PERGAMON

Phytochemistry 60 (2002) 533–540

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Flavonoids from two *Lonchocarpus* species of the Yucatan Peninsula

Rocío Borges-Argáez[a,b], Luis M. Peña-Rodríguez[a], Peter G. Waterman[b,*]

[a]*Grupo de Química Orgánica, Unidad de Biotecnología, Centro de Investigación Científica de Yucatán, Calle 43 No. 130, Colonia Chuburná de Hidalgo, Mérida, Yucatán 97200, Mexico*

[b]*Centre for Phytochemistry, Southern Cross University, PO Box 157, Lismore, NSW 2480, Australia*

Received 21 January 2002; received in revised form 21 March 2002

Abstract

Leaves, stem bark and root of *Lonchocarpus xuul* and *Lonchocarpus yucatanensis* were studied separately. A chalcone, 2′,4-dimethoxy-6′-hydroxylonchocarpin (**1**), and the flavones 5,4′-dihydroxy-3′-methoxy-(6:7)-2,2-dimethylpyranoflavone (**2**) and 5,4′-dimethoxy-(6:7)-2,2-dimethylpyrano-flavone (**3**), together with the known carpachromene (**4**), were isolated from the leaves of both species. Similarly, the previously reported flavans xuulanin (**5**) and 3β-methoxyxuulanin (**6**), together with the novel 3β,4β,5-trimethoxy-4′-hydroxy-(6:7)-2,2-dimethylpyranoflavan (**7**), 3-hydroxy-4,5-dimethoxy-(6:7)-2,2-dimethyl-pyranoflavan (**8**), and 3,4-dihydroxy-5-methoxy-(6:7)-2,2-dimethylpyranoflavan (**10**), were isolated from the stem bark and root of both species. Finally, the known 2′,4′-dihydroxy-3′-(3-methylbut-2-enyl) chalcone (**13**) was obtained from the root of *L. xuul* only. The structures of the various metabolites were established by interpretation of their spectroscopic data. © 2002 Published by Elsevier Science Ltd.

Keywords: *Lonchocarpus xuul*; *Lonchocarpus yucatanensis*; Leguminosae-Papilionoideae; Flavonoids; Flavan-3, 4-diol; Flavones; Chalcone

1. Introduction

Lonchocarpus xuul Lundell and *Lonchocarpus yucatanensis* Pittier are quasi-endemic and endemic trees of the Yucatan peninsula, respectively (Durán et al., 1998). The former is known as ''xuul'', ''kan-xuul'' or ''yaax-xuul'' and the wood is used locally for construction. The latter is known as ''Balché chi'', ''box xuul'' and ''balche keh'' and its flowers are greatly appreciated for honey production (Mendieta and Del Amo, 1981).

We have previously reported the results of a study on the stem bark of *L. xuul*, which led to the isolation of two novel flavans named xuulanin (**5**) and 3β-methoxyxuulanin (**6**), together with the known flavanone, spinoflavanone-B (**10**) (Borges-Argáez et al., 2000). To date, there are no phytochemical studies reported on *L. yucatanensis*. We wish to report herein on the flavonoids present in the leaves, stem bark and roots of both of these *Lonchocarpus* species.

2. Results and discussion

The hexane-soluble portion of the leaf methanolic extracts of both *L. xuul* and *L. yucatanensis* were separately subjected to a succession of chromatographic procedures, including vaccum liquid chromatography (VLC), gel permeation chromatography using Sephadex LH-20, and preparative TLC to afford four pure metabolites (**1**–**4**) from both samples.

Metabolite **1** appeared as an intense deep brown spot when observed on TLC under UV light. On fuming with ammonia, the colour of the spot changed to a deep red suggesting a chalcone (Harborne, 1998). This was confirmed by the UV spectra of **1** which showed a dominant band I absorption at 364 nm and a relatively minor band II at 289 nm, and by the absorption bands at 1630 and 1604 cm^{-1} in its IR spectrum, indicating the presence of a carbonyl group and an aromatic ring, respectively. The molecular formula of **1** was determined to be $C_{22}H_{22}0_5$ by HR-EIMS. Support for a chalcone structure for **1** was provided by the signals observed in its ^1H NMR (Table 1). In addition to two *trans*-olefinic protons at δ 7.79 and 8.04 ($J = 15.6$Hz), readily assigned to the β and α positions, respectively, the spectrum displayed signals for two methoxyl groups,

* Corresponding author. Tel.: +61-2-6622-3211; fax: +61-2-6622-3459.

E-mail address: pwaterma@scu.edu.au (P.G. Waterman).

0031-9422/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0031-9422(02)00131-0

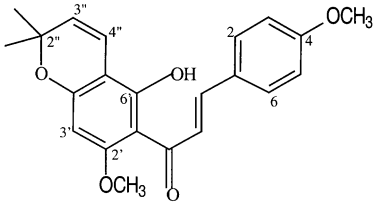
an isolated aromatic proton and four aromatic protons showing the A_2B_2 pattern typical of a 4-substituted B ring. The presence of two *cis*-coupled olefinic protons, together with two methyl group resonances and a quaternary carbon at δ 78.0 in the ^{13}C NMR spectrum, indicated a 2,2-dimethylpyran system.

The arrangement of the substituents and the placement of the pyran at the 4′/5′ positions was established from the results of NOE and HMBC experiments. In the former, irradiation of the methoxyl resonances at C-2′ and C-4 yielded a significant enhancement of the H-3′ and H-3/H-5 signals, respectively, while in the latter (Table 1), the methoxyl group in ring-A showed the expected ^3J correlation between its protons and C-2′ (δ 161.3). The HMBC experiment showed a clear ^3J correlation between H-4″ and C-6′ (δ 155.9) and between H-3′ and C-5′ (δ 103.3); while H-3′ showed the expected ^2J correlations with C-2′ (δ 161.3) and C-4′ (δ 167.7). This data supported the identification of **1** as 2′,4-dimethoxy-6′-hydroxylonchocarpin, a chalcone that does not appear to have been reported to date.

