Electron Spin Resonance Study of Radicals obtained from the Oxidation of Naturally Occurring Hydroxypyrones

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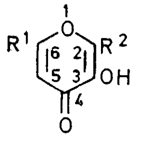
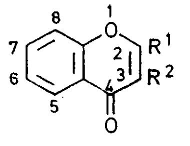
Many hydroxylated derivatives of γ-pyrone such as kojic acid, maltol, flavones, and also coumarins, may be oxidised by Ce^{IV} in acidic solutions to give radicals analogous to phenoxyl radicals. These have been observed by e.s.r. spectroscopy: the patterns of coupling constants obey some simple rules which are given in terms of non-bonding orbital coefficients. Addition of ·OH to 4-hydroxy-6-methylpyran-2-one gives a novel type of radical, 2,3,4-trihydroxy-6-methylpyranyl, whose structure is confirmed theoretically.

DURING the course of our investigations of substituted phenoxyl radicals [1] and also of tropolonyl radicals,[2] we developed theories which enabled us to rationalise the coupling constants observed in their e.s.r. spectra. The understanding we gained from these studies led us to predict alternating spin densities in other types of oxygenated organic radicals such as those derived from hydroxylated pyrones. These compounds occur widely in nature, the monocyclic derivatives such as kojic acid (Ia) and maltol (Ib) being found in certain fungal metabolites [3] and the benzopyrones such as the flavones (IIa), isoflavones (IIb), and coumarins (III) being distributed throughout the plant kingdom.[4]



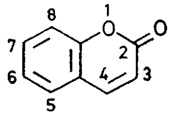
(I)

a; R^1 = CH_2OH, R^2 = H
b; R^1 = H, R^2 = CH_3



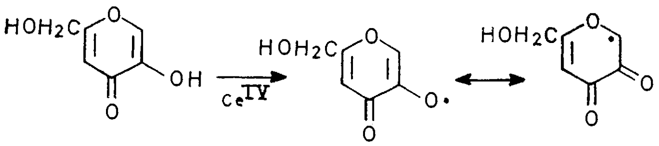
(II)

a; R^1 = Ar, R^2 = H
b; R^1 = H, R^2 = Ar



(III)

Like tropolone, kojic acid exhibits a number of phenolic properties,[3] for it is strongly acidic and gives a characteristic colour with ferric chloride. It was not surprising, therefore, that these oxygen heterocyclic compounds could also be oxidised by cerium(IV), or by hydroxyl radicals generated in the Ti^{III}–H_2O_2 system,[5] to give radicals analogous to phenoxyl [6] (Scheme 1).



SCHEME 1

Using a flow system [7] we were able to obtain well-resolved e.s.r. spectra (see Figure 1) of these radicals and the coupling constants are given in Table 1.

Assignment of Coupling Constants.—Some of the coupling constants are easily assigned because of the siting of the substituents in our chosen examples. However, in order to make a more complete assignment it is necessary to turn to theory as a guide. We have

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[1] W. T. Dixon, M. Moghimi, and D. Murphy, *J.C.S. Faraday II*, 1974, 1713.

[2] W. T. Dixon and D. Murphy, *J.C.S. Perkin II*, 1974, 1430.

[3] A. Beélik, *Adv. Carbohydrate Chem.*, 1956, **11**, 145.

[4] T. A. Geissman and D. H. G. Grout, 'Organic Chemistry of Secondary Plant Metabolism,' Freeman Cooper, San Francisco, 1969.

[5] W. T. Dixon and R. O. C. Norman, *J. Chem. Soc.*, 1963, 3119.

[6] T. J. Stone and W. A. Waters, *J. Chem. Soc.*, 1964, 213.

[7] W. T. Dixon, W. E. J. Foster, and D. Murphy, *J.C.S. Perkin II*, 1973, 2124.

