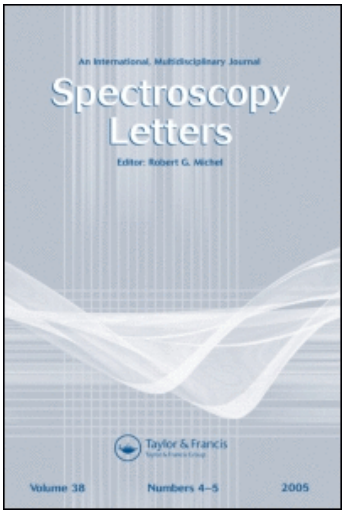

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Structural Elucidation of Isomeric Pyranoflavones by 2D-Noesy

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STRUCTURAL ELUCIDATION OF ISOMERIC PYRANOFLAVONES BY 2D-NOESY

Key Words: 2D-NOESY, Differentiation of isomeric pyranoflavones, carpachromene, atalantoflavone and racemoflavone.

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ABSTRACT:

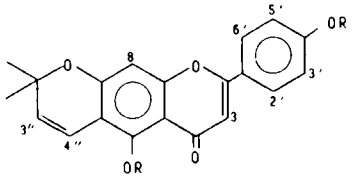
Unequivocal assignments of the uncoupled protons at H-3 and H-6/H-8 of pyranoflavones by 2D-NOESY experiments have made it possible to distinguish linear and angular isomers. This study also suggests that the linear structure (carpachromene, 1) assigned to a pyranoflavone isolated from *Atalantia ceylanica* should be revised to angular form (atalantoflavone, 4). 2D-NOESY is also useful for differentiating 3'4'- and 2'4'- disubstituted B-ring flavones.

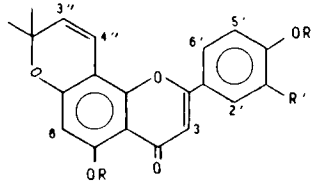
INTRODUCTION

1D-^{1}H NMR spectroscopy has been extensively utilized in the structural elucidation of flavonoids and related compounds. However, the assignment of signals of uncoupled protons, H-3 and H-6/H-8 present in A-ring trisubstituted flavonoids are difficult due to their close or often overlapping chemical shifts[1,2]. This has resulted in the erroneous assignments of several structures as in isomeric pyranoflavones where distinction between linear and angular isomers were not possible by 1D-^{1}H NMR spectroscopy[3,4]. Such a situation calls for chemical degradation[5] or unequivocal synthesis[6,7] for the establishment of structures. Aromatic solvent induced shift is a useful complementary technique but there too the assignments are based on empirical considerations[1,8]. We have explored the utility of 2D-NOESY as an aid for elucidation of structures of isomeric pyranoflavones.

RESULTS AND DISCUSSIONS

During the course of structural elucidation of two new pyranoflavones *viz.* atalantoflavone (4) and racemoflavone (7), isolated from *Atalantia racemosa*[6], several discrepancies were noticed in the reported physical and spectral properties of pyranoflavone 1 (FIG. 1). Dreyer and Park assigned linear structure 1 to a pyranoflavone isolated from *Psoralea missionis*[3] on the basis of spectroscopic evidences and color reaction. Inspite of significant differences in physical and spectral properties (Tables 1 and 2), pyranoflavones isolated from *Atalantia ceylanica*[4] and *Flindersia laevicarpa*[5] have also been attributed the same structure 1 on the basis of color test, spectroscopic data and chemical





1. R = H
2. R = Me
3. R = Ac

4. R = R' = H
5. R = Me, R' = H
6. R = Ac, R' = H
7. R = H, R' = OMe
8. R = Me, R' = OMe

FIG. 1: Structures of Pyranoflavones.

degradation. The confusion in their structural assignments mainly stems from the interchange of assignments of uncoupled protons H-3 and H-6/H-8 by the various workers.

