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FLAVONOIDS FROM *TEPHROSIA*—VII[1]

THE CONSTITUTION AND ABSOLUTE CONFIGURATION OF LUPINIFOLIN AND LUPINIFOLINOL, TWO FLAVANONES FROM *TEPHROSIA LUPINIFOLIA* BURCH (DC)

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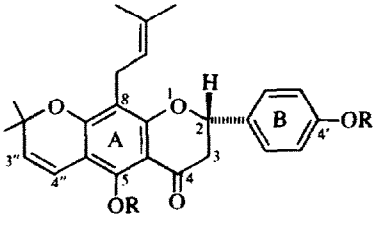
Abstract—The structure and absolute configuration of lupinifolin, (2*S*) - 4',5 - dihydroxy - 8 - (3''' - methyl - 2''' - butenyl) - 2'',2'' - dimethylpyrano[5''.6'' - g]flavanone, and lupinifolinol, (2*R*,3*R*) - 8 - (3''' - methyl - 2''' - butenyl) - 3,4',5 - trihydroxy - 2'',2'' - dimethylpyrano[5''.6'' - g]flavanone, have been deduced from spectroscopic and chemical evidence.

The root of *Tephrosia lupinifolia* Burch (DC) has been employed by members of primitive societies in Southern Africa as an abortifacient and to commit suicide.[2] As *Tephrosia* species are known to be a source of flavanoids[1,3-6] we undertook the phytochemical investigation of *T. lupinifolia*.

The dichloromethane extract of the aerial parts and roots gave after chromatographic separation, two new flavanones which we have named lupinifolin and lupinifolinol.

Lupinifolin, $[\alpha]_D^{24} - 8{\cdot}7°$ (*c* 1·15 in $CHCl_3$) analysed for $C_{25}H_{26}O_5$ and is assigned structure (1) [(2*S*) - 4',5 - dihydroxy - 8 - (3''' - methyl - 2''' - butenyl) - 2'',2'' - dimethylpyrano[5''.6'' - g]flavanone].

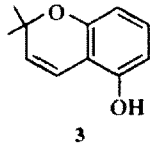
The IR spectrum showed strong OH absorption at 3250 cm^{-1}. The presence of one or more phenolic OH groups was indicated by the strong coloration with ethanolic ferric chloride. The band at 1620 cm^{-1} was assigned to the chelated flavanone CO group.[7]



1: R = H
2: R = Me
4: R = Ac

The nature of the groups present in lupinifolin was indicated by its NMR spectrum (Table 1). The doublets at τ 3·37 and τ 4·52 (J = 10·0 Hz), each equivalent to one proton, and the singlet at τ 8·56 (6H) are characteristic of the *cis* double bond and *gem*-dimethyl group of a 2,2 - dimethylchromene moiety.[8,9] The presence of a C-γ,γ-dimethylallyl group was inferred from the singlet at τ 8·36 (6H), the doublet at τ 6·80 (2H, J = 7·0 Hz) and the triplet at τ 4·86 (1H, J = 7·0 Hz).[10-13] Signals due to four aromatic protons were discernible at τ 2·72 (2H, J = 8·5 Hz) and τ 3·16 (2H, J = 8·5 Hz) and these could be readily analysed in terms of a *p*-disubstituted benzene ring. The salient feature of the NMR spectrum of lupinifolin is the ABX system, diagnostic for the C_2 and C_3 protons of a flavanone.[14,15] The C_2 proton, the X part, appears as a double doublet at τ 4·70 (J_{AX} = 12·7, J_{BX} = 3·3 Hz) while the C_3 protons, the AB part, appear at τ 6·97 and τ 7·22 (J_{AB} = 17·3, J_{AX} = 12·7, J_{BX} = 3·3 Hz). The singlets at τ −2·20 (1H) and τ 3·70 (1H), which both disappeared upon addition of D_2O, were assigned to the two phenolic protons.

Chemical evidence for the presence of two phenolic OH groups in lupinifolin (1) was provided by methylation to give the dimethyl ether (2).



