Metabolism of the Proximate Carcinogen 1′-Hydroxysafrole and the Isomer 3′-Hydroxyisosafrole

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Associating physical, chemical properties and molecular reactivity with the biological effects of a compound is one of the prerequisites to understanding the mechanism of action of carcinogenic chemicals. MILLER and MILLER (1971) proposed the electrophilic theory which states that the ultimate reactive form of a carcinogen should be strongly electrophilic, thereby enabeling it to attack tissue nucleophiles such as DNA, RNA, and proteins.

Safrole (3,4-methylenedioxyallylbenzene) has been recognized as a low-grade hepatic carcinogen and subsequently banned as a food additive in the United States. Safrole is relatively non-electrophilic. High dietary levels of safrole are required to produce a relatively low carcinogenic response. BORCHERT et al. (1971, 1973a, 1973b) have proposed that safrole may be metabolized to an electrophilic component, and have described 1'-hydroxysafrole as a metabolite of safrole. These workers have also demonstrated that 1'-hydroxysafrole shows a higher incidence of hepatic carcinomas than safrole when fed at the same dietary level. HAGAN et al. (1965) have shown that neither allylbenzene nor methylenedioxybenzene show any appreciable effects on the livers of mice, when compared to safrole. TAYLOR et al. (1964) have suggested that both the methylenedioxy ring and the allylbenzene moiety must be necessary for the hepatotoxicity to occur.

The present investigation describes the metabolism of the proximate carcinogen 1'-hydroxysafrole and the isomer 3'-hydroxyisosafrole in the rat. We offer a mechanism for the carcinogenic action of 1'-hydroxysafrole which supports the electrophilic theory and associates chemical reactivity with biological response.

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MATERIALS AND METHODS

Diazomethane was prepared from N-methyl-N'-nitro-N-nitrosoguanidine. 1'-Hydroxysafrole, 1'-hydroxyallylbenzene, and 3'-hydroxyisosafrole were prepared by the method of BORCHERT et al. (1971). Animals were given compounds by intraperitoneal injection as the neat liquid or as a suspension in trioctanoin. Control animals were given trioctanoin.

Metabolites were isolated and were identified by gas chromatography/mass spectroscopy as described earlier (PEELE and OSWALD 1977). Identification was facilitated by use of the Mass Spectral Search System on the NIH PDP-10 computer (Division of Computer Research and Technology, NIH, Bethesda, Md.) using the Biemann search system (HERTZ et al. 1971).

Allylic isomerization reactions were measured by the method of BRAUDE et al. (1946). The acid catalyzed rearrangement of 1'-hydroxyallylbenzene to cinnamyl alcohol was measured spectrophotometrically in 60% dioxane 40% 3 N HCl at 251 nm. The molar extinction coefficient for 1'-hydroxyallylbenzene in this solvent system was 306. The molar extinction coefficient for cinnamyl alcohol in the same system was 1.7×10^4. The acid catalyzed conversion of 1'-hydroxysafrole to 3'-hydroxyisosafrole was measured at 266 nm where the molar extinction coefficient was 1.33×10^3 for 1'-hydroxysafrole and 1.1×10^4 for 3'-hydroxyisosafrole. Kinetic constants were not calculated because accurate control of cuvette temperature was unavailable.

RESULTS

The neutral urinary extracts from animals injected with 1'-hydroxysafrole contained the unmetabolized compound and the isomer 3'-hydroxyisosafrole. This agrees with BORCHERT et al. (1971) who have shown that 1'-hydroxysafrole is excreted unchanged and will rearrange under the conditions of incubation with β-Glucuronidase to yield small quantities of 3'-hydroxyisosafrole. 3,4-Methylenedioxyphenyl vinyl ketone was also present in the neutral extract. Two other neutral metabolites, which have not been fully characterized, were present. The first of these had an apparent molecular weight of 180 and yielded a compound with an apparent molecular weight of 194 when treated with diazomethane. This could be the native material with partial cleavage of the methylenedioxy ring to yield methoxy and phenol groups. The other neutral metabolite had an apparent molecular weight of 226 and yielded a derivative with an apparent molecular weight of 240 when treated with diazomethane. The nature of this metabolite is uncertain.

The basic urinary fraction from animals given 1'-hydroxysafrole contained the dimethylamino and piperidyl Mannich base metabolites as seen for safrole (OSWALD et al. 1971).

This observation is consistent with the proposed mechanism for the formation of Mannich base metabolites from allylbezene (PEELE and OSWALD 1977). The only observed acidic metabolite was 3,4-methylenedioxyhippuric acid.

