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^{13}C NMR STUDIES OF SOME COMPLEX NATURAL OXYGEN HETEROCYCLICS

STRUCTURE OF MILLETTIN, A NOVEL ISOFLAVONE ISOLATED FROM *MILLETTIA AURICULATA*

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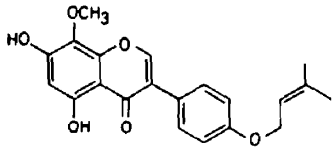
Abstract—^{13}C NMR spectra for a variety of isoflavones are presented and analysed. The novel isoflavone, Millettin (Xa) is shown to be closely related to Auriculatin (II). While comparison of the ^{13}C NMR spectra did not readily prove the orientation of the fused pyran ring, the use of acetylation shifts showed unambiguously that the fusion in linear, rather than angular.

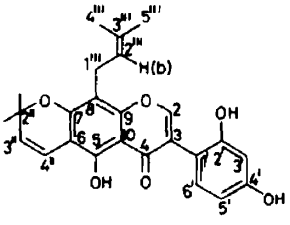
Earlier the roots[1,2] and leaves[3] of *Millettia auriculata* (Leguminosae) were examined and several new isoflavones were reported. Recently aurmillone (I), auriculatin (II), auriculasin (III) and sumatrol (IV) were reported from the seeds of *Millettia auriculata*.[4] A reinvestigation of the seeds of *Millettia auriculata* resulted in the isolation of an additional compound, designated as millettin (Xa) m.p. 237–38°.

Millettin (Xa), $C_{25}H_{22}O_6$, (M^+418) is soluble in 2% aqueous potassium hydroxide solution and gave a green colour with alcoholic ferric chloride indicating its phenolic nature. The IR and UV spectral data of millettin (Xa) are similar to those of auriculatin (II) (Table 1) and suggest that it is an isoflavone. As with auriculatin[1], aluminium chloride–hydrochloric acid produced and bathochromic shift of the 280 nm band in the UV spectrum of millettin, indicating the presence of a chelated OH group.[5] Sodium acetate did not affect the UV absorption maxima of either compound so suggesting the absence of C7-OH group.[5] Millettin formed a diacetate (Xb), m.p. 142°, $C_{29}H_{26}O_8$ (M^+502) indicating the presence of two OH's, while auriculatin was found to possess three OH groups.[1]

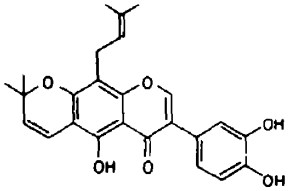
The ^{1}H NMR spectrum of millettin (Xa), recorded in DMSO-d_6, is very similar to that of auriculatin (II) (Table 2). A set of peaks characteristic of a fused 2,2-dimethylpyran unit[6] and a C-3-methylbut-2-enyl group[6] are observed in its NMR spectrum in the same region as in the spectrum of auriculatin. In the aromatic region two doublets are found at 7.65δ (1H, d, J = 8 Hz) and 6.82δ



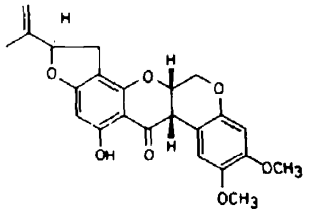
(I)



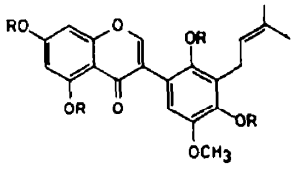
(II)



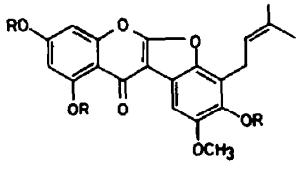
(III)



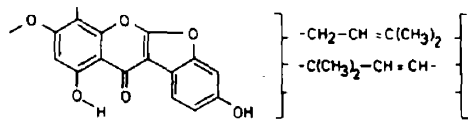
(IV)