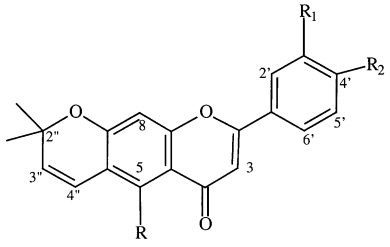
Table 1
^1H NMR, ^{13}C NMR and HMBC spectral data of metabolite **1** (δ values in $CDCl_3$)

Position	^1H[a]	^{13}C	^2J	^3J
1		128.7		
2/6	7.57 *d* (8.5)	130.2		C-β, C-4
3/5	6.95 *d* (8.5)	114.7	C-4	
4		161.6		
β	7.79 *d* (15.6)	142.6		C=O, C-2/6
α	8.04 *d* (15.6)	125.4	C=O	C-1
C=O		193.0		
1′		106.6		
2′		161.3		
3′	6.07 *s*	92.9	C-2, C-4	C-1′, C-5′
4′		167.7		
5′		103.3		
6′		155.9		
2″		78.0		
3″	5.46 *d* (9.9)	124.8	C-2″	C-5′
4″	6.60 *d* (9.9)	117.0		C-2″, C-6′
2″-Me_2	1.56 *s*	28.2	C-2″	C-3″
2′-Ome	3.86 *s*	55.9		C-2′

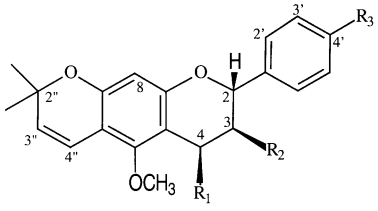
[a] Figures in parentheses = *J* values in Hz.



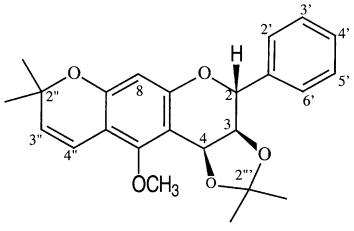
1



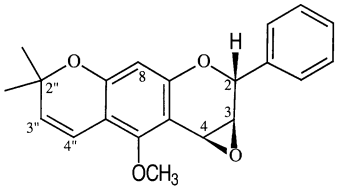
2 R=OH R_1=OCH_3 R_2=OH
3 R=OCH_3 R_1=H R_2=OCH_3
4 R=OH R_1=H R_2=OH



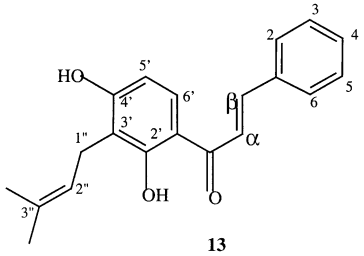
5 R_1=OCH_3 R_2=H R_3=H
6 R_1=OCH_3 R_2=OCH_3 R_3=H
7 R_1=OCH_3 R_2=OCH_3 R_3=OH
8 R_1=OCH_3 R_2=OH R_3=H
9 R_1=OCH_3 R_2=OAc R_3=H
10 R_1=OH R_2=OH R_3=H



11



12



13

Metabolite **2** was found to have an molecular formula of $C_{21}H_{18}O_6$ from the HR-EIMS. Its UV spectrum showed the typical absorptions of a flavone at 264 and 347 nm and its IR spectrum revealed bands for hydroxyl groups (3400 cm^{-1}), a carbonyl function (1651 cm^{-1}) and an aromatic system (1588, 1456 cm^{-1}). (Harborne, 1998).

The ^1H NMR of **2** revealed resonances for a methoxyl group, a hydrogen bonded hydroxyl proton, a 2,2-dimethylpyran ring and four aromatic protons, of which three formed an ABD system and the other a single A-ring proton. The HMBC correlations observed from H-8 (to C-6, C-7, C-8a and C-4a), from the 5-OH (to C-4a, C-5 and C-6), and the ^3J correlation between H-4″ and both C-5 and C-7 required that the 2,2-dimethylpyran group was connected to the A-ring in a linear manner. The substitution pattern on the B-ring was established from an NOE experiment with irradiation of the methoxyl group causing enhancement of H-2′. On this basis **2** was unambiguously identified as the novel 5,4′-dihydroxy-3′-methoxy-(7,6: 2″,3″)-6″,6″-dimethylpyrano flavone.

Metabolite **3** showed spectral characters similar to **2**. The molecular formula ($C_{22}H_{20}O_5$), obtained from HR-EIMS, indicated an additional methoxyl but one less oxygen than **2**. The ^1H NMR spectrum of revealed the presence of two methoxyl groups, five aromatic protons with four forming an A_2B_2 system and the other being a single A-ring proton, together with signals corresponding to a 2,2-dimethylpyran ring. Irradiation of the methoxyl signal at δ 3.89 enhanced the H-3′/-5′ resonances requiring placement at C-4′ while irradiation of the other methoxyl induced a significant NOE at H-4″ of the 2,2-dimethylpyran system. In the absence of a strongly deshielded resonance for an H-bonded 5-OH and because the ^{13}C NMR resonance of this methoxyl occurred at δ 63,1, requiring that both ortho positions and substituted (Panichpol and Waterman, 1978), this second methoxyl must be assigned to C-5. This allowed the identification of **3** as 5,4′-dimethoxy-(7,6:2″,3″)-6″,6″-dimethylpyranoflavone, a new natural product although previously reported as a synthetic derivative (Jain et al., 1978).