In this communication, we report the application of 2D-NOESY for the correct assignments of these protons (H-3 and H-6/H-8). Three compounds *viz.* atalantoflavone dimethyl ether (5), carpachromene dimethyl ether (2) and racemoflavone dimethyl ether (8) whose structures were unequivocally established by synthesis[6,7] served as models for our studies. Since H-3 and H-6/H-8 protons in these compounds lack scalar couplings, it was thought that the nOe studies would be useful for their unambiguous assignments. Thus 2D-NOESY spectral studies of these compounds were carried out.

TABLE 1

Reported Melting Points and UV Spectral Data of Pyranoflavones 1, 4 and their Derivatives.

SR. NO.	COMPOUND (ASSIGNED STRUCTURE)	MELTING POINT (°C)	UV	REFERENCES
1.	Carpachromene from *P. laevicarpa* (1)	239-41	234 (4.48), 300 sh (4.49), 308 (4.50) and 344 (4.44)	5
2.	Carpachromene dimethyl ether (2)	156	225 (4.52), 267 (4.37), 327 (4.47)	5
3.	Carpachromene diacetate (3)	239-41	222 (4.51), 265 (4.46), 317 (4.35)	5
4.	Pyranoflavone from *P. missionis* (1)	276-78	236, 273, 279, 313, 330, 355	3
5.	Diacetate derivative of pyranoflavone from *P. missionis* (3)	216-19	228, 283, 322	3
6.	Pyranoflavone from *A. ceylanica* (1)	268-70	233 (3.75), 275 (3.66), 310 (3.47), 327sh (3.36)	4
7.	Atalantoflavone from *A. racemosa* (4)	289-90	233 (4.51), 277 (4.45) 312 (4.42), 328sh (4.27)	6
8.	Atalantoflavone dimethyl ether (5)	207-09	231 (4.41), 275 (4.45) 302 (4.25), 348sh (4.27)	6
9.	Atalantoflavone diacetate (6)	221-23	232 (4.50), 271 (4.42),	6

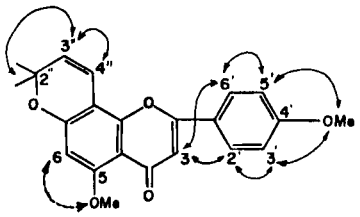
TABLE 2

Reported ^{1}H NMR Spectral Data of Pyranoflavones 1, 4 and their Derivatives.

SR. NO.	COMPOUND (ASSIGNED STRUCTURE)	H-3"	H-4"	H-3	H-6	H-8	GEM DIMETHYL	H-2' H-6'	H-3' H-5'		REFERENCES
1.	Carpachromene from *F. laevicarpa* (1)	5.64	6.67	6.53	-	6.40	1.46	7.75	6.95	OH: 13.6 9.75	5
2.	Carpachromene dimethyl ether (2)	5.72	6.75	6.55	-	6.70	1.46	7.79	6.99	OMe 3.87 3.94	5
3.	Carpachromene diacetate (3)	5.77	6.51	6.54	-	6.80	1.48	7.84	7.24	OAc 2.32, 2.46	5
4.	Diacetate of pyranoflavone from *P. missionis* [$CDCl_3$] (3)	5.49	6.58	6.28	-	6.24	1.44	7.56	6.96	OAc 2.23 2.32	3
5.	Pyranoflavone from *A. ceylanica* [acetone-d_6] (1)	5.76	6.89	6.15	-	6.66	1.45	7.97	7.03	C-5 (OH) 13.15	4
6.	Atalantoflavone from *A. racemosa* acetone - d_6 (4)	5.78	6.93	6.70	6.19	-	1.49	8.01	7.01	-	6
7.	Atalantoflavone dimethyl ether [$CDCl_3$] (5)	5.62	6.84	6.58	6.32	-	1.50	7.81	7.01	OMe 3.88 3.95	6
8.	Atalantoflavone diacetate [acetone - d_6] (6)	5.72	6.84	6.56	6.50	-	1.51	7.86	7.25	OAc 2.34 2.42	6

