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FLAVANONES FROM *LONCHOCARPUS MINIMIFLORUS*

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Key Word Index—*Lonchocarpus minimiflorus*; Papillionaceae; flavanones; leafcutter ants; *Atta cephalotes*; ant-repellent.

Abstract—Five novel flavanones have been isolated from the leaves of the Costa Rican tree *Lonchocarpus minimiflorus*, and characterized on the basis of their spectral features. Three of these compounds contain an uncommon prenyl cyclization, new to flavanone chemistry.

INTRODUCTION

As part of our systematic study [1–5] of Central American plants which escape attack of the leafcutter ant *Atta cephalotes*, we have screened the leaves of *Lonchocarpus minimiflorus*. In our study, fractionation of the *L. minimiflorus* extracts was guided by our ant-repellency bioassay [6], which measures the feeding preferences of a captive leafcutter colony. From the active hexane extract of these leaves, sitosterol and stigmasterol were isolated, along with mixtures of fats which were not further investigated. The marginally active chloroform extract was the major source of five flavanones, which were isolated by a combination of column chromatography and preparative radial thin-layer chromatography. These compounds were characterized by spectral analysis as the prenylated flavanones, lonchocarpol A (**1**), B (**3**), C (**4**), D (**6**), and E (**7**). Although a recent phytochemical examination of the seeds of this plant reported three flavanones [7], the products of this study of the leaves include three examples of an uncommon prenyl cyclization, apparently without precedent in flavanone chemistry.

RESULTS AND DISCUSSION

Compound *A*, the most abundant of the isolated flavanones, gave a high resolution mass spectrum (HRMS) which showed peaks up to *m/z* 408, suggesting a molecular formula of $C_{25}H_{28}O_5$. The UV spectrum of this compound had maxima at 297 and 345 nm, indicating the presence of a flavanone nucleus [8]. The ^{1}H NMR spectrum (Table 1) showed a downfield resonance at δ12.28, attributed to a chelated hydroxyl proton, while two doublets in the aromatic region (at δ7.27 and 6.85, each 2H, J = 8.5 Hz) suggested the presence of a para-substituted aromatic ring. Four methyl singlets (δ1.68, 1.69, 1.73, 1.80), two methylene doublets (centered at δ3.33, 3.28), and two triplets for vinylic hydrogen (δ5.17, 5.22), indicated the presence of two prenyl units. Three signals (δ5.28, 3.03, 2.78, each 1H, *dd*) were assigned to the C-2 and C-3 hydrogens of a flavanone C-ring. Upon treatment with acetic anhydride, a diacetate retaining the chelated hydroxyl proton signal was obtained, thus indicating the presence of three hydroxyl groups in compound *A*. However, upon further reflux a tetraacetate was obtained, presumably through rupture of the C-ring. From the summation of these data, structure **1** was assigned to compound *A*.

Compound **1** has been prepared as an intermediate towards the total synthesis of lupinifolin [9], but it apparently has not been observed as a natural product [10] and the previous spectral characterization was very brief. Accordingly, final confirmation of our proposed structure was obtained from its cyclization upon treatment with DDQ to the natural product lupinifolin (**10**).

Compound **B** gave an electron-impact mass spectrum with peaks up to *m/z* 442, fitting the molecular formula $C_{25}H_{30}O_7$. Although the ^{1}H NMR spectrum of this compound had many similar features to that of compound **1**, new signals included a triplet centered at δ3.84 (1H), an upfield shift of two of the four methyl resonances (to δ1.35, 1.39), and the presence of a new multiplet (δ2.57–2.86). That all of these new features indicate a doubly hydroxylated prenyl unit, is clear from comparisons with literature values [11, 12]. The position of the oxidized prenyl unit is more difficult to establish. In compound **1**, assignment of resonances to the two prenyl units was based on previously reported data for C-prenylated flavanones, which showed H-1″ and H-2″ resonances to be slightly downfield from the H-1‴ and H-2‴ signals [10]. Because the resonances (δ3.28 *d*, and 5.17 *t*) assigned to the C-8 prenyl group of compound **1** are not observed in the spectrum of compound **3**, it is reasonable to assign the oxidized prenyl group to the C-8 position. The alternative isomer cannot be ruled out however.

