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UNUSUAL FLAVONOIDS FROM *LONCHOCARPUS OROTINUS* SEEDS

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Key Word Index—*Lonchocarpus orotinus*; Leguminosae; Papilionaceae; 2',6'-hydroxylated flavonoids; orotinin; orotinichalcone; orotinin-5-methyl ether; 4,6'-epoxyorotiniflavan-4-ol.

Abstract—Seeds of *Lonchocarpus orotinus* have yielded five flavonoids the identities of which have been elucidated by spectroscopic analysis. In addition to the known minimiflorin three novel flavonoids, characterized by the presence of 2',6'-dihydroxy substitution of the B-ring, were isolated and identified as orotinin (6'-hydroxyminimiflorin), orotinin-5-methyl ether and orotinichalcone. A further compound, which was obtained only in a mixture with orotinin-5-methyl ether, was tentatively identified as the 4,6'-epoxy derivative of the flavan-4-ol of the orotinin series.

INTRODUCTION

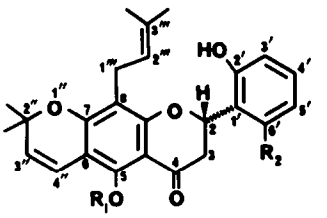
Lonchocarpus orotinus Pittier (Leguminosae–Papilionoideae) is a small forest tree, up to 8 m tall, found in the forests of Costa Rica [1, 2]. As part of an investigation of the flavonoids occurring in a number of sympatric species of *Lonchocarpus* growing in the Santa Rosa National Park, Costa Rica [3–5] we have examined a small sample of seeds from *L. orotinus*.

RESULTS AND DISCUSSION

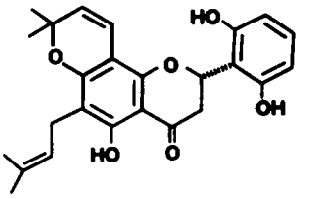
Sequential extraction with petrol (bp 40–60°) and then chloroform revealed the presence of several compounds with similar fluorescence in both extracts. Column chromatography of the concentrated extracts over silica gel gave, in each case, four flavonoid fractions, of which three were single compounds. One of these, the first to be eluted (yield 0.37%), proved to be identical to minimiflorin (**1**), which had previously been identified from the sympatric species *L. minimiflorus* Donn-Smith [5].

The second compound to be eluted (yield 0.5%) analysed for $C_{25}H_{26}O_6$. It was optically active, being strongly laevorotatory as is typical for 2(*S*) flavanones. The UV spectrum gave the anticipated bands for a flavanone and revealed the presence of a 5-hydroxy substituent through a bathochromic shift on the addition of $AlCl_3$. The ^1H NMR spectrum accounted for all 26 protons as (i) a 3,3-dimethylallyl substituent, (ii) a 2,2-dimethylpyran substituent, (iii) the normal ABX system for H-2 and H-3 of a flavanone C-ring with H-2 axial, (iv) signals at δ6.43 (2H, *d*) and δ7.02 (1H, *t*) for three adjacent protons on an aromatic nucleus, and (v) three phenolic protons, one H-bonded resonating at δ12.19 (H-5).

These data required that the flavanone had 3,3-dimethylallyl and 2,2-dimethylpyran substituents on ring A and hydroxy substituents on C-2' and C-6' of ring B so suggesting structure **2** or its isomer **3**. This was confirmed by the mass spectrum which showed major ions at *m/z* 271 (**4**) and at *m/z* 136 (**5**) and *m/z* 135 (**6**) for fission of the flavanone in ring C. Other major ions were based on fragmentation of *m/z* 404 (**7**) which is formed via a



1 $R_1 = R_2 = H$
2 $R_1 = H, R_2 = OH$
11 $R_1 = Me, R_2 = OH$



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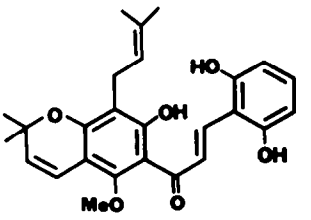

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rearrangement that has been noted previously by Pelter and Stainton [6]. The linear annulation of the pyran ring, and hence the structure **2**, was established by formation of the triacetate in which a shielding of 0.29 ppm for H-4″ and a corresponding deshielding of 0.11 ppm for H-3″ in the ^{1}H NMR spectrum must be attributed to the introduction of the *peri* C-5 acetoxy group. Another feature of the spectrum of the triacetate was the strong shielding of the equatorial H-3 proton (*ca* 0.3 ppm) and comparable deshielding of the axial H-3 proton through the influence of the 2′ and 6′ acetoxy groups.

