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2-Carbomethoxyoxepin: 1-Carbomethoxybenzene 1,2-Oxide. A Metabolite of *Phellinus tremulae*.

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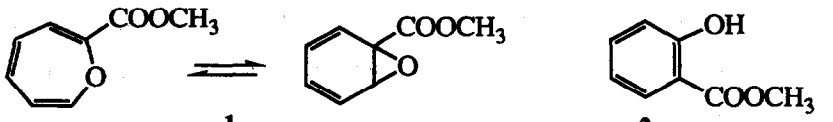
Abstract: The valence tautomer 2-carbomethoxyoxepin: 1-carbomethoxybenzene 1,2-oxide (**1**) has been isolated from liquid cultures of the wood-rotting fungus *Phellinus tremulae*. Methyl salicylate **2**, a rearrangement product of the arene oxide **1**, is produced by the same fungus. The biogenetic implications of these findings are discussed.

Arene oxides have been postulated as biosynthetic intermediates in the monooxygenase–catalyzed formation of phenolic secondary metabolites from aromatic substrates.[1] The involvement of arene oxides is based on observation of substituent migration using labelled or strategically substituted aromatic compounds,[1] as well as on the isolation of some arene oxides from natural sources.[1,2] In a very elegant study, Boyd and Berchtold prepared a number of 1-carboxy- and 1-carbomethoxybenzene oxides and showed that these undergo facile acid–catalyzed rearrangement to the corresponding phenols.[3] In particular, they showed that 1-carbomethoxybenzene 1,2-oxide (**1**) is readily transformed to methyl salicylate (**2**) *via* an NIH shift involving migration of the carbomethoxyl group.[3] In this paper we report the isolation of 2-carbomethoxy-oxepin: 1-carbomethoxybenzene 1,2-oxide (**1**) from liquid cultures of the fungus *Phellinus tremulae*.



1 **2**

Phellinus tremulae (Bond.) Bond. and Borisov (= *Fomes igniarius* var. *populus*) is a wood-rotting fungus occurring in various species of aspen (*Populus*) in Europe, Asia, and North America.[4,5] *P. tremulae* causes white soft-rot of the heartwood and leaves a wintergreen odor which has been attributed to methyl salicylate (**2**).[6]

P. tremulae was grown in shake culture on 2% malt extract broth for 16 days in the presence of DIAION HP 20®,[7a] a nonionic highly porous resin useful in removing nonpolar metabolites from aqueous media.[7b] The resin was separated from the culture broth and mycelium and was eluted with dichloromethane. Flash chromatography of the crude metabolites obtained in this way provided 2-carbomethoxyoxepin (**1**)[8] (13 mg from a 4 liter culture).[9] The ^{1}H NMR and IR spectra are identical with those of authentic 2-carbomethoxyoxepin.[3] Addition of a drop of trifluoroacetic acid to a solution of **1** of $CDCl_3$ in an NMR tube at room temperature lead to complete rearrangement to methyl salicylate (**2**) within one hour, in accord with the previous observation.[3]

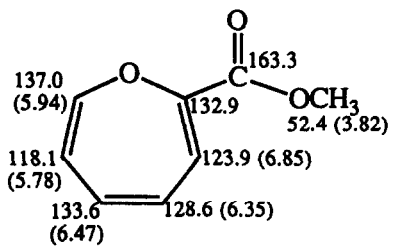
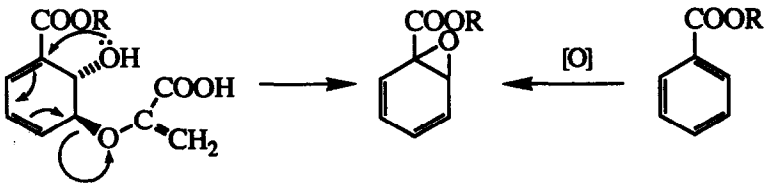


Fig. 1. ^{13}C NMR shifts and ^{1}H NMR shifts (in brackets)[3] for 2-carbomethoxyoxepin (**1**) in $CDCl_3$.

Berchtold has suggested that **1** exists predominantly as the oxepin valence tautomer, based mainly on the long-wavelength (318 nm, CH_3OH) absorption in the UV spectrum.[3] The ^{13}C NMR spectrum of **1** at room temperature (Figure 1) also favors the oxepin tautomer. The ^{13}C NMR shift assignments in Figure 1 are based on HMQC experiments utilizing the previously assigned ^{1}H NMR shifts. INAPT experiments correlated the H–3 proton (δ 6.85) with the carbonyl carbon and the C–2 carbon, and the H–7 proton (δ 5.94) with C–2 as well as C–4 and C–5. Oxepin itself shows carbon signals at δ 141.8 (C–2,7), 117.6 (C–3,6) and 130.8 (C–4,5).[10] The C–1,2 signals in benzene oxide appear at δ 56.6.[10] Since the signal for C–7 in **1** (δ 137.0) is little shifted from the corresponding signal in oxepin, it appears that the benzene oxide tautomer makes a very small contribution in $CDCl_3$ at room temperature.

Before concentration, when the *P. tremulae* culture broth (without added DIAION HP 20®) was extracted with ether and the concentrated ether extract was subjected to GC–MS the previously reported[6] metabolites methyl benzoate and methyl salicylate were detected.

Scheme 1



The biosynthesis of salicylic acid and by analogy, methyl salicylate, is generally regarded as proceeding *via* chorismic and isochorismic acid,[1,1] and the possibility that the 1,2-arene oxide of benzoic acid is an intermediate has been considered.[3,12] The arene oxide could arise from isochorismic acid (Scheme 1) or by direct monooxygenase-catalyzed oxidation of benzoic acid. The identification of 2-carbomethoxyoxepin (**1**) as a metabolite of a fungus which produces methyl salicylate strongly suggests that the arene oxide is, at least in this case, an intermediate in the biosynthesis.

In addition to compound **1**, several sesquiterpenes are produced when *P. tremulae* is grown in liquid culture. The structures of these sesquiterpenes, which appear to be new, will be reported in a subsequent paper.

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References and Notes

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(8) Compound **1** was obtained as a liquid which showed a single spot on silica gel tlc (R_f 0.4, CH_2Cl_2 : hexanes : CH_3OH (50:50:1)): ms; 152.0474 (M^+, 100%, calc'd for $C_8H_8O_3$; 152.0474), 121 (M^+ -OCH_3, 8%), 93 (M^+ -CO_2CH_3, 19%), 77 ($C_6H_5^+$, 21%), 65 ($C_5H_5^+$, 82%). ^{13}C NMR; 163.3 (s), 137.0 (d), 133.6 (d), 132.9 (s), 128.6 (d), 123.9 (d), 118.1 (d), 52.4 (q). UV λ_{max} (ε); MeOH; 253 (2130), 313 (1860). IR, ^1H NMR, identical with those previously reported.[3]

(9) Conventional workup of the culture broth without the use of DIAION HP 20® and extraction with CH_2Cl_2 provided a very small amount (2.7 mg from 6 liter culture) of compound **1**. When the culture broth was concentrated (40°) under reduced pressure before extraction, compound **1** was not isolated.

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