Chalaurenol: a Novel Product from Enzymic Oxidation of 2′,4,4′-Trihydroxychalcone

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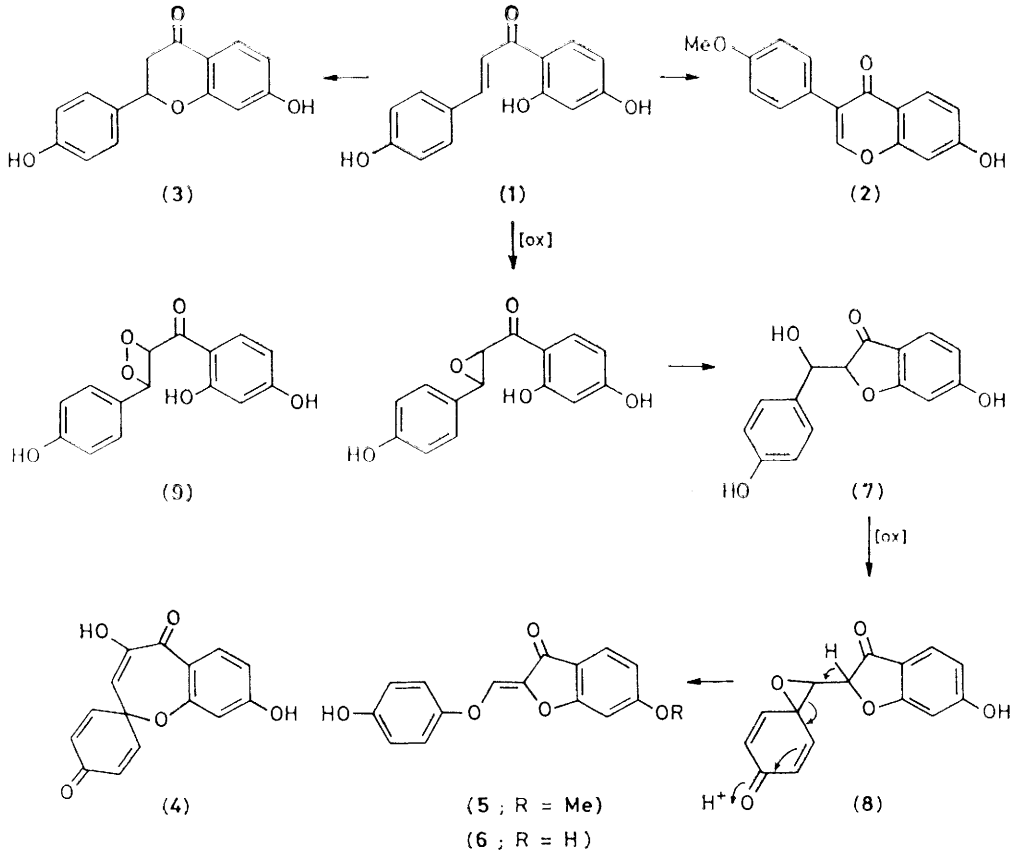
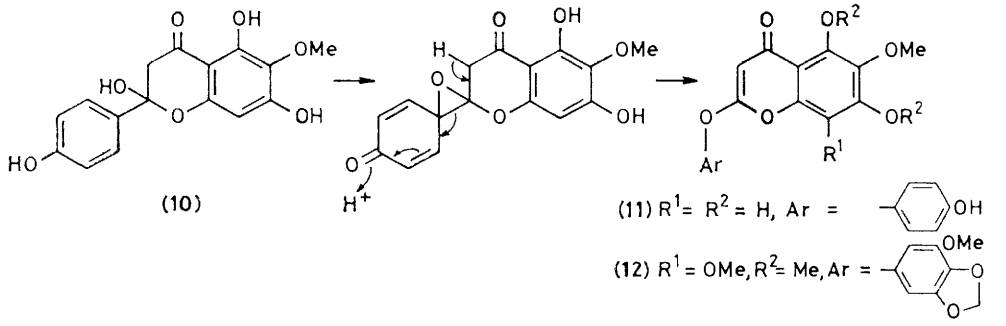
Peroxidase enzymes from various sources convert 2′,4,4′-trihydroxychalcone (**1**) into the novel quinol ether of 2-formyl-6-hydroxycoumaranone (**6**), the structure of which was determined by *X*-ray diffraction; a biosynthetic pathway is suggested and capillarisin (**11**) may arise by a similar route.