This flavanone appears to be novel and has been assigned the trivial name orotinin. The ^{13}C NMR spectrum of **2** was recorded and compared with that of **1** (Table 1). The major differences were the strong shielding of C-1′ and C-5′ caused by the presence of the additional hydroxy function at C-6′. Alkaline degradation of **2** with 70% KOH under N_2 gave resorcinol rather than 2,6-dihydroxybenzoic acid, presumably through decarboxylation of the latter.

The final eluant from the column was obtained as an orange solid (yield 0.081%) which analysed for $C_{26}H_{28}O_6$. Both the UV and ^{1}H NMR spectra were typical of a 2′-hydroxychalcone, the latter also revealing the presence of a single methoxy substituent. The identity of the chalcone as **8** (orotinichalcone) was confirmed by the mass spectrum which gave major fragments at *m/z* 285 (**9**) and *m/z* 418 (**10**), corresponding to **4** and **7**, respectively. Acetylation again yielded a triacetate, but in this case chemical shift values for H-3″ and H-4″ were not significantly changed, so establishing the position of the methoxy substituent as adjacent to the pyran ring. The ^{13}C NMR spectrum was recorded (Table 1) and showed agreement with previously published data for chalcones [7].

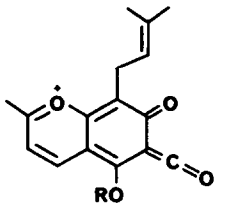
The penultimate eluant from the column was obtained as a yellow solid (yield 0.13%) which analysed for $C_{26}H_{28}O_6$. The UV spectrum suggested a phenolic flavanone and the IR spectrum that the C-4 carbonyl (1680 cm^{-1}) was not H-bonded. Both ^{1}H and ^{13}C NMR spectra suggested that this material was a mixture, the former indicating from relative integration of two methoxy signals a ratio of about 55:45 for two components and the latter showing the presence of 49 carbon resonances including three attributable to two carbons each. All attempts to separate the two components failed. Following acetylation a re-examination of the ^{1}H NMR spectrum now showed a *ca* 4:1 mixture, suggesting that one of the components was converted into the other during the acetylation process. In an attempt to fully convert the mixture to a single compound a portion was refluxed in pyridine for 1 hr, whereupon it yielded orotinichalcone **8** as the sole product.

A detailed analysis of the ^{1}H NMR spectrum of the original mixture allowed the signals for the major isomer to be abstracted. These were comparable in all respects to

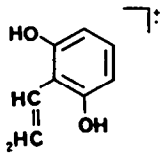
Table 1. ^{13}C NMR chemical shift values for the flavanones **1**, **2**, **11** and **12** and the chalcone **8**

Carbon no.	1	2	11	12	Carbon no.	8
2	76.8	75.9	76.2	66.4	C=O	194.7
3	41.9	41.2	43.0	33.8	C-α	128.2
4	196.4	196.6	191.1	95.4	C-β	136.3
4a	103.4*	103.9*	109.5*	113.8*	1′	107.5
5	158.8	158.2	155.8	151.8**	2′	157.7
6	108.9	109.3**	109.1**	108.3***	3′	109.2
7	159.8	159.8	155.4	160.2	4′	157.0
8	102.4*	102.6*	109.0*	113.7*	5′	111.4
8a	156.8	157.0	157.9	151.6**	6′	164.6
1′	124.5	110.5**	110.5**	110.5***	1	113.0
2′	153.7	154.7	154.7	155.0	2	159.1
3′	116.8	108.9	108.6	108.8	3	107.6
4′	129.9	130.1	130.7	129.7	4	131.7
5′	120.9	108.9	108.6	107.6	5	107.6
6′	126.2	154.7	154.7	152.1	6	159.1
2″	78.3	78.5	77.8	75.8		77.6
3″	115.7	115.6	116.1	115.9		116.9
4″	126.9	126.5	128.6	128.4		128.0
2″-Me$_2$	28.5	28.4	28.3	28.2		27.9
	28.4	28.4	28.1	27.9		27.9
1‴	21.5	21.5	22.0	21.9		21.6
2‴	122.4	122.0	122.9	121.4		122.9
3‴	131.7	132.2	132.6	130.8		130.5
3‴-Me$_2$	25.7	25.7	25.6	25.6		25.3
	17.8	17.8	17.7	17.7		17.5
OMe	—	—	63.7	62.2		63.3

