On the Mechanism of Formation of Mannich Bases as Safrole Metabolites

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Safrole (1-allyl-3,4-methylenedioxybenzene) is the simplest member of the allyl and propenyl benzenes that has the methylenedioxyphenyl moiety. Members containing the methylenedioxy group are widely distributed in essential oils, alkaloids, and other physiologically active compounds of natural and synthetic origin. Compounds of the methylenedioxyphenyl type are extensively used in food products and additives, perfumes, medicinals and topical preparations, and as insecticides and insecticidal synergists. In order to account for various observed physiological responses after administration of myristicin and other propenyl benzene derivatives, it was suggested (1) that the substituted benzene derivatives may be converted biologically to amphetamines which in turn would be responsible for the psychoactive properties. Investigations concerning the metabolism of safrole (2), myristicin and other allyl benzene derivatives have revealed that basic ninhydrin positive metabolites are formed; however, these nitrogen-containing metabolites are tertiary aminopropiophenones (Mannich base) and are not amphetamines. Since no nitrogen-containing metabolites were found for dihydrosafrole (3), and since no chemical structurally equivalent metabolites were found for isosafrole, it seems that the allyl double bond is a prerequisite for the formation of the Mannich bases. Furthermore, since these Mannich base metabolites were quite unique, it was of immediate interest to investigate their mechanism of formation.

Materials and Methods

Melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. IR-spectra were obtained with Perkin-Elmer Model 337 and 621 spectrophotometers. The NMR spectra were obtained with a Varian T-60 spectrometer using tetramethylsilane (TMS) as an internal reference. Gas chromatography was done on a Varian Aerograph Model 1868-40 chromatograph equipped with a flame ionization detector. The low resolution mass spectra were obtained with a Perkin-Elmer Model 270 gas chromatograph mass spectrometer via the direct probe inlet. Thin layer chromatography was done on Uniplate, Silica Gel GF plates 250 and 500 microns in thickness and were developed in methylene chloride. The bands were detected with UV light or the chromotropic spray reagent previously reported (3). The synthetic

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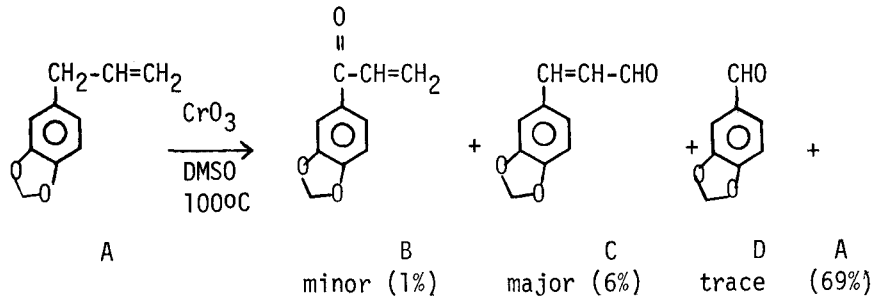
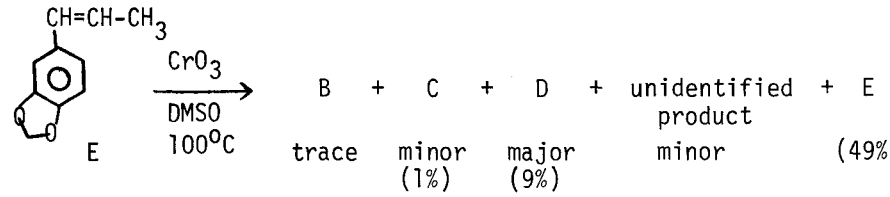
methods given in the following discussion if not previously reported are described with sufficient detail.

Results and Discussion

The most plausible explanation for the formation of Mannich base metabolites was that safrole (A) first undergoes an allylic-benzylic oxidation to afford a vinyl ketone (B) [1-(3',4'-methylenedioxyphenyl)-3-propen-1-one] which in turn can condense with an available amine in a conjugate addition (Michael condensation) to produce the Mannich base. Safrole (A) was subjected to a variety of oxidizing conditions in an effort to demonstrate the feasibility of an allylic oxidation. Safrole (A) was reproducibly oxidized in low yield (about 1%) to the vinyl ketone B by heating a solution of safrole in dimethylsulfoxide (DMSO) in the presence of anhydrous chromium trioxide (CrO_3). The major isolated oxidation product[1] of this reaction was piperonyl acrolein (C) along with trace amounts of piperonal (D). In comparison, isosafrole (E) was oxidized under these conditions largely to piperonal (D) with minor amounts of piperonyl acrolein (C) and an unidentified component along with trace amounts of vinyl ketone B.