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The substitution pattern of lupinifolin was determined from NMR and mass spectral data. The presence of a chelated C_5–OH was evident from the low field position (τ-2·20) of the phenolic proton resonance. The non-chelated OH group (τ 3·7)

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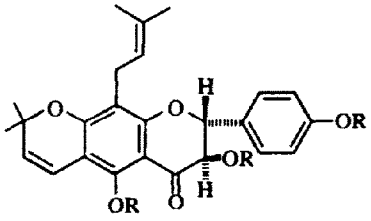
Table 1. Chemical shifts (τ) and multiplicities* (J in Hz) in the NMR spectra of the flavanones

	2-H	3-H	3-OH	5-OH	5,4'-OAc	5,4'-OMe	B-ring H	4'-OH	2''-*gem*-Me_2	3''-H	4''-H	1'''-H	2'''-H	3'''-*gem*-Me_2
Lupinifolin (1)	4·70 dd $J_{2,3ax}$ 12·7 $J_{2,3eq}$ 3·3	6·97 dd 7·22 dd $J_{3ax,3eq}$ 17·3 $J_{2,3ax}$ 12·7 $J_{2,3eq}$ 3·3		−2·20			2·72 d 3·16 d J 8·5	3·7	8·56	4·52 d $J_{3'',4''}$ 10·0	3·37 d $J_{3'',4''}$ 10·0	6·80 d J 7·0	4·86 t J 7·0	8·36
5,4'-O,O-Dimethyl-lupinifolin (2)	4,65 dd $J_{2,3ax}$ 12·8 $J_{2,3eq}$ 3·2	7·03 dd 7·22 dd $J_{3ax,3eq}$ 17·0 $J_{2,3ax}$ 12·8 $J_{2,3eq}$ 3·2				6·17	2·64 d 3·06 d J 8·5		8·56	4·42 d $J_{3'',4''}$ 10·0	3·38 d $J_{3'',4''}$ 10·0	6·73 d J 7·0	4·82 t J 7·0	8·34
Lupinifolin diacetate (4)	4·58 dd $J_{2,3ax}$ 13·1 $J_{2,3eq}$ 3·2	7·06 dd 7·22 dd $J_{3ax,3eq}$ 16·5 $J_{2,3ax}$ 13·1 $J_{2,3eq}$ 3·2			7·60 7·70		2·56 d 2·88 d J 8·5		8·56	4·39 d $J_{3'',4''}$ 10·0	3·65 d $J_{3'',4''}$ 10·0	6·71 d J 7·0	4·85 t J 7·0	8·34
Lupinifolinol (5)	5·05 d J 12·0	5·50 d J 12·0	6·2	−2·38			2·65 d 3·23 d J 8·5	3·9	8·55	4·50 d $J_{3'',4''}$ 10·0	3·37 d $J_{3'',4''}$ 10·0	6·84 d J 7·0	4·89 t J 7·0	8·36 (3H) 8·41 (3H)
Lupinifolinol triacetate (6)	4·65 d J 12·0	4·36 d J 12·0	3-OAc 7·99		7·60 7·70		2·53 d 2·86 d J 8·5		8·54	4·39 d $J_{3'',4''}$ 10·0	3·65 d $J_{3'',4''}$ 10·0	6·75 d J 7·0	4·90 t J 7·0	8·35 (3H) 8·41 (3H)

*d = doublet, dd = double doublet, t = triplet



5: R = H
6: R = Ac

could thus be located in either the A- or the B-ring. The mass spectrum of lupinifolin (Scheme 1) showed that the fragments at *m/e* 271 (25%) and *m/e* 120 (8%) could be rationalized only if the non-chelated OH group and the γ,γ-dimethylallyl side-chain were assigned to the B- and A-ring, respectively. The non-chelated OH group is therefore located at $C_{4'}$, in agreement with the NMR data (see above) while the γ,γ-dimethylallyl side-chain could be located at either C_6 or C_8.

