# THE SYNTHESIS OF $^{14}$C-SAFROLE, MYRISTICIN AND ELEMICIN LABELED IN THE ALLYL SIDE CHAIN

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SUMMARY

*The preparation of 1-[(3'-$^{14}$C)-Allyl]-3,4-methylene dioxy-, 1-[(3'-$^{14}$C)-allyl]-3-methoxy-4,5-methylene dioxy-, and 1-[(3'-$^{14}$C)-allyl]-3,4,5-trimethoxy benzenes is described. The $^{14}$C-label was introduced into the 3'-carbon of the allylic side chain via the Wittig reaction.*

## INTRODUCTION

Substituted allyl- and, propenyl benzene derivatives are widely distributed in the environment. These families of compounds have been isolated and identified in many oils and flavoring agents with which individuals have daily contact[1]. Some of the allyl benzenes have been shown to possess significant pharmacological activity with accompanying pathological changes in a number of species[2-4]. Furthermore, studies at this laboratory and by P. Borchert et al. indicate that a "metabolic activation" of the allylic side chain takes place to form metabolites which have an even greater pathogenic character, acting in some instances as potent hepatocarcinogens[5-8]. Our

previous studies using $^{14}C$-safrole and myristicin labeled in the methylene dioxy ring confirm the results of J. E. Casida et al. as to the metabolic lability of the methylene dioxy moiety[9-10]. The reactivity of the methylene dioxy ring prompte the positioning of the $^{14}C$-label in the allylic side chain since such a system would be a more stable and desirable biological tracer with which to study the "metabolic activation" of these compounds and their in vivo distribution. The synthesis of [3'-$^{14}C$]-safrole, myristicin and elemicin was undertaken to facilitate further biological study.

## DISCUSSION

Commercial grade safrole was used as a starting material (J. T. Baker) to prepare safrole glycol. Myristicin and elemicin were prepared synthetically from 4-allyl-2,6-dimethoxy phenol (Aldrich Chemical Co.). This phenol was partially demethylated (details to be published) to give hydroxy eugenol (3,4-dihydroxy-5-methoxy-1-allyl benzene) in moderate yields (55%) and uncontaminated with isomers. This procedure avoids the production, difficult separation, and rearrangement of undesirable isomeric mono allyl ethers of pyrogallol-1-methyl ether associated with the Claisen rearrangement[11-12]. The hydroxy eugenol was converted to myristicin (Ib) on treatment with methylene iodide and anhydrous potassium carbonate by the method of Perkin and Trikojus[13]. Elemicin (Ic) was derived by methylation of 4-allyl-2,6-dimethoxy phenol.

The reaction sequence described (Scheme I) involves conversion of the respective allyl benzenes to their glycols, phenyl acetaldehydes, and back to the [3'-$^{14}C$] labeled safrole, myristicin, and elemicin. This procedure is identical for each allyl benzene.

The glycols (Ia,b,c) were prepared by the method of R. Criegee et al. which requires the reaction of the respective olefin (allyl benzenes) with

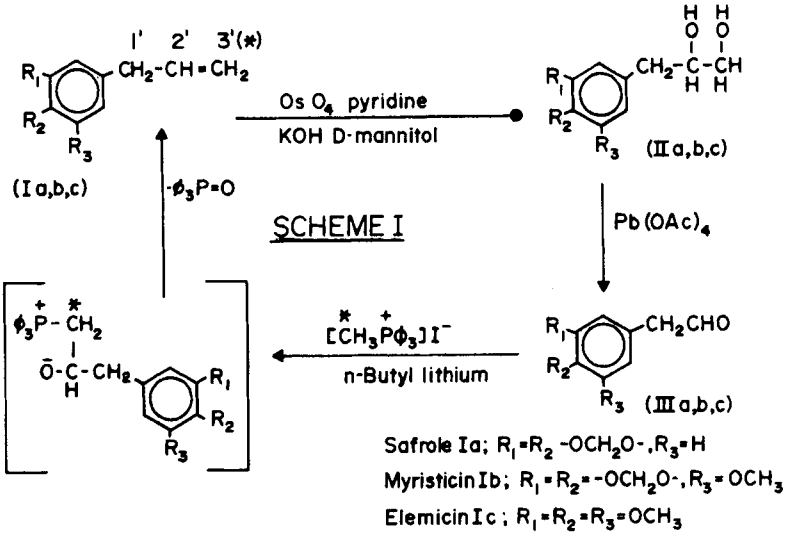
osmium tetroxide in the presence of pyridine followed by reductive hydrolysis of the osmate ester with alkaline-D-Mannitol[14]. The phenyl acetaldehydes (IIa,IIb,IIc) were produced in turn from the vic-glycols via oxidative cleavage with lead tetra-acetate[15]. Labeling was introduced in the last step when excess $^{14}C$-methyl triphenyl phosphonium iodide (Wittig salt) was reacted with aldehydes (IIIa,IIIb,IIIc) in the presence of 0.9 equivalents of n-butyl-lithium[16]. Labeled experiments utilized 4.0 to 4.4 mmoles $^{14}C$-methyl Wittig salt with total activities ranging from 4.36 to 5.0 mci. The labeled products were purified by column chromatography and had both a chemical and radiochemical purity of 99% or greater as determined by GC, NMR, TLC and MS.