All spectra run in $CDCl_3$ except for **8** which was run in $(CD_3)_2CO$. All spectra run at 90.56 MHz except for **1** and **2** which were obtained at 62.6 MHz. Signals in each column with the same number of asterisks are interchangeable.



4 R = H
9 R = Me



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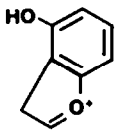

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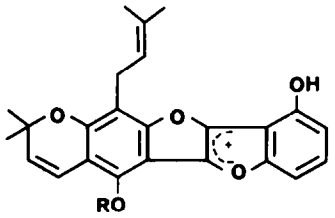


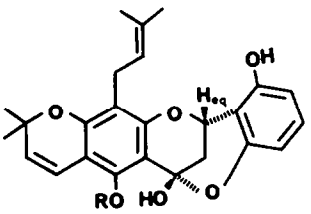
7 R = H
10 R = Me

the spectrum of **2** except for the addition of a methoxy substituent at C-5 indicating that this was the 5-methyl ether of orotinin. The position of the pyran ring was confirmed by an NOE of 8% between the OMe and H-4″. A similar analysis of ^{13}C NMR signals (Table 1) also revealed a close relationship in chemical shifts to **2**, with variations for C-4, C-4a, C-6 and C-8 of the order anticipated to result from methylation of C-5 [8, 9]. The major component of the mixture must therefore be orotinin-5-methyl ether, which has the typical equatorially substituted B-ring, i.e. structure **11**. This hypothesis was supported by the mass spectrum which gave major fragments for **6**, **9** and **10**. One interesting feature of the ^{1}H NMR spectrum of **11** was the non-equivalence of the protons of 1‴-CH_2 due, presumably, to restricted rotation of the B-ring resulting in a preferred conformation for the C-8 side-chain.

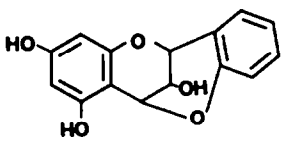
A study of the ^{1}H NMR spectra of the second component of the mixture revealed signals comparable to **11** for 3,3-dimethylallyl, 2,2-dimethylpyran, methoxy and 2′,6′-oxygenated B-ring substituents. There was again a positive NOE (11%) between the OMe and H-4″ resonances. Major differences in the ^{1}H NMR spectra were associated with the ABX system for H-2 and H-3, all three signals being markedly shielded with the X-proton now occurring as a triplet (J = 2.9 Hz) indicating an equatorial configuration and the A and B protons strongly shielded in comparison to **11** but still showing marked non-equivalence.

The ^{13}C NMR spectrum of this component (Table 1) also showed signals comparable to **11** for pyran and prenyl substituents. By contrast, appreciable variation was found for some carbons associated with rings A and B and major differences in respect of ring-C. With reference to the latter both C-2 and C-3 occur *ca* 10 ppm shielded in comparison with **11** whilst no carbonyl can be observed for C-4, it apparently being replaced by a quaternary resonance at 95.4 ppm, suggesting a carbon carrying two oxy-substituents.

