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STEMPHONE: A NEW TYPE OF NATURAL QUINONE

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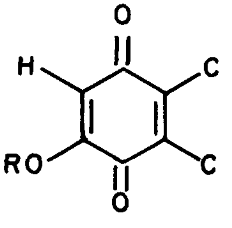
Stemphone is a yellow pigment from *Stemphylium sarcinaeforme* (Cav.) Wiltshire, a fungal pathogen for red clover[1]. It crystallized as needles from hexane, mp 161.5°, $[\alpha]_D$ +146° (c=1 in ethanol). It had ν_{max} (CCl_4) 3578, 3510, 1735, 1673, 1645, 1620 and 1596 cm^{-1} and λ_{max} (MeOH) 267 nm (9890) and 390 nm (1030) (no shift in alkali). The empirical formula $C_{30}H_{42}O_8$ was supported by analysis, mass spectrum (M^+530) and molecular weight in chloroform (found 523).

Further evidence that it was a benzoquinone was obtained. It was reduced by hydrogen over Pd/C to a colorless dihydro compound, mp 138° with ν_{max} ($CHCl_3$) 3545, 3377, 1720, 1636 and 1594 cm^{-1}, and λ_{max} (EtOH) 295 nm (6000), reoxidizable to stemphone by $FeCl_3$ in alcohol. The dihydro compound when treated with methyl iodide and potassium carbonate in acetone gave a monomethyl ether mp 219°, M^+546, and a dimethyl ether mp 131°, ν_{max} ($CHCl_3$) 3560 and 1725 cm^{-1} and λ_{max} 288 nm (3200). The latter gave ^1H nmr signals at δ3.79 and 3.89 (3H singlets).

Treatment of stemphone with zinc in acetic anhydride-pyridine gave a leuco triacetate with mp 222° which analysed for $C_{36}H_{50}O_{11}$. It had ν_{max} (CCl_4) 3580, 2978, 2950, 2875, 1763, 1733, 1621 and 1577 cm^{-1}. This evidence that stemphone contained a secondary and tertiary hydroxyl group was supported by ^1H nmr evidence (see below).

A sharp signal in the ^1H nmr spectrum of stemphone at δ6.45 corresponded to a proton on the quinone ring. The absence of coupling with other protons suggested that the adjacent carbon was substituted by oxygen or a quaternary carbon. The absence of a shoulder or maximum around 430 nm suggested that the quinone ring carried an oxygen substituent, and this was further supported by the ^{13}C nmr spectrum (see below). We hence postulated the part structure **1**.



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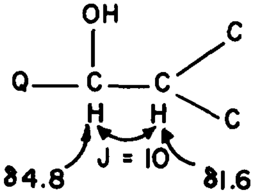

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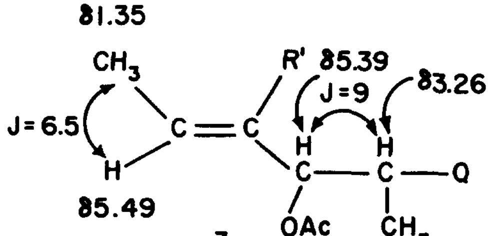

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Treatment of the monomethyl ether of dihydro stemphone with 3N sulfuric acid in tetrahydrofuran gave a dehydration product mp 210°, M^+528, λ_{max} (EtOH), 290 nm (15,300), 304 nm (10,340), 314 nm (sh., 5060). The extended conjugation, and the very low-field resonance of the new vinyl hydrogen (δ approx. 6.8) were consistent with a styrene system. Hence the arrangement shown in 2 must be present in stemphone. Hydrolysis of this product gave 1.4 equivalents of volatile acid. The product had M^+486 indicating loss of acetic acid. A 1H doublet at δ5.4 in the spectrum of the parent ether moved upfield to δ4.1 on the hydrolysis product, and a 3H singlet at δ1.8 disappeared. This, and the M-60 peaks in the mass spectra of stemphone and derivatives confirmed that stemphone contained a secondary acetoxy group. The low field resonance of the proton on this carbon required that it also be in an allylic or benzylic position.

The ^{13}C nmr spectrum of stemphone showed the presence of a carbon-carbon double bond (see below). This, and evidence from chemical shifts and coupling of protons provided by a 220 MHz spectrum of stemphone in benzene, enabled postulation of the sequence shown in 3. The ^{13}C nmr spectrum showed the presence of six saturated carbons carrying oxygen (δ63 to 85 ppm). Hence in addition to the acetoxyl, secondary hydroxyl and tertiary hydroxyl groups, two ether oxygens are present, one of which is attached to the quinone ring or an olefinic carbon.

No reasonable structure could be written incorporating the above features. The limited amount of material available[2], and the low yields in other reactions studied, prevented solution of the structural problem. However an X-ray analysis was successful.

