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Flavonoids from the stem bark of *Lonchocarpus xuul*

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Abstract

The stem bark of *Lonchocarpus xuul* (Leguminosae) has yielded four flavonoids which have been identified by spectroscopic methods as the novel 4β,5-dimethoxy-6″,6″-dimethyl-2H-pyrano-(2″,3″:7,6)-flavan (xuulanin), 3β,4β,5-trimethoxy-6″,6″-dimethyl-2H-pyrano-(2″,3″:7,6)-flavan (3β-methoxyxuulanin), 4β-ethoxy-5-methoxy-6″,6″-dimethyl-2H-pyrano-(2″,3″:7,6)-flavan (4β-demethylxuulanin-4β-ethyl ether), and the known 5,7-dihydroxy-6,8-di(3-methylbut-2-enyl)flavanone (spiniflavanone-B). The ethyl derivative is considered likely to be an artefact.

Keywords: *Lonchocarpus xuul*; Leguminosae–Papilionoideae; Flavonoids; Flavan-4-ol; Flavan-3,4-diol; Flavanone

1. Introduction

The genus *Lonchocarpus*, Leguminosae subfamily Papilionoideae, consists of about 100 species distributed in tropical America, Africa, Madagascar and Australia (Allen and Allen, 1981). The genus is known for its insecticidal and pesticidal properties and most of the species studied have been shown to contain flavonoids of a wide range of structural types (Bisby et al., 1994).

Lonchocarpus xuul Lundell is a tree endemic to the Yucatan Peninsula of Mexico where it is known as *xuul*, *kan-xuul* or *yaax-xuul* and the wood is used locally for construction (Mendieta and Del Amo, 1981). An investigation of the seeds (Delle Monache et al., 1978) yielded three chalcones: lonchocarpin, derricidin and 4-hydroxylonchocarpin. As part of a study of some Leguminosae of Yucatan we now report the results of an investigation of the stem bark of *L. xuul*.

2. Results and discussion

A ^{1}H NMR spectrum of the crude hexane-soluble portion of the methanol extract of *L. xuul* stem bark revealed the presence of signals suggesting the occurrence of flavonoids. The extract was subjected to a succession of chromatographic separations using vacuum liquid chromatography (VLC) over silica gel, followed by gel permeation chromatography with Sephadex LH-20 and finally preparative TLC on silica gel to afford four compounds. These have been characterised as **1**–**4**, three of which appear to be novel.

The most abundant of the isolated compounds (**1**) was obtained as a pale yellow oil, the HREI-mass spectroscopy of which gave a molecular ion at *m*/*z* 352, solving for $C_{22}H_{24}O_4$. The UV spectrum gave a single maximum at 260 nm, suggesting a simple aromatic chromophore. The ^{1}H NMR spectrum accounted for all 24 protons, which were recognised as two methoxyls, two methyls, one isolated and two *ortho*-coupled aromatic or olefinic protons, five unresolved aromatic protons indicative of a mono-substituted aromatic ring and a four proton (ABMX) spin system for –O–CH(R)–CH_2–CH(R)–O–. The ^{13}C NMR spectrum (Table 1) confirmed the presence of

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eight sp^2 and two sp^3 methines, a methylene, four methyls and seven quaternary carbons.

The occurrence of the *ortho*-coupled olefinic protons at δ 5.54 and 6.54, together with the methyl resonances at δ 1.40 and 1.43 and a quaternary carbon at 76.1 ppm in the ^{13}C NMR spectrum, was typical for a 2,2-dimethylpyran ring system. Given the presence of signals for an unsubstituted B-ring and a single proton attributable to the A-ring, **1** must be substituted in the A-ring with the pyran and a methoxyl. The second methoxyl must be associated with the ABMX spin-system.

