The Cell Research Institute and Department of Botany, The University of Texas and Department of Chemistry, Aligarh Muslim University, Aligarh, India

NMR Analysis and Synthesis of 6- and 8-*C*-Methylisoflavones

K. R. Markham (1), W. Rahman (2), Sardar Jehan (2), and T. J. Mabry (1)

NMR data for eighteen 6- and 8-*C*-methylisoflavones provide conclusive evidence that in isoflavones, the C-8 proton and C-8 methyl signals occur downfield from the C-6 proton and C-6 methyl signals. Other useful NMR correlations and the synthesis of five new C-methylisoflavones are described.

Flavonoids often occur as natural products with the 6 and 8 positions carbon-carbon linked to a variety of substituents including the C-methyl, $\gamma\gamma$-dimethylallyl, C-glycosyl, and flavonyl (as in biflavonyls). The 5,6,7- and 5,7,8-substitution patterns that are usually present in these 6 and 8 substituted flavonoids were difficult to distinguish prior to the application of NMR spectroscopy. Early NMR work established that in 5,7-dihydroxyflavone derivatives, the resonance signal for the C-8 proton occurs about 0.1-0.15 ppm downfield from the signal of the C-6 proton (3), and a number of structural assignments have since been made on this basis. These include the isomeric pairs of 6 and 8 substituted C-glycosides, vitexin and isovitexin (4), and orientin and isoorientin (5). In some instances additional support for the structural assignments was provided by the Gibbs indophenol reaction (4) and by NMR studies of the rotational isomerism of C-glucosylflavonoid acetates (6); thus the original C-6, C-8 ring proton assignments for flavones are now well established. In contrast, the C-substituted isoflavones have received little attention. In 5,7-dihydroxyisoflavones the C-8 ring proton has been assigned the downfield NMR signal relative to the signal ascribed to the C-6 proton (7,8,9), but these assignments were based largely on the earlier flavone work. We now present NMR data (Tables I and II) on eighteen 6- and 8-*C*-Methylisoflavones, with 5,6,7- and 5,7,8-substitution patterns, which provide confirmation that in 5,7-dihydroxyisoflavones the C-8 proton signal occurs downfield from that of the C-6. The syntheses of isoflavones I-XIV have been previously reported by Rahman *et al.* (10,11), and the syntheses of the remainder (XV-XIX) are described here.

Inspection of Table I reveals that, in the isomeric pairs I/II, III/IV, and XI/XII, of known substitution patterns (10,11), the H-8 signal is found 0.2-0.3 ppm downfield from that of the H-6. This difference in chemical shift is observed for completely trimethylsilylated and completely methylated derivatives in both deuterochloroform and carbon tetrachloride and apparently is not affected by substitution at C-2 or in the B-ring. This 0.2-0.3 ppm difference applies to unsubstituted 5,7-dihydroxyisoflavones as well as 6- or 8-*C*-methyl derivatives since in the latter compounds the C-methyl substituent has little effect on the chemical shift of the remaining A-ring proton. For example:

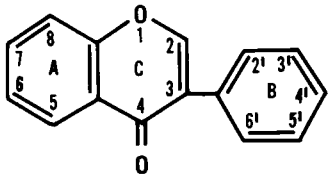
	H-6	H-8
4'-Methoxy-5,7-dihydroxyisoflavone (12)	6.15	6.35 ppm
6-C-Methyl derivative (I)		6.36 ppm
8-C-Methyl derivative (II)	6.16	ppm

Complete trimethylsilylation of the hydroxyl groups in the isoflavones is necessary if the above relationship is to hold, but this is not always readily achieved. Compounds I, VII, IX, XI, and XIII for example, all possess a 6-C-methyl group and because of the steric hindrance this substituent imposes, the 5-hydroxyl group in these isoflavones will not trimethylsilylate significantly under the usual conditions (13). This is in contrast to the rapid and complete trimethylsilylation of the 8-C-methyl isomers. This difference in reactivity provides additional evidence for the assignment of either substitution pattern. The extent of trimethylsilylation can be determined by the presence or absence of the 5-hydroxyl proton signal (near 12.6 ppm) and also by the position of the H-8 signal which moves about 0.15 ppm upfield on trimethylsilylation of the 5-hydroxyl group, *cf.* flavones (14).