The molecular formula for **4** was found to be $C_{20}H_{16}O_5$ by HR-EIMS. The ^1H NMR spectrum did not contain signals for methoxyl groups but was otherwise similar to that of **3**. All spectroscopic ividence indicated that **4** was 5,4′-dihydroxy-(7,6:2″,3″)-6″,6″-dimethylpyranoflavone, a metabolite previously reported, as carpachromene, from *Flindersia laevicarpa* (Jain et al., 1978).

Extraction of the root and stem bark of *L. xuul* with MeOH, followed by refluxing of the methanolic extract using hexane, then CH_2Cl_2 and MeOH led to the isolation from the hexane fraction of products **5, 6, 7, 8** and **10**. The same metabolites and a further chalcone, **13**, were obtained when the root and stem bark of *L. yucatanensis* were subjected to this procedure.

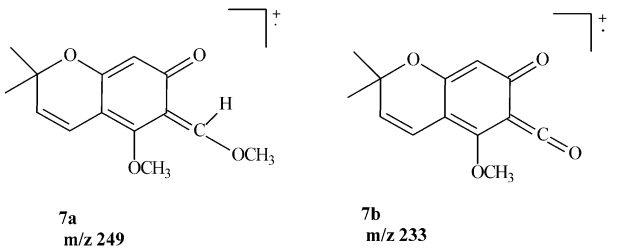
Two of these metabolites, the flavans xuulanin (**5**) and 3β-methoxyxuulanin (**6**), have previously been reported by us from *L. xuul* (Borges-Argáez et al., 2000). The additional compounds **7**, **8** and **10** were all flavans and displayed the same 2,2-dimethylpyran moiety as other metabolites reported here.

Metabolite **7** showed a molecular ion peak at M^+ 398 from LR-EIMS, corresponding to a molecular formula of $C_{23}H_{26}O_6$, with two prominent mass spectral fragments **7a** and **7b** (Fig. 1), resulting from the retro Diels–Alder cleavage of ring-C in a flavan skeleton. The ^1H NMR spectrum (Table 2) displayed two (2H) doublets for an A_2B_2 system corresponding to the four protons of a B-ring substituted at 4′, a single A-ring proton, three methoxyl resonances and three oxymethine protons suggesting H-2, H-3 and H-4 protons in a 3,4-dioxygenated flavan. The presence of methoxyl substituents at C-3, C-4 and C-5 was established from an HMBC study and so C-4′ must carry a hydroxyl group.

The 3,4-dioxygenated flavan skeleton was confirmed by signals in the ^{13}C NMR spectrum (Table 3) at δ 75.4, 82.0 and 68.6 attributable to C-2, C-3 and C4, respectively. A large coupling constant between H-2 and H-3 ($J = 10.3$ Hz) required a diaxial interaction and consequently the 3-methoxyl group must be in the equatorial (β) orientation. By contrast the small coupling constant between H-3/H-4 meant H-4 must be equatorial so the 4-methoxyl must, once more, be β. Comparison with the previously reported xuulanin (**5**) (Borges-Argáez et al., 2000) confirms the identification of **7** as 3β,4β,5-trimethoxy-4′-hydroxy-(7,6:2″,3″)-6″,6″-dimethylpyranoflavan.

Metabolite **8** was obtained as white needles after crystallization from hexane. The HR-EIMS spectrum revealed a molecular formula of $C_{22}H_{24}O_5$. The ^1H (Table 2) and ^{13}C NMR (Table 3) spectra of **8** were very similar to those of **7** indicating a 3,4-dioxygenated flavan. The main differences observed in the ^1H NMR spectrum was the presence of a multiplet corresponding to the five aromatic protons of an unsubstituted B-ring, and only two methoxyl group signals, one at C-5 and the other at C-3 or C-4.

Treatment of **8** with acetic anhydride and pyridine yielded a monoacetate **9**, which showed a parent ion at



7a m/z 249 **7b** m/z 233

Fig. 1. Fragment ions observed in the mass spectra of **7**.

Table 2
^{1}H NMR (500 MHz, $CDCl_3$) spectral data of metabolites **7–10**[a]

H	7	8	9	10
2	5.17 *d* (10.3)	5.05 *d* (10.3)	5.33 *d* (10.7)	5.01 *d* (9.9)
3	3.51 *dd* (10.3, 2.9)	3.97 *m*	5.25 *dd* (10.7, 3.4)	3.96 *m*
4	4.74 *d* (2.9)	4.60 *d* (3.4)	4.69 *d* (3.4)	5.02 *d* (3.8)
8	6.18 *s*	6.21 *s*	6.21 *s*	6.22 *s*
2′/6′	7.35 *d* (8.5)	7.49 *m*	7.49 *m*	7.49 *m*
3′/5′	6.86 *d* (8.5)	7.49 *m*	7.49 *m*	7.49 *m*
4′		7.49 *m*	7.49 *m*	7.49 *m*
3″	5.55 *d* (10.0)	5.56 *d* (9.9)	5.56 *d* (10.0)	5.57 *d* (10.0)
4″	6.52 *d* (10.0)	6.53 *d* (9.9)	6.52 *d* (10.0)	6.52 *d* (10.0)
2″-Me_2	1.43/1.41 2×*s*	1.43 *s*	1.42 *s*	1.43 *s*
3-OMe	3.16 *s*			
4-OMe	3.62 *s*	3.60 *s*	3.58 *s*	
5-OMe	3.86 *s*	3.83 *s*	3.84 *s*	3.92 *s*
Ac			1.89 *s*	

[a] Figures in parentheses = *J* values in Hz.

m/*z* 410 in its LR-EIMS and no absorption bands in the hydroxyl region of its IR spectrum. The ^{1}H NMR spectrum of **9** (Table 2) showed a large downfield shift (δ 5.25) for the oxymethine H-3 proton. The relative stereochemistry of C-2, C-3 and C-4 positions were established by comparison with those for **7**. This was further supported by the interaction observed between H-3 and H-4 in the NOESY spectrum of **8** (Fig. 2). On the basis of these observations **8** was identified as the novel 4β,5-dimethoxy-3β-hydroxy-(7,6:2″,3″)-6″,6″-dimethylpyranoflavan.