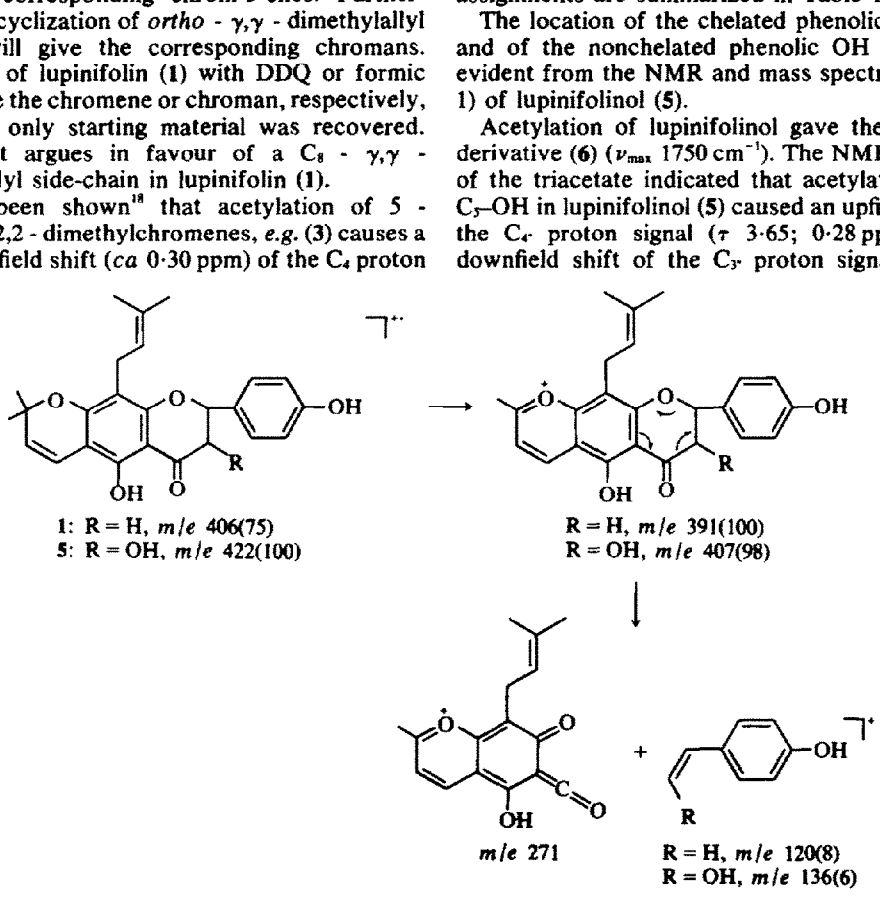
Cardillo *et al.*[16,17] have shown that cyclodehydrogenation of *ortho* - γ,γ - dimethylallyl phenols with 2,3 - dichloro - 5,6 - dicyanobenzoquinone (DDQ) yield the corresponding chrom-3-enes. Furthermore acid cyclization of *ortho* - γ,γ - dimethylallyl phenols will give the corresponding chromans. Treatment of lupinifolin (**1**) with DDQ or formic acid to give the chromene or chroman, respectively, failed and only starting material was recovered. This result argues in favour of a C_8 - γ,γ - dimethylallyl side-chain in lupinifolin (**1**).

It has been shown[18] that acetylation of 5 - hydroxy - 2,2 - dimethylchromenes, *e.g.* (**3**) causes a marked upfield shift (*ca* 0·30 ppm) of the C_4 proton signal while the C_3 proton signal suffers a small downfield shift (*ca* 0·10 ppm). Acetylation of lupinifolin (**1**) gave the diacetate **4** (ν_{max} 1760 cm^{-1}). The NMR spectrum of the diacetate derivative (**4**) shows that the signal of the $C_{4'}$ proton (τ 3·65, d) is shifted upfield (0·28 ppm) compared with that in lupinifolin (**1**) (τ 3·37, d). The signal of the $C_{3'}$ proton (τ 4·39, d) suffered a small downfield shift (0·13 ppm). The γ,γ-dimethylallyl side-chain is therefore located at C_8 and the structure **1** is assigned to lupinifolin.

Lupinifolinol (**5**), $C_{25}H_{26}O_6$, $[\alpha]_D^{24}$ + 26·8° (*c* 1·12 in $CHCl_3$) is (2*R*,3*R* - 8 - (3‴ - methyl - 2‴ - butenyl) - 3,4',5 - trihydroxy - 2″,2″ - dimethylpyrano[5″.6″-g]flavanone. The NMR spectrum (Table 1) indicated the presence of a chelated phenolic proton (τ − 2·38), and a non-chelated phenolic proton (τ 3·9). The singlet at τ 6·2 (1H, D_2O exchangeable) is assigned to the C_3–OH. The doublets at τ 5·05 (1H) and τ 5·50 (1H), J = 12·0 Hz, are characteristic of the antiperiplanar conformation of the C_2 and C_3 protons of a 3-hydroxyflavanone.[19] The remainder of the NMR spectrum was similar to that of lupinifolin (**1**) and assignments are summarized in Table 1.

The location of the chelated phenolic OH at C_5 and of the nonchelated phenolic OH at $C_{4'}$ was evident from the NMR and mass spectra (Scheme 1) of lupinifolinol (**5**).

Acetylation of lupinifolinol gave the triacetate derivative (**6**) (ν_{max} 1750 cm^{-1}). The NMR spectrum of the triacetate indicated that acetylation of the C_5–OH in lupinifolinol (**5**) caused an upfield shift of the $C_{4'}$ proton signal (τ 3·65; 0·28 ppm) and a downfield shift of the $C_{3'}$ proton signal (τ 4·39;



1: R = H, *m/e* 406(75)
5: R = OH, *m/e* 422(100)

R = H, *m/e* 391(100)
R = OH, *m/e* 407(98)

m/e 271

R = H, *m/e* 120(8)
R = OH, *m/e* 136(6)

SCHEME 1. Mass spectral fragmentation of lupinifolin and lupinifolinol.