The neutral urinary extract from rats given 3'-hydroxyisosafrole contained the unmetabolized parent compound, 3,4-methylenedioxyacetophenone, and traces of 1'-hydroxysafrole. The basic fraction contained no metabolites. The acidic fraction contained 3,4-methylenedioxyacetophenone and 3'-(3,4-methylenedioxyphenyl)-3'-hydroxypropionic acid. In addition traces of piperonylic acid and 3,4-methylenedioxyhippuric acid were present.

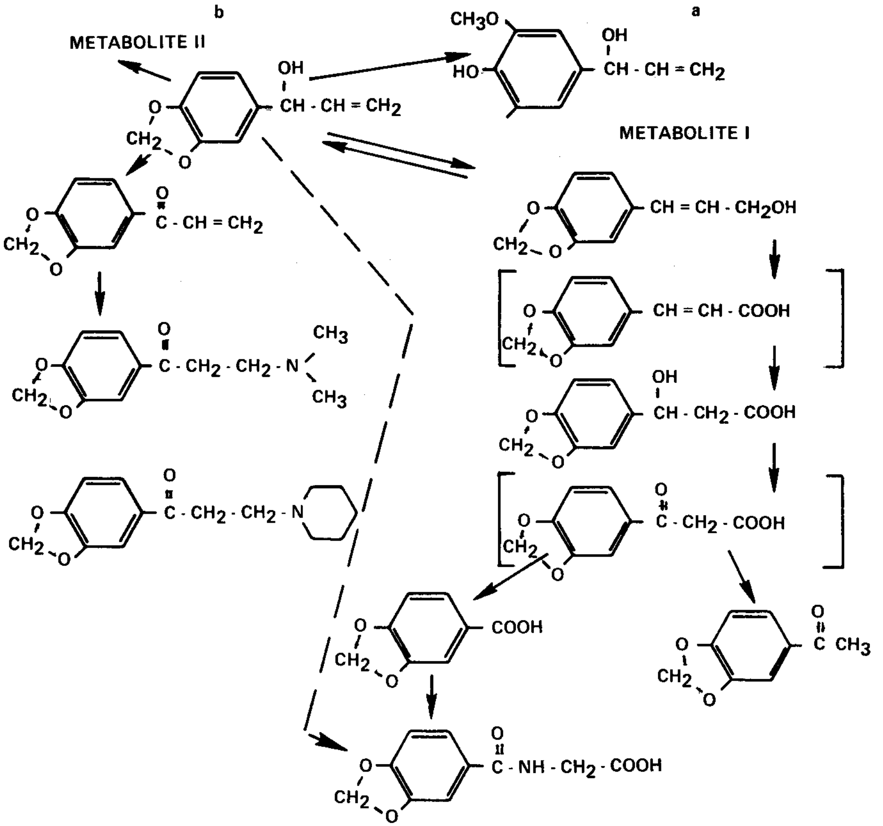
Since 1'-hyroxysafrole has been implicated as the proximate carcinogen of safrole (BORCHERT et al. 1973b), we studied the reactivity of 1'-hydroxysafrole by the acid catalyzed conversion of 1'-hydroxysafrole to the isomer 3'-hydroxyisosafrole. We compared the rearrangement of the methylenedioxy substituted derivative to the rearrangement of the unsubstituted analog (1'-hydroxyallylbenzene).

The unsubstituted compound reaches equilibrium in 2-2.5 hours in 60% dioxane in 3N HCl. Three determinations of the final percent product in the reaction mixture gave values of 80.6, 80.0, and 75.2%. In contrast, 1'-hydroxysafrole spontaneously rearranged to 3'-hydroxyisosafrole in less than one minute under the same conditions. The final percent of 3'-hydroxyisosafrole present in the reaction mixture was 100, 100, and 97.0% for three determinations. Both reactions were pseudo first order. Reduction of the acid concentration by one-third still showed 1'-hydroxysafrole to reach equilibrium with 3'-hydroxyisosafrole in about 20 minutes, and the reaction was essentially 100% complete.

DISCUSSION

The metabolites of 1'-hydroxysafrole and 3'-hydroxyisosafrole are shown in Figure 1. The metabolism of the proximate carcinogen is consistent with the pathways decribed for 1'-hydroxyallylbenzene (PEELE and OSWALD 1977). Mannich base metabolites were observed for 1'-hydroxysafrole but not for 3'-hydroxyisosafrole. Partial cleavage of the methylenedioxy ring was observed for 1'-hydroxysafrole. Rearrangement of 1'-hydroxysafrole to 3'-hydroxyisosafrole occurred to give propenyl type metabolites from an allylic compound. There is no evidence for direct isomerization of the allylic group of safrole to a propenyl group prior to oxidative metabolism.

