Carbon-13 Magnetic Resonance Spectra of Natural Rotenoids and their Relatives

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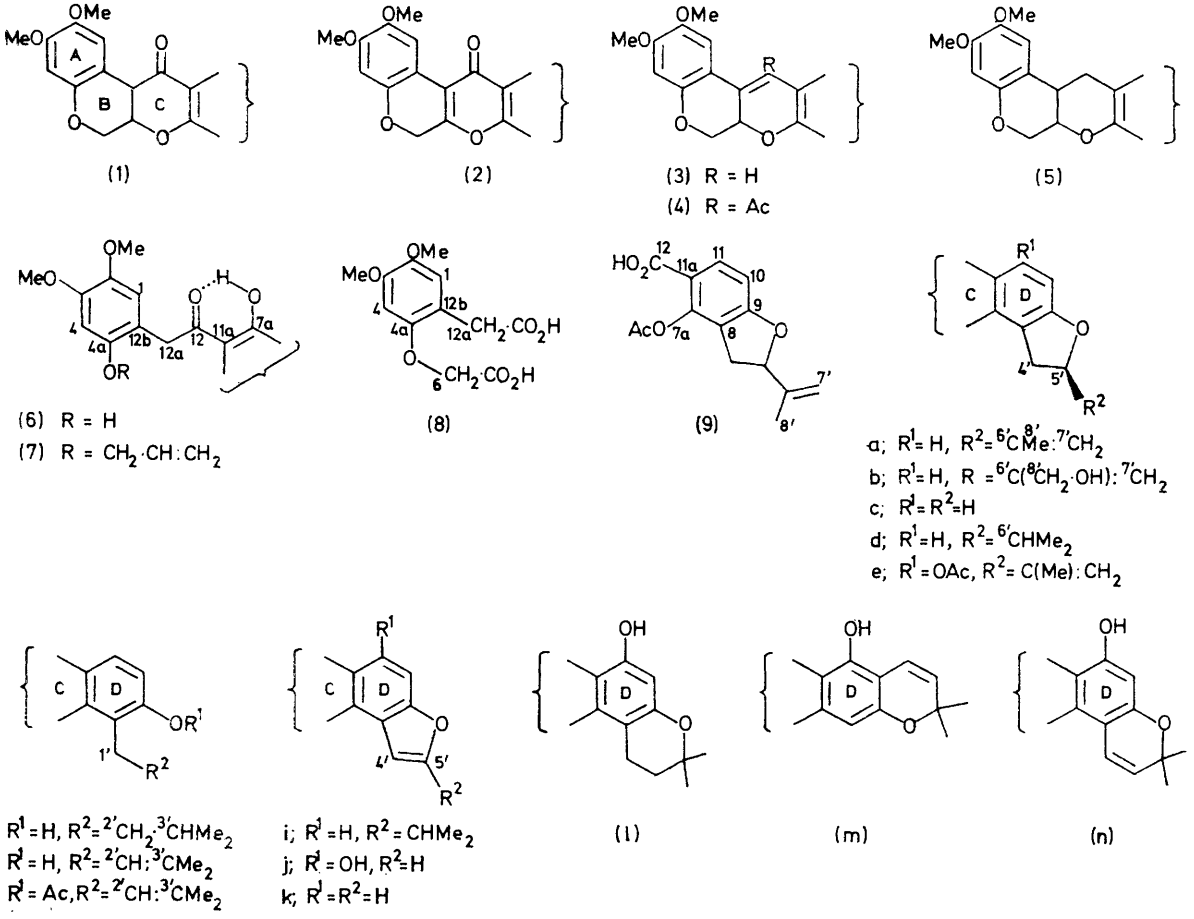
Natural abundance ^{13}C n.m.r. spectra of six rotenoids and eighteen related structures have been measured by pulse-Fourier transform techniques. From use of noise-decoupling. and single frequency off-resonance decoupling, a self-consistent series of assignments has emerged.

INCREASING knowledge of ^{13}C chemical shift data and advances in magnetic resonance technique have enabled substantially complete analyses of the ^{13}C n.m.r. of various rather complex natural products [1] to be performed. Such information is useful in structure determination [2] and, increasingly, for the detection of labelled sites in biosynthetic experiments,[3] especially those employing micro-organisms. The lower precursor incorporations common in higher-plant biosynthesis makes such experimentation possible, but more exacting.

