2′-diacetyl-6,7-(2″,2″-dimethylcromene)-8-(3‴,3‴-dimethylallyl)-dihydrofavonol) in the usual way with anhydrous acetic acid in pyridine. Yellow oil, purified by pTLC (Silica gel0.25 mm, petrol ether–ethyl acetate, 9:1); $[\alpha]_D^{23}$ = −0.0049° ($CHCl_3$; *c* 2.45); EIMS 70 eV, *m*/*z* (rel. int): 432 (12.8) $[M]^+$ ($C_{27}H_{28}O_5$), 390 $[M-C_2H_3O]^+ = A^+$ (50), 375 $[A^+-CH_3]$ (100), 347 (6), 335 (4.2), 322 (6), 319 (7), 307 (17.8), 285 (3.5), 271 (8.5), 243 (6.4) 215 (15.7), 203 (9.2); ^1H NMR spectral data 300 MHz ($CDCl_3$/TMS): 5.43 (1H, *dd*, *J* = 3.0 and 13.0 Hz, H-2), 2.97 (1H, *dd*, *J* = 13.0 and 16.6 Hz, H-3 ax), 2.73 (1H, *dd*, *J* = 3.2 and 16.6 Hz, H-3 ec), 7.35–7.46 (Ar, *m*, 5H), 5.63 (1H, *d*, *J* = 10.4 Hz, H-3″), 6.38 (1H, *d*, *J* = 10.1 Hz, H-4″), 3.29 (2H, *d*, *J* = 7.4 Hz, H-1‴), 5.16 (1H, *tt*, *J* = 1.4, 7.4 Hz, H-2‴), 1.64 (3H, *d*, *J* = 1.0 Hz, CH_3-Pr), 1.65 (3H, *d*, *J* = 1.2 Hz, CH_3-Pr), 1.44 (3H, *s*, CH_3-DMP), 1.45 & 2.41 (3H, *s*, CH_3-DMP).

Table 2
Inhibition of *Postia placenta* mycelial growth by *L. oaxacensis* flavonoids (0.25 mg/ml)

Compound	Growth (cm)[a]	% Inhibition
Control	4.97±0.23	0.0
1	2.89±0.09	41.85
2	4.58±0.12	7.84
3	4.70±0.41	5.43
4	4.49±0.08	9.65
Pentachlorophenol	0.00±0.00	100.0

[a] Mean of five replicates ± standard deviation.

3.6. Minimiflorin (*4*) (3,5-dihydroxy-6,7-(2″,2″-dimethyl cromene)-8-(3‴,3‴-dimethylallyl)-flavanone)

Yellow oil, purified by TLC (petrol ether–ethyl acetate, 85:15); $[\alpha]_D^{23}$ = −27.62° ($CHCl_3$; *c* 2.1), reported $[\alpha]_D^{23}$ = −66.0° ($CHCl_3$; *c* 1.00) (Van Zyl et al., 1979); ^{13}C NMR spectral data 125 MHz, ($CDCl_3$/TMS): C-6 (103.16), C-7 (159.97), C-8 (108.85), C-8a (159.05), other data as reported (Mahmoud and Waterman, 1985).

3.7. Antifungal activity

Bioassays were carried out as described in Reyes-Chilpa et al. (1997). Each compound was dissolved in Me_2CO. A portion (0.5 ml) of the tested solution was poured into a petri dish (60×15 mm) and immediately 6 ml of hot sterile growth medium (malt-agar 1.5%) was added. Control plates were treated with solvent only. The plates were left to stand overnight inside a sterile hood to remove residual solvent. Each plate was inoculated with a plug (6 mm ϕ) of *Postia placenta* mycelium taken from the edge of a 7 day culture. Five replicates per treatment were run simultaneously, incubating at 25° for 7 days. Inhibition was determined by measuring the diameter of the mycelial mat.

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