TABLE 1

Coupling constants in radicals by one electron oxidation of pyrone derivatives

Starting material	Coupling constants (10^{-4}T) a_2	a_3	a_4	a_5	a_6	a_7	a_8
5-Hydroxy-2-hydroxymethylpyran-4-one (kojic acid)	a_{OH_2} 1·38 [−2·0]	0·35 [0·63]			13·2 [−7·4]		
3-Hydroxy-2-methylpyran-4-one	a_{OH_3} 11·75 [−7·4]			0·25 [0·63]	1·38 [−2·0]		
7-Hydroxycoumarin (umbelliferone)		5·6 [−8·0]	1·9 [1·9]	2·1 [0·63]	5·6 [−3·3]		6·8 [−6·8]
7-Hydroxy-4-methylcoumarin		5·6 [−8·0]	a_{CH_3} 1·9 [1·9]	1·9 [0·63]	5·6 [−3·3]		6·3 [−6·8]
6-Hydroxy-4-methylcoumarin		0·4 [0·4]	a_{CH_3} 0 (−0·66]	5·2 [−7·8]		7·3 [−2·0]	1·1 [1·2]
5,7-Dihydroxyflavone (chrysin)		1·0 [2·4] [−2·0]			8·2 [−5·52] [−8·7] *		11·25 [−15·1] [−13·0] *
3′,5,7-Trihydroxy-4′-methoxyflavone 7-rhaminosylglucoside (hesperidin)		1·5 [−2·0]			9·0 [−8·7]		11·5 [−13·0]
5,7-Dihydroxy-4′-methoxyisoflavone (biochanin A)	0·0 [0·2] [−0·3] *				7·8 [−5·52] [−8·7] *		10·75 [−15·1] [−13·0] *
7,8-Dihydroxy-4-methylcoumarin (4-methyldaphnetin)		0·0 [−0·8]	a_{CH_3} 0·0 [0·06]	6·6 [−5·16]	1·3 [1·4]		
6,7-Dihydroxy-4-methylcoumarin (4-methylaesculetein)		2·4 [−2·22]	a_{CH_3} 0·8 [0·24]	2·4 [−2·9]			0·0 [0·8]
6,7-Dihydroxycoumarin (aesculetin)		2·62 [−2·22]	1·0 [0·24]	2·62 [−2·9]			0·25 [0·8]
5,7-Dihydroxy-4-methylcoumarin		5·45 [−6·0] ‡	a_{CH_3} 1·30 [1·2] †		4·25 [−3·5]		10·5 [−11·2] †

Calculated values are in square brackets. Q value for methyl protons is taken to be the same as that for aromatic protons but is of opposite sign (Q_H −30).

* Values calculated for the radical with the 7-oxygen protonated. † Values calculated for equilibrium between radicals protonated at 5- or 7-O.[7]

previously been able to account for the hyperfine splittings in a variety of aryloxyl radicals[1] by means of McLachlan's simplified form of SCF theory,[8] using as oxygen parameters $\alpha_O = \alpha_C + 1{\cdot}6\beta_{CC}$ and $\beta_{CO} = 1{\cdot}3\beta_{CC}$. The theoretical results given in Table 1 were calculated using this method, and the agreement with experiment is encouraging, considering the restricted class of compounds for which the semi-empirical theory was developed. We feel, from this, reasonably confident that we have correctly interpreted the nature of the radicals and the main features of the spin distribution within them.

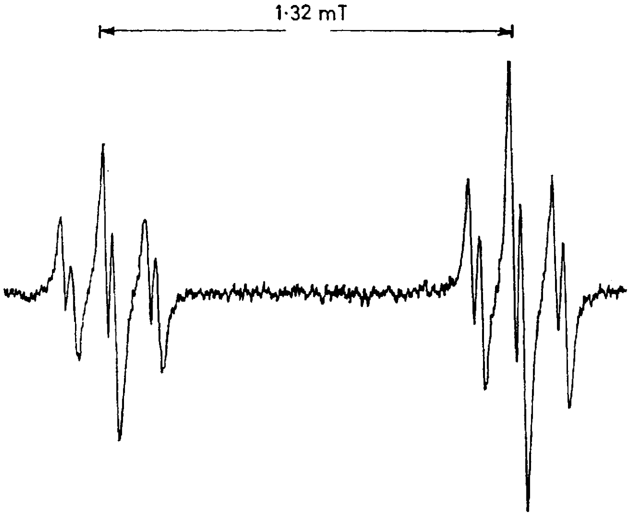

FIGURE 1 E.s.r. spectrum of radical from kojic acid

DISCUSSION

This investigation of radicals from pyrones was a natural continuation of our work on other hydroxylated aromatic systems. The general pattern of spin densities in radicals related to phenoxyl can often be predicted from two very simple MO models, both involving an odd electron in a non-bonding orbital.[2,9] In the first model the extranuclear oxygen atoms are treated effectively as carbon atoms, and in the second we consider the fragment left after removing these oxygen atoms and the adjacent carbons.[10] Both models give the same pattern of NBO coefficients and it is assumed that where zeros occur near to large spin densities in the theory, appropriate (smaller) negative spin densities will result in practice.

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[8] A. D. McLachlan, *Mol. Phys.*, 1960, **3**, 233.

[9] N. V. Riggs, ' Quantum Chemistry,' Macmillan, 1969, ch. 5, p. 160.

[10] W. T. Dixon, W. E. J. Foster, and D. Murphy, *Mol. Phys.*, 1974, **27**, 1709.

Intelligent application of this crude theory often gives better, as well as more rapid, predictions than much more sophisticated calculations. There have, of course been many other similarly successful applications of simple MO theories in the past.[11] It is an illustration of the usefulness of these models that we did correctly forecast (before the experiments) the number and positions of relatively high spin density in most of the radicals in Table 1. The best match with experiment was obtained when the models were modified by excluding the nuclear oxygen (see Figure 2).