In connection with our work on rotenoid biosynthesis [4] we have undertaken analysis of the ^{13}C magnetic resonances of rotenone (1a) and five other natural rotenoids [amorphigenin (1b), rotenonic acid (1g), elliptone (1k),



Numbering in formulae (6)—(9) employed to clarify tabulation

[1] *Inter alia* R. H. Levin, J.-Y. Lallemand, and J. D. Roberts, *J. Org. Chem.*, 1973, **38**, 1983; E. Wenkert, D. W. Cochran, E. W. Hageman, F. M. Schell, N. Neuss, A. S. Katner, P. Potier, C. Kan, M. Plat, M. Koch, H. Mehri, J. Poisson, N. Kunesch, and Y. Rolland, *J. Amer. Chem. Soc.*, 1973, **95**, 4990; G. Lukacs, F. Piriou, S. D. Gero, and D. A. Van Dorp, *Tetrahedron Letters*, 1973, 515; B. Birdsall, N. J. M. Birdsall and J. Feeney, *J.C.S. Chem. Comm.*, 1972, 316; A. Allerhand and D. Doddrell, *J. Amer. Chem. Soc.*, 1971, **93**, 2777; E. Wenkert, D. W. Cochran, E. W. Hageman, R. B. Lewis, and F. M. Schell, *ibid.*, p. 6271.

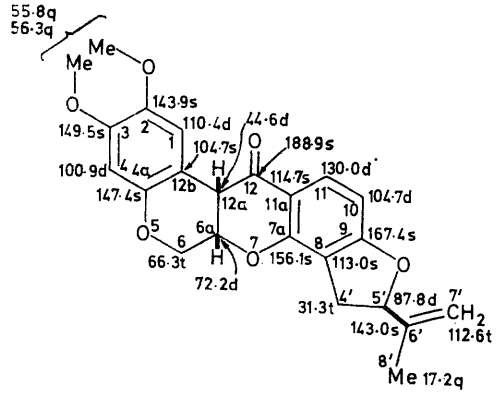
[2] A. Rabaron, M. Koch, M. Plat, J. Peyroux, E. Wenkert, and D. W. Cochran, *J. Amer. Chem. Soc.*, 1971, **93**, 6270; N. S. Bhacca, R. A. Wiley, N. H. Fischer, and F. W. Wehrli, *J.C.S. Chem. Comm.*, 1973, 614; A. J. Jones and M. H. Benn, *Tetrahedron Letters*, 1972, 4351.

[3] *Inter alia* M. Tanabe, T. Hamasaki, H. Seto, and L. Johnson, *Chem. Comm.*, 1970, 1539; R. J. Cushley, D. R. Anderson, S. R. Lipsky, R. J. Sykes, and H. H. Wassermann, *J. Amer. Chem. Soc.*, 1971, **93**, 6284; A. L. Burlingame, B. Balogh, J. Welch, S. Lewis, and D. Wilson, *J.C.S. Chem. Comm.*, 1972, 318; A. R. Battersby, J. Moron, E. McDonald, and J. Feeney, *ibid.*, p. 920.

[4] L. Crombie, P. M. Dewick, and D. A. Whiting, *J.C.S. Perkin I*, 1973, 1285, and references cited therein.

toxicarol (1n), and malaccol (1j)], the acetate of sumatrol (1e), and seventeen derivatives. Noise-decoupled spectra were recorded for all compounds, usually together with single-frequency off-resonance (s.f.o.r.) spectra.

Assignments for rotenone itself are shown in the Figure; multiplicities observed under s.f.o.r. conditions are also shown. The order of chemical shift from Me_4Si of the sp^3-hybridised carbon atoms is expected to be $8' < 4' < 12a < MeO < 6 < 6a < 5'$, from simple