The HR-EIMS of metabolite **10** suggested a molecular formula $C_{21}H_{22}O_5$, and a ^{1}H NMR spectrum very similar to that of **8**, strongly suggesting a 3,4-dioxygenated flavan. The presence of a single methoxyl group signal (δ 3.92) in the ^{1}H NMR (Table 1) and δ 63.5 (Table 2) suggested placement at C-5. with C-3 and C-4 both carrying hydroxyl groups. The coupling constants between protons H-2 and H-3 and H-3 and H-4 were identical to those of **8**, thus allowing the relative stereochemistry of ring C in **10** to be established as 2,3-*trans*-3,4-*cis*. The presence of a *cis*-diol in **10** was confirmed by preparing the ketal derivative **11**, which showed a parent ion peak at *m*/*z* 394 in its HR-EIMS, a lack of bands in the hydroxyl region of its IR spectrum and the

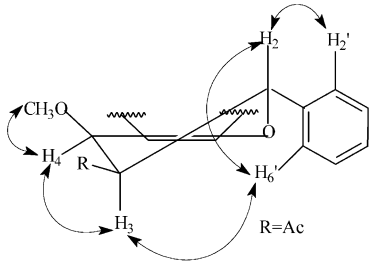


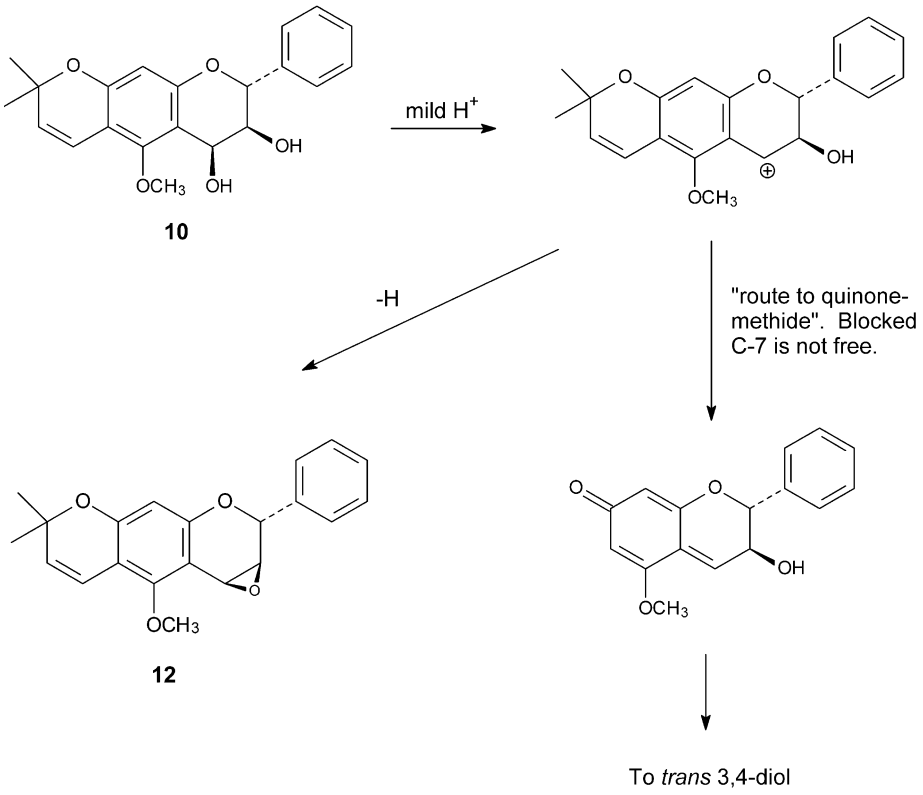
Fig. 2. Selected NOESY interactions for metabolite **9**.

presence of an additional CH_3 signal at δ 1.40 (6H, *s*) in the ^{1}H NMR spectrum.

When **10** was left standing in $CDCl_3$ over a period of about 2 h, it underwent a spontaneous transformation to yield the epoxide **12** as the only product. The identity of the new product was supported by a lack of bands in the hydroxyl region of its IR spectrum and the molecular ion peak at *m*/*z* 336, corresponding to the formula of $C_{21}H_{20}O_4$. In the ^{1}H NMR of **12** the change of conformation due to the formation of the epoxide, resulted in small variations on the chemical shift values of most of the protons when compared to those in **10**. However, the coupling constants between protons H-2 and H-3 and H-3 and H-4 remained unchanged, indicating that the stereochemistry at these centres was still the same as in diol **10**.