Both compounds *C* and *D* gave high resolution electron impact mass spectra with highest mass peaks at *m/z* 424, corresponding to molecular formulae of $C_{25}H_{28}O_6$. From the ^{1}H NMR spectra, it was clear that both compounds had the same essential nucleus as compounds **1** and **3**, but that they had undergone further cyclization. For example, in the ^{1}H NMR spectrum of compound *C*, one prenyl unit was still observed, but the two other methyl singlets were shifted upfield (to δ1.21, 1.33), and one of the two olefinic signals had been replaced by two new signals (δ4.73, *t*, 1H; and δ3.09, *d*, 2H). When double irradiation experiments confirmed the coupling between these later two signals, this fact, together with their

Table 1. ^{1}H NMR of compounds 1, 3–10

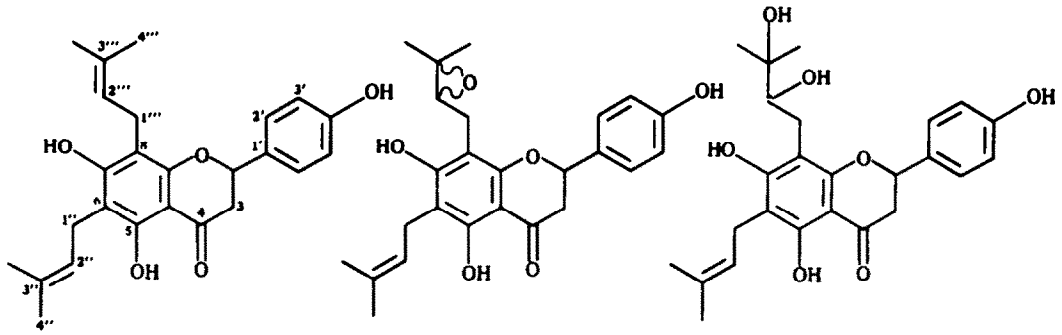
	1	3	4	5	6
2 *ax.*	5.28 *dd* (12.83, 2.98)	5.28 *dd* (13.0, 2.6)	5.30 *dd* (12.8, 3.2)	5.42 *dd* (13.5, 3.0)	5.32 *dd* (12.2, 3.1)
3 *ax.*	3.03 *dd* (17.12, 12.83)	2.94 *dd* (13.1, 16.3)	3.04 *dd* (17.1, 12.8)	3.20–2.89 *m*	3.03 *dd* (17.2, 12.2)
3 *eq.*	2.78 *dd* (17.13, 2.98)	2.72 *dd* (16.3, 2.6)	2.78 *dd* (17.1, 3.4)	2.73 *dd* (17.0, 3.1)	2.75 *dd* (17.2, 3.1)
2′, 6′	7.27 *d* (8.54)	7.29 *d* (8.5)	7.31 *d* (8.4)	7.45 *d* (8.4)	7.30 *d* (7.9)
3′, 5′	6.86 *d* (8.54)	6.90 *d* (8.5)	6.87 *d* (8.4)	7.14 *d* (8.4)	6.87 *d* (7.9)
1″	3.33 *d* (7.1)	3.34 *d* (7.0)	3.09 *d* (9.2)	3.20–2.89 *m*	3.24 *d* (7.0)
1‴	3.28 *d* (6.97)	2.57–2.86 *m*	3.19 *d* (7.0)	3.26 *d* (7.5)	3.03 *d* (8.8)
2″	5.22 *t* (7.1)	5.21 *t* (7.0)	4.73 *t* (9.2)	4.71 *t* (8.6)	5.24 *t* (7.0)
2‴	5.17 *t* (6.97)	3.84 *t* (6.0)	5.17 *t* (7.0)	5.19 *t* (7.5)	4.70 *t* (8.8)
4″, 4‴	1.73, 1.80 1.68, 1.69	1.70, 1.71 1.35, 1.39	1.33, 1.21 1.66, 1.64	1.64, 1.67 1.21, 1.32	1.33, 1.20 1.69, 1.77
C-5 OH	12.28	12.29 *s*	12.10 *s*	—	12.47 *s*
Ac Me	—	—	—	2.32, 2.39	

*Literature values [10].