On this basis, compound **1** must be a 4-methoxyflavan. A comparison with the known compound methylhildgardtol-A (**5**), reported from *Tephrosia hildebrandtii* (Delle Monache et al., 1986), revealed considerable discrepancies in the values observed for the A-ring methine carbon (100.8 ppm in **1**; 92.9 ppm in **5**). This suggests placement of the methine carbon at C-8 in **1**. The linear arrangement for the pyran (between C-6 and C-7), rather than an annular arrangement (between C-6 and C-5), is confirmed by the deshielded resonance position for the methoxyl resonance (62.9 ppm). This requires that both *ortho* positions should be substituted (Panichpol and Waterman, 1978), which would not be the case if the methoxyl was placed at C-7. An HMBC experiment established the ^{13}C NMR assignments and an NOE experiment confirmed the proximity between the 5-methoxyl and H-4″.

Table 1
^{13}C NMR data for compounds **1**–**3**[a]

Position	1	2	3
2	73.3	68.5	73.5
3	34.1	82.1	34.8
4	68.2	75.8	66.8
4a	108.6*	nd[b]	109.1*
5	156.0**	nd	156.1**
6	108.4*	nd	108.6*
7	156.3**	nd	155.3**
8	100.8	100.8	101.1
8a	155.1	nd	156.5**
1′	141.2	nd	141.5
2′/6′	128.0	128.0	126.6
3′/5′	128.5	128.5	128.8
4′	128.0	128.2*	126.6
2″	76.1	76.4	76.2
3″	117.0	117.2	117.3
4″	126.4	128.5	126.5
2″-Me_2	27.7	28.0	27.8
	28.0	28.0	28.3
3-OMe		58.3*	
4-OMe	55.8	58.8*	
5-OMe	62.9	63.2	63.2
4-OCH_2CH_3			63.7
4-OCH_2CH_3			16.0

[a] Values in the same column with the same number of asterisks are interchangeable.
[b] Not detected.

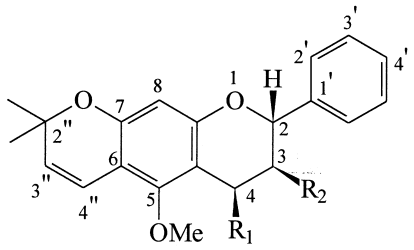
The relative stereochemistry at C-2 and C-4 was resolved from the NMR coupling and chemical shift data (Table 1). The proton H-2 must be axial due to the large coupling shown by one H-3 proton. By contrast the couplings between H-4 and the two H-3 protons are both bound to be small because of the conformation of the C-ring (Magãlhaes et al., 1996) and H-4 could exist either *cis* or *trans* to the H-2 proton. Following the arguments of Delle Monache et al. (1986), the ^{13}C NMR resonances for C-2 and C-4 clearly indicate that the protons are *trans* leading to the assignment of the compound as 4β,5-dimethoxy-6″,6″-dimethyl-2H-pyrano-(2″,3″:7,6)-flavan (**1**), to which we have assigned the trivial name xuulanin.

The ^{1}H NMR spectrum of **2** was comparable to that of **1** but with an additional methoxyl resonance at δ 3.54 and the methylene replaced by another oxymethine, requiring that this be a 3,4-dimethoxyflavan with substituents otherwise identical to **1**. A large coupling constant between H-2 and H-3 allowed the 3-methoxyl to be assigned the equatorial (β) configuration, but the relative stereochemistry of H-4 cannot be determined in this manner (Magalhães et al., 1996). For similar compounds H-3/H-4 stereochemistry has been established by nuclear Overhauser and chiroptical studies (Magalhães et al., 1996). On the basis of chemical shift values (Table 1) for the three oxymethine carbons C-2 (68.5 ppm), C-3 (82.1 ppm) and C-4 (75.8 ppm), and their comparison with data published by Magalhães et al. (1996), **2** can be assigned a 2,3-*trans*-3,4-*cis* configuration and characterised as 3β,4β,5-trimethoxy-6″,6″-dimethyl-2H-pyrano-(2″,3″:7,6)-flavan, trivial name 3β-methoxyxuulanin.