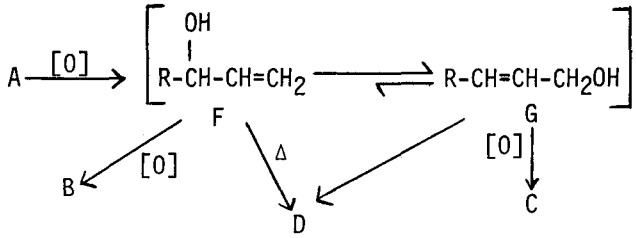


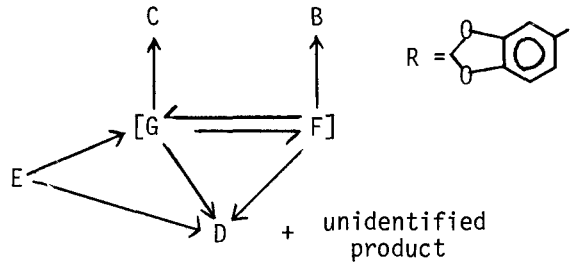


Oxidations of safrole (A) and isosafrole (E) under these conditions give qualitatively but not quantitatively the same products

[1]The terms major, minor and trace are used only in a relative sense. No attempt was made to isolate water soluble products.

implying that common intermediates may exist. However, the formation of an unidentified component only from isosafrole indicates that there is at least one oxidative pathway which is not common to both. Rapid isomerization of safrole to isosafrole can be ruled out since the reaction products are not quantitatively the same, in particular the poor yield of piperonal (D) from safrole (A) as compared to its predominance in the case of isosafrole (E). The following intermediates are most tenable with our results.





Allylic oxidation of safrole (A) would be expected to proceed via initial formation of the allyl alcohol F which would equilibrate in favor of the conjugated alcohol G. Further oxidation of the conjugated alcohol G would yield piperonyl acrolein (C); oxidation of the allyl alcohol F would yield the vinyl ketone B. The piperonal (D) detected may be the result of disproportionation of allyl alcohol F or oxidative cleavage of the conjugated alcohol G. In the case of isosafrole (E), the equilibrating alcohols generated by oxidation of the terminal methyl group would afford largely the conjugated aldehyde C with some vinyl ketone B. However, oxidative cleavage of the double bond might be expected to compete with the terminal methyl oxidation and would ultimately lead to piperonal (D) and possibly the unidentified material[2]. Nevertheless,

[2]The material was isolated by preparative TLC along with C. Gas chromatography indicated that it was relatively non-polar, and infrared analysis indicated a split carbonyl (1705-1715 cm^{-1}). Although there was insufficient sample for identification, it may be an α-diketone from oxidation of an intermediate glycol.

it is necessary to invoke an alternative pathway[3] leading to piperonal (D) since it is formed in greater yield from isosafrole (E).

The feasibility of forming a Mannich[4] base by a Michael condensation was demonstrated by reacting piperidine with the vinyl ketone B. The crude Mannich base (H) [3-piperidyl-1-(3',4'-methylenedioxyphenyl)-1-propanone] was obtained by heating a solution of the vinyl ketone B in piperidine at 80°C for 3 hrs. followed by removal of the excess piperidine by pumping in vacuo. The purified Mannich base[5] was obtained in a 45% yield by vacuum distillation at temperatures up to 190°C and pressures as low as 0.1 mm. The unusual thermal stability of this Mannich base prompted us to look further at its decomposition properties (5) as well as the reactivity of the vinyl ketone B. The Mannich base H resisted decomposition at elevated temperatures and reduced pressures but, in the form of its hydrochloride, mp 210-211°, decomposed on steam distillation to vinyl ketone B, although an appreciable amount of undistillable material remained behind. This latter material consisted primarily of the keto alcohol I, mp 77-78°C, which could be formed quantitatively from vinyl ketone B by heating (100°C) the finely dispersed aqueous solution of the vinyl ketone from steam distillation in a closed container. When the vinyl ketone B was isolated and redissolved in protic solvents, it formed dimers and polymers[6], and its monomeric reactions were minimized. Solvolysis of the vinyl ketone B in glacial acetic acid in the presence of sodium acetate produced the acetate J, mp 93-95°C, in good yield which could be reconverted to vinyl ketone B by refluxing in pyridine. The acetate J was also formed quantitatively from the keto alcohol I by acetylation with acetic anhydride and pyridine at low temperatures.