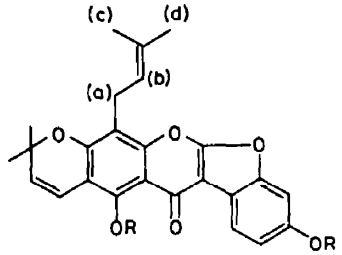
(Va) R=H
(Vb) R=CH_3
(Vc) R=$COCH_3$



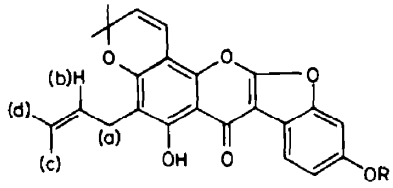
(VIa) R = H
(VIb) R = CH_3
(VIc) R = $COCH_3$



(VII) (VIII) (IX)



(Xa) R = H
(Xb) R = $COCH_3$



(XI)

(1H, d, J = 2 Hz) and a quartet at 7.02δ (1H, q, J = 8 Hz and 2.0 Hz) which may be assigned to a 2', 4'-dioxy-generated isoflavone B-ring[1]. However in contrast to auriculatin, millettin (Xa) lacks the characteristic low field C-2 proton singlet of isoflavones and thus must possess a modified isoflavonoid skeleton.

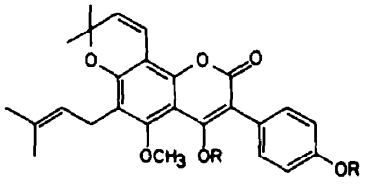
Ollis *et al.*[7] reported the isolation of 2'-hydroxy-isoflavone (piscerythrone, Va) along with the corresponding closely related coumarano (2',3':2,3) chromone, lisetin(VIa) from *Piscidia erythrina*. The spectral data of piscerythrone (Va) is similar to that of lisetin (VIa) (Table 1), the major difference being the lack of a C-2 proton signal in lisetin. Further, the chemical shift of the C-6'-proton in millettin (7.65δ) is as expected,[7] downfield from that in auriculatin (7.25δ) due to diamagnetic deshielding by the nearby CO group. Therefore, a coumarano (2', 3':2, 3) chromone skeleton (VII) would be consistent with the data for millettin. A coumestan skeleton (VIII) was excluded since the lactone CO in coumestans[8] at 1700–1740 cm^{-1} was not present in the IR of millettin. Further, UV spectral data for milletin differed considerably from those of coumestans (IR and UV spectral data of a typical coumestan, psoralidin (IX)[9] are given in Table 1).

Two isomeric structures (Xa and XI) can thus be considered for millettin. The co-occurrence of millettin with auriculatin (II) suggests a linear fusion of the pyran unit as in Xa, but the alternative structure XI, possessing an angularly fused pyran unit can not be ruled out. In order to decide on the orientation of the pyran ring the ^{13}C NMR spectra of millettin, auriculatin (II) and lonchocarpic acid methyl ether (XIIIb) with a linearly fused pyran ring, and scanden in methyl ether (XIIb) with angular ring fusion, were recorded. The spectrum of trapezifolixanthone (XIV), a more closely related model compound has been assigned previously.[15]

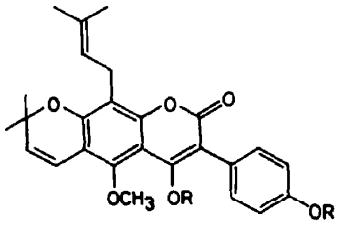
The high degree of substitution of these compounds made some assignments of skeletal carbons (C-2 to 10) ambiguous. By the use of substituent effects[11–13] obtained from similar systems, and by analogy, many signals were assigned.

The spectra of the compounds studied are presented in Table 3. There are no significant shifts of signals of the substituent (C-2" to 4", C-2"-Me, and C-1'" to C-5'") carbons on change of attachment site in both coumarin (XIIb and XIIIb) and isoflavone (II, XIIIb and XIV) systems, so isomer identification must be based entirely on skeletal carbons, particularly those of ring A. A direct comparison of shifts of A ring resonances caused by differences in isomerism of the coumarins, (XIIb) and (XIIIb), and the isoflavones (II, XIIb and XIV) is difficult.

