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Spectral Assignments and Reference Data

Complete ^{13}C and ^{1}H NMR spectral assignments of two isoflavones from the roots of *Dalbergia horrida*

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Two methoxylated isoflavones were isolated form the roots of *Dalbergia horrida*. These compounds show great promise as pharmaceutical agents. The ^{1}H and ^{13}C NMR spectra of the compounds were completely assigned by using a combination of 2D NMR experiments which included ^{1}H–^{1}H COSY, HMQC and HMBC studies.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; 2D NMR; HMQC; HMBC; *Dalbergia horrida*; isoflavones; flavonoids

INTRODUCTION

Plants belonging to the genus *Dalbergia* are known to be rich sources of isoflavones and neoflavones.[1,2] The phytochemical and pharmacological studies of *Dalbergia* species have been reviewed.[3] *Dalbergia horrida* Dennst. (*Leguminosae*; sub-family, *papilionaceae*) is a straggling shrub armed with thorns with strap-shaped pods that grows in southern India. As there is no previous phytochemical report on *D. horrida,* we have systematically examined the roots. We report here the complete ^{1}H NMR and ^{13}C NMR spectra of dalspinin (**1**) and dalspinosin (**2**). In the case of 5,7-dihydroxyflavonoids, a few reported techniques have been useful in determining the positions of the substituents at C-6 or C-8 of flavonoids, including the Gibb's test,[5] aluminium chloride-induced UV shift,[6,7] chemical shift of the 5-OH signal[8–10] and the chemical shift of the lone A-ring proton.[11] In the present study, the complete assignments of the protonated and non-protonated carbons and the position of the methoxyl group at C-6 in both **1** and **2** were assigned by interpretation of data from 2D NMR experiments such as HMQC and HMBC.

RESULTS AND DISCUSSION

Compound **1** was isolated as a cream-colored solid, m.p. 186–187 °C, and **2** a pale yellow solid, m.p. 165 °C. The ^{1}H NMR data for both compounds were in accordance with those reported in the literature.[12] The carbon signals at δ 130.34 and 93.19, due to C-6 and C-8, respectively, could not be specified as they were found to be interchangeable by a Wessely–Moser rearrangement[13] and so were the ^{1}H NMR signals of the corresponding protons due to substitution at either C-6 or C-8.

The HMQC NMR study revealed that the proton signal at δ 6.52 showed a correlation with the carbon signal at δ 93.19, assigned to the lone A-ring proton, attached to either C-6 or C-8. The methoxyl-substituted carbon was found to occur downfield. The HMBC NMR study clearly indicated that the unambiguous 5-hydroxy signal appearing at δ 13.35 showed multiple bond coupling to carbon signals observed at δ 152.90, 108.46 and 130.34, of which the first two were assigned to C-5 and C-10, respectively. The other signal was thus assigned to C-6 as this corresponded to a three-bond coupling with the C-6 carbon. Hence the methoxyl group was confirmed to be at the C-6 position. The presence of a methoxyl group at C-6 was also confirmed by the downfield shift of the C_5-OH proton at δ 13.30, the position of the lone A-ring proton at δ 6.52 and the bathochromic shift in the UV spectrum on addition of $AlCl_3$. The complete assignments for both **1** and **2** were determined by 2D NMR spectroscopy and are given in Table 1.

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EXPERIMENTAL

Product isolation

The roots of *D. horrida* were collected in collaboration with the Kerala Forest Research Institute (KFRI), Peechi, Trichur District of Kerala, India, and the authenticity was confirmed by KFRI (specimen voucher No. 6699, Renuka and Vijayakumaran). The shade-dried roots (1.2 kg) were extracted successively with light petroleum (b.p. 60–80 °C), benzene, chloroform, ethyl acetate and ethanol (6 × 6 h each). The benzene extract was taken up and the solvent was evaporated to give a brown residue (6 g). This was column chromatographed on silica gel with benzene to yield **1** (30 mg) and on using benzene–ethyl acetate (9 : 1) afforded **2** (25 mg).