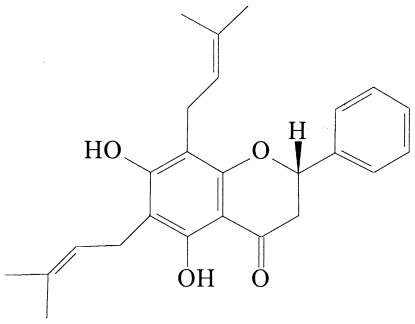
In compound **3** the ^{1}H NMR spectrum showed the same A-ring substituents and ABMX system as **1**, but one methoxyl resonance was absent and replaced by signals attributable to an ethoxyl. Compound **3** must, therefore, be either the 4-ethoxy or 5-ethoxy analogue of **1**. As the ^{13}C NMR spectrum (Table 1) continued to show the resonance for the sterically-hindered 5-methoxyl (63.2 ppm), the ethoxyl must be assigned to C-4 and **3** is 4β-ethoxy-5-methoxy-6″,6″-dimethyl-2H-pyrano-(2″,3″:7,6)-flavan. As ethoxyl derivatives are highly unusual among flavonoids it is thought unlikely that **3** is a genuine metabolite, but is probably an artefact of the isolation process.

The final compound (**4**) showed a molecular ion at m/z 392 ($C_{25}H_{28}O_4$) in the HREI mass spectrum. The UV spectrum was indicative of a flavanone (Mabry et al., 1970), and this was confirmed by the presence of an ABX system for H-2 and H_2-3 in the ^{1}H NMR spectrum together with a carbonyl resonance at 196.4 ppm in the ^{13}C NMR spectrum. Other features of the ^{1}H NMR spectrum were signals for a monosubstituted

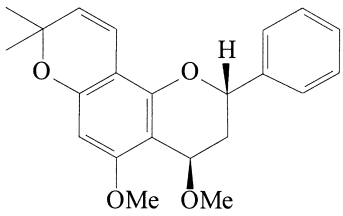
B-ring and two series of signals attributable to 3-methylbut-2-enyl side-chains. In the absence of any A-ring protons these two substituents must be placed at C-6 and C-8 and **4** can be identified as 5,7-dihydroxy-6,8-di(3-methylbut-2-enyl)flavanone which has previously been reported from *Tephrosia spinosa* and named spinoflavanone-B (Venkata Rao and Prasad, 1993). The corresponding 4′-hydroxyflavanone was isolated from *Lonchocarpus minimiflorus* (Roussis et al., 1987).



	R_1	R_2
1	OMe	H
2	OMe	OMe
3	OEt	H



(4)



(5)

3. Experimental

3.1. General

UV: MeOH. FT-IR: film. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra: $CDCl_3$. EI-mass spectra: 70 eV between 120 and 140°C. All TLC was carried out using coated silica gel F_{254}, 0.20 mm thick plates. CC: silica gel 60 (230–400 mesh) was used. Gel filtration: Sephadex LH-20 Sigma (bead size 25–100).

3.2. Plant material

The bark of *L. xuul* was collected in June 1997 from a tree growing in cultivated ground at the 9 km post of the Yokdzonot-Pisté highway, Yucatán, México. Voucher specimens are deposited in the Herbarium of the Unidad de Recursos Naturales of Centro de Investigación Científica de Yucatán (CICY) under the collection number 1089. After sun and oven drying at a temperature not greater than 60°C, the material was ground using a Brabender Dusiburg (880804 type) mill and collected using a No. 2 sieve.