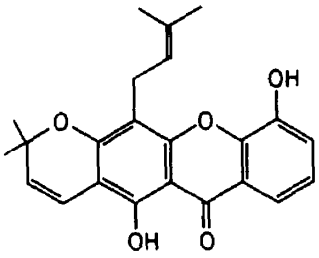
A comparison of the A rings of 4,4'-dimethoxylonchocarpic acid (XIIIb) and 4,4'-dimethoxyscandenin (XIIb) shows similarities to resonance differences of β-toxicarol and α-toxicarol[14] respectively, that is, the C-8 signal of 4,4'-dimethoxyscandenin (XIIb) is significantly down field to that of 4,4'-dimethoxylonchocarpic acid (XIIIb), and the C-6 signal is found at significantly higher field. On the other hand, this large difference between the C-6 and C-8 shifts in 4,4'-dimethoxyscandenin (XIIb) is not reflected in any of the other compounds studied, including trapezifolixanthone (XIV), indicating that millettin probably is Xa rather than XI. However, a clear decision



(XIIa) R = H
(XIIb) R = CH_3



(XIIIa) R = H
(XIIIb) R = CH_3



(XIV)

between the two structures can not be made on this basis alone.

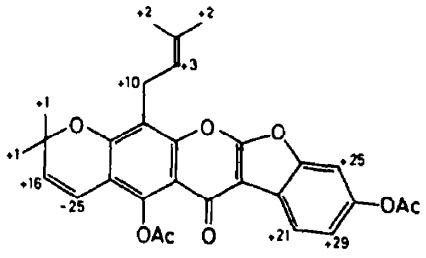
The C-2 oxygenation causes widespread changes in resonance values throughout the entire skeleton (A, B and C rings) of millettin, preventing a direct comparison of skeletal resonances of millettin and auriculatin. Therefore in order to confirm the previous assignments and gain further information by the use of ^{13}C-^{1}H coupling constants, the spectra were recorded under conditions where the coupling to protons was not removed by noise decoupling, but the n.O.e. was retained ("gated decoupling").

This technique confirmed the assignments of signals in the decoupled spectrum and also permitted the analysis of structural details which were not apparent in the noise decoupled spectra. In auriculatin, the C-2 signal was of high intensity and exhibited a primary coupling of 198 Hz indicating the presence of a directly bonded hydrogen. In millettin, this signal is of such low intensity that its assignment is easily confused with the resonances of C-7 and C-9, there being no primary coupling. This is a good indication that there is a change in substitution at C-2 in millettin in comparison with auriculatin.

In millettin, the signal at 107.6 ppm, assigned to C-8, appears as a doublet with coupling of 3.2 Hz. This value is associated with benzylic coupling of the carbon concerned. Proof that millettin is (Xa) rather than (XI) was provided by comparison of the proton NMR spectra of (Xa) and (Xb). The signs and magnitudes of these shift changes ((acetylation shifts)[16] are in excellent agreement with those found in auriculatin.[1] It is clear that acetylation of the 5-OH group affected the protons on C-4″ and C-3″ much more than those on C-1‴ or C-2‴, hence the 5-OH must be peri to the pyran ring.

The mass spectral fragmentation pattern of millettin (XIa) (Scheme 1) is very similar to that of auriculatin (II)[1] and the corresponding ions of millettin (Xa) differ by two mass units from those of II. The base peak is due to the ion at *m/e* 403, which arises due to the loss of Me radical from the 2,2-dimethylpyran unit of the molecular ion. Ions due to loss of C_4H_7 (M-55) and C_3H_7 (M-43) are also observed.

The occurrence of a rotenoid (sumatrol, IV)[4], a 2′-hydroxy-isoflavone, auriculatin, (II) and millettin (Xa) in addition to the other isoflavones aurmillone (I) and auriculasin (III) in the seeds of *Millettia auriculata* is biogenetically interesting and is consistent with the biogenetic scheme suggested by Ollis that 2′-hydroxy-isoflavones are the precursors for rotenoids and coumarano (2′, 3′:2, 3) chromones.[7,8]



^{1}H Acetylation shifts of (Xb).
(δppm, 100 MHz, in $CDCl_3$ solution)

EXPERIMENTAL

M.ps are uncorrected. UV spectra were recorded on a Hilger–Watts spectrophotometer, IR spectra (in KBr) on Perkin–Elmer Infracord-337, NMR spectra on a Varian A-60D or Jeol P-100 FT spectrometer with TMS as internal standard, and mass spectra on a RMU 6L mass spectrometer. Silica gel, 200 mesh (ACME) was used for column chromatography.