In the 2D-NOESY spectrum of atalantoflavone dimethyl ether (5, FIG. 2), the doublet (1H, J = 10Hz) at δ 5.62 displayed interactions with the doublet (1H, J = 10Hz) at δ 6.84 and a singlet (6H) at δ 1.50. Therefore the resonances at δ 5.62 and δ 6.84 were ascribed to H-3" and H-4" of the 2,2-dimethylpyran ring. The doublet (2H, J = 8.5Hz) at δ 7.01 showed interactions with the doublet (2H, J = 8.5Hz) at δ 7.81 and one of the methoxyl resonances at δ 3.88. Therefore these resonances at δ 7.01, 7.81 and 3.88 were attributed to H-3' and H-5', H-2' and H-6' and 4'-methoxyl protons of the B-ring respectively. The other singlet (3H) at δ 3.95 was ascribed to methoxyl at position 5. The crucial assignments of the uncoupled resonances (1H each) at δ 6.58 and 6.32 were achieved by observing their individual nOe interactions. The signal at δ 6.58 showed cross peaks with a doublet (2H, J = 8.5Hz) at δ 7.81, which has been already assigned to H-2' and H-6', thus the former resonance was attributed to H-3 proton. The other spatial interaction between the singlet at δ 6.32 and the C-5 methoxyl proton (δ 3.95) establishes the position of the former as 6. This interaction is therefore of diagnostic value in assigning the angular structure 5 to atalantoflavone dimethyl ether.

The 2D-NOESY spectral studies (FIG. 3) of carpachromene dimethyl ether (2), the linear isomer of atalantoflavone dimethyl ether (5) showed interactions of 2,2-dimethylpyran ring protons [δ 1.48 (Gem dimethyl) «--» 5.72 (H-3") «--» 6.74 (H-4")], B-ring protons [3.87 (OMe) «--» 6.99 (H-3' and H-5') «--» 7.79 (H-2' and H-6')] and H-3 proton at δ 6.55 with a doublet (2H, J = 8.2Hz) at δ 7.79 (H-2' and H-6'). The two important noticeable differences



5

(Gem dimethyl) 1.50 ⟷ 5.62 (H-3'')
(H-3'') 5.62 ⟷ 6.84 (H-4'')
(C-4', OMe) 3.88 ⟷ 7.01 (H-3', H-5')
(C-5, OMe) 3.95 ⟷ 6.32 (H-6)
(H-3) 6.58 ⟷ 7.81 (H-2', H-6')
(H-3', H-5') 7.01 ⟷ 7.81 (H-2', H-6')