Another distinguishing feature between the C-6 and C-8 methyl isomeric pairs of trimethylsilylated isoflavones is the difference in chemical shifts observed for their C-methyl groups. For example, in the five isomeric pairs I/II, VII/VIII, IX/X, XI/XII and XIII/XIV, the C-6 methyl signal is found at 2.04-2.06 ppm, consistently 0.1-0.18 ppm upfield

TABLE I (a)

NMR Spectra of Known *C*-Methylisoflavones and Their Trimethylsilyl Ethers



	A-Ring		C-Ring	B-Ring		C-Methyl		Methoxyl		Hydroxyl
	H-6	H-8	H-2	H-3', 5'	H-2', 6'	6	8	4'	Other	5
5,7-Dihydroxy-4'-methoxy-6-methylisoflavone (I) (b)		6.22	7.72	6.82 (*d*)	7.38 (*d*)	2.04		3.75		12.95
(I) (c)		6.36	7.64	6.82 (*d*)	7.38 (*d*)	2.04		3.75		
5,7-Dihydroxy-4'-methoxy-8-methylisoflavone (II) (c)	6.16		7.75	6.84 (*d*)	7.38 (*d*)		2.14	3.77		
5,7,4'-Trimethoxy-6-methylisoflavone (III) (d)		6.62	7.80	6.96 (*d*)	7.48 (*d*)	2.18		3.82	3.85 3.88	
5,7,4'-Trimethoxy-8-methylisoflavone (IV) (d)	6.40		7.80	6.90 (*d*)	7.50 (*d*)		2.17	3.80	3.89 3.93	
5-Hydroxy-7,8-dimethoxyisoflavone (V) (d)	6.44		7.96	5 protons 7.30-7.70					3.89 3.92	12.60
5,7-Dimethoxy-8-methylisoflavone (VI) (d)	6.42		7.85	5 protons 7.30-7.70			2.19		3.92 3.95	
2-Carboxy-5,7-dihydroxy-4'-methoxy-6-methylisoflavone (VII) (b)		6.36		6.88 (*d*)	7.20 (*d*)	2.06		3.82		
(VII) (c)		6.51		6.88 (*d*)	7.20 (*d*)	2.06		3.82		
2-Carboxy-5,7-dihydroxy-4'-methoxy-8-methylisoflavone (VIII) (c)	6.22			6.86 (*d*)	7.15 (*d*)		2.18	3.82		
2-Carbethoxy-5,7-dihydroxy-4'-methoxy-6-methylisoflavone (IX) (d,e)		6.49		6.97 (*d*)	7.15 (*d*)	2.14		3.82		12.72
(IX) (b,e)		6.32		6.87 (*d*)	7.19 (*d*)	2.04		3.81		12.67
2-Carbethoxy-5,7-dihydroxy-4'-methoxy-8-methylisoflavone (X) (d,e)	6.30			6.95 (*d*)	7.21 (*d*)		2.22	3.82		12.45
(X) (c,e)	6.20			6.86 (*d*)	7.14 (*d*)		2.20	3.80		
2-Carbethoxy-5,7-dihydroxy-6-methylisoflavone (XI) (b,e)		6.34		5 protons 7.32		2.04				12.62
(XI) (c,e)		6.50		5 protons 7.32		2.04				
2-Carbethoxy-5,7-dihydroxy-8-methylisoflavone (XII) (c,e)	6.20			5 protons 7.30			2.22			

TABLE I (Continued)

	A-Ring		C-Ring	B-Ring		C-Methyl		Methoxyl		Hydroxyl
	H-6	H-8	H-2	H-3', 5'	H-2', 6'	6	8	4'	Other	5
2-Carboxy-5,7-dihydroxy-6-methylisoflavone (XIII) (b)		6.38		5 protons 7.32		2.05				
2-Carboxy-5,7-dihydroxy-8-methylisoflavone (XIV) (c)	6.26			5 protons 7.30			2.18			

(a) Spectra were determined in carbon tetrachloride on a Varian A-60 spectrometer unless otherwise noted and values are given in ppm relative to tetramethylsilane as an internal standard. Singlets are unmarked and (*d*) = doublet with J = about 8.5 cps. Compound V is not a C-methyl derivative. (b) Partially trimethylsilylated (C-5 OH free). (c) Fully trimethylsilylated. (d) Determined in deuterochloroform. (e) Ethoxyl protons observed at about 4.1 (quartet) and 1.0 (triplet) ppm.