The formation of the epoxide can be readily explained. Foo and Wong (1986) have proposed that under acidic conditions, such as can occur in chloroform solutions, 3,4-*cis* diols will lose the 4-substituent to form a carbonium ion. This is normally stabilised by formation of a quinone-methide, which involves a free hydroxyl at either C-7 or C-5 of the flavan nucleus and this can subsequently undergo solvolysis to give the conformationally preferred 3,4-*trans* product (Scheme 1). In **10** the route to a quinone-methide is blocked as C-5 is methylated and C-7 involved in the pyran ring and so formation of the epoxide represents an alternative mechanism to eliminate the carbonium ion. It is interesting to note that Clark-Lewis and Williams (1967) considered formation of an epoxide as an alternative intermediate in the conversion of *cis* to *trans* flavan diols. However, compound **12** was found to be stable.

Finally, metabolite **13,** obtained as yellow needles, proved to be identical to isocordoin [2′,4′-dihydroxy-3′-(3-methylbut-2-enyl)chalcone], previously reported from *Cordoa piaca* (Leguminosae) (De Lima et al., 1973).



Scheme 1. Proposed mechanism for formation of the epoxide derivative **12**.

A number of biological activities have been attributed to the flavonoids, including antioxidant, anticancer and anti-inflammatory properties (see for example Antus et al., 1996). However, none of the metabolites isolated proved to be active when tested against a series of microorganisms including *Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeroginosa*, *Escherichia coli* and *Baccilus subtillis*.

3. Experimental

3.1. General experimental procedures

Melting points (uncorrected) were determined on a Fluka 70/20 series II Holster C70G apparatus. IR spectra (film) were recorded on a Nicolet FT–IR spectrometer. UV spectra were recorded on a Beckman DU-65 spectrophotometer using MeOH as the solvent. NMR spectra (both 1D and 2D) were obtained on a Bruker DRX NMR (500 MHz) spectrometer, using the residual solvent peaks as internal standards. HR-EIMS and LR-EIMS were determined on a LC–MS and a Hewlett Packard GC Mass selective detector, respectively. Vacuum-liquid chromatography (VLC) was carried out using Merck Si gel 60H, while column chromatography was carried out using Merck Si gel 60 (70–230 mesh) or Sephadex LH-20 (sigma bead size 25–100). Flash chromatography was performed using Merck Si gel 60 (230–400 mesh) and preparative TLC were run using glass-coated silica gel F254 (0.20 mm thick) plates. Analytical TLC was carried out on precoated aluminum plates using Merck Si gel F254; the plates were visualized under UV light (λ 254 and 366 nm) and by spraying with phosphomolybdic acid reagent, followed by gentle heating.

3.2. Plant material

Bark, roots and leaves of both *Lonchocarpus* spp. were collected in June 1997 from trees growing at the 9 km post of the Yokdzonot-Pisté highway (*L. xuul*) and at the 7 km post of the Mérida-Holca highway (*L. yucatanensis*), in Yucatán, México. Voucher specimens were deposited in the Herbarium of the Unidad de Recursos Naturales of Centro de Investigación Científica de Yucatán (CICY) under collection numbers 1089 (*L. xuul*) and 1103 (*L. yucatanensis*). After drying the plant material at room temperature (1 week), and in an oven at a temperature not greater than 60 °C (72 h), the material was ground using a Brabender Duisburg mill (880804 type) and collected using a No. 2 sieve.

3.3. Leaf extraction and purification of leaf extract

Powdered leaves of *L. xuul* (854 g) and *L. yucatanensis* (423 g) were Soxhlet extracted separately with MeOH. After evaporation of the solvent under reduced pressure, the MeOH crude extract of *L. xuul* (116.2 g) was subjected to successive hot extractions under reflux with hexane, CH_2Cl_2 and MeOH. The hexane extract was fractionated by VLC eluting with hexane and hexane:EtOAc mixtures of increasing polarity to produce nine fractions (1A–1H). Fraction 1B (477.8 mg) was further purified using flash chromatography (hexane:acetone 85:15) to yield **1** (120 mg). Fraction 1H (1.08 g) was passed through a Sephadex LH-20 column, eluting with $CHCl_3$ and $CHCl_3$:MeOH 9:1, to give 7 fractions (5A–5G). Fraction 5B (138.1 mg) was successively purified by CC (hexane:EtOAc 8:2) and preparative TLC (benzene: EtOAc 9:1, multiple elution, two times) to give **3** (5.3 mg). Fraction 5C (98.6 mg) was similarly purified to yield **2** (4.4 mg). Finally, succesive CC (hexane:EtOAc 8:2) and preparative TLC (benzene:EtOAc 9:1, double elution) of fraction 5G (66.6 mg) produced pure **4** (10.7 mg). Purification of the leaf MeOH extract from *L. yucatanensis* (58 g) following a similar procedure, yielded additional amounts of the metabolites **1** (1.4 mg), **3** (9.2 mg) and **4** (6.2 mg).

3.4. Root and stem bark extraction and purification

Powdered roots (1.3 kg) of *L. yucatanensis* were Soxhlet extracted with MeOH and the resulting MeOH extract (162 g) was subjected to succesive hot extractions under reflux with hexane, CH_2Cl_2 and MeOH. Initial fractionation of the hexane extract (10 g) by VLC, eluting with hexane and hexane:EtOAc mixtures of increasing polarity, furnished 10 major fractions (12A–12J). Fraction 12B (3.06 g) was further purified by CC (hexane:EtOAc 95:5) to afford xuulanin (**5**) (366 mg), 3β-methoxyxuulanin (**6**) (158 mg) and **8** (50 mg). Fraction 12F (239.8 mg) was further purified by CC (hexane:EtOAc 8:2) to afford **7** (186 mg), while fraction 12G (541 mg) was subjected to CC (hexane:EtOAc 7:3) to produce **10** (55 mg). Metabolites **5**–**8** and **10** were also found in the root extract of *L. xuul* and in the stem bark of both species. The root extract of *L. xuul* yielded an additional metabolite **13** (44.3 mg), after successive purifications of the hexane extract.