Compound 1.
5,7-Dihydroxy-3′,4′-methylenedioxyisoflavone (dalspinin), pale yellow solid, m.p. 186–187 °C. UV (MeOH), λ_{max} (nm) 268, 293sh, +$AlCl_3$ 274, +NaOAc 273; EIMS, *m/z* (relative intensity, %) 329 (25), 328 (100), 285 (55), 89 (15), 69 (25); IR (KBr), ν_{max} (cm^{-1}) 3380 (OH), 1660 (C═O), 1626, 1583, 1507, 1493, 1379, 1330, 1241, 1104, 1033 (OCH_2O), 1061, 876; ^{1}H and ^{13}C NMR data are given in Table 1

Compound 2.
5,7-Dihydroxy-6,3′,4′-trimethoxyisoflavone (dalspinosin), cream-colored amorphous powder, m.p. 165 °C. UV (MeOH), λ_{max} (nm) 265, 290sh,

Table 1. NMR spectral data for compounds **1** and **2**

	Compound **1**		Compound **2**	
Position	$\delta(^{1}H)$	$\delta(^{13}C)$	$\delta(^{1}H)$	$\delta(^{13}C)$
2	7.85, 1H, s	152.50	7.89, 1H, s	152.62
3	—	123.18	—	123.31
4	—	181.10	—	181.37
5	—	152.90	—	152.96
6	—	130.34	—	130.40
7	—	155.10	—	155.17
8	6.52, 1H, s	93.19	6.50, 1H, s	93.17
9	—	153.30	—	153.42
10	—	108.52	—	106.46
1′	—	124.30	—	123.31
2′	7.04, 1H, d (J = 2 Hz)	114.05	7.02, 1H, d, (J = 2Hz)	112.45
3′	—	147.87	—	147.8
4′	—	147.87	—	147.8
5′	6.86, 1H, d (J = 8Hz)	109.60	6.93, 1H, d (J = 8Hz)	111.35
6′	6.94, 1H, d (J = 8Hz)	122.46	7.09, 1H, d (J = 8Hz)	121.31
Ome	4.03, 3H, s	60.77	3.92, 3.93, 4.03, each 3H, s	60.87, 56.00
OCH_2O	5.99, 2H, s	101.20	—	—
OH	13.35, 1H, s	—	13.10	—

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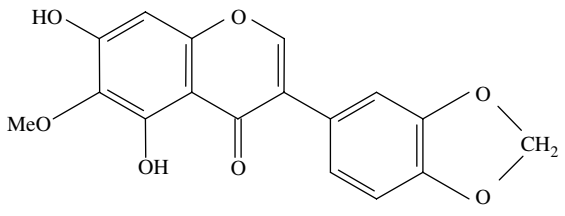
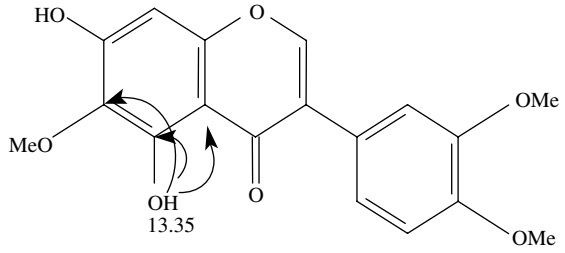
Spectral Assignments and Reference Data



Dalspinin



Dalspinosin

HMBC correlation

+$AlCl_3$ 272, +NaOAc 271; EIMS, m/z (relative intensity, %) 345 (30), 344 (100), 301 (50), 229 (35), 69 (20); IR (KBr), ν_{max} (cm^{-1}) 3354 (OH), 2937, 1662 (C═O), 1580, 1518, 1456, 1368, 1256, 1117, 1071, 1022 (OCH_2O) and 1000; ^1H and ^{13}C NMR data are given in Table 1

