Major Flavanones from *Lonchocarpus guatamalensis*

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The flavanones 2S-lupinifolin and 2R:3R-lupinifolinol have been isolated as major components from a methanolic extract of *Lonchocarpus guatamalensis* root bark. These known compounds occur together with a minor flavanone (2R:3R-3-O-methyl-lupinifolinol) not previously recognized as a natural product.

Introduction

Several species from the genus *Lonchocarpus* (Leguminosae-Papilionoideae; tribe Tephrosieae) have been found to contain flavanones with alkyl (*e.g.* prenyl and dimethylpyrano) side attachments [1−4]. These compounds include isolonchocarpin obtained from the seeds of *L. sericeus* [1] and *L. eriocaulinalis* [1], the latter species also affording 3-hydroxyisolonchocarpin [1], and lonchocarpols A−E from *L. minimiflorus* leaves [4]. As part of a continuing study of flavonoids and isoflavonoids from leguminous plants, we recently examined the methanol-soluble root bark constituents of *L. guatamalensis*, a medium-sized tree (up to ~ 15 m) native to Central America. Apart from 2S-lupinifolin (**1**) and 2R:3R-lupinifolinol (**2**), two rare flavanones originally isolated from *Tephrosia lupinifolia* [5], the root bark of *L. guatamalensis* yielded small quantities of the new natural product 3-O-methyl-lupinifolinol (**3**). The identification of flavanones **1−3** is described in this report.

Results and Discussion

The air-dried root bark of *Lonchocarpus guatamalensis* Benth. was powdered, and then extracted over a period of 15 days with 90% aqueous MeOH (3×2.2 l). The combined extracts were worked up as described in the Experimental section to afford three phenolic compounds designated LG-1 (approx. 17 g/kg of dry root material), LG-2 (21 g/kg) and LG-3 (1.8 g/kg). All three compounds afforded a purple-pink colour, characteristic of a flavanone, when treated in MeOH with Mg powder and conc. HCl (Shinoda test) [6]. Additionally, AlCl$_3$-induced UV shifts, and the detection of a very low-field ^{1}H NMR singlet between δ 11.30 and 12.30 (Table I), indicated the presence in each flavanone of an H-bonded C-5 OH group [7, 8].

The ^{1}H NMR spectrum of LG-1 revealed both a prenyl side-chain (δ 1.65 s, 6H, 2× CH$_3$; δ 3.21 d, 2H, −CH$_2$−; δ 5.15 t, 1H, −CH=) and a dimethylpyrano attachment (δ 1.44 s, 6H, 2× CH$_3$; δ 5.50 d and 6.63 d, both 1H, HC=CH) with the remaining signals being attributable to the protons of a flavanone nucleus. Thus, two *ortho*-coupled doublets centred at δ 6.87 (2H) and 7.32 (2H) were assigned to the protons of a *para*-disubstituted benzene ring, whilst a complex series of signals at δ 5.33 (1H, dd), and 2.77 and 3.09 (2H, two double doublets), were attributed, respectively, to H-2 and H-3 of ring C. Compound LG-1 had M$^+$ 406 with a major fragment

Table II. Effect of acetylation on the chemical shift values observed for the olefinic protons H$_A$ (1‴) and H$_B$ (2‴) of the *Lonchocarpus* flavanones[a,b,c].