Final yields based on n-butyl lithium were:

[3'-$^{14}C$]-safrole 37%, specific activity 0.89 mCi/mmole

[3'-$^{14}C$]-myristicin 12%, specific activity 0.79 mCi/mmole

[3'-$^{14}C$]-elemicin 24%, specific activity 0.88 mCi/mmole



The experimental procedure outlined for [3'-$^{14}$C]-safrole is generally applicable to the [3'-$^{14}$C] labeling of both myristicin and elemicin.

## EXPERIMENTAL

Safrole glycol (IIa): A solution of 14.06 g (0.055 mole) of osmium tetroxide in 500 ml absolute ether was added to a solution of 8.95 g (0.055 mole) of safrole (Ia) and 28 ml pyridine in 250 ml absolute ether with vigorous stirring in a 3 l beaker, producing a red-brown precipitate almost immediately. After 2 hours stirring the ether was slowly decanted off the safrole-osmium tetroxide-pyridine solid adduct. This complex was dissolved in 300 ml methylene chloride and mixed vigorously with a solution of 19.5 grams (0.35 mole) potassium hydroxide and 195 grams (1.06 mole) D-mannitol in 300 ml water. Gradually (30-40 min.) the organic phase becomes lighter in color as the aqueous phase becomes progressively darker. The clear methylene chloride layer was removed, placed in a separatory funnel, washed with one 100 ml portion of 0.2N sulfuric acid, two 100 ml portions of water, dried over anhydrous sodium sulfate and evaporated to yield a clear oily residue. Distillation of the crude glycol product under reduced pressure (0.2 mm Hg) on a "Kugel Rohr" apparatus (Büchi, Switzerland) gave a forerun of unreacted safrole (100-130°C/0.2 mm Hg) and 5.7 grams (57%) of glycol (IIa) (130-230°C/0.2 mm Hg). The glycol (IIa) crystallized on standing (m.p. 77-78°C) and was identical in all respects to an authentic sample prepared by the method of D. Swern et al.[17].

Homopiperonal [3,4-methylene dioxyphenyl acetaldehyde (IIIa)]: To a boiling solution of 3.92 g safrole glycol (0.020 mole) in 160 ml dry benzene contained in a 1500 ml beaker was introduced 9.75 g (0.022 mole) of lead tetraacetate in small portions with vigorous stirring over a 3-4 minutes period. After addition was complete, the reaction mix was allowed to cool to room temperature with stirring. The lead acetate was filtered off under

vacuum, and washed with two 50 ml portions of hot benzene. The combined extracts were shaken once with saturated sodium carbonate, and washed twice with water. The organic phase was dried over anhydrous sodium sulfate and evaporated to yield 2.2 grams (67%) of a clear oil with a distinctly musty odor. The homopiperonal was identical in all respects (NMR, GC, TLC, and IR) to an authentic sample prepared by other less convenient means (to be reported elsewhere).

One gram (6.0 mmoles) of the aldehyde (IIIa) was dissolved in 10 ml of tetrahydrofuran (dried over 3Å molecular sieves) in a 12 ml vial which had been sealed with a serum cap and repeatedly flushed with dry nitrogen.

[3'-$^{14}$C]-Safrole: $^{14}$C-methyl triphenyl phosphonium iodide (Mallinckrodt; 1.617 mg, 1.09 mCi/mmole) was transferred to a 30 ml reaction vial (in a dry $N_2$ atmosphere) and sealed with a serum cap. The vial was evacuated, flushed with dry nitrogen, and evacuated once again. Dry tetrahydrofuran (7 ml) was introduced via a hypodermic syringe and the resulting mixture was cooled (-76°C dry ice bath), quickly removed from the ice bath and 1.6 ml (3.5 mmoles) of n-butyllithium (Alfa Inorganics) injected while shaking vigorously. Immediately most of the Wittig-Salt went into solution to form the yellow-red $^{14}$C-methylene triphenyl phosphorane. Agitation was continued 3-4 minutes during which time the reaction mixture slowly warmed to between 0-10°C. The reaction vial was once again cooled to -76°C and evacuated, followed by addition of 1.0 g homopiperonal in 10 ml of tetrahydrafuran using a syringe and a 25 GA. hypodermic needle. Shaking was continued during addition of the aldehyde and for an additional 3.5 minutes during which time the temperature reached 10°C. Finally 5 ml water was injected with shaking to "quench"[18] the reaction. After 3-4 minutes agitation, the mixture was extracted with two 25 ml portions of benzene and the combined benzene extracts washed with two 25 ml portions of water. The solution was dried over anhydrous sodium sulfate and evaporated to give a crude oil which was purified by column chromatography on Florisil. The crude labeled product was placed on a 45 cm x 1 cm column (packed to 40 cm) with a minimum amount of

benzene and eluted with hexane (200 ml) yielding 213 mg of pure [3'-$^{14}$C] safrole identical in all respects to an authentic sample obtained commercially.

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