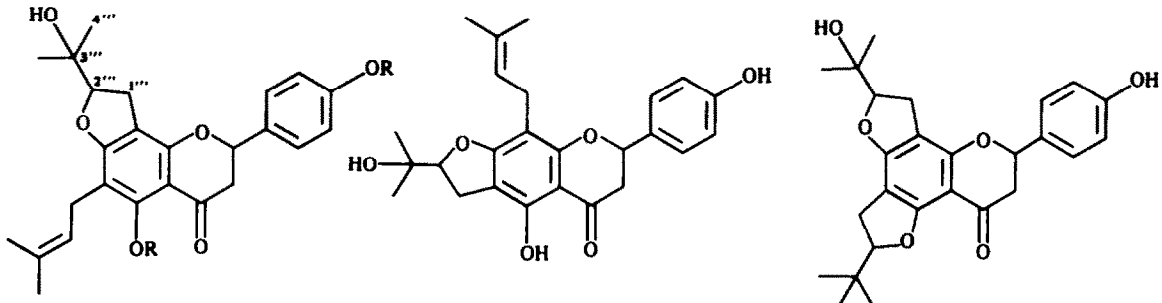
	7	8	9	10	Lupinifolin*
2 *ax.*	5.34 *dd* (12.8, 2.9)	5.42 *dd* (13.2, 2.9)	7.49 *d* (16.1)	5.33 *dd* (12.8, 3.1)	5.30 *dd*
3 *ax.*	3.10–2.91 *m*	3.07 *dd* (17.1, 13.2)	6.86 *d* (16.1)	3.04 *dd* (17.1, 12.8)	3.03 *dd*
3 *eq.*	2.78 *dd* (17.0, 3.0)	2.87 *dd* (17.1, 3.0)		2.72 *dd* (17.1, 3.1)	2.78 *dd*
2′, 6′	7.30 *d* (8.5)	7.45 *d* (8.5)	7.57 *d* (8.5)	7.31 *d* (8.5)	7.28 *d* (8.5)
3′, 5′	6.87 *d* (8.5)	7.15 *d* (8.5)	7.12 *d* (8.5)	6.87 *d* (8.5)	6.84 *d* (8.5)
1″	3.10–2.91 *m*	3.24–3.09 *m*	3.12 *br d* (6.0)	6.65 *d* (10.0)	6.63 *d*
1‴	3.10–2.91 *m*	3.24–3.09 *m*	3.12 *br d* (6.0)	3.20 *d* (7.3)	3.20 *d* (7.0)
2″	4.98–4.89 *m*	5.11 *t* (7.1)	5.01 *t* (6.0)	5.50 *d* (10.0)	5.48 *d*
2‴	4.69 *t* (8.7)	5.06 *t* (8.3)	5.01 *t* (6.0)	5.14 *t* (7.3)	5.14 *t* (7.0)
4″, 4‴	1.20, 1.19 1.36, 1.33	1.65, 1.58 1.74, 1.68	1.67 *s*	1.65, 1.44, 1.43	1.44, 1.64
C-5 OH—	—	12.05 *s*		12 24 *s*	12.20 *s*
Ac Me	—	2.31, 2.32	2.27, 2.31	—	—

chemical shifts, suggested a prenyl cyclization to a furan ring [13, 14]. In the spectrum of compound *C* the hydrogen-bonded hydroxyl resonance was still present. Therefore, the C-7 hydroxyl group must be involved in the cyclization. Because the spectrum of compound *D* contained these same characteristics, we concluded both were cyclized derivatives of compound 1, one linearly fused and one angularly fused.

Upon treatment of compound *C* with acetic anyhydride and pyridine, a diacetate was obtained, with acetylation at the C-5 and C-4′ hydroxyl groups. Because acetylation at C-5 produced a slight shift in the resonance assigned to the prenyl methylene, we hypothesized that compound *C* was the angularly fused isomer. More substantive evidence was obtained through derivatization. Treatment of compound *C* with DDQ resulted in the expected cycliz-

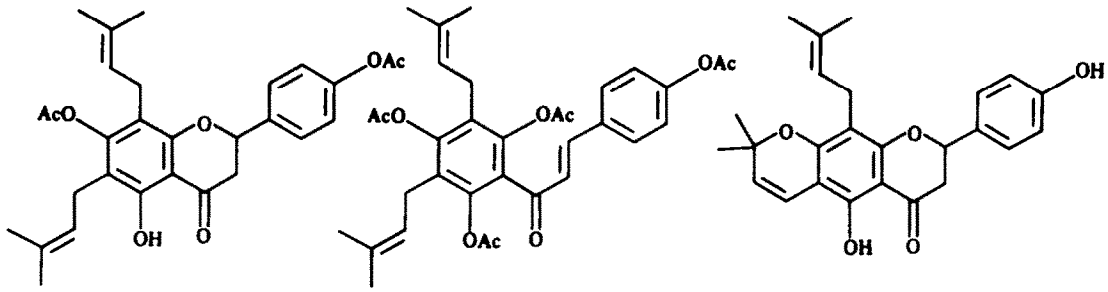


1 2 3



4 R = H
5 R = Ac

6 7



8 9 10

ation of the prenyl group with the C-5 hydroxyl. Accordingly, compound *C* was assigned the angular structure **4**, and the isomeric compound *D* was assigned as the linear isomer **6**.