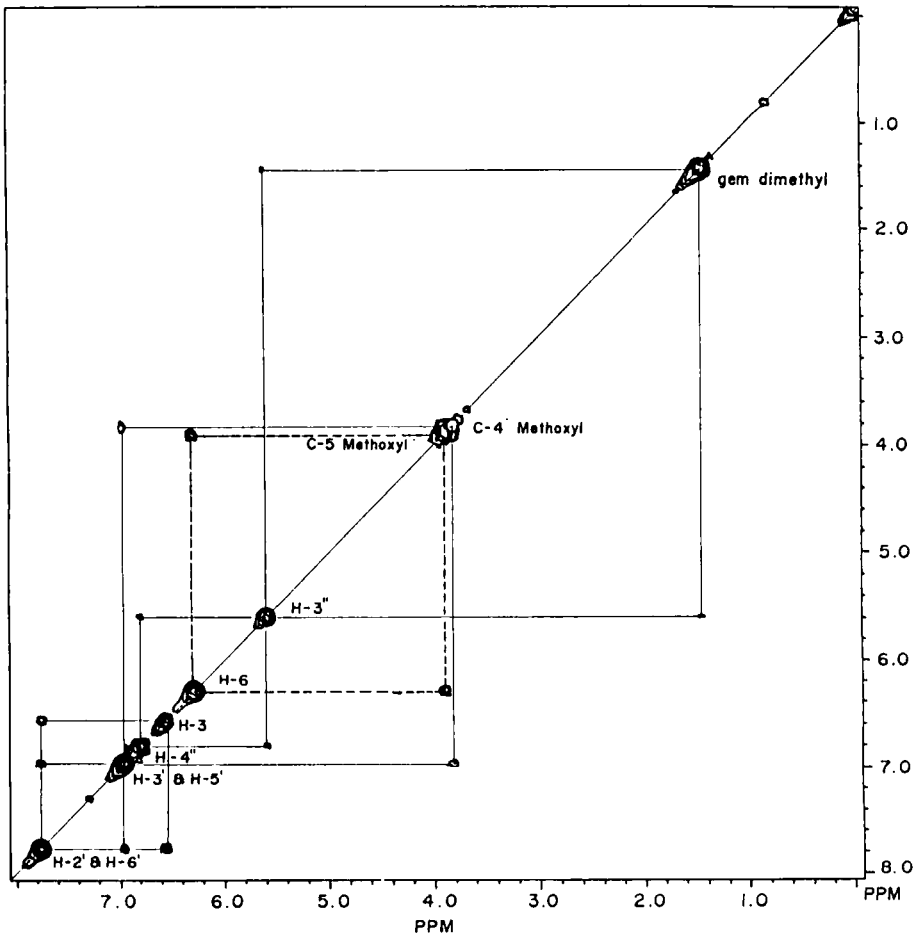


FIG. 2: Symmetrized 2D-NOESY Spectrum of Atalantoflavone Dimethyl Ether (5) in $CDCl_3$.

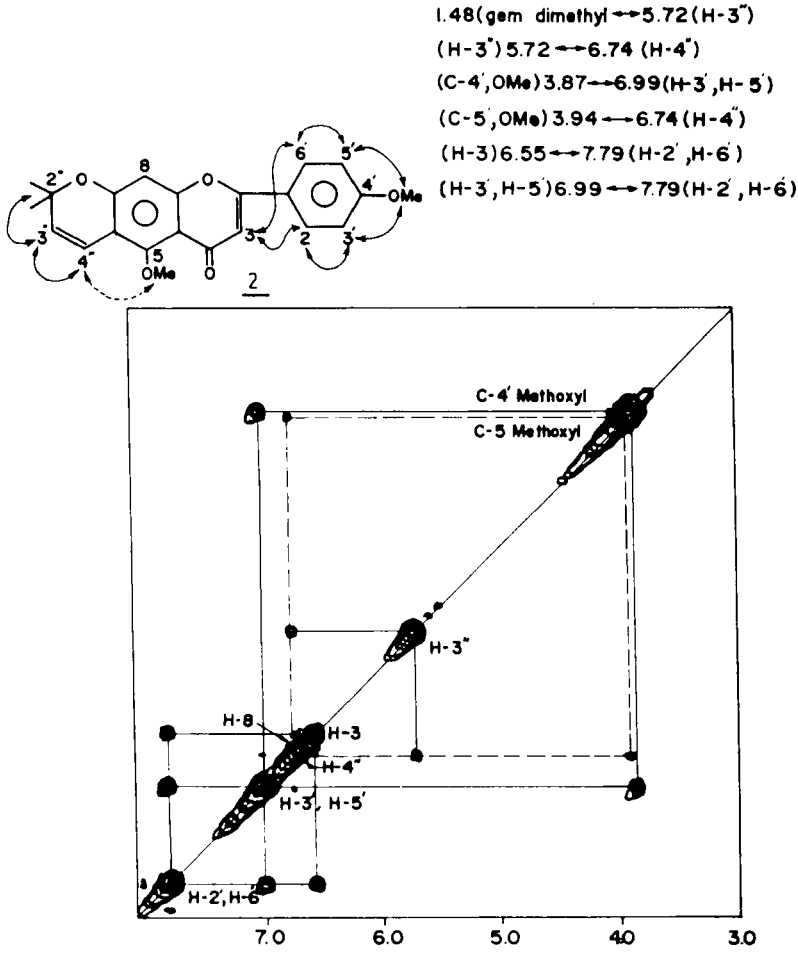


FIG. 3: Symmetrized 2D-NOESY Spectrum of Carpachromene Dimethyl Ether (2) in $CDCl_3$.