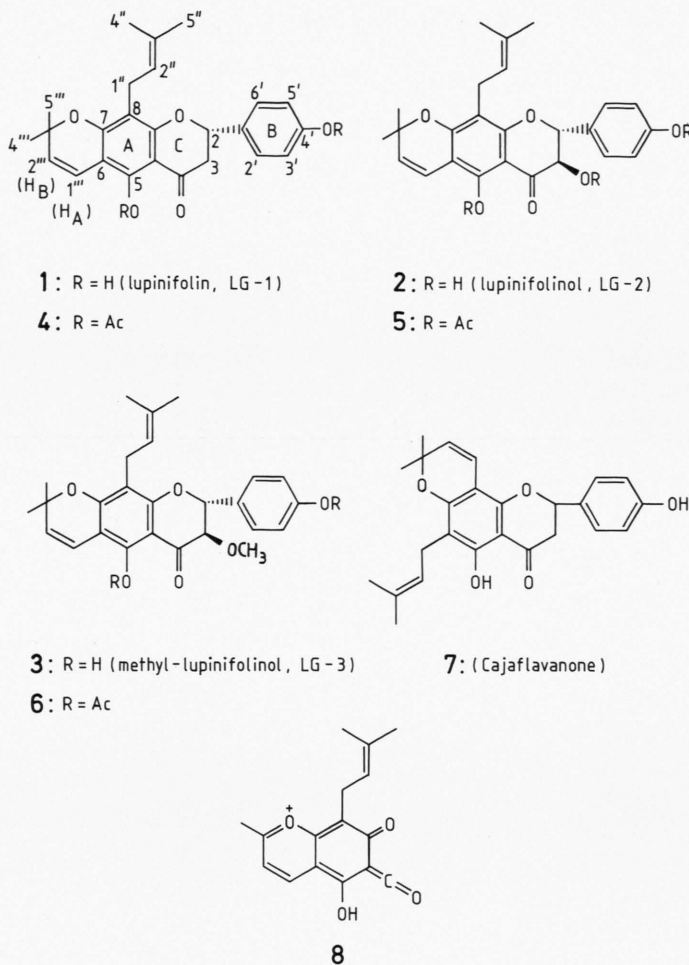| Olefinic proton | Δδ Value | | |
	1 → 4	2 → 5	3 → 6
H$_A$ (H-1‴)	−0.24	−0.27	−0.25
H$_B$ (H-2‴)	+0.14	+0.12	+0.14

[a] **1** = LG-1 (lupinifolin), **2** = LG-2 (lupinifolinol), and **3** = LG-3 (methyl-lupinifolinol). Compounds **4**, **5** and **6** are the acetate derivatives of **1**, **2** and **3**, respectively.
[b] Spectra were recorded in CDCl$_3$ at 100 MHz (TMS reference).
[c] Actual δ values of H-1‴ (H$_A$) and H-2‴ (H$_B$) before and after acetylation are given in Table I. See text and ref. [10] for further discussion.

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1 : R = H (lupinifolin, LG-1)

4 : R = Ac

2 : R = H (lupinifolinol, LG-2)

5 : R = Ac

3 : R = H (methyl-lupinifolinol, LG-3)

6 : R = Ac

7 : (Cajaflavanone)

8

at m/z 271 (**8**) confirming that the prenyl substituent was located on the same aromatic ring (A) as the dimethylpyrano side structure, and an OH (C-5) group. Ring A of LG-1 is therefore fully substituted. In order to satisfy the MS and ^{1}H NMR data, a single OH group must also be present at C-4' (ring B).

Since LG-1 is hydroxylated at C-5, the pyrano attachment must be cyclized in either a linear (C-6 → C-7 [0]) or an angular (C-8 → C-7 [0]) fashion, with the remaining position (C-6 or C-8) being occupied by the prenyl side-chain as in **1** (= lupinifolin [5]) or its isomer **7** (= cajaflavanone [9]). As discussed earlier [10] the angular or linear disposition of a pyrano substituent on the A-ring of a 5-hydroxylated flavonoid/isoflavonoid can be established from the ^{1}H NMR spectrum of the acetate derivative. In a linear compound, the olefinic H_A signal shifts to sig-

nificantly higher field upon acetylation whereas the H_B signal moves slightly in the opposite direction. For angular compounds, acetylation causes both H_A and H_B to resonate at a slightly lower field. Based on data in Table I, it is clear from Table II that after acetylation the chemical shift of H_A (= H-1''', δ 6.63 → 6.39, $\Delta\delta$ − 0.24) and H_B (= H-2''', δ 5.50 → 5.64, $\Delta\delta$ + 0.14) is entirely consistent with the presence of a linear side structure allowing LG-1 to be formulated as **1** (lupinifolin). The $2S$ absolute stereochemistry of *Lonchocarpus*-derived lupinifolin was confirmed by its CD curve in MeOH which exactly resembled that of $2S$-lupinifolin previously obtained from the roots of *Tephrosia lupinifolia* [5].

As shown in Table I, the ^{1}H NMR spectrum of LG-2 was virtually identical with that of lupinifolin, the only difference being that in the former com-

Table I. ¹H NMR data (δ values) for the *Lonchocarpus* flavanones (**1–3**) and their acetate derivatives (**4–6**)[a,b,c].