TABLE II (a)

NMR Spectra of New *C*-Methylisoflavones and Their Trimethylsilyl Ethers

	A-Ring		C-Ring	B-Ring	C-Methyl		Methoxyl	Carbomethoxyl		Hydroxyl
	H-6	H-7	H-8	H-2		6	8	5	6	8	5
5-Carbomethoxy-2,4-dihydroxy-deoxybenzoin (XV)			7.24	8.00	6 protons (incl. H-5) 7.40-7.60				3.92		
6-Carbomethoxy-5-hydroxy-8-methylisoflavone (XVI)		8.06		8.06	5 protons 7.47		2.36		3.94		13.82
8-Carbomethoxy-5-hydroxy-6-methylisoflavone (XVII)		8.09		8.09	5 protons 7.47	2.27				3.90	13.80
(XVII) (b)		8.14		8.73	5 protons 7.50-7.70	2.22				3.87	13.80
6-Carbomethoxy-5-methoxy-8-methylisoflavone (XVIII)		7.92		7.98	5 protons 7.50		2.45	4.00	3.94		
(XVIII) (b)		7.99		8.57	5 protons 7.56		2.45	3.91	3.87		
5-Methoxy-8-methylisoflavone (XIX)	6.74 (*d*)			7.91	6 protons (incl. H-7) 7.20-7.70		2.37	3.92			

(a) Spectra were determined in chloroform on a Varian A-60 spectrometer unless otherwise noted. Values are given in ppm relative to tetramethylsilane as internal standard. Singlets are unmarked and (*d*) = doublet with J = about 8.5 cps. (b) Hexadeutero-dimethylsulfoxide as solvent.

from that of the C-8 methyl. The signals of the C-6 and C-8 methyl groups thus show the same general relationship to one another as do the H-6 and H-8 signals which were discussed above. This relationship has been used here to tentatively distinguish between the isomeric isoflavones XVI and XVII, the NMR spectra of which are otherwise almost identical (Table II).

Several other useful NMR correlations, not directly relevant to the distinction of 5,6,7- and 5,7,8-substitution patterns are also evident from the data presented in Tables I and II.

(a) Methoxyl groups in the 4'-position all show signals in the 3.75-3.82 ppm range in both deuterochloroform and carbon tetrachloride, while methoxyl groups at positions 5, 7, and 8 appear further downfield in the 3.85-3.95 ppm range.

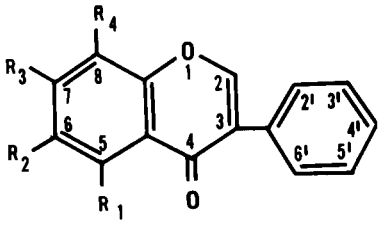
(b) In the 4'-methoxyisoflavones I-IV, the H-2',6' signals appear between 7.38 and 7.5 ppm, but in the same compounds possessing 2-carboxyl substituents (VII-X) the H-2',6' signals are found in the range 7.15-7.21 ppm. The upfield shift of these signals in compounds VII-X could be the result of positive shielding due to the diamagnetic anisotropy of the carbonyl group, or of loss of coplanarity of the B-ring with the rest of the molecule.

(c) The presence of the C-6 and C-8 carboxyl functions in compounds XVI and XVII (Table II) cause a 1 ppm downfield shift of the hydrogen-bonded 5-hydroxyl proton signal.