3.5. 2′-Methoxy-4,6′-dihydroxylonchocarpin (*1*)

Orange needles from $CHCl_3$; mp 153–156 °C ; UV λ_{max} (MeOH) nm (log ε): 364 (4.16), 289 (3.93) nm . IR ν_{max} (film) cm^{-1}: 3426, 2924, 1731, 1608, 1454. ^1H NMR: see Table 1. ^{13}C NMR: see Table 1. HR-EIMS *m*/*z* (rel. int) 366 (7) [M^+], 365 (26), 299 (8), 234 (18), 232 (25), 186 (32), 141 (18), 136 (39), 134 (100); calcd. for $C_{22}H_{22}O_5$ 366.1467; found 366.1465.

3.6. 5, 4′-Dihydroxy- 3′- methoxy-(7,6:2″,3″)- 6″,6″-dimethylpyranoflavone (*2*)

Yellow amorphous powder; mp 218–222° C. UV λ_{max} (MeOH) nm (log ε): 347 (4.14), 264 (4.15); (+ $AlCl_3$) 379 (4.19), 304 (4.10). IR ν_{max} (film) cm^{-1}: 3400, 1651, 1588, 1454. ^1H NMR (500 MHz, $CDCl_3$) δ 1.48 (6H, *s*, 2×Me), 4.01 (3H, *s*, 3′-OMe), 5.62 (1H, *d*, *J* = 10 Hz, H-3″), 6.43 (1H, *s*, H-8), 6.55 (1H, *s*, H-3), 6.73 (1H, *d*, *J* = 10 Hz, H-4″), 7.03 (1H, *d*, *J* = 8.5 Hz, H-5′), 7.33 (1H, *d*, *J* = 2.0 Hz, H-2′), 7.47 (1H, *dd*, *J* = 8.5, 2.0 Hz, H-6′), 13.09 (1H, *s*, 5-OH). ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.3 (2″-Me), 56.1 (3′-OMe), 78.0 (C-2″), 95.0 (C-8), 104.3 (C-3), 105.4 (C-6), 108.3 (C-4a), 115.0 (C-2′/C-5′), 115.5 (C-4″), 120.7 (C-6′), 123.5 (C-1′), 128.0 (C-3″), 146.8 (C-3′), 149.2 (C-4′), 156.5 (C-5), 157.0 (C-8a), 159.4 (C-7), 163.8 (C-2), 182.4 (C-4). HR-EIMS *m*/*z* (rel. int): 366 (100), 349 (18), 301 (15; calcd. for $C_{21}H_{18}O_6$ 366.1103 ; found 366.1104.

3.7. 5,4′-Dimethoxy-(7,6:2″,3″)-6″,6″-dimethylpyranoflavone (*3*)

Yellow needles from $CHCl_3$; mp 137–140 °C. UV λ_{max} (MeOH) (log ε) nm: 353 (3.66), 349 (3.65). IR ν_{max} (film) cm^{-1}: 2971, 2918, 2832, 1632, 1597, 1454. ^1H NMR (500 MHz, $CDCl_3$): δ 1.48 (6H, *s*, 2×Me), 3.89 (3H, *s*, 4′-OMe), 3.93 (3H, *s*, 5-OMe), 5.72 (1H, *d*, *J* = 10.1 Hz, H-3″), 6.67 (1H, *s*, H-3), 6.72 (1H, *s*, H-8), 6.74 (1H, *d*, *J* = 10.1 Hz, H-4″), 7.01 (2H, *d*, *J* = 8.6 Hz, H-3′, H-5′), 7.83 (2H, *d*, *J* = 8.6 Hz, H-2′,H-6′). ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.6 (2″-Me), 55.7 (4′-OMe), 63.1 (5-OMe), 78.0 (C-2″), 101.0 (C-8), 107.0 (C-3), 112.4 (C-4a), 113.5 (C-6), 114.7 (C-3′/C-5′), 116.3 (C-4″), 124.0 (C-1′), 128.0 (C-2′/C-6′), 131.0 (C-3″), 155.5 (C-5), 158.5 (C-7), 159.0 (C-8a), 161.6 (C-2), 162.5 (C-4′), 177.2 (C-4). HR-EIMS *m*/*z* (rel. int.%) 364 [M^+] (41), 303 (2), 235 (2; calcd. for $C_{22}H_{20}O_5$ 364.1310, found 364.1296.

3.8. 5,4′-Dihydroxy-(7,6:2″,3″)-6″,6″-dimethylpyranoflavone (*4*)

Yellow amorphous powder. UV, IR, ^1H NMR and EIMS, in agreement with those previously published (Jain et al., 1978).

3.9. 3β, 4β-5-Trimethoxy-4′-hydroxy-(7,6: 2″,3″)-6″,6″-dimethylpyranoflavan (*7*)

Needles from hexane; mp 199–201 °C; UV λ_{max} (MeOH) (log ϵ) nm: 310 (3.43), 276 (3.85). IR ν_{max} (film) cm^{-1}: 3318, 2934, 2842, 1608, 1567, 1454. ^1H NMR: see Table 2. ^{13}C NMR: see Table 3. HR-EIMS

