On the Biosynthesis *in Vitro* of Usnic Acid

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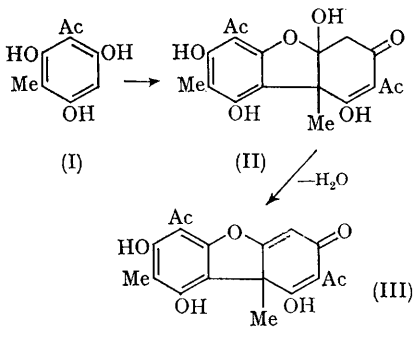
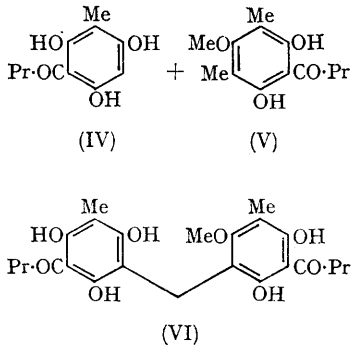
THE lichen constituent, usnic acid (III) and its hydration product (II) have been the subject of considerable investigation.[1] Barton *et al.*[2] have reported their chemical syntheses from methylacetylphloroglucinol (I) by oxidation with ferricyanide.



We have recently[3] been concerned with a related oxidative coupling of acylphloroglucinols such as (IV) and (V) in the presence of peroxidase and hydrogen peroxide to form the naturally occurring methylenebisacylphloroglucinols, *e.g.*, (VI), found in *Dryopteris* species.



It occurred to us that compounds related to usnic acid have never been observed in *Dryopteris*

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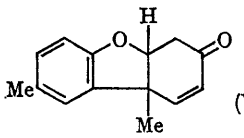
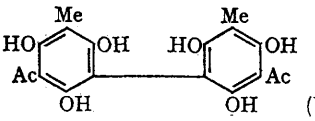
species, even though methylbutyrylphloroglucinol (IV), a demonstrated precursor of the methylenebisacylphloroglucinol (VI),[3] is a simple homologue of (I). The oxidative coupling resulting in formation of (VI) obviously follows a different path and, to our knowledge, is not brought about by ferricyanide. However, the fact that Pummerer's ketone (VII) *is* a peroxidase product of *p*-cresol[2] led us to re-investigate the enzymatic oxidation of (I). Although Dean has reported that the reaction does not occur,[4] we find that methylacetylphloroglucinol (I)[5] oxidizes smoothly at pH 7·8 under the same conditions[3] in the presence of horseradish peroxidase, forming (II) (> 30%) identified by mixed melting point and identical i.r. spectra with chemically prepared (II).[2] The products (II) and (III) were racemic and even the crude reaction mixture showed no rotation.[6]



(VII)

During this reaction we isolated the same by-product (m.p. 291—292°) described earlier.[2] It was found to be an isomer of (II) with a molecular weight of 362·1025 ($C_{18}H_{18}O_8$ required 362·1001, C = 12·0000, determined by high-resolution mass spectrometry). In contrast to (II) it was recovered unchanged from concentrated sulphuric acid. Its n.m.r. spectrum exhibited only two bands in pyridine, one for the two acetyl methyls at δ 2·72 and one for the toluene methyls at δ 2·20 [compare compound (I), δ 2·88 and δ 2·43] indicating that the molecule is symmetrical and very likely has the structure (VIII), a simple analogue of the *oo'*-dicresol found by oxidation of *p*-cresol.[7]



(VIII)

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[1] F. M. Dean, "Naturally Occurring Oxygen Ring Compounds," Butterworths, London, 1963, p. 148.
[2] D. H. R. Barton, A. M. Deflorin, and O. E. Edwards, *J. Chem. Soc.*, 1956, 530.
[3] A. Penttila and H. M. Fales, *J. Amer. Chem. Soc.*, **1966**, **88**, 2327.
[4] Ref. 1, p. 151.
[5] Prepared according to F. H. Curd and A. Robertson, *J. Chem. Soc.*, **1933**, 437.
[6] To our knowledge no optically active oxidation product has ever been obtained from peroxidase systems.
[7] W. W. Westfield and C. Lowe, *J. Biol. Chem.*, **1942**, **145**, 463.