^{13}C N.m.r. data of rotenone

electronegativity considerations, and the observed peaks fall into this pattern, with the correct multiplicities. Comparison with derivatives (see below) adds further confirmation. Recognition of the two olefinic signals is assisted by their absence in the spectrum of dihydrorotenone (1d) which otherwise, relative to rotenone, has almost unchanged sp^2-hybridised carbon resonances. The 12 aromatic carbons, giving relatively closely spaced resonances (and of importance in biosynthesis), are less easily assigned. As a starting point, the substituent shifts for benzene derivatives collated by Levy and Nelson [5] and Stothers [6] were assumed to be additive and were used to calculate the resonance parameters. 'Calculated' shifts are shown for the aromatic carbons of rotenone in Table 2: the assignments of the Figure were then made, taking into account the observed multiplicities and changes in chemical shift resulting from structural variation in the rotenoid congeners. The main points of ambiguity lie in allocation of the pair of signals * at 114.7 and 113.0 † to C-8 and C-11a an the pair at 147.4 and 149.5 to C-3 and C-4a. In the first case, the order shown in the Figure (the same as that calculated) is suggested by the shifts induced in ring D carbon signals by C-11 acetoxylation, as in sumatrol acetate (1e): 113.0 ⟶ 110.9 for C-8 (−2.1) and 114.7 ⟶ 105.6 for C-11 (−9.1), reasonably for carbons *ortho* and *para* to the acetoxy-group.[6] For C-3 and C-4a the 'calculated' order is probably incorrect, with the C-3 signal at 149.5 and that of C-4a at 147.4, thus rationalising the change in the latter signal in isoderritol (6i) (+2.0 p.p.m.) and dihydrorotenone enol acetate (4d) (−3.4 p.p.m.). The former resonance is little altered (149.4 and 150.0, respectively).

Data for the rotenoid relatives are collected in Tables 1 (carbon atoms other than rings A and D) and 2 (aromatic ring A and D carbon atoms), and provide a useful aid to structure determination as well as to biosynthetic experimentation.

The two methoxy-carbon atoms give rise to very close signals [coincident in (1e) and (1n)] in the range 55.7—56.7 p.p.m. In the BC system, C-12 (carbonyl) absorbs close to 189 p.p.m., except if chelated with a hydroxy-group at C-11 or -7a [(6i), (7i), 1n), (1l), and (1m) when it shifts downfield from Me_4Si in line with previous reports.[6] Malaccol (1j) with an 11-OH and a furanoid ring E is an exception. Reduction of the C(12)=O to $C(12)H_2$ (5d) diminishes the shielding at C-12a (−12.8) and also at C-6a (−2.5). Introduction of a 12,12a-double bond [as in (3a), (4a), and (4h)] has little effect on the shift of C-6 or -6a, nor is there significant change in the C-6 signal in compounds with a 6a,12a-double bond (2a) and d).

The nature of ring E, or other attachments to ring D, is clearly characterised in these spectra. The difference between amorphigenin and rotenone is shown not only by the change of status of C-8', but also by the reduced shielding (−5.8) of C-6' in the 8'-hydroxy-compound. Formal ring closure of tetrahydrorotenone (1f) to dihydrorotenone (1d) is reflected in the (+68.2) shift of C-2' on oxygen substitution.

Methyl carbon signals (C-7' and C-8') are separated in dihydrorotenone (adjacent to chiral C-5') but not in tetrahydrorotenone. The *cis*- and *trans*-methyl groups of rotenonic acid (1g) can be linked with the 17.8 and 25.8 resonances, respectively, in accord with similar (terpenoid) systems.[6] C-1' in rotenonic acid is also shielded (δ 22.1). Usefully, the *gem*-dimethyl groups in six-membered ring E compounds (1n, l, and m) resonate at significantly higher field (26.4—28.2) than those in acyclic structures.

Chemical shifts of the aromatic carbon atoms (C-2, C-3, and C-4) of ring A are rather constant in this series of compounds, being insensitive to ring BC changes. The C-1 and C-4a resonances also vary little; the former shows slight increase in BC-cleaved compounds (8), (6i), and (7i), and the latter responds to altered electron release in (6i) and (7i). The C-12b peak remains within a narrow range in compounds with the rotenoid BC structure, but is more deshielded in compounds with BC opened, or with the 12-oxo-group removed, or with a 6,6a- or 12,12a-double bond. In ring D, the C-7a signal is shifted in compounds lacking the 12-oxo-group, or

* Data throughout given in p.p.m. from tetramethylsilane.

† *Note added in proof:* The C-8 signal (113.0) lies close to that of C-7' (112.6) and the multiplicity of the latter is not readily observed. However assignments can be made on a basis of relaxation times (T_1): 6.27 and 5.35 s for C-7' and -8 respectively (measured at 450 mg cH^{-3}, $CDCl_3$).