Proton		Compound					
		Lupinifolin (**1** = LG-1)	Lupinifolin acetate (**4**)	Lupinifolinol (**2** = LG-2)	Lupinifolinol acetate (**5**)	Methyl-lupinifolinol (**3** = LG-3)	Methyl-lupinifolinol acetate (**6**)
H-2		5.33 dd (12.0, 3.9)	5.43 dd (12.3, 4.0)	4.99 d (11.8)	5.32 d (12.2)	5.15 d (10.1)	5.24 d (10.6)
H-3	ax	3.09 dd (17.1, 12.0)	2.97 dd (16.7, 12.3)	4.50 d (11.8)	5.65 d (12.2)	4.02 d (10.1)	3.91 d (10.6)
	eq	2.77 dd (17.1, 3.9)	2.70 dd (16.7, 4.0)				
5-OH		12.24 s	–	11.37 s	–	11.99 s	–
H-2'/6' [2H]		7.32 d (8.6)	7.47 d (8.6)	7.41 d (8.6)	7.48 d (8.6)	7.35 d (8.6)	7.50 d (8.7)
H-3'/5' [2H]		6.87 d (8.6)	7.14 d (8.6)	6.85 d (8.6)	7.15 d (8.6)	6.86 d (8.6)	7.14 d (8.7)
H-1″ [2H]		3.21 br d (7.3)	3.29 br d (7.1)	3.17 br d (7.3)	3.24 br d (7.1)	3.17 br d (7.3)	3.25 br d (7.3)
H-2″		5.15 br t (7.3)	5.15 br t (7.1)	5.12 br t (7.3)	5.11 br t (7.1)	5.12 br t (7.3)	5.12 br t (7.3)
H-4″/5″		1.65 s [6H]	1.66 s [6H]	1.60 s [3H] 1.63 s [3H]	1.59 s [3H] 1.64 s [3H]	1.62 s [6H]	1.63 s [6H]
H-1‴ (H$_A$)		6.63 d (10.0)	6.39 d (10.0)	6.64 d (10.0)	6.37 d (10.3)	6.63 d (10.0)	6.38 d (10.0)
H-2‴ (H$_B$)		5.50 d (10.0)	5.64 d (10.0)	5.53 d (10.0)	5.65 d (10.3)	5.50 d (10.0)	5.64 d (10.0)
H-4‴/5‴		1.44 s [6H]	1.45 s [6H]	1.45 s [6H]	1.45 s [6H]	1.43 s [3H] 1.45 s [3H]	1.44 s [3H] 1.45 s [3H]
3-OCH$_3$ [3H]		–	–	–	–	3.39 s	3.32 s
3-OAc [3H]		–	–	–	2.01 s	–	–
5-OAc [3H]		–	2.41 s	–	2.41 s	–	2.42 s
4'-OAc [3H]		–	2.32 s	–	2.32 s	–	2.32 s

[a] Spectra were determined in CDCl₃ at 100 MHz (TMS reference).
[b] Except where indicated by [2H, 3H or 6H], all the signals integrated for 1 proton.
[c] Coupling constants (*J* in Hz) are given in parentheses.

pound two 1H doublets (*J* = 11.8 Hz) at δ 4.99 and 4.50 were attributed respectively to H-2 and H-3 of a *trans*-3-hydroxyflavanone (dihydroflavonol) [6]. The high *J* value is indicative of a *trans* stereochemistry [11, 12]. Chemical shift measurements on the acetate derivative of LG-2 established that the pyrano side structure was located linearly on ring A (see Table II, and the earlier discussion). Thus, LG-2 has the same structure (**2**) as lupinifolinol from *T. lupinifolia* [5]. CD data confirmed that, like *Tephrosia* lupinifolinol, the *Lonchocarpus* compound had the

2*R*:3*R* absolute configuration. Apart from *T. lupinifolia*, lupinifolinol has also been previously isolated from *Millettia pachycarpa* [13].

Unlike compounds **1** and **2**, the third *Lonchocarpus* flavanone (LG-3) was found to contain a methoxyl substituent (δ 3.39 s, 3H). In all other respects, however, the ¹H NMR spectrum closely resembled that of lupinifolinol, allowing the OCH₃ group to be placed at C-3. Further support for 3-methoxylation was provided by the H-3 NMR signal (Table I) which appeared at higher field (δ 4.02)

than in the corresponding *trans*-3-hydroxyflavanone (lupinifolinol **2**; δ 4.50) [12]. As in **2**, *trans*-orientation of the C-2 and C-3 protons of LG-3 was evident from the high $J_{2,3}$ value (10.1 Hz) [11, 12], whilst the 2*R*:3*R* absolute configuration was established by a CD comparison with lupinifolinol of known stereochemistry. Since the dimethylpyrano side structure of LG-3 is linearly disposed (Table II), the compound can be formulated as the new flavanone **3** for which we suggest the common name *methyl-lupinifolinol*.