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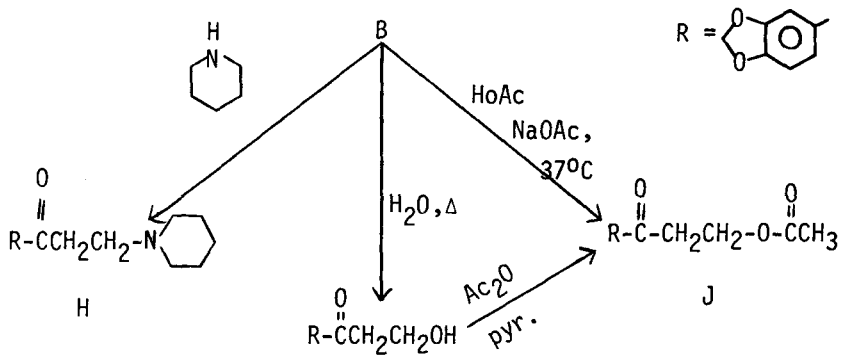
[3] CrO_3 has been shown (4) to form an unstable peracid in the presence of H_2O_2 which oxidizes isosafrole in 68% yield to piperonal via a glycol intermediate. A peracid may exist in DMSO solutions of CrO_3 since dimethyl sulfone is present.

[4] The conceivable formation of the Mannich base H from interaction of piperonyl acrolein (C) and piperidine is unlikely since C gave no detectable Mannich base when subjected to the same conditions of Michael condensation.

[5] The authenticity of this compound and others (B-D) was established by direct synthesis.

[6] Phenyl vinyl ketone behaves similarly (6).



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Allylic oxidation of safrole (A)[7] to the vinyl ketone B and Michael condensation of B with an amine is chemically feasible. In addition, the vinyl ketone B seems to be uniquely reactive[8] in a Michael manner since it will react with water having a poor nucleophilicity.[9] Its reactivity is approaching that of a strong alkylating agent[9]. The fate of the vinyl ketone B in a biological system would depend on whether or not it is enzyme bound and its proximity to stabilizing functional groups (possibly electrophiles as well as nucleophiles) which may or may not be bound. Although it is likely that the methylenedioxyphenyl compounds of increased substitution on the phenyl ring (usually methoxy groups) will vary [10] in their capability to form vinyl ketones as intermediary metabolites, it appears that increased substituency of this type enhances the reactivity toward 1,2-addition to the conjugated double bond as previously predicted (11).

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[7]Although safrole has been previously (7) subjected to allylic-benzylic oxidizing conditions, allylic oxidation was not clearly demonstrated since the expected direct product, vinyl ketone B, was not isolated nor otherwise detected.

[8]Although β-hydroxy propiophenones are common, we could find no reports of their facile formation from vinyl ketones; i.e., the related compound, 3,4-MeO(OH)C_6H_6COCH=CH_2, was converted to its keto alcohol in the presence of sodium hydroxide (8).

[9]The kinetics encompassing the alkylating properties of β-amino ketones (Mannich bases) via elimination-addition reactions, have been previously studied (9); however, a detailed study separating inductive, resonance, and steric effects on the reactivity of the intermediary vinyl ketones has not been made.

[10]Electron donating groups in the aromatic ring have been shown (10) to increase the oxidation rate of benzylic secondary alcohols.

References

1. Shulgin, A. T., Nature 210, 380 (1966).

2. Oswald, E. O., Fishbein, L., Corbett, B. J., and Walker, M. P., Biochim. et Biophys. Acta 230, 237 (1971).

3. Oswald, E. O., Fishbein, L., and Corbett, B. J., J. Chromatog., 45, 437 (1969).

4. Milas, N. A., U. S. 2,414,385 (1947).

5. Blicke, F. F., "Organic Reactions," 3rd. ed., Vol. 1, Chapter 10 (1947), John Wiley and Sons, New York.

6. Allen, C. F. H. and Bridges, M. P., J. Amer. Chem. Soc., 51, 2151 (1929).

7. Kuraska, T., Nippon Kagaku Zasshi, 82, 50 (1961).

8. Freudenberg, R., Lautenschlaeger, L., and Tausend, H., Ann. Chem., 685, 139 (1965).

9. Carsky, P., Zuman, P., and Horak, V., Czechoslov. Chem. Commun., 29, 3044 (1964).

10. Kwart, H. and Francis, P. S., J. Amer. Chem. Soc., 77, 4907 (1955).

11. Kossanyi, J., Bull. Soc. Chim. France, 704 (1965); Polansky, O., Monatsh. Chem. 88, 91 (1957).