From these data the second component of the mixture is tentatively identified as **12**, a hypothesis supported by its conversion into **8** under alkaline conditions. Stereochemistry is relative but **12** has been drawn in the most likely form for an assumed derivation from **11**. The proposed structure could be envisaged to arise via the concerted rearrangement of **11** in which H-2 becomes equatorial but C-2 retains *S*-configuration. The compound cyanomaclurin (**13**) from *Artocarpus integrifolia* [10] represents a precedent for this type of rearrangement but differs in the absence of the 4-hydroxy substituent. Published ^{1}H NMR data for **13** and allied synthetic products [10, 11] suggest comparable stereochemistry in **12** and **13**.



12 R = Me
15 R = H



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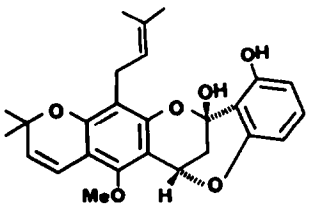

14

A possible alternative structure from the spectroscopic evidence would be **14**. This is not supported by acetylation during which about half of **12** is converted to the diacetate of **11** but the remainder is acetylated unchanged. For the unchanged portion the X proton of the ABX system is shielded by about 0.25 ppm, which must be due to the proximity of an acetate moiety. This effect could be rationalized in **12** by the introduction of the 2'-acetoxy group. However, there is no obvious reason for similar shielding to occur in **14**. Furthermore in **14** H-4 would be expected to show an NOE with the 5-OMe, but none was observed.

Attempts to synthesize **12** from **8** or its demethylated analogue **15** from **2** by treatment with acid [10] were unsuccessful. It is presumed that **12** is present in the seeds prior to extraction and that it does represent a true natural product but this requires to be substantiated.

Flavonoids oxygenated at 2' and 6' are extremely rare in nature [12, 13]. Although a number of 2'-hydroxy compounds are known from the Tephrosieae [14] this appears to be the first example of the 2',6'-pattern within this taxonomic group.

EXPERIMENTAL

UV: EtOH, IR: KCl discs, ^{1}H and ^{13}C NMR: run in $CDCl_3$ with TMS as int. standard; field strength given in text, EIMS: direct probe insert at 70 eV, petrol refers to petroleum spirit (bp 40–60°).

Plant material. Seeds of *L. orotinus* were collected in the Santa Rosa National Park, Guanacaste Province, Costa Rica. A voucher sample of the seeds has been deposited in the Carpological Collection of the Royal Botanic Gardens, Kew, London, U.K. and a voucher for the species is deposited at the Herbarium of the Missouri Botanic Gardens, St. Louis, U.S.A. as part of the collection of the flora of Santa Rosa National Park.

Isolation of flavonoids. Ground seeds (42 g) were extracted separately and successively with petrol, $CHCl_3$ and MeOH. TLC revealed several UV fluorescent compounds in the $CHCl_3$ extract which was concd and subjected to CC over silica gel, eluting with petrol followed by petrol containing increasing amounts of EtOAc. Elution with 10% EtOAc gave **1** (28 mg). From 15% EtOAc a mixture of two compounds was obtained and these were separated by centrifugal PTLC (Si gel, toluene–EtOAc–AcOH 95:5:0.5) to give further **1** (8 mg) followed by **2** (127 mg). From 25% EtOAc a mixture of **11** and **12** (19 mg) was obtained whilst further elution with 30% EtOAc gave another mixture which on separation by centrifugual PTLC (toluene–EtOAc–AcOH 92:7:1) gave **8** (18 mg) and further **11/12** (16 mg). Similar treatment of the petrol extract gave **1** (121 mg), **2** (85 mg), **8** (16 mg) and **11/12** (20 mg).

Minimiflorin (**1**). **1** and its diacetate were identical in all respects (TLC, UV, IR, ^{1}H NMR, EIMS, mmp) to **1** previously isolated from *L. minimiflorus* [5].