The yellow acicular crystals of stemphone have orthorhombic symmetry. The space group is $P2_12_12_1$ and the cell dimensions are a = 6.526, b = 19.071, c = 23.285 Å. There are four molecules per unit cell. The cell dimensions and the relative intensities of 2845 independent reflexions were measured on a Picker 4-circle diffractometer with Ni-filtered Cu radiation. The structure was solved by direct methods, and refined by block-diagonal least-squares calculations to an R value of 0.062.

The molecular structure is shown in Fig. 4. The fused tetracyclic ring system appears to be unique among naturally-occurring quinones. The B/C and C/D ring junctions are both trans, and rings C and D are chair-shaped. Ring B has a half-chair form. The quinone system is markedly non-planar; both carbonyl groups are bent out of the plane through the four central atoms, in the same direction. The side-chain and its attached acetate group are twisted around so that the carbonyl bond in the acetate group lies below the quinone ring as shown in 4. Both hydroxyl groups are involved in intra-molecular hydrogen bonds, the terminal OH to its neighbouring ring oxygen atom, and the OH at C-7 to the carbonyl oxygen atom at C-1.

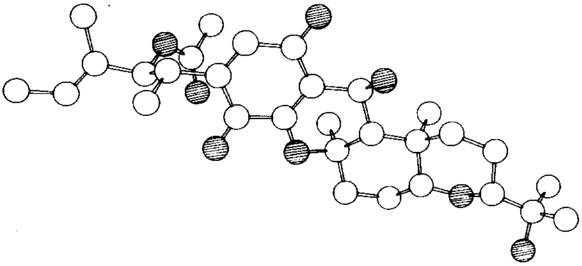
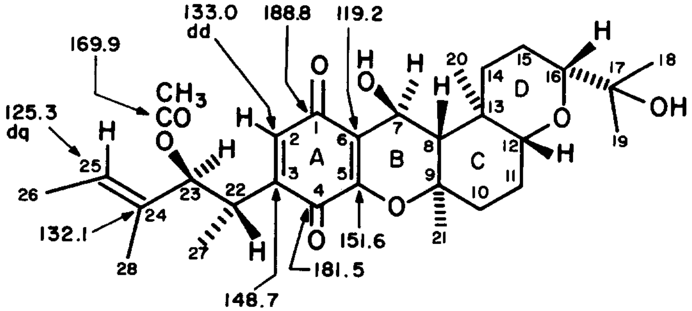


Fig. 4. Structure shown by X-ray analysis. Shaded circles represent oxygen atoms.



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Assignment of the low-field ^{13}C nmr signals, based in part on the assignment to perezone[3] and supported by the multiplicity due to coupling with protons, is indicated in 5. A signal at 51 ppm is unusual. It is coupled to one proton, and assignment to C-8 which is attached to two carbons carrying oxygen seems reasonable.

Clearly the fact that the proton on the quinone nucleus was not coupled to other protons was misleading, and partial structure 1 was wrong. In the 220 MHz proton spectrum of stemphone the protons coupling with those resonating at δ3.02 (dd) and 2.78 (dd) could not be located in spin-decoupling studies. However the assignment to the tertiary hydrogens on C-12 and C-16 seems sound. The origin of a one-proton distorted doublet at δ2.51 however is an intriguing question. The equatorial hydrogen on C-10 is in the plane of one ether oxygen and the quinone ring, so might be deshielded to this extent. Although the protons of the acetyl group of stemphone are only slightly shielded (δ1.95 in $CDCl_3$) the chemical shift of this group in dihydrostemphone and its mono- and dimethyl ethers and in the leuco triacetate was near δ1.8 in $CDCl_3$. This suggests a high population of rotamers with this group shielded by the π-electrons of the double bond and aromatic ring. The signal for the 7-hydroxyl proton was a doublet with J = 1.5 Hz at δ3.76 in $CDCl_3$. Hence it is not exchanging with other protons, and the hydrogen bonding found in the crystal also exists in solution. The coupling constant is in accord[4] with the HOCH dihedral angle of 65.6° found in the X-ray analysis.

The biosynthetic origin of the fused ring system is clearly a triprenyl chain alkylating the quinone ring. The 7-carbon chain appears to arise from a rearranged isoprene unit linked to an ethyl side chain. A similar feature occurs in perezone (3), in which an isoprene unit is coupled with an isopropyl side chain.

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References

1. P.M. Scott and J.W. Lawrence, Can. J. Microbiol. 14, 1015 (1968).
2. A strain of *Stemphylium sarcinaeforme* obtained from the American Type Culture Collection failed to yield stemphone when grown on the medium described in ref. 1.
3. P. Joseph-Nathan, M.P. González, L.F. Johnson and J.N. Shoolery, Org. Magn. Resonance 3, 23 (1971).
4. N.L. Bauld and Y.S. Rim, J. Org. Chem. 33, 1303 (1968).