Flavanone **3** was also obtained following extraction of *Lonchocarpus* root bark with 95% aqueous ethanol. Moreover, pure lupinifolinol **2** (the demethyl analogue of **3**) was recovered unchanged from aqueous methanol (90%) maintained at room temperature for 2–3 weeks. Lupinifolin (**1**) was similarly unaffected by aqueous MeOH. These results indicate that methyl-lupinifolinol (**3**) occurs naturally in *L. guatamalensis* and is not an artifact formed during MeOH extraction of the root bark (see Experimental).

Experimental

Plant material

Root bark of *Lonchocarpus guatamalensis* Benth. was collected in 1987 by Dr. C. E. Hughes (Oxford Forestry Institute, England) from a tree growing near Champoton, Campeche Province, Mexico. Reference material (C. E. Hughes 937) has been placed in the Forest Herbarium, Oxford University (FHO).

Isolation and purification of Lonchocarpus flavanones

The air-dried, powdered root bark of *L. guatamalensis* (approx. 225 g) was steeped (room temp.) over a 15 day period in 90% MeOH (3 × 2.2 l). The three extracts (6.6 l) were then combined and concentrated under reduced pressure (40 °C) to give an orange-yellow solution (200 ml) which was shaken with EtOAc (3 × 300 ml). After washing with 5% aqueous NaHCO$_3$ (2 × 150 ml), the combined EtOAc fractions were reduced to dryness, and a portion of the resulting material (corresponding to 34 g of dried root bark) was then chromatographed (Si gel PTLC; Merck plates, F-254, layer thickness 0.5 mm) in CHCl$_3$–MeOH (25:1). Major fluorescence-quenching bands were visible under short wave-

length UV light at R_F 0.72 (Band B 1) and 0.43 (B 2). Elution (EtOAc) and subsequent Si gel PTLC (benzene–EtOAc, 5:1, ×3) of the B 1 material gave compound LG-1, **1** (= lupinifolin, 604 mg, upper zone) and LG-3, **3** (= methyl-lupinifolinol, 61.5 mg, lower zone). Re-PTLC of the B 2 component in CHCl$_3$–acetone–conc. ammonia water (35:30:1) afforded only LG-2, **2** (= lupinifolinol, 732 mg) at R_F 0.52.

Acetylation of Lonchocarpus flavanones

Each flavanone (10–13 mg) was acetylated over a 20 h period (room temp.) in a mixture of acetic anhydride and pyridine (1:1, 1 ml). After diluting with toluene, the solution was reduced to dryness (*in vacuo*, 40 °C), and the product was then chromatographed (Si gel TLC) in *n*-hexane–EtOAc (13:7) to give between 10 and 12 mg of the desired acetate. R_F values in *n*-hexane–EtOAc (13:7) were as follows: lupinifolin (LG-1) acetate **4**, 0.37; lupinifolinol (LG-2) acetate **5**, 0.31; methyl-lupinifolinol (LG-3) acetate **6**, 0.35.

Shinoda test

The Shinoda test for flavanones was undertaken as previously described [6].

Lupinifolin 1 (LG-1)

Pale yellow, semi-solid gum. Colour given (TLC plates) with diazotized *p*-nitroaniline reagent [14], orange-brown. Fluorescence on Si gel thin-layer plates viewed under long wave-length (365 nm) UV light, purple-black. UV: λ$_{max}$, nm: MeOH 225, 267 sh, 276, 301, 313 sh, 362 (br); +NaOMe 247, 266 sh, 275, 293 sh, 313, 341, 390 sh; +AlCl$_3$ 228, 262 sh, 276, 287, 341 (br), 360 sh, 412 sh. The MeOH spectrum was unaffected by NaOAc. MS (rel. int.): *m/z* 406 (M$^+$, 69), 392 (29), 391 (M$^+$ − 15, 100), 351 (13), 271 (A-ring fragment with OH, intact prenyl side-chain and pyran ring − CH$_3$, **8**, 42), 243 (23), 231 (19), 216 (11), 215 (73), 91 (10), 43 (15), 41 (16). ^{1}H NMR: see Table I. CD (MeOH, 23 °C, rel. [θ]): 220 nm +0.66, 250 nm +0.21, 260 nm 0, 280 nm −0.79, 296 nm −1.00, 315 nm 0, 323 nm +0.16, 365 nm +0.21.