were the weak interaction between C-5 methoxyl protons (δ 3.94) and H-4" (δ 6.74), and the absence of any nOe interaction of the singlet (1H) at δ 6.69. Therefore the singlet at δ 6.69 was attributed to H-8. The latter two interactions confirm the linear structure 2 to carpachromene dimethyl ether.

A critical examination of the ^{1}H NMR data of the pyranoflavones and their derivatives presented by different workers (Table 2) revealed interesting findings. Structure 1 was wrongly assigned to the compound isolated from *A. ceylanica* [4] due to the erroneous assignments of uncoupled protons at δ 6.15 and 6.66. Infact the ^{1}H NMR data are in agreement with those of authentic atalantoflavone (Table 2, entries 5 and 6). Therefore the structure of the former compound should be revised from 1 to 4. This contention is supported by identical UV data (Table 1, entries 6 and 7) for both the compounds. The discrepancy in the melting points may be due to the presence of some impurity in the sample obtained from *A. ceylanica*. The data (mp, UV, IR, ^{1}H NMR, MS) for dimethyl ether derivative of carpachromene isolated from *Flindersia laevicarpa* [5] agree very well with those of the synthetic sample[7] of carpachromene dimethyl ether and hence these results confirms the assigned structure of carpachromene. The physical and spectral data of compound from *Paubrus nissionis* to which structure 1 (carpachromene) was assigned do not tally with either of 1 or 4 and therefore needs revision.

The NOESY spectrum of racemoflavone dimethyl ether (8) displayed interactions of the 2,2-dimethylpyran ring (FIG. 4). The methoxyl signals (δ 3.97-3.98, 9H, 3 X OMe) which did not