Hydroxylated chalcones are key intermediates in the biosynthesis of flavanoids and their diverse relatives, including rotenoids. In the latter case, 2′,4,4′-trihydroxychalcone (**1**) is converted into 7-hydroxy-4′-methoxyisoflavone (**2**) *via* an aryl migration apparently closely connected with *O*-methylation.[1,2] In studying this process using cell-free preparations of *Amorpha fruticosa* seedlings we have observed two enzymes, a chalcone–flavanone isomerase, and a peroxidase. The former yields (**3**) and shows maximal activity in dark-grown seedlings at 3 days from germination, when chalcone synthesis is also near its peak; it has been partially purified, is not inhibited by low concentrations of cyanide, and consists of at least two iso-enzymes (*M* 50000 and 45500 Daltons). On the other hand, peroxidase activity is inhibited by cyanide and is maximal at 6 days. It is the novel structure (**6**) formed by this enzyme which is the subject of the present communication.

Incubation of 2′,4,4′-trihydroxychalcone with a homogenate of dark-grown *A. fruticosa* seedlings (partially purified by chromatography on Sephadex G-150) gave a mixture containing the flavanone (**3**), 4-hydroxybenzaldehyde, 2,4-dihydroxyacetophenone, various minor products, and, as the major component, a labile phenol which we named chalaurenol. This was best purified by Sephadex LH-20 chromatography followed by C_{18}-reversed-phase h.p.l.c., and from the few mg isolated it was recognized to be the same as the compound isolated by Wong and Wilson when chalcone (**1**) was treated



Scheme 1



Scheme 2

with horseradish peroxidase.[3] Only minor differences were observed in the ^{1}H n.m.r., i.r.,† u.v., and mass spectra of the compounds and their monomethyl ethers. A spirobenzoxepinone structure (**4**) has been assigned to the substances on spectroscopic grounds,[3] but since the data were not definitive for the structure proposed, further evidence was sought.

Several incubations of chalcone (**1**) with horseradish peroxidase were carried out using catalytic quantities of hydrogen peroxide and the accumulated product was methylated (diazomethane). Chalaurenol methyl ether (8 mg) was isolated by h.p.l.c. and crystallised from ethanol.

Crystal data: single crystal, $C_{16}H_{12}O_5$, M = 284.27, orthorhombic, space group *Pbca*, a = 7.473(1), b = 27.646(2), c = 12.911(1) Å. Direct methods[4] were used and the structure was refined to R 5.55 (539 observed reflections) giving the mole-

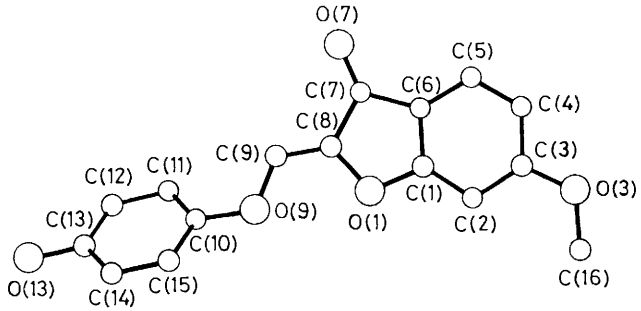


Figure 1. *X*-Ray structure of chalaurenol.

† We thank Dr. E. Wong for the full i.r. spectrum.

cular structure (**5**) as illustrated in Figure 1.‡ Chalaurenol is thus the novel quinol ether of 2-formyl-6-hydroxycoumaranone (**6**) rather than the spiro-oxepine (**4**). It is of interest that hydrolysis of the enol ether (**6**) would lead initially to a formylcoumaranone in which the aliphatic three-carbon unit of phenylalanine is transferred to an acetate-derived aromatic ring; this would be a source of biogenetic confusion.

A plausible course for the conversion of (**1**) into (**6**) is shown in Scheme 1. This invokes the chalcone epoxide ring opening to give the ketol (**7**), which undergoes oxidative phenolic coupling to give the spiroepoxide (**8**). This then rearranges as shown in Scheme 1 to form chalaurenol. Diastereomers of the ketol (**7**) have been isolated from the oxidation of chalcone (**1**) by a peroxidase from *Soja hispida*,[5] and by horseradish peroxidase.[3] Other products observed on peroxidase oxidation of (**1**) include 3,4′,7-trihydroxyflavanone,[6] 3,4′,7-trihydroxyflavone,[6,7,8] and 4′,6-dihydroxyaurone.[7,9] Treatment of the latter with horseradish peroxidase did not produce chalaurenol; thus a pathway to (**7**) involving hydration of the aurone is not supported. It is noted that the ^{1}H n.m.r. data recorded by Wong and Wilson[3] for a labile intermediate which was further transformed into (**6**), and ascribed to the dioxetan (**9**), would more satisfactorily accord with our proposed alternative (**8**).

‡ The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Rd., Cambridge, CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

Investigation of the extractives of *Artemisiae capillaris* Herba and *Ageratum conyzoides* have recently led to the isolation of the flavonoid quinol ethers capillarisin (**11**)[10] and conyzorigun (**12**).[11] Such compounds could originate from the 2-hydroxyflavanone (**10**) by a pathway similar to that discussed above, as indicated in Scheme 2.

We thank the S.E.R.C. for support.

Received, 1st September 1982; Com. 1045

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