Lupinifolin acetate (4)

Colourless glassy solid. UV: λ$_{max}$, nm: MeOH 215 sh, 256 sh, 261, 291 sh, 342 (br). MS (rel. int.):

m/z 490 (M^+, 13), 449 (21), 448 (64), 434 (31), 433 (100), 406 (3), 405 (8), 285 (12), 271 (21), 243 (13), 215 (41), 44 (13), 43 (32). ^{1}H NMR: see Table I.

Lupinifolinol 2 (LG-2)

Pale yellow needles, m.p. 130–131.5 °C (lit. 121–123 °C [5]). Colour with diazotized *p*-nitroaniline reagent, and appearance on chromatograms viewed under long wave-length UV light, as given for lupinifolin (1). UV: λ_{max}, nm, ε: MeOH 225 (20,800), 268 (37,100), 276 (40,500), 305 sh (11,000), 316 (13,000), 364 (3000); +NaOMe 236–240 (br), 270 sh, 275, 292 sh, 318 sh, 398 (br); +AlCl₃ 229, 260, 276, 288, 342. The MeOH spectrum was unaffected by NaOAc. MS (rel. int.): m/z 422 (M^+, 92), 408 (27), 407 (M^+ − 15, 100), 351 (16), 288 (17), 287 (87), 271 (A-ring fragment 8, 58), 260 (17), 245 (68), 243 (36), 231 (87), 215 (47), 189 (38), 107 (40), 77 (18), 44 (35), 43 (28), 41 (28), 40 (20). ^{1}H NMR: see Table I. $[\alpha]_D^{23}$ +34° (c = 0.113 in MeOH; lit. $[\alpha]_D^{24}$ +26.18°, c = 1.12 in CHCl₃ [5]). CD (MeOH, 23 °C, rel. [θ]): 225 nm +0.65, 250 nm +0.76, 278 nm 0, 285 nm −0.65, 303 nm −1.00, 322 nm 0, 330 nm +0.15, 360 nm +0.28.

Lupinifolinol acetate (5)

Colourless gum. UV: λ_{max}, nm: MeOH 216 sh, 255 sh, 261, 292 sh, approx. 343 (br). MS (rel. int.): m/z 548 (M^+, 3), 506 (23), 492 (8), 491 (27), 431 (11), 286 (16), 285 (10), 271 (28), 243 (12), 231 (9), 215 (24), 150 (30), 108 (13), 107 (100), 77 (9), 43 (71).

Methyl-lupinifolinol 3 (LG-3)

Pale yellow gum. Colour with diazotized *p*-nitroaniline reagent, and long wave-length UV fluorescence, as given for lupinifolin. UV: λ_{max}, nm: MeOH 226, 268 sh, 276, 302 sh, 316, 367; +NaOMe 240 sh, 248, 268 sh, 276, 293 sh, 315 sh, 392; +AlCl₃ 229, 267 sh, 276, 288 sh, 323 (br), 343, 444 sh. The MeOH spectrum was unaffected by NaOAc. MS (rel. int.): m/z 436 (M^+, 83), 422 (31), 421 (M^+ − 15, 100), 330 (26), 329 (43), 287 (12), 285 (10), 272 (12), 271 (A-ring fragment 8, 69), 243 (31), 231 (35), 215 (51), 150 (25), 135 (15), 107 (26), 77 (11), 43 (17), 41 (17). ^{1}H NMR: see Table I. CD (MeOH, 23 °C, rel. [θ]): 225 nm +1.00, 250 nm +0.40, 265 nm 0, 284 nm −0.96, 304 nm −1.00, 323 nm 0, 337 nm +0.48.

Methyl-lupinifolinol acetate (6)

Colourless gum. UV: λ_{max}, nm: MeOH 256 sh, 262, 292 sh, approx. 344 (br). MS (rel. int.): m/z 520 (M^+, 6), 479 (20), 478 (61), 464 (32), 463 (100), 329 (35), 271 (51), 243 (23), 231 (16), 215 (45), 150 (23), 135 (11), 107 (20), 60 (21), 45 (26), 44 (25), 43 (69). ^{1}H NMR: see Table I.

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