0·11 ppm); the γ,γ-dimethylallyl side-chain is thus located at C_8.[18] The structure **5** is therefore assigned to lupinifolinol.

Absolute configuration of lupinifolin and lupinifolinol

The CD spectra of (−)-2*S*-flavanones and (+)-(2*R*,3*R*)-3-hydroxyflavanones, having the 2-aryl group substituted equatorially to the dihydro-γ-pyrone ring in the former or having the 2,3-groups substituted equatorially in the latter, exhibit a positive Cotton effect due to the $n \rightarrow \pi^*$ transition (320–330 nm) and a negative Cotton effect in the $\pi \rightarrow \pi^*$ region (270–290 nm).[20] In view of the high value of the coupling constant ($J_{2,3} \sim 12$ Hz) between protons in the 2 and 3 position of the heterocyclic ring, it has been concluded[19] that all natural flavanones and 3-hydroxyflavanones exist in the thermodynamically favoured conformation with the 2 or 2,3-substituents equatorial.

Since the 2-aryl group in (−)-lupinifolin (**1**) is equatorial ($J_{2,3ax} = 12·7$ Hz) the positive Cotton effect at 322 nm ($\Delta\epsilon + 1·5$) and the negative Cotton effect at 297 nm ($\Delta\epsilon - 6·4$) (Fig. 1) allows the assignment of the *S* configuration at C-2 in (−)-lupinifolin (**1**).

The CD spectrum of (+)-lupinifolinol (**5**) (Fig. 1) shows a positive Cotton effect at 327 nm ($\Delta\epsilon + 1·0$) and a negative Cotton effect at 300 nm ($\Delta\epsilon - 5·2$). Since the 2,3-substituents are equatorial ($J_{2,3} = 12·0$ Hz) the (2*R*, 3*R*) configuration is assigned to lupinifolinol (**5**).

Varian HA-100 spectrometer with TMS as internal standard. Optical rotations were measured with a Perkin-Elmer 411 polarimeter. CD spectra were measured for solns in MeOH (JASCO J-20 spectropolarimeter). Silica gel (0·05–0·20 mm) was used for column chromatography.

Isolation of lupinifolin and lupinifolinol. The sun-dried and ground plant material (1·3 kg) was extracted with CH_2Cl_2 for 24 hr in a Soxhlet apparatus. The CH_2Cl_2 extract was evaporated and the residue dissolved in MeOH:H_2O (9:1, 2 l). The aqueous MeOH soln was extracted with n-hexane (20 × 250 ml). Water was added to the aqueous MeOH until the ratio of MeOH to H_2O was 3:1. The resulting soln was extracted with benzene (10 × 400 ml). The combined benzene extracts yielded a brown gum (11·8 g). The gum was dissolved in $CHCl_3$ and fractionated by column chromatography using $CHCl_3$ as eluent. Fractions (100 ml) were collected and appropriate fractions (TLC, silica gel; $CHCl_3$:MeOH, 96:4 v/v) were combined.

Fraction 1. Rechromatography on SiO_2 with $CHCl_3$ and crystallization from benzene-n-hexane gave **1** (3·5 g), m.p. 117–119°; $[\alpha]_D^{24}$ −8·7° (*c* 1·15 in $CHCl_3$); λ_{max} 224 sh, 267 sh, 275, 297 sh, 314 and 364 nm (log ε 4·38, 4·68, 4·72, 4·17, 4·15 and 3·55); ν_{max} 3250 (OH) and 1620 (CO) cm^{-1}; *m/e* 406 (75), 391 (100), 363 (4), 351 (5), 335 (2), 285 (8), 271 (25), 243 (8), 215 (40) and 120 (8); $\Delta\epsilon_{414}$ 0, $\Delta\epsilon_{364} + 1·6$, $\Delta\epsilon_{330} + 0·8$, $\Delta\epsilon_{322} + 1·5$, $\Delta\epsilon_{314}$ 0, $\Delta\epsilon_{297} - 6·4$, $\Delta\epsilon_{264}$ 0, $\Delta\epsilon_{230} + 4·8$ and $\Delta\epsilon_{230} + 7·0$ (Found: C, 74·08; H, 6·48. $C_{25}H_{26}O_5$ requires: C, 73·87; H, 6·45%).