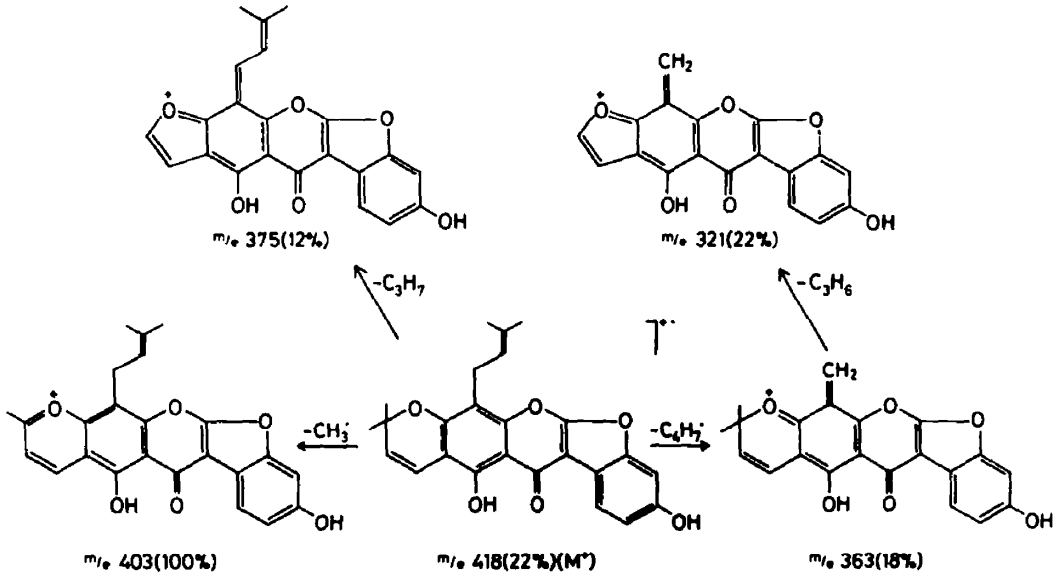


Table 1. UV spectral data

Compound	MeOH $\lambda_{max.}$ nm	log.E	Ir $\nu^{max}_{C=O}$ cm^{-1}
Millettin (Xa)	280	4.46	1650
	354	3.81	
Auriculatin (II)	226	4.48	1650
	280	4.65	
Lisetin[7] (VIa)*	258		1653
	284		
	338		
Piscerythrone[7] (Va)*	265		1655
	294		
Psoralidin (coumestan) (IX)[9]	208		1710
	244		
	305		
	347		

*U.V. recorded in EtOH

Table 2. ^{1}H-NMR spectral data of millettin (Xa) and auriculatin (II) (δ ppm, J in Hz)

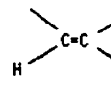
Protons	Auriculatin(II) (in acetone-d_6)	Millettin (Xa) (in DMSO-d_6)
C2-H	8.30	-
2,2-Dimethylpyran unit:		
C2"-$(CH_3)_2$	1.49 (6H.s)	1.45 (6H,s)
C3"-H	5.85 (1H,d,J=10)	5.75 (1H,d,J=10)
C4"-H	6.75 (1H,d,J=10)	6.60 (1H,d,J=10)
C-3-methyl-2-butenyl group:		
 $\underline{CH_3}$ (trans)(c)	1.65 (3H,s)	1.65 (3H,s)
$\underline{CH_3}$ (cis)(d)	1.85 (3H,s)	1.85 (3H,s)
$-CH_2-$(a)	3.45 (2H,d,J=7)	3.30 (2H,d,J=7)
-CH=(b)	5.36 (1H,m)	5.15 (1H,m)
Aromatic protons:		
C3'-H	6.45-6.65 (2H,m)	6.82 (1H,d,J=2)
C5'-H		7.02 (1H,q,J=8 & J=2
C6'-H	7.25 (1H,d,J=8)	7.65 (1H,d,J=8)

Table 3.