3.3. Extraction and isolation

Powdered stem bark (1.5 kg) was extracted with MeOH in a Soxhlet. After evaporation of solvent under reduced pressure the MeOH extract (286.83 g) was subjected to successive hot extraction under reflux with hexane, EtOAc, Me_2CO and MeOH. The hexane extract was subjected to VLC eluting with hexane and then hexane:EtOAc mixtures in increasing polarity, furnishing 17 fractions. Fraction 1D (999.7 mg), obtained from 5% EtOAc, was subjected to Sephadex LH-20 gel filtration eluting with $CHCl_3$ and then further purified by preparative TLC (hexane:EtOAc 9:1) to give **1** (90 mg) and **3** (2.7 mg). From fraction 1E (eluted with 8% EtOAc in hexane) **4** (2 mg) was obtained by preparative TLC (multiple elution, hexane:EtOAc 9:1). Fraction 1H (368 mg), obtained from 10% EtOAc, was passed through a Sephadex LH-20 column ($CHCl_3$) to eliminate chlorophylls and then rechromatographed by VLC using hexane:EtOAc mixtures of increasing polarity. The fraction 15D (33 mg) showed an orange spot after sprayed with vanillin and was purified by prepararative TLC (multiple elution with hexane:EtOAc 9:1) to give **2** (6.6 mg).

3.4. 4β,5-Dimethoxy-6″,6″-dimethyl-2H-pyrano-(2″,3″:7,6)-flavan (*1*)

Yellow oil; UV λ_{max} nm: 225, 260. IR ν_{max} cm^{-1}: 3062, 3033, 2893, 1615, 1385. ^{1}H NMR δ 1.39, 1.40 (2 × 3H, 2 × *s*, 2″-Me_2), 1.87 (1H, *ddd*, $J = 14.0$, 12.0, 3.0 Hz, H-3_{ax}), 2.34 (1H, *ddd*, $J = 14.0$, 2.2, 2.2 Hz, H-

3_{eq}), 3.47 (3H, *s*, 4-OMe), 3.87 (3H, *s*, 5-OMe), 4.50 (1H, *dd*, $J = 2.7$, 2.7 Hz, H-4), 5.20 (1H, *dd*, $J = 12.0$, 2.1 Hz, H-2), 5.53 (1H, *d*, $J = 9.9$ Hz, H-3″), 6.21 (1H, *s*, H-8), 6.52 (1H, *d*, $J = 9.9$ Hz, H-4″), 7.40 (5H, *m*, H-2′-H-6′). ^{13}C NMR: see Table 1. EIMS *m*/*z* (rel. int.) calcd. for $C_{22}H_{24}O_4$ 352.1674; found 352.1678 $[M]^+$ (12), 337 (58), 321 $[M - H_2O]^+$(20), 305 $[M - Me–MeOH]^+$ (58), 280 (12), 233 (75), 124 (10), 121 (15), 115 (10), 69 (100).

3.5. 3β,4β,5-Trimethoxy-6″,6″-dimethyl-2H-pyrano-(2″,3″:7,6)-flavan (**2**)

Oil; UV λ_{max} nm: 225, 259, 277, 284, 311 sh. IR ν_{max} cm^{-1}: 2915, 1612, 1457. ^{1}H NMR δ 1.41, 1.43 ($2 \times 3H$, $2 \times s$, 2″-Me_2), 3.13 (3H, *s*, 3-OMe), 3.54 (1H, *dd*, $J = 10.3$, 2.8 Hz, H-3), 3.62 (3H, *s*, 4-OMe), 3.87 (3H, *s*, 5-OMe), 4.75 (1H, *d*, $J = 2.8$ Hz, H-4), 5.23 (1H, *d*, $J = 10.3$ Hz, H-2), 5.54 (1H, *d*, $J = 9.9$ Hz, H-3″), 6.22 (1H, *s*, H-8), 6.54 (1H, *d*, $J = 9.9$ Hz, H-4″), 7.40 (5H, *m*, H-2′-H-6′). ^{13}C NMR: see Table 1. EIMS *m*/*z* (rel. int.) calcd. for $C_{23}H_{26}O_5$ 382.1780; found 382.1785 $[M]^+$ (100), 367 (97), 350(4), 335 (11), 305 (6).