[5] G. C. Levy and G. L. Nelson, 'Carbon-13 Nuclear Magnetic Resonance for Organic Chemists,' Wiley–Interscience, New York, 1972.

[6] J. B. Stothers, 'Carbon-13 N.m.r. Spectroscopy,' Academic Press, London, 1972.

TABLE 1

^{13}C N.m.r. assignments for non-benzenoid carbons of rotenoids

Compound		C-6	C-6a	C-12	C-12a	C4′ * C1′ †	C5′ * C2′ †	C6′ * C3′ †	C7′ * C4′ †	C8′ * C5′ †	OCH_3	OCH_3	Others
Rotenone	(1a)	66.3	72.2	188.9	44.6	31.3	87.8	143.0	112.6	17.2	55.8	56.3	
	(1d)	66.2	72.2	188.9	44.6	29.3	90.8	33.2	17.9	17.6	55.8	56.3	
Amorphigenin	(1b)	66.8	73.1	189.3	45.3	32.5	86.2	147.8	112.7	63.5	56.1	56.9	
Rotenonic acid	(1g)	66.3	72.1	188.9	44.2	22.1	121.1	134.3	17.8	25.8	55.8	56.2	
	(1f)	66.3	72.0	190.5	44.3	28.0	22.6	38.0	20.6	20.6	55.8	56.3	
Elliptone	(1k)	65.1	71.8	186.6	44.0	103.0	142.5				54.9	55.5	
	(1c)	66.3	72.3	188.9	44.6	26.3	73.0				55.8	56.3	
Derric acid	(8)	66.3			34.6						55.7	56.1	
Tubaic acid acetate	(9)			170.2		31.6	87.7	142.8	112.9	16.9			Ac 168.8, 20.8
	(6i)			205.1	40.0	98.0	165.0	28.1	20·8	20.8	55.8	56.7	
	(7i)	70.4		204.0	39.8	98.0	164.5	28.2	20.8	20.8	56.3	56.7	CH_2·CH:CH_2 133.6, 117.3
	(4d)	67.6	72.4		135.3	29.7	89.6	33.2	17.7	18.1	55.8	56.1	Ac 167.6, 20.8
Sumatrol acetate	(1e)	65.9	71.6	187.5	45.4	31.2	88.2	142.6	112.7	17.1	56.0	56.0	
α-Toxicarol	(1n)	65.8	71.7	194.1	43.4			78.1	28.2	28.2	56.1	56.1	
	(1l)	66.1	71.8	194.1	43.5	16.1	31.8	76.3	26.4	27.1	55.8	56.3	
Malaccol	(1j)	65.8	72.5	183.9	44.1	104.3	143.9				55.8	56.3	
	(5d)	65.6	69.7	30.87	31.8	29.4	89.1	33.2	17.7	18.2	55.8	56.0	
	(3a)	67.9	71.1	105.3	123.3	31.7	86.6	143.8	112.0	17.3	56.0	56.0	
	(4h)	67.7	72.7		132.0	23.2	121.3	134.4	17.8	25.7	56.0	56.3	2 × Ac 167.6, 169.2, 20.9
	(4a)	67.7	72.5		135.2	31.6	86.8	143.6	112.1	17.2	55.8	56.2	Ac 167.7, 20.9
	(2a)	64.8	156.1	174.2	118.8	31.4	87.9	142.8	112.9	17.1	55.8	56.3	
	(2d)	64.8	156.0	174.2	118.5	29.5	90.8	33.2	18.0	17.6	55.8	56.2	
	(1i)	66.2	72.7	190.0	44.7	97.8	165.2	28.1	20.8	20.8	55.8	56.3	
β-Toxicarol	(1m)	66.0	71.8	194.3	43.7			78.4	28.5	28.5	55.8	56.3	

* Rotenoids with ring E. † Rotenonic acid relatives.