Table 3
^{13}C NMR (125 MHz, $CDCl_3$) spectral data of metabolites **7–10**

C	7	8	9	10
2	75.4	77.2	74.6	76.5
3	82.0	71.4	73.5	71.1
4	68.6	70.7	70.0	62.1
5	156.0	156.2	156.0	155.6
6	108.3	108.3	108.7	109.8
7	156.0	155.9	155.3	157.4
8	100.8	100.9	100.4	100.7
8a	156.0	155.9	155.9	156.5
4a	108.1	107.2	107.5	106.8
1′	131.5	138.7	137.6	138.4
2′/6′	129.5	128.1	128.0	128.1
3′/5′	115.5	128.7	128.5	128.7
4′	156.0	128.8	128.9	129.0
2″	76.4	76.5	76.5	76.5
3″	128.3	128.3	128.4	128.0
4″	117.1	117.3	117.0	116.8
2″-Me_2	27.9	28.1	28.0	28.0
	27.9	28.2	28.0	28.4
3-OMe	58.4			
4-OMe	58.8	58.3	59.0	
5-OMe	63.1	63.1	63.1	63.5
COMe			169.8/21.0	

m/z (rel. int.%) 421 (100) [M^+ + Na], 367 (45), 335 (6), 303 (6), 261 (6), 137 (13); calcd. for $C_{23}H_{26}O_6Na$ 421.1626; found 421.1627.

3.10. 4β,5-Dimethoxy-3β-hydroxy-(7,6:2″,3″)-6″,6″-dimethylpyranoflavan (**8**)

Needles from hexane, mp 104–106 °C; UV λ_{max} (MeOH) (log ϵ) nm: 295 (3.83), 246 (3.84). IR ν_{max} (film) cm^{-1}: 3015, 2983, 1621, 1576, 1473, 1118; ^{1}H NMR: see Table 2. ^{13}C NMR: see Table 3; LR-EIMS *m/z* (rel. int) 368 [M^+] (55), 353 (100), 233 (62), 219 (24), 91 (12).

3.11. 3β-Acetoxy-4β,5-dimethoxy-(7,6: 2″,3″)-6″,6″-dimethylpyranoflavan (**9**)

A mixture of **8** (40.5 mg), acetic anhydride (2 ml), and pyridine (1 ml) was allowed to stir overnight at room temperature. The reaction mixture was poured over water (60 ml) and the resulting suspension extracted with EtOAc three times. The organic layer was washed successively with water (1:1, v/v), 5% HCl, 2.5% NaOH, and NaCl saturated solution. Treatment of the solvent with anhydrous Na_2SO_4 followed by filtration and evaporation yielded 30.2 mg (67%) of crude acetylated product. TLC: Rf 0.77 (hexane:EtOAc 70:30); UV λ_{max} (MeOH) (log ε) nm: 292 (3.43), 260 (3.44), 244 (3.45). IR ν_{max} (film) cm^{-1}: 2932, 1740, 1610,1586, 1480, 1379, 1235, 1143, 1091. ^{1}H NMR: see Table 2. ^{13}C NMR: see Table 3. LR-EIMS (rel. int): 410 [M^+] (17), 395 (100), 321 (7), 233 (39), 121 (23), 91 (10), 77 (6), 43 (30).

3.12. 3β, 4β-Dihydroxy-5-methoxy-(7,6:2″,3″)-6″,6″-dimethylpyranoflavan (**10**)

Needles from hexane, mp 152–155 °C; UV λ_{max} (MeOH) (log ε) nm: 310 (3.67), 273 (3.99), 244 (4.01). IR ν_{max} (film) cm^{-1}: 3457, 1618, 1572, 1470, 1142, 1081. ^{1}H NMR: see Table 2. ^{13}C NMR- see Table 3. LR-EIMS *m/z* (rel. int.): 354 [M^+] (33), 339 (80), 219 (100), 176 (14), 91 (10).

3.13. 3β,4β-Dihydroxy-5-methoxy-(7,6:2″,3″)-dimethyl pyranoflavan-3,4-acetonide (**11**)

A mixture of **10** (23.3 mg), acetone (10 ml, HPLC grade) and *p*-toluenesulphonic acid (catalytic amount) was allowed to stir at room temperature The reaction was monitored by TLC and after 48 h no starting material was observed. The reaction mixture was diluted with Et_2O (50 ml) and washed with saturated $NaHCO_3$ (3×30 ml). The organic extract was dried (dry Na_2SO_4), filtered, and conc. to give 10.5 mg (40%) of a colourless oil identified as **11**. TLC: R_f 0.62 (hexane:acetone 70:30); UV λ_{max} (MeOH) (log ε) nm: 309 (3.34), 274 (3.69), 241 (3.73) nm. IR ν_{max} (film) cm^{-1}: 2986, 1710, 1608, 1577, 1470. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.40 (6H, *s*, 2×Me), 1.49 (3H, *s*, Me), 1.58 (3H, *s*, Me), 4.21 (1H, *dd*, *J* = 9.9, 5.0 Hz, H-3), 4.61 (1H, *d*, *J* = 9.9 Hz, H-2), 5.22 (1H, *d*, *J* = 5.0 Hz, H-4), 5.56 (1H, *d*, *J* = 9.9 Hz, H-3″), 6.26 (1H, *s*, H-8), 6.54 (1H, *d*, *J* = 9.9 Hz, H-4″), 7.58 (5H, *m*, H2′-H6′). ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.1 (Me), 27.7 (Me), 28.0 (2‴-Me), 28.7 (2‴-Me), 63.5 (5-OMe), 68.0 (C-4), 75.2 (C-3), 76.3 (C-2), 77.4 (C-2″), 101.0 (C8/C2‴), 106.5 (C-6), 108.9 (C4a), 116.8 (C-4″), 127.5 (C3′/C5′), 128.5 (C2′/ C4′/ C6′), 137.8 (C-1′), 155.5 (C-7), 156.5 (C-8a), 156.8 (C-5); LR-EIMS (rel. int.): 394 [M^+] (23), 379 (100), 219 (100), 103 (22), 43 (27).

