Ergosterol Peroxide from *Rhizoctonia repens:* Composition, Conformation, and Origin

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Summary Ergosterol peroxide (ep), synthetic or from fungal extracts, is shown to be a mixture of isomers arising from α- and β-attack by $O_2(^1\Delta_g)$ on ergosterol; the stereochemistry of the peroxide bridge is assigned.

The origin of ergosterol peroxide (ep), a relatively rare sterol isolated from a number of fungal extracts,[1a,b] has been questioned. The gummy residue from extractions contains pigments which may function as photosensitizers in the presence of light and air, generating singlet oxygen $(^1\Delta_g)$,[1] after which ep is produced by symmetry-allowed attack[2] on endogenous ergosterol. The hypothesis was verified for two systems (*Piptoporus betulinus* and *Daedalea quercina*) and it was suggested that ep may be an artifact rather than a natural product.[1b] We now report new information which supports this contention.

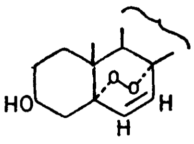
Extraction of fungal mats of *Rhizoctonia repens* M 32 yielded ep (0·03% t.l.c., u.v.) When crude extracts are left, the yield rises, but some ep is always present, even when extraction is followed immediately by analysis.

The stereochemistry of peroxide attachment is known to be 5α, 8α as originally inferred from the rule of rear attack.[3,4]

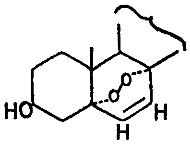
However, if an electrophilic reagent has relatively low steric requirements, C-5 approach is not exclusively from the α face.[5] *A priori*, it seemed reasonable that singlet oxygen ($^1\Delta_g$) would be small enough to permit attack from both faces. Ep was prepared[6] to give a product which melted sharply and had the properties reported. Signals of the ring B vinyl protons were two superimposed AB patterns (*J* 9 Hz for both) centred at δ ($CDCl_3$ *vs* Me_4Si) 6·33 (large) and 6·38 p.p.m. (small). We atrributed these two patterns to the 5α, 8α and 5β, 8β isomers respectively. In spectra run at 220 MHz, the two patterns were partially resolved. Integration showed the minor component to constitute 16% of the mixture.

The n.m.r. data could have had an alternate explanation: *viz.*, conformers of the peroxide bridge, (**1**) and (**2**), both derived from α-attack, which interconvert at a rate slow on the n.m.r. time scale. Models suggested (**1**) and (**2**) to be of comparable energy as required by this hypothesis. (A 15:85 mixture corresponds to a difference of one kilocalorie per mole in free energy at 25°.)

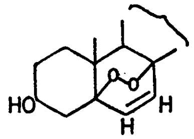
By the addition of dilute solutions of Eu(dpm)$_3$,[7] shifts of as much as 40 Hz in the C-6 and C-7 ep signals could be obtained, and the upfield doublet was always shifted more than the downfield doublet in both AB patterns. Such behaviour is not consistent with a mixture of (**1**) and (**2**) but would be expected from (**1**) and (**3**) if the proton closest to oxygen is always the downfield one, *i.e.* if the proximate C–O bond is deshielding.[8] Such is the case for ascaridole, (**4**),[9] where the vinyl protons are shifted some 0·2 p.p.m. downfield from the vinyl absorption in the model compound, bicyclo[2,2,2]octene.[9b] Since the C-6 (more shifted) proton corresponds to the upfield resonance in the AB pattern, the stereochemistry of the peroxide bridge is as in (**1**) and (**3**).



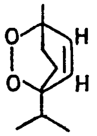
(1)



(2)



(3)



(4)

Comparison with "naturally occurring" ep shows that both samples are the same mixture. This finding strongly suggests that the "natural" material also arises from attack of singlet oxygen on ergosterol and hence is an artifact.

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