Finally, compound **7** gave a high resolution mass spectrum with a molecular ion at *m/z* 440, fitting the molecular formula of $C_{25}H_{28}O_7$. The fundamental differences between the ^{1}H NMR spectrum of this compound and those of compounds **4** and **6** were the absence of resonances for the chelated hydroxyl group and vinyl methyl groups. Instead, four singlets were observed (δ1.20, 1.19, 1.36, and 1.33; each 3H), together with a new triplet (δ4.69; 4H) and a multiplet (δ2.90–3.10; 4H). These new features, and the ^{13}C NMR spectrum (Table 2), indicated cyclization of both prenyl units in this compound to produce structure **7**.

The prenyl cyclization observed in compounds **4**, **6**, and **7**, appears to be unique in flavonoids, with the closest analogy reported only very recently [15]. However, it has been observed several times in coumarins, rotenoids, and chromones [16–19]. Several review articles [20, 21] include comments on the biosynthesis of these compounds. Most agree that the furan rings originate via oxidation of a prenyl double bond to a diol or epoxide (e.g. compound **2**), followed by cyclization. The fact that *L. minimiflorus* contains the parent prenylated flavanone,

Table 2. ^{13}C NMR of compounds 1, 4, 7 and 9

	1	4	7	9
2	78.57	78.79	78.87	145.00
3	43.13	43.12	43.22	120.77
4	196.79	196.36	195.59	204.73
5	156.19	155.97	171.10	145.17
6	107.41	103.63	112.23	129.73
7	157.89	157.69	171.10	145.17
8	106.65	105.07	112.23	129.73
8a	159.35	155.97	156.26	145.17
4a	102.88	103.46	104.23	129.73
1′	130.82	129.01	130.79	132.39
2′, 6′	127.66	127.65	127.80	129.73
3′, 5′	115.61	115.53	115.67	122.24
4′	162.49	160.00	160.66	152.49
1″, 1‴	25.76	26.83, 25.73	27.03, 23.95	24.74, 25.55
2″, 2‴	121.77, 122.00	91.36, 122.02	87.27, 91.47	126.09, 126.35
3″, 3‴	133.80, 134.56	72.13, 127.83	72.33, 72.00	132.39
4″, 4‴	17.80, 17.85	17.72, 22.14	27.15, 24.07	21.14, 17.89
	21.33, 21.93	23.89, 25.73		15.28
Ac–C=O	—	—	—	168.39, 169.04
Ac Me	—	—	—	20.58, 20.49

a diol, and the cyclized products, may provide support for this theory, but our efforts to locate the intermediate epoxide 2 went unrewarded.

EXPERIMENTAL

The NMR spectra were obtained on a Bruker WM-360 instrument in $CDCl_3$. Chemical shifts are reported in ppm downfield from TMS. Low resolution MS were recorded with a Hewlett-Packard 5985B instrument operating at 70 eV. High resolution MS were obtained on a Kratos MS-50 instrument at the Midwest Center for the Mass Spectrometry, Lincoln NB.

Isolation. The isolation sequence was guided by a bioassay [6] that measured repellency by monitoring ant choices among an array of treated and control food flakes. Approximately 1.0 kg *L. minimiflorus* leaves were collected from Santa Rosa National Park, Costa Rica, air-dried, and kept in sealed plastic bags until extracted. This plant material was successively extracted with hexane, $CHCl_3$, and MeOH in a Soxhlet apparatus. The extracts were concentrated and each was bioassayed. The majority of the ant repellency was found to reside in the hexane extract, which, after several chromatographic separations and purifications yielded only the common plant sterols, stigmasterol and sitosterol.

Column chromatography (EtOAc and hexane gradient, silica gel, 63-200 mesh) was used to fractionate the marginally active $CHCl_3$ extract. Between 40% and 70% EtOAc, compounds **1**, **3**, **4**, **6**, and **7** were eluted, in that order. Final purification was achieved by radial chromatography (Chromatotron) on silica gel using toluene–EtOAc–AcOH (80:19:1). Although not significantly active in the ant-repellency bioassay, compound **1** was found to be fungistatic when tested against the attine fungus, *Rhozites gongylophora*.