3.14. 3β,4β-Epoxy-5-methoxy-(7,6:2″,3″)-6″,6″-dimethylpyranoflavan (**12**)

Amorphous solid. UV λ_{max} (MeOH) (log ε) nm: 239 (3.6), 275 (3.4), 310 (3.0). IR ν_{max} (film) cm^{-1}: 2970, 1613, 1577, 1460, 1367, 1147, 1096, 1024. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.36, 1.40 (6H, *s*, 2×Me), 3.16 (3H, *s*, 5-OMe), 4.30 (1H, *dd*, *J* = 10.0, 5.0 Hz, H-3), 4.90 (1H, *d*, *J* = 10.0 Hz, H-2), 5.08 (1H, *d*, *J* = 5.0 Hz, H-4), 5.49 (1H, *d*, *J* = 10.0 Hz, H-3″), 6.18 (1H, *s*, H-8), 6.39 (1H, *d*, *J* = 10.0 Hz, H-4″), 7.39 (5H, *m*, H2′-H6′); ^{13}C NMR (125 MHz, $CDCl_3$): δ 27.6, 28.2 (2″-Me), 62.0 (C-4), 63.1 (5-OMe), 75.8 (C-3), 76.3 (C-2), 76.5 (C-2″), 100.5 (C-8), 106.5 (C-4a), 109.6 (C-6), 116.5 (C-4″), 128.1 (C-

2′,C-3′,C-5′,C-6′), 128.3 (C-4′, C-3″), 138.2 (C-1′), 155.4 (C-5), 156.3 (C-7), 157.2 (C-8a). HR-EIMS *m/z* (rel. int.): 337 (33) $[M+1]^+$, 303 (3), 217 (3), 190 (2); calcd. for $C_{21}H_{21}O_4$ 337.1439, found 337.1403.

3.15. 2′.4′-Dihydroxy-3′-(3-methylbut-2-enyl)chalcone (*13*)

Yellow amorphous solid. UV, IR and HR-EIMS in agreement with published data (De Lima et al., 1973). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.78 (3H, *s*, 3″-Me), 1.85 (3H, *s*, 3″-Me), 3.49 (2H, *d*, $J=7.0$ Hz, H-1″), 5.31 (1H, *t*, $J=7.0$ Hz, H-2″), 6.44 (1H, *d*, $J=8.9$ Hz, H-5′), 7.43 (5H, *m*, B ring protons), 7.60 (1H, *d*, $J=15.4$ Hz, Hα), 7.74 (1H, *d*, $J=8.9$ Hz, H-6′), 7.89 (1H, *d*, $J=15.4$ Hz, Hβ), 13.76 (1H, *s*, OH). ^{13}C NMR (125 MHz, $CDCl_3$): δ 18.2 (3″-Me), 22.0 (C-1″), 26.0 (3″-Me), 108.1 (C-5′), 114.3 (C-1′), 114.4 (C-3′), 120.8 (C-α), 121.3 (C-2″), 128.7 (C-2/C-6), 129.2 (C-3/C-5), 129.6 (C-6′), 130.8 (C-4), 135.1 (C-1) 136.1 (C-3″), 144.5 (C-β), 162.0 (C-4′), 164.2 (C-2′), 192.3 (C=O).

Acknowledgements

The authors wish to thank Filogonio May-Pat for locating and identifying the plant material and Angélica Flores Pérez and Fatima Alejos-González for technical assistance. R.B. acknowledges the British Council and the National Council of Science and Technology (CONACYT-Mexico) for financial support.

References

Antus, S., Gábor, M., Vetschera, K., 1996. Flavonoids and Bioflavonoids 1995. Akadémiai Kiadó, Budapest.

Borges-Argáez, R., Peña-Rodríguez, L.M., Waterman, P.G., 2000. Flavonoids from the stem bark of *Lonchocarpus xuul*. Phytochemistry 54, 611–614.

Clark-Lewis, J.W., Williams, L.R., 1967. Flavan derivatives. XVII. Epimerization of the benzylic 4-hydroxyl group in flavan-3,4-diols and the formation of the 4-alkyl ethers by solvolysis. Australian Journal of Chemistry 20, 2151–2167.

Durán, R., Trejo-Torres, J.C., Ibarra-Manríquez, G., 1998. Endemic phytotaxa of the Peninsula of Yucatan. Harvard Papers in Botany 3, 265–316.

De Lima, O.G., Marini-Bettolo, G.B., De Méllo, J.F., Delle Monache, F., De Barros Coélho, J.S., De Andrade Lyra, F.D., Fernandes de Alburquerque, M.M., 1973. *C*- and *O*-prenylated chalcones from *Cordoa piaca*. Gazzetta Chimica Italiana 103, 771–777.

Foo, L.Y., Wong, H., 1986. Diastereoisomeric leucoanthocyanidins from the heartwood of *Acacia melanoxylon*. Phytochemistry 25, 1961–1965.

Harborne, J.B., 1998. Phytochemical Methods, 3rd ed. Chapman and Hall, London.

Jain, A.C., Khazanchi, R., Kumar, A., 1978. Synthesis of carpachromene and related isopentenylated derivatives of apigenin. Tetrahedron 34, 3569–3573.

Mendieta, R.M., Del Amo, R.S., 1981. Plantas Medicinales del Estado de Yucatan. CECSA, Xalapa, Veracruz.

Panichpol, K., Waterman, P.G., 1978. Novel flavonoids from the stem of *Popowia cauliflora*. Phytochemistry 17, 1363–1367.