	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-1'	C-2'	C-3'	C-4'
AURICULATIN (II)	155.3	120.5	180.9	158.7[a]	105.4	154.1	108.8	158.5[a]	100.4	111.6*	155.0	103.1	156.3
MILLETTIN (Xa)	nv[a]	122.3*	178.1	157.2	105.4	154.3	107.6	155.4	103.4*	113.1	149.9	98.6	156.3
4,4'-DIMETHOXY-SCANDENIN (XIIb)	161.2	112.1	163.9	154.9[a]	107.8	153.9[a]	119.9	147.4	103.8	124.1	131.7	113.3	158.7
4,4'-DIMETHOXY-LONCHOCARPIC ACID (XIIIb)	161.3	111.9	164.1	151.5[a]	112.2	153.6[a]	113.3	150.5	104.5	124.0	131.6	113.3	158.7
TRAPEZIFOLIXANTHONE[15] (XIV)	144.5[f]	120.9	180.9	158.2	104.7	153.6	107.0	156.0	103.4	144.5	119.7	123.9	115.7

C-5'	C-6'	C-2"	C-3"	C-4"	C-2"Me	C-1"'	C-2"'	C-3"'	C-4"'	C-5"'	4-OMe	4'-OMe	5-OMe
106.6	132.0	77.8	114.9	128.6	27.7	21.0	121.9	131.0	17.5	25.3	-	-	-
113.7	120.9	77.8	114.5	128.6	27.6	21.0	121.3	131.2	17.5	25.1	-	-	-
113.3	131.7	77.4	114.3	129.7	27.5	21.8	122.4	130.5	17.6	25.2	60.8	55.0	62.5
113.3	131.6	77.1	115.6	130.6	27.5	21.3	121.2	131.8	17.6	25.2	60.8	55.0	62.9
-	-	78.3	116.7	127.4	28.3	21.7	122.7	131.4	17.9	25.6	-	-	-

*Uncertain assignment.

a,b shift values with same superscript may be interchangeable.

f Isoflavone, rather than the xanthone numbering has been used to better show similarities with other compounds.

Extraction of seeds of Millettia auriculata [*Isolation of auriculasin* (III), *millettin* (Xa), *aurmillone* (I) *and auriculatin* (II). Finely powdered seeds (0.4 Kg) of *Millettia auriculata* were successively extracted with pet ether (60–80°) (2 l) ether (2 l) and $CHCl_3$ (1 l) at room temp. The combined product (29 g) was macerated with pet ether (1 l) and the resulting colourless solid (6.1 g) was found to contain (I[4] and II.[4]

The pet ether soluble portion, concentrated to 200 ml and kept in a refrigerator for 10 days, yielded a pale yellow solid (2.3 g). This was chromatographed over silica gel (200 g) and successively eluted with benzene (fractions 1–5) and $CHCl_3$ (6–20) and $CHCl_3$–EtOAc v/v 9:1 (21–25) (each fraction 200 ml). The product (0.2 g) obtained from fractions 6–8, was rechromatographed over silica gel (30 g) by eluting with benzene (portions 1–3, each 100 ml). Portion 2 yielded auriculasin (0.03 g) m.p. 176–78°. It formed a triacetate, m.p. 174–76°, with Ac_2O–NaOAc. Fractions 9–10 yielded a pale yellow solid (0.2 g) which was crystallised from benzene to yield Xa (0.05 g), m.p. 237–38° (Found: C, 71.65; H, 5.32; $C_{25}H_{22}O_6$ Requires: C, 71.76; H, 5.26%), Mass spectrum: *m/e* 418 (M^+). Fractions 11–15 yielded a yellow product (0.4 g) which crystallised from pet ether-$CHCl_3$ to give aurmillone (0.2 g) (I) as pale yellow needles, m.p. 157–58°, identical with an authentic sample.[4] Fractions 21–25 yielded II (0.2 g) m.p. 138°, identical with an authentic sample.[1]

Diacetate of millettin (Xb). Xa (0.01 g), Ac_2O (1 ml) and NaOEt (0.5 g) were refluxed in an oil bath for 4 hr and the mixture was poured over ice (10 g) to give a colourless solid which crystallised from MeOH to give Xb (0.006 g), m.p. 142°. (Found: C, 69.25; H, 5.32; $C_{29}H_{26}O_8$ Requires: C, 69.33; H, 5.18%), Mass spectrum *m/e* 502 (M^+).

Compounds XIIa and XIIIa were isolated from the roots of *Derris scandens* (collected from the forests of Warangal, Andhra Pradesh) and subsequently methylated to give XIIb and XIIIb respectively according to a known procedure.[10]

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