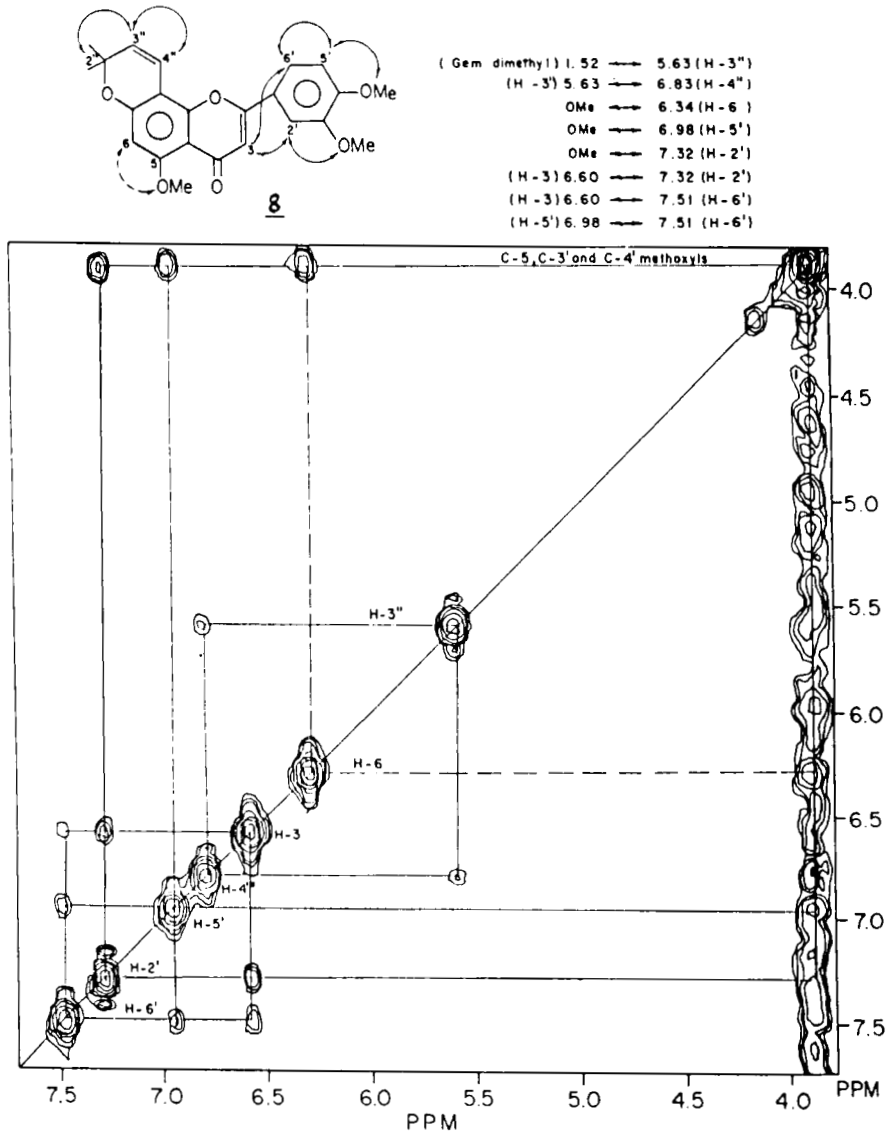


FIG. 4: 2D-NOESY Spectrum of Racemoflavone Dimethyl Ether (6) in $CDCl_3$.

resolve showed nOe interaction with doublet (1H, J = 8.5Hz) at δ 6.98. The latter signal further displayed interaction with the doublet (1H, J = 8.5Hz) at δ 7.51. Thus these resonances were attributed to H-5' and H-6' respectively. Cross peaks between methoxyl and a broad singlet at δ 7.32 were also observed. The singlet at δ 6.60 showed interactions with the broad singlet at δ 7.32 and the doublet at δ 7.51 (H-6'), it was therefore attributed to H-3. The interactions of H-3 proved beyond doubt that C-2' position is unsubstituted and assigned the signal at δ 7.32 for the same. These interactions showed the presence of 3',4'-disubstituted B-ring and differentiated it from 2',4'-disubstituted B-ring flavonoids, which also has same ABM spin system. The interaction of the signal at δ 6.34 with methoxyl proton helped in assigning the former resonance to H-6 and thereby angular structure 8 to racemoflavone dimethyl ether.

CONCLUSIONS

The 2D-NOESY spectroscopy offers a method for the unequivocal assignments of H-3 and H-6/H-8 protons, thereby providing an unique alternative method for elucidating structures of isomeric 5,6,7- and 5,7,8-trisubstituted A-ring flavones; it also distinguishes between 3',4'and 2',4'-disubstituted B-ring flavones without recourse to tedious chemical degradation and elaborate synthesis. Comparison of UV and ^{1}H NMR data of pyranoflavones isolated from *A. ceylanica* and atalantoflavone suggests that both the compounds are identical. This study therefore calls for revision of structure of former compound to 4. In addition, it also postulates that the compound isolated from *P. missionis* does not resemble either carpachromene or atalantoflavone and therefore needs revision of structure.

EXPERIMENTAL

Compounds 2, 5 and 8 were synthesized by the unambiguous route and their structures were established by physical and spectral data[6,7]. The 2D-NOESY spectra were recorded on a Bruker AM 500 FT-NMR spectrometer. NOESY experiments were performed with 256t_1 and 1K t_2 points. The mixing time τ_m was 1 sec. The time domain data were zero filled to 512 points along the t_1 axis. The data were multiplied by sine square bell and sine bell window functions along the t_2 and t_1 axes respectively, prior to respective Fourier transformations. Digital resolution in all the spectra is 15 Hz/point along both $_1$ and $_2$ axes. Absolute value spectra have been presented.

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