(d) The H-2 signals in the isoflavone spectra, with chemical shifts in the 7.65-8.1 ppm region, are generally downfield from the A- and B-ring aromatic proton peaks and are thus readily distinguished. If however there is difficulty in distinguishing the H-2 signal, as in compounds XVI-XVII, then it can be identified by examining the compound first in either deuterochloroform or carbon tetrachloride and then in hexadeuterodimethylsulfoxide. In the latter solvent, the H-2 signal occurs 0.6-0.75 ppm downfield from its position in the former solvents (15). The magnitude of this shift appears to be good diagnostic evidence for the absence of a C-2 substituent in isoflavones, since the H-2 signal is the only ring proton signal that shifted more than 0.13 ppm in the isoflavones investigated (see Table II and reference 15).

Syntheses.

Acylation of methyl 2,4-dihydroxy-5-methylbenzoate with phenylacetyl chloride gave 5-carbomethoxy-2,6-dihydroxy-3-methyldeoxybenzoin, which was subsequently cyclized using Venkataraman's variation (16) of the ethyl formate-sodium synthesis. The two isomeric isoflavones so formed (XVI and XVII) were distinguished by NMR spectroscopy and XVI was then converted to the methyl ether (XVIII). The above deoxybenzoin, after decarboxylation and monomethylation, yielded 2-hydroxy-6-methoxy-3-methyldeoxybenzoin, which was cyclized with ethyl formate to give 5-methoxy-8-methylisoflavone (XIX). 6-Carbomethoxy-7-acetoxyisoflavone (XV) was synthesized by the same method as were isoflavones XVI and XVII but using methyl β-resorcylate as starting material.



	R_1	R_2	R_3	R_4
XV	H	$COOCH_3$	$OCOCH_3$	H
XVI	OH	$COOCH_3$	H	CH_3
XVII	OH	CH_3	H	$COOCH_3$
XVIII	OCH_3	$COOCH_3$	H	CH_3
XIX	OCH_3	H	H	CH_3

It is of interest to note that four of the new isoflavones reported here (XVI-XIX) contain carboxyl functions in the A-ring, a feature as yet unobserved in any natural or synthetic isoflavones. Previously 2-carboxyisoflavones, commonly isolated as intermediates in the ethoxyalylation process (17), were the only known carboxylisoflavones.

EXPERIMENTAL

The fully trimethylsilylated isoflavones, as well as those in which the C-5 hydroxyl group was not trimethylsilylated, were prepared by the procedures previously described by Mabry *et al.*, (13,14). Microanalyses were carried out by Drs. Weilers and Strauss, Oxford, England.

The synthesis and characterization of compounds I-XIV were reported earlier (10,11).

5-Carbomethoxy-2,4-dihydroxydeoxybenzoin.

To a solution of methyl-β-resorcylate (12 g.) in nitrobenzene (45 ml.) at 5° was added powdered aluminum chloride (30 g.) followed by phenylacetyl chloride (8 ml.) in nitrobenzene (20 ml.). The mixture was kept at 30° for 12 days and the subsequent removal of nitrobenzene gave a solid which crystallized from ethyl alcohol as colorless needles (8.2 g.), m.p. 98-99°.

Anal. Calcd. for $C_{16}H_{14}O_5$: C, 67.13; H, 4.92. Found: C, 66.82; H, 4.51.

6-Carbomethoxy-7-hydroxyisoflavone.

To pulverized sodium (1 g.) cooled to 0° was added, with vigorous stirring, a suspension of 5-carbomethoxy-2,4-dihydroxydeoxybenzoin (1.0 g.) in freshly distilled ethyl formate (20 ml.) over a period of 30 minutes. After storage of the reaction mixture for 48 hours at 0°, crushed ice (60 g. and concentrated hydrochloric acid (40 ml.) were added. Unreacted ethyl formate was distilled off *in vacuo*. The resultant solid was filtered off and crystallized first from methanol and then from ethyl acetate to give colorless needles (0.61 g.), m.p. 168-170°.

Anal. Calcd. for $C_{17}H_{12}O_5$: C, 68.91; H, 4.08. Found: C, 68.59; H, 3.83.

6-Carbomethoxy-7-acetoxyisoflavone (XV).

6-Carbomethoxy-7-hydroxyisoflavone (1.5 g.) was allowed to react with acetic anhydride (8 ml.) in pyridine (8 ml.) at 100° for 2 hours. The product (XV) crystallized from ethanol as white needles (0.32 g.), m.p. 188-190°.