Orotinin (**2**). Yellow needles from petrol–EtOAc, mp 186°, $[\alpha]_D^{21}$ −220° (*c*. 1.00, $CHCl_3$). Found: M^+ 422.1723; $C_{25}H_{26}O_6$ requires 422.1729. UV λ_{max}^{MeOH} nm: 273, 309. IR ν_{max} cm^{-1}: 3425, 1630, 1600, 1460, 1380, 1140, 880, 785. ^{1}H NMR (90 MHz): δ1.44 (6H, *s*, 2''-Me_2), 1.65 (6H, *s*, 3'''-Me_2), 2.80 (1H, *dd*, *J* = 18, 5 Hz, H-3_{eq}), 3.12 (1H, *dd*, *J* = 18, 13 Hz, H-3_{ax}), 3.22 (2H, *d*, *J* = 8 Hz, 1'''-CH_2), 5.05 (1H, *t*, *J* = 8 Hz, H-2'''), 5.52 (1H, *d*, *J* = 10 Hz, H-3''), 5.98 (1H, *dd*, *J* = 13, 5 Hz, H-2), 6.43 (2H, *d*, *J* = 8 Hz, H-3', H-5'), 6.64 (1H, *d*, *J* = 10 Hz, H-4''), 7.02 (1H, *t*, *J* = 8 Hz, H-4'), 12.19 (1H, *s*, 5-OH). ^{13}C NMR—see Table 1. EIMS *m/z* (rel. int.): 422 $[M]^+$ (100), 407 (76), 404 (8), 389 (37), 361 (30), 349 (11), 333 (19), 271 (14), 243 (5), 231 (6), 215 (28), 135 (3), 107 (2). Acetylation of **2** (23 mg) using Ac_2O in pyridine followed by normal work-up gave *orotinin triacetate* (18 mg), mp 66–68°. Found: M^+ 548.2045; $C_{31}H_{32}O_9$ requires 548.2046. IR ν_{max} cm^{-1}: 1780, 1690, 1600, 1460, 1380, 1200. ^{1}H NMR (90 MHz): δ1.41, 1.45 (2''-Me_2), 1.63, 1.69 (2'''-Me_2), 2.21 (6H, *s*, 2'-, 6'-AcO), 2.40 (3H, *s*, 5-AcO), 2.51 (H-3_{eq}), 3.18 (1'''-CH_2), 3.49 (H-3_{ax}), 5.02 (H-2'''), 5.52 (H-2), 5.63 (H-3''), 6.35 (H-4''), 7.03 (H-3', H-5'), 7.40 (H-4'). EIMS *m/z* (rel. int.): 506 $[M]^+$ (100), 491 (96), 285 (15), 271 (12), 215 (25), 136 (4), 109 (8), 43 (54).

Alkaline degradation. **2** (127 mg) was dissolved in MeOH (5 ml) and 75% alc. KOH (15 ml) was added. The mixture was refluxed under N_2 for 18 hr, cooled, acidified with 2 N HCl and extracted into Et_2O. The ethereal layer was dried over Na_2SO_4 and evaporated to give resorcinol (27 mg) identical with an authentic sample (UV, IR, ^{1}H NMR, mmp).

Orotinichalcone (**8**). Red needles from Me_2CO, mp 166°. Found: M^+ 436.1881; $C_{26}H_{28}O_6$ requires 436.1886. UV λ_{max}^{MeOH} nm: 275, 368. IR ν_{max} cm^{-1}: 3400, 1620, 1465, 1380, 1100. ^{1}H NMR (250 MHz, $(CD_3)_2CO$): δ1.44 (6H, *s*, 2''-Me_2), 1.63, 1.77 (2 × 3H, 2 × *s*, 3'''-Me_2), 3.27 (2H, *d*, *J* = 7 Hz, 1'''-CH_2), 3.76 (3H, *s*, 6'-OMe), 5.19 (1H, *t*, *J* = 7 Hz, H-2'''), 5.71 (1H, *d*, *J* = 10 Hz, H-3''), 6.49 (2H, *d*, H-3, H-5), 6.59 (1H, *d*, *J* = 10 Hz, H-4''), 7.04 (1H, *t*, *J* = 8 Hz, H-4), 8.49, 8.56 (2H, *ABq*, *J* = 16 Hz, H-α, H-β), 14.55 (1H, *s*, 2'-OH). EIMS *m/z* (rel. int.): 436 $[M]^+$ (56), 421 (19), 418 (19), 403 $[C_{25}H_{23}O_5]^+$ (100), 375 (39), 363 (14), 265 (23), 215 (3), 107 (7), 95 (21). Acetylation of **8** (15 mg) using the same procedure as above gave the triacetate (10 mg), mp 92°. IR ν_{max} cm^{-1}: 1760, 1665, 1600, 1460, 1380. ^{1}H NMR (250 MHz, $(CD_3)_2CO$): δ1.45 (2''-Me_2), 1.64, 1.74 (3'''-Me_2), 2.15 (3H, *s*, 2'-AcO), 2.29 (6H, *s*, 2-, 6-AcO), 3.16 (1'''-CH_2), 3.65 (6'-OMe), 5.07 (H-2'''), 5.82 (H-3''), 6.59 (H-4''), 7.09 (H-α), 7.13 (H-3, H-5), 7.35 (H-β), 7.47 (H-4).