NMR Spectra

All NMR spectra were obtained on a Bruker-400 FT NMR spectrometer equipped with a ^1H/multinuclear computer-switchcable 5 mm probe (^1H, 400 MHz, 90° pulse width 14 μs; ^{13}C, 100.6 MHz, 90° pulse width 11 μs). A sample (15–20 mg) taken in 0.7 ml of $CDCl_3$ was purged with argon to remove oxygen and sealed in a 5 mm NMR tube. Using the variable-temperature Unit, the temperature was maintained constant. The spectra were recorded using $SiMe_4$ ($\delta = 0$ ppm) and the centre line of the $CDCl_3$ triplet ($\delta = 77.0$ ppm) as internal standards. Initial assignments were carried out with the help of ^1H spectra and regular ^{13}C NMR spectra with a ^1H spectral width of 5600 Hz and a ^{13}C spectral width of 23 000 Hz, using 32K data points (zero filled to 64K) in each case. Coupling constants in the ^{13}C and ^1H spectra are given in hertz with d indicating a doublet. The complete assignments were made with the aid of 1D NOE, HMQC and HMBC 2D NMR experiments.

One-dimensional steady-state NOE spectra were acquired at 400 MHz with a sweep width of 6400 Hz. The experiment was carried out with the following parameters: time domain size 16K (number of data points of the FID), 8 scans, high-power level on the f_1 channel −3dB, power level for NOE buildup 70 dB (this was optimized by changing the power level), as 90° high-power pulse on the f_1 channel 7 μs, relaxation delay 1 s and irradiation time 50 ms.

In HMQC, the time domain size was 1 K with 16 scans with a high-power level on the f_1 and f_2 channels of −3dB; 90° and 180° high-power pulses of 7 and 14 μs, respectively, were used. The relaxation delay D1 was 1.5 s and the delay for the creation of anti-phase magnetization ($1/2J_{XH}$) was 3.45 ms and a short delay of D13 = 3 μs was given to acquire the spectrum. The composite pulse decoupling sequence was GARP.

In HMBC, the time domain size used was 4K with 64 scans and using a high-power level on the f_1 and f_2 channels of −3dB. The 90° and 180° high-power pulses were 7 and 14 μs, respectively. The delay for evolution of long-range coupling was 50 ms. A relaxation delay of 1.5 s was used. The delay for the creation of anti-phase magnetization ($1/2J_{XH}$) was 3.45 ms The spectral width in the f_1 channel was 260 ppm.

Acknowledgements

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REFERENCES

1. Narayanan V, Seshadri TR. *Indian J. Chem.* 1971; **9**: 14.
2. Harborne JB, Mabry TJ, Mabry H. *The Flavonoids*. Chapman and Hall: London, 1970; 747, 804.
3. Khan MSY, Javed K. *Indian Drugs* 1997; **34**: 7.
4. Nair KKN. *Taxon* 1981; **30**: 46.
5. King FW, King TJ, Manning LC. *J. Chem. Soc.* 1957; 562.
6. Sherif EA, Gupta RC, Krishnamurti M. *Tetrahedron Lett.* 1980; **21**: 641.
7. Amaro-Luis JM, Delgado-Mendez P. *J. Nat. Prod.* 1993; **56**: 610.
8. Bohlmann F, Zdero C, Robinson H, King RM. *Phytochemistry* 1981; **20**: 2245.
9. Ruangrungsi N, Iinuma M, Tanaka T, Ohyama M, Yokoyama J, Miizuno M. *Phytochemistry* 1992; **31**: 999.
10. Tahara S, Ingham J, Hanawa F, Mizutani J. *Phytochemistry* 1991; **30**: 5, 1683.
11. Dauguet J-C, Bert M, Dolley J, Bekeart A, Lewin G. *Phytochemistry* 1993; **33**: 6, 1503.
12. Gandhidasan R, Nagarajan NS, Narayanan V, Neelakantan S, Raman PV. *Indian J. Chem., Sect. B* 1982; **21**: 385.
13. Shirataki Y, Yokoe I, Endo M, Komatsu M. *Chem. Pharm. Bull.* 1985; **33**: 444.