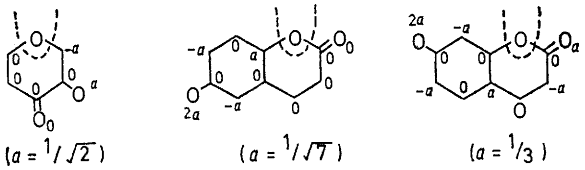
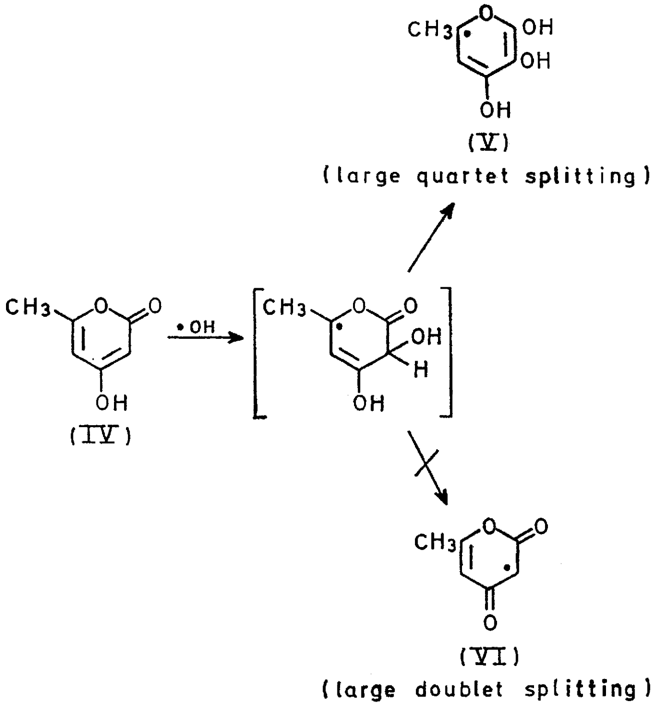


FIGURE 2 Model systems for some of the radicals, with NBO coefficients

Results from the hydroxyflavone derivatives were unexpected in the sense that two large coupling constants were observed where, by analogy with the radical from resorcinol,[12] one would have expected only one. However, comparison with the result from hesperidin suggests that under the strongly acidic conditions, the oxygen attached to C-7 must be preferentially protonated. This was confirmed by calculation, using parameters found previously [1] to be suitable for the hydroxy-group, *i.e.* $\alpha_{OH} = \alpha_C + 1{\cdot}5\beta_{CC}$, $\beta_{C-OH} = 0{\cdot}6\beta_{CC}$.

Reactions using Hydroxyl Radicals.—A large unanalysable mixture of radicals was obtained when kojic acid was present during the Ti^{III}–H_2O_2 reaction, though especially at high acidities, the spectrum of the radical from kojic acid, given in Figure 1, could be picked out. In contrast to this, a pure spectrum was obtained when maltol was the substrate, for in this case the most reactive position is blocked. Similarly there was a remarkably intense spectrum when the substrate was 4-hydroxy-6-methylpyran-2-one (IV), which like 4-hydroxycoumarin would not react with cerium(IV). The coupling constants observed were $0{\cdot}75 \times 10^{-4}$ T (doublet) and $6{\cdot}54 \times 10^{-4}$ T (quartet) and we ascribe the spectrum to the novel radical (V). We suggest that it arises from initial addition of ·OH followed by rapid enolisation in preference to acid-catalysed loss of water.[13]

The McLachlan-type calculations, using the parameters already given for ·OH and O and neglecting the effects of the methyl group, are given in Table 2 and it is clear that, whereas the predicted values for (V) are almost exactly the same as those observed, those for (VI) do not resemble the experimental splittings at all. Thus we confirm the qualitative suggestions of simple valence-bond theory and it seems very probable that we have correctly deduced the nature of the observed radical.



SCHEME 2 Possible reaction schemes for reaction of (IV) with most significant canonical structures

TABLE 2

Comparison of calculations with results of oxidation of 4-hydroxy-6-methylpyran-2-one

	Coupling constants (10^{-4}T)		
	a_3	a_5	$a^{\dagger}_{CH_3}$
Experimental	0 *	0·75 *	6·55
Calculation for (V)		1·7	7·2
Calculation for (VI)	17·6	3·3	3·5

* These are of course ambiguous. † $Q_{CH_3} = -Q_H$ in the calculations.

In a number of flavones in which there were hydroxy-substituents in the phenyl ring, mixtures of radicals were observed showing oxidation at competing sites in these molecules.

EXPERIMENTAL

The experiments were carried out with the flow system described previously.[7] In the ceric oxidations concentrations were of the order of 10^{-2}M and in the Ti^{III}–H_2O_2 system, substrate concentrations were 0·1M except for 4-hydroxy-6-methylpyran-2-one which was *ca.* 0·02M.

[4/1430 *Received*, 15*th July*, 1974]

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[11] A. Carrington, *Quart. Rev.*, 1963, **17**, 67.

[12] T. J. Stone and W. A. Waters, *J. Chem. Soc.*, 1964, 4302.

[13] C. R. E. Jefcoate and R. O. C. Norman, *J. Chem. Soc.* (*B*), 1968, 48.