Fraction 2. Rechromatography on SiO_2 with $CHCl_3$ and crystallization from acetone-n-hexane gave **5** (1·1 g), m.p. 121–123°; $[\alpha]_D^{24}$ +26·8° (*c* 1·12 in $CHCl_3$); λ_{max} 225 sh, 267 sh, 275, 296 sh, 316 and 364 nm (log ε 4·26, 4·53, 4·57, 4·04, 4·04 and 3·36); ν_{max} 3450 (OH) and 1620 (CO) cm^{-1};

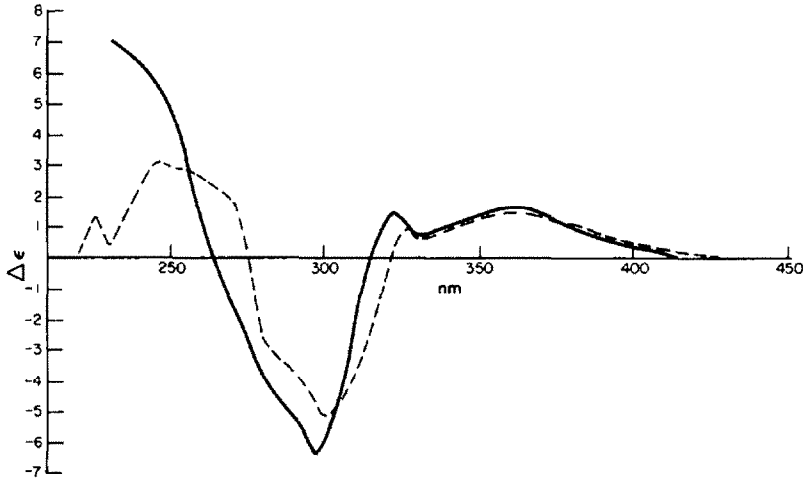


Fig 1. CD spectra of lupinofolin (**1**) (—) and lupinifolinol (**5**) (---).

EXPERIMENTAL

M.ps were determined on a Kofler hot-stage apparatus. UV absorptions were measured for solns in MeOH (Unicam SP 800 spectrometer). IR were recorded on a Unicam SP 200 spectrometer using KBr. Mass spectra were taken on an A.E.I. MS9 double-focusing spectrometer. NMR spectra were recorded for solns in $CDCl_3$ on a

m/e 422 (100), 407 (98), 287 (90), 271 (57), 245 (71), 243 (42), 231 (85), 215 (50), 189 (42), 136 (5) and 107 (34); $\Delta\epsilon_{430}$ 0, $\Delta\epsilon_{364} + 1·5$, $\Delta\epsilon_{330} + 0·6$, $\Delta\epsilon_{327} + 1·0$, $\Delta\epsilon_{321}$ 0, $\Delta\epsilon_{300} - 5·2$, $\Delta\epsilon_{275}$ 0, $\Delta\epsilon_{243} + 3·2$, $\Delta\epsilon_{230} + 0·4$, $\Delta\epsilon_{225} + 1·4$ and $\Delta\epsilon_{220}$ 0 (Found: C, 71·15; H, 6·24. $C_{25}H_{26}O_6$ requires: C, 71·07; H, 6·20%).

Methylation of lupinifolin. A mixture of **1** (100 mg),

anhyd K_2CO_3 (1 g) and MeI (1 ml) in acetone (20 ml) was refluxed for 4 hr to give **2** (68 mg), m.p. 98–100° (from light petroleum 40–60°); ν_{max} 1645 (CO) cm^{-1} (Found: C, 74·72; H, 7·02. $C_{27}H_{30}O_5$ requires: C, 74·63; H, 6·96%).

Lupinifolin diacetate (**4**). Acetylation of **1** (100 mg) with Ac_2O (3 ml) and pyridine (1 ml) gave **4** (96 mg), m.p. 105–106° (from light petroleum 40–60°); λ_{max} 261, 296 and 345 nm (log ϵ 4·64, 4·09 and 4·04); ν_{max} 1760 (acetate CO) and 1680 (CO) cm^{-1} (Found: C, 70·92; H, 5·96. $C_{28}H_{30}O_7$ requires: C, 71·01; H, 6·06%).

Lupinifolinol triacetate (**6**). Acetylation of **5** (100 mg) with Ac_2O (3 ml) and pyridine (1 ml) gave **6** (85 mg) as a colourless oil; $\nu_{max}^{CHCl_3}$ 1750 (acetate CO) and 1690 (CO) cm^{-1} (Found: M^+, 548·2044. $C_{31}H_{32}O_9$ requires: M, 548·2046).

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