Compound **1**. Isolated as green oil; $[\alpha]^{34} = -4.7°$; UV λ_{max}^{MeOH} nm: 217, 297, 345; $\lambda_{max}^{MeOH+NaOH}$ nm: 211, 240, 297, 335. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2950, 2350, 1650, 1450, 1385, 1270; MS *m/z* (rel. int.): 408 $[M]^+$ (47), 393 (7), 365 (6), 353 (26), 337 (25), 297 (20), 273 (16), 245 (15), 217 (41), 189 (76), 177 (47), 120 (62), 91 (86), 69 (46), 41 (100); HRMS: found, *m/z* 408.1917; calcd for $C_{25}H_{28}O_5$, 408.1937.

Compound **3**. Isolated as a greenish-yellow oil; MS *m/z* (rel. int.): 442 $[M]^+$ (1), 424 (3), 369 (31), 297 (11), 249 (24), 231 (11), 189 (19), 177 (35), 147 (10), 120 (58), 91 (46), 71 (83), 59 (100).

Compound **4**. Isolated as a yellow oil; $[\alpha]^{34} = -7.75°$; UV λ_{max}^{MeOH} nm: 235, 300, 345 nm; MS *m/z* (rel. int.): 424 $[M]^+$ (9), 409 (4), 369 (5), 289 (9), 189 (25), 147 (13), 120 (81), 107 (29), 91 (57), 59 (100); HRMS: found, *m/z* 424.1880; calcd for $C_{25}H_{28}O_6$, *m/z* 424.1886. When a small sample (5 mg) was treated with DDQ in benzene at reflux, cyclization of the prenyl group with the C-5 hydroxyl group resulted ^1H NMR: δ1.26, 1.25, 1.17, 1.19 (each 3H, *s*); MS, *m/z* (rel. int.): 422 $[M]^+$ (1), 407 (1), 374 (2), 230 (37), 228 (58), 200 (47), 91 (100).

Compound **5**. Compound **4** (10 mg) was dissolved in redistilled acetic anhydride (5 ml), dry pyridine (2 ml) was added, and the resulting mixture was stirred overnight at room temp. Addition of Et_2O (20 ml) was followed by aq. workup, employing aq. nickelous chloride to remove the pyridine. Flash CC (toluene–EtOAc–AcOH, 90:9.5:0.5) yielded pure compound **5** (5 mg). MS *m/z* (rel. int.): 520 $[M]^+$ (1), 477 (1), 410 (4), 165 (10), 105 (9), 91 (33), 69 (42), 43 (100).

Compound **6**. Isolated as a yellow oil. MS *m/z* (rel. int.): 424 $[M]^+$ (65), 409 (23), 369 (42), 353 (13), 289 (45), 231 (51), 203 (35), 189 (87), 149 (26), 135 (52), 91 (100), 69 (75).

Compound **7**. Isolated as an orange-yellow oil. UV λ_{max}^{MeOH} nm: 214, 299, 346; MS *m/z* (rel. int.): 440 $[M]^+$ (8), 422 (5), 407 (3), 369 (100), 297 (19), 249 (55), 231 (25), 189 (30), 177 (83), 147 (15), 120 (33), 91 (27), 59 (21); HR-MS: found, *m/z* 440.1814; calcd for $C_{25}H_{28}O_7$, 440.1835.

Compound **8**. Compound **1** (20 mg) was treated with acetic anhydride–pyridine as described above to give, after column chromatography, the diacetate **8** (15 mg). MS *m/z* (rel. int.): 492 $[M]^+$ (12), 449 (7), 433 (10), 421 (8), 393 (42), 379 (9), 351 (8), 231 (48), 217 (20), 189 (52), 177 (23), 135 (14), 91 (19), 43 (100).

Compound **9**. Compound **8** (15 mg) was treated with acetic anhydride–pyridine at reflux overnight. The usual work up, followed by flash chromatography (toluene–EtOAc–AcOH, 80:19:1), gave the tetraacetate **9** (10 mg). UV λ_{max}^{MeOH} nm: 207, 226, 308; MS, *m/z* (rel. int.): 576 $[M]^+$ (2), 533 (7), 491 (7), 449 (4), 433 (7), 407 (2), 393 (150), 231 (28), 215 (16), 147 (18), 91 (22), 43 (100).

Compound **10**. Compound **1** (50 mg) was added to a soln of DDQ (50 mg) in dry benzene (20 ml). The reaction mixture was heated at reflux for 4 hr, allowed to cool, and then purified through flash CC (toluene–EtOAc–AcOH, 90:9.5:0.5) to give lupinifolin (30 mg) [10].

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