Orotinin-5-methyl ether (**11**) *and 4,6'-epoxyorotiniflavan-4-ol* (**12**). A yellow solid, mp 82°. Found: M^+ 436.1881; $C_{26}H_{28}O_6$ requires 436.1886. UV λ_{max}^{MeOH} nm: 264. IR ν_{max} cm^{-1}: 3450, 1670, 1660, 1600, 1460, 1380. EIMS *m/z* (rel. int.): 436 $[M]^+$ (98), 421 (25), 418 (18), 403 $[C_{25}H_{23}O_5]^+$ (100), 375 (36), 363 (17), 285 (30), 257 (17), 245 (20), 135 (41), 107 (7). ^{1}H NMR of **11** (360 MHz): δ1.44, 1.45 (2 × 3H, 2 × *s*, 2''-Me_2), 1.67 (6H, *s*, 3'''-Me_2), 2.85 (1H, *dd*, *J* = 17.2, 2.8 Hz, H-3_{eq}), 3.06 (1H, *dd*, *J* = 17.2, 13.9 Hz, H-3_{ax}), 3.29 (2H, *d*, *J* = 6.7 Hz, 1'''-CH_2), 3.85 (3H, *s*, 5-OMe), 5.07 (1H, *t*, *J* = 6.7 Hz, H-2'''), 5.62 (1H, *d*, *J* = 10 Hz, H-3''), 6.02 (1H, *dd*, *J* 13.9, 2.8 Hz, H-2), 6.46 (2H, *d*, *J* = 8.2 Hz, H-3', H-5'), 6.64 (1H, *d*, *J* = 10 Hz, H-4''), 7.04 (1H, *t*, *J* = 8.2 Hz, H-4'). ^{13}C NMR of **11**—see Table 1. ^{1}H NMR of **12** (360 MHz): δ1.33, 1.38 (2 × 3H, 2 × *s*, 2''-Me_2), 1.62, 1.73 (2 × 3H, 2 × *s*, 3'''-Me_2), 2.24 (1H, *dd*, *J* = 13.4, 2.9 Hz, H-3_{eq}), 2.53 (1H, *dd*, *J* = 13.4, 2.9 Hz, H-3_{ax}), 3.20 (2H, *m*, 1'''-CH_2), 3.91 (3H, *s*, 5-OMe), 5.07 (1H, *t*, H-2'''), 5.51 (1H, *d*, *J* = 10 Hz, H-3''), 5.74 (1H, *t*, *J* = 2.9 Hz, H-2), 6.39, 6.45 (2H, 2 × *dd*, *J* = 7.9, 1.0 Hz, H-3' and H-5'), 6.41 (1H, *d*, *J* = 10 Hz, H-4''), 7.02 (1H, *t*, *J* = 7.9 Hz, H-4'). ^{13}C NMR of **12**—see Table 1. Acetylation of the mixture of **11** and **12** following the same procedure as above gave an oil. ^{1}H NMR (250 MHz) of the resulting mixture gave the following significant resonances. For **11**: δ6.73 (H-2), 3.52 (H-3_{ax}), 2.63 (H-3_{eq}); for **12**: δ5.51 (H-2).

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