TABLE 2

^{13}C N.m.r. assignments for sp^2-hybridised carbon atoms of rings A and D

Compound		C-1	C-2	C-3	C-4	C-4a	C-12b	C-7a	C-8	C-9	C-10	C-11	C-11a
Rotenone	(1a)	110.4	143.9	149.5	100.9	147.4	104.7	156.1	113.0	167.4	104.7	130.0	114.7
	(1d)	110.2	143.9	149.3	100.8	147.4	104.7	157.9	112.5	167.7	104.7	129.9	113.3
Amorphigenin	(1b)	111.7	145.2	150.4	101.6	148.3	105.2	158.6	113.5*	167.6	105.2	130.5	114.6
Rotenonic acid	(1g)	110.4	143.6	149.3	100.8	147.6	104.7	162.2	112.6	160.1	110.8	127.0	114.7
	(1f)	110.5	143.7	149.2	100.9	147.7	104.8	161.4	112.6	160.6	110.5	126.6	118.7
Elliptone	(1k)	109.1	141.7	147.4	99.6	145.2	103.0	157.6	111.7	159.0	104.9	121.9	115.0
	(1c)	110.4	143.9	149.5	100.9	147.4	104.8	158.0	113.3	167.9	104.8	129.8	113.3
Derric acid	(8)	106.8	143.0	150.1	99.8	148.2	116.3						
Tubaic acid acetate	(9)							148.6	114.2*	165.7	107.0	134.4	121.6
	(6i)	114.3	143.0	149.4	102.2	149.4	112.9	159.1	111.5	160.0	104.0	126.0	118.9
	(7i)	114.5	143.7	150.9	99.8	149.1	115.4	158.4	113.7	159.5	103.3	125.9	118.6
	(4d)	109.1	143.5	150.0	100.9	144.0	110.9	162.0	113.4	162.2	102.7	121.8	113.4
Sumatrol acetate	(1e)	110.3	143.8	149.5	100.8	147.0	104.4	157.7	110.9	166.2	99.9	160.0	105.6
α-Toxicarol	(1n)	110.1	143.7	149.4	101.0	147.1	104.3	162.6	101.0	164.3	97.5	155.7	101.5
	(1l)	110.3	143.8	149.5	100.7	147.3	104.7	162.2	100.7	163.5	97.7	159.0	100.7
Malaccol	(1j)	110.1	143.9	149.8	101.1	147.4	104.3	161.9	102.6	160.8	93.0	160.8	101.1
	(5d)	111.0	143.6	150.0	100.6	147.8	111.0	149.0	111.0	160.1	101.8	128.2	113.0
	(3a)	112.0	144.7	149.4	101.0	147.7	110.9	150.2	112.8*	161.2	102.8	126.8	115.2
	(4h)	109.7	144.2	149.6	101.0		108.5		118.9		115.7	121.8	119.4
	(4a)	109.5	144.0		100.9		111.1		113.7*	162.0	102.8	121.9	113.0
	(2a)	109.9	143.9	148.8	100.3	146.2	110.5	152.2		164.7	108.6	127.7	
	(2d)	109.9	143.9	148.8	100.3	146.1	110.6	152.2		165.0	108.6	127.5	
	(1i)	110.3	143.8	149.5	100.9	147.4	104.6	155.2	113.3	160.0	106.2	122.9	118.2
β-Toxicarol, calculated for rotenone	(1m)	110.3	143.9	149.6	101.0	147.3	104.5	161.5	96.2	162.6	103.2	159.1	101.0
		116.8	140.8	146.3	100.6	154.0	116.3	161.7	108.6	165.8	106.3	127.7	115.4

* Signals from C-8 and -7′ are very close and assignments are tentative.

when ring C has γ-pyrone character (2a and d). Where ring E is opened to form a free 9-OH (1g and f), C-10 is less shielded (*ca.* +6 p.p.m.); when ring D carries an 11-OH (1j, n, l, and m) the C-11 signal is moved downfield as expected, while C-8 and C-11a are more shielded (*ca.* +10 p.p.m.). C-11 Absorption is also responsive to a furan ring E (1k and j), which induces additional shielding at that position.

EXPERIMENTAL

The ^{13}C n.m.r. spectra were obtained by using a JEOL JNM-PS-100 spectrometer at 25.15 MHz, interfaced with a Nicolet 1085 20 K computer. Deuterium lock was provided by the solvent ($CDCl_3$). The pulse width was 3 μs (22° tip) and the F.I.D.'s were compiled by using 8 K data points over a spectral width of 6 000 Hz.

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