Anal. Calcd. for $C_{19}H_{14}O_6$: C, 67.45; H, 4.17. Found: C, 67.56; H, 4.02.

5-Carbomethoxy-2,6-dihydroxy-3-methyldeoxybenzoin.

To a solution of methyl 2,4-dihydroxy-5-methylbenzoate (9 g.) in nitrobenzene (60 ml.) at 5° was added powdered aluminum chloride (22 g.) followed by phenylacetyl chloride (6.5 ml.) in nitrobenzene (25 ml.). The mixture was kept at 30° for 12 days. The usual work up gave a solid from which 5-carbomethoxy-2,6-dihydroxy-3-methyldeoxybenzoin was extracted with ether. This compound crystallized from ethyl acetate as fine needles (7.9 g.), m.p. 168-170°.

Anal. Calcd. for $C_{17}H_{16}O_5$: C, 68.00; H, 5.36. Found: C, 68.24; H, 5.38.

6-Carbomethoxy-5-hydroxy-8-methylisoflavone (XVI) and 8-Carbomethoxy-5-hydroxy-6-methylisoflavone (XVII).

5-Carbomethoxy-2,6-dihydroxy-3-methyldeoxybenzoin (4.0 g.) in ethyl formate (100 ml.) was added with stirring to pulverized sodium (4 g.) at ice temperature over a period of 20 minutes. The reaction mixture was then stored for 48 hours at 0°. Work up gave a brown solid which was partially purified by crude crystallization from ethanol. Further crystallization of the crude material from a large volume of ethyl acetate gave yellow needles (1.67 g.), m.p. 204-206°. Gradual concentration of the mother liquor gave a second product in the form of yellow prisms (0.93 g.), m.p. 167-169°.

Anal. Calcd. for $C_{18}H_{14}O_5$: C, 69.67; H, 4.54. Found for compound m.p. 204-206°: C, 70.10; H, 4.52 and for compound m.p. 167-169°; C, 69.88; H, 4.63.

6-Carbomethoxy-5-methoxy-8-methylisoflavone (XVIII).

Compound XVI (0.2 g.), dry acetone (100 ml.), methyl sulfate (0.2 ml.) and potassium carbonate (2 g.) were refluxed for 60 hours. The resultant oil crystallized from ethanol in fine needles (0.16 g.), m.p. 156-158°.

Anal. Calcd. for $C_{19}H_{16}O_5$: C, 70.37; H, 4.97. Found: C, 70.29; H, 5.01.

2-Hydroxy-6-methoxy-3-methyldeoxybenzoin.

A solution of 5-carbomethoxy-2,6-dihydroxy-3-methyldeoxybenzoin (5 g.) in ethanol (38.5 ml.) and water (38.5 ml.) containing potassium hydroxide (5.4 g.) was refluxed for 4 hours. 2,6-Dihydroxy-3-methyldeoxybenzoin was obtained and it crystallized from benzene-petroleum ether as fine needles (1.52 g.), m.p. 135-136°.

Anal. Calcd. for $C_{15}H_{14}O_3$: C, 74.38; H, 5.82. Found: C, 74.46; H, 5.88.

Methylation of this deoxybenzoin (1 g.) with dimethyl sulfate (4 ml.) and potassium carbonate (2 g.) in boiling acetone (25 ml.) gave 2-hydroxy-6-methoxy-3-methyldeoxybenzoin, which crystallized from ethanol as needles (0.78 g.), m.p. 80-82°.

Anal. Calcd. for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found: C, 75.26; H, 5.95.

5-Methoxy-8-methylisoflavone (XIX).

2-Hydroxy-6-methoxy-3-methyldeoxybenzoin (1.5 g.) was condensed with ethyl formate (40 ml.) in the presence of sodium (1.5 g.) under the usual conditions. The product was heated for 30 minutes with acetic acid (10 ml.) and then crystallized from ethyl acetate-petroleum ether to give colorless needles (1.0 g.), m.p. 138°.

Anal. Calcd. for $C_{17}H_{14}O_3$: C, 76.69; H, 5.29. Found: C, 76.66; H, 5.20.

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