Since both 1'-hydroxysafrole and 1'-hydroxyallylbenzene are metabolized similarly, the reactivity of these compounds was investigated as possible means for explaining the noncarcinogenicity of allylbenzene as compared to the low carcinogenicity of safrole. The acid catalyzed rearrangement of 1'-hydroxysafrole was much faster and more complete than the rearrangement of the unsubstituted analog under identical conditions. A mechanism for this difference in reactivity would involve the stabilization of a carbonium ion intermediate by the resonance effect of the methylenedioxy group. Such a mechanism is shown in Figure 2. Resonance stabilization could increase the electrophilicity of the molecule, allowing it to attack tissue nucleophiles. Estragole (p-methoxyallylbenzene) has also been shown to be weakly carcinogenic (DRINKWATER 1976) and an increase in the reactivity may be rationalized by the proposed mechanism. MILLER and MILLER (1971) have proposed that the reactive forms of chemical carcinogens appear to be strong electrophiles. WISLOCKI et al. (1976) have shown that administration of tritiated 1'-hydroxysafrole to rats yielded hepatic DNA, RNA, and protein bound tritium.



[a]PROPOSED STRUCTURE FOR METABOLITE I
[b]METABOLITE II HAS NOT BEEN CHARACTERIZED

Figure 1. Summary of Metabolites determined for 1'-Hydroxysafrole and 3'-Hydroxyisosafrole.

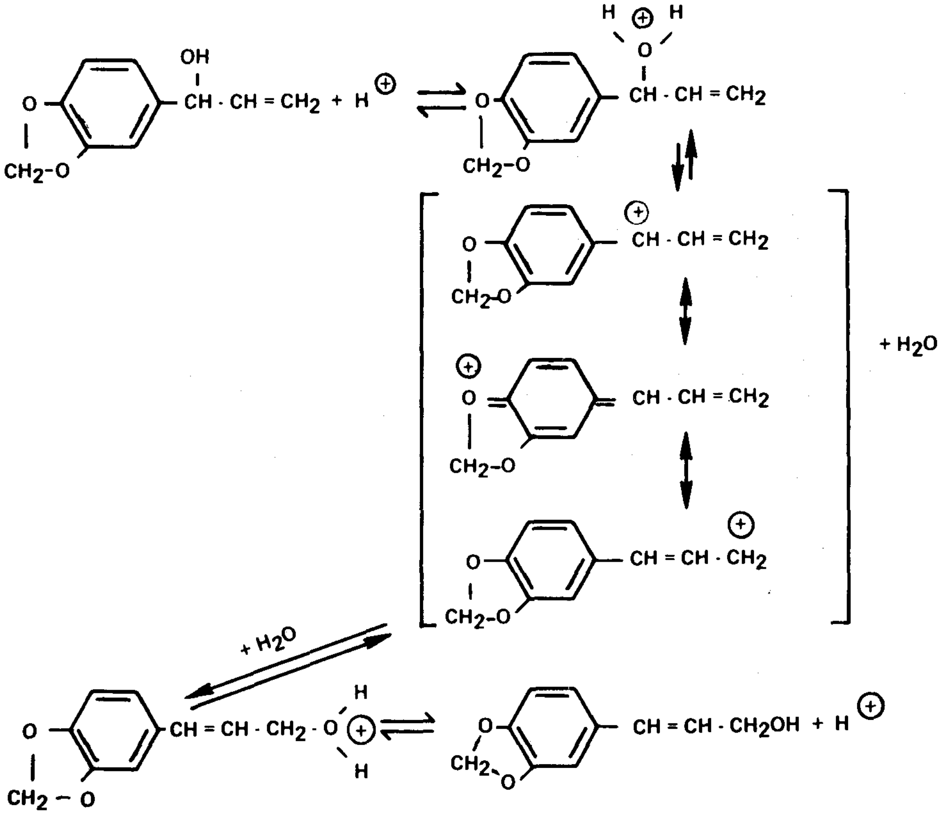


Figure 2. Proposed Mechanism for Allylic Rearrangement of 1'-Hydroxysafrole.

Inclusion of a 3,4-methylenedioxy group on the phenyl ring enhanced the acid catalyzed rearrangement of 1'-hydroxyallylbenzene to cinnamyl alcohol by resonance stabilization of a carbonium ion intermediate. The influence of aromatic substitution on electorphilicity may be a means for explaining why safrole was carcinogenic but allylbenzene was not. This mechanism may also serve as a basis for predicting the carcinogenic activity of other naturally occurring allylbenzenes.

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