3.6. 4β-Ethoxy-5-methoxy-6″,6″-dimethyl-2H-pyrano-(2″,3″:7,6)-flavan (**3**)

Yellow oil; UV λ_{max} nm : 227, 276. IR ν_{max} cm^{-1}: 2969, 2927, 1616. ^{1}H NMR δ 1.30 (3H, *t*, $J = 7$ Hz, OCH_2CH_3), 1.39, 1.43 ($2 \times 3H$, $2 \times s$, 2″-Me_2), 1.88 (1H, *ddd*, $J = 13.0$, 12.8, 2.9 Hz, H-3_{ax}), 2.33 (1H, *ddd*, $J = 14.0$, 2.1, 2.1 Hz, H-3eq), 3.66 (1H, *dd*, $J = 9.0$, 7.0 Hz, OCH_2CH_3), 3.78 (1H, *dd*, $J = 9.0$, 7.0 Hz, OCH_2CH_3), 3.87 (3H, *s*, 5-OMe), 4.64 (1H, *dd*, $J = 2.6$, 2.6 Hz, H-4), 5.26 (1H, *dd*, $J = 12.0$, 1.6 Hz, H-2), 5.55 (1H, *d*, $J = 9.9$ Hz, H-3″), 6.20 (1H, *s*, H-8), 6.53 (1H, *d*, $J = 9.9$ Hz, H-4″), 7.40 (5H, *m*, H-2′-H-6′). ^{13}C NMR: see Table 1. EIMS *m*/*z* (rel. int.) calcd. for $C_{23}H_{26}O_4$ 366.1831; found 366.1819 $[M]^+$ (48), 351 (100), 305 (62), 291 (15), 247 (37), 219 (76), 176 (23), 128 (11).

3.7. 5,7-Dihydroxy-6,8-di(3-methylbut-2-enyl)flavanone (**4**)

Oil; UV λ_{max} nm: 294. IR ν_{max} cm^{-1}: 3394, 2966, 1631, 1446, 1376. ^{1}H NMR δ 1.72, 1.72, 1.76, 1.82 ($4 \times 3H$, $4 \times s$, $2 \times Me_2$), 2.85 (1H, *dd*, $J = 17.0$, 3.2 Hz, H-3_{eq}), 3.05 (1H, *dd*, $J = 17.0$, 12.8 Hz, H-3_{ax}), 3.32 (1H, *d*, $J = 7.2$ Hz, H-1‴), 3.35 (1H, *d*, $J = 7.5$ Hz, H-1″), 5.25 (1H, *t*, $J = 7.04$ Hz, H-2″), 5.21 (1H, *t*, $J = 7.1$ Hz, H-2‴), 5.40 (1H, *dd*, $J = 12.8$, 3.1 Hz, H-2), 7.40 (5H, *m*, H2′–H-6′), 12.33 (1H, *s*, 5-OH). ^{13}C NMR ppm 18.0, 18.1, 26.0, 26.1 (C-3″, C-3‴, C-4″, C-4‴), 21.5, 22.2 (C-1″, C-1‴), 43.7 (C-3), 79.0 (C-2), 103.0 (C-4a), 106.7 (C-8), 107.5 (C-6), 122.0, 122.2 (C-2″, C-2‴), 126.2 (C-3′, C-5′), 128.7 (C-4′), 129.0 (C-2′, C-6′), 134.2, 135.0 (C-3″, C-3‴), 139.1 (C-1′), 157.8 (C-8a), 159.5 (C-5), 162.5 (C-7), 196.4 (C-4). EIMS *m*/*z* (rel. int.) calcd. for $C_{25}H_{28}O_4$: 392.1988; found 392.2033 $[M]^+$ (24), 320 (100), 292 (54), 280 (74), 256 (88), 216 (88), 188 (96), 166 (98).

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