Synthesis of *N*-Acetyl-1-^{14}C Zectran

(4-Dimethylamino-3,5-xylyl-*N*-acetyl-1′-^{14}C Methylcarbamate) by Microacetylation

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A microacetylation method for synthesizing *N*-acetyl-1-^{14}C Zectran (4-dimethyl-3,5-xylyl-*N*-acetyl-1′-^{14}C methylcarbamate) from 4-nitro-3,5-xylyl *N*-methylcarbamate and radiolabeled acetic anhydride is presented. The method overcomes the inefficient method of direct acetylation of Zectran. The radiolabeled acetyl derivative of Zectran is used in the metabolism study of *N*-methylcarbamate insecticides in insects and mammals. Acetylation of Zectran with acetyl chloride gave two products.

N-Acetyl-1-^{14}C Zectran (I) was synthesized for the study of the metabolism of Zectran (4-dimethylamino-3,5-xylyl methylcarbamate, Dow Chemical Co.) and its derivatives in insects and mammals (Miskus *et al.*, 1968) (Figure 1). This derivative of Zectran was necessary in the U. S. Forest Service's continuing research to develop safe, short-lived chemical treatments for use against forest pests.

The nonradioactive acetyl Zectran was first reported by Fraser *et al.* (1965). Their method of acetylation of Zectran with acetic anhydride or acetyl chloride did not lend itself to the synthesis of radioactive acetyl Zectran because a large excess of the acetylating agent is necessary. Labeled acetyl Zectran can be synthesized starting from 4-nitro-3,5-xylyl-*N*-methylcarbamate (II). Acetylation of this carbamate in acetic acid solvent and catalyzed with sulfuric acid proceeded with only a slight excess of acetic anhydride to give the *N*-acetyl-4-nitro-3,5-xylyl-*N*-methylcarbamate (III). Acetyl exchange of the solvent with the anhydride was small under the conditions employed in view of the minor dilution of the specific activity of the product. Acetylation reactions in other solvents such as xylene or dimethylformamide were unsuccessful. Catalytic hydrogenation of III in the presence of formaldehyde gave I. The authenticity of nonlabeled I was established by thin-layer cochromatography, infrared spectra, and mixed melting points of the product made by this method with a known sample of acetyl Zectran (Fraser *et al.*, 1965).

Acetylation of Zectran with acetyl chloride in xylene with *N*,*N*-dimethylaniline gave a mixture of I and another product, identification of which has not been fully established.

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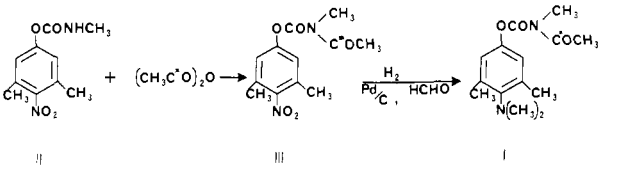

Figure 1. Synthesis of *N*-acetyl-^{14}C Zectran

EXPERIMENTAL

Preliminary experiments and characterizations were carried out with nonlabeled material and products. Identity of radioactive materials with nonlabeled products was determined by cochromatography by thin-layer chromatography on silica gel G plates with two solvent systems (ether-hexane, 1 to 2, and ether-hexane-ethanol, 70:20:3). The acetic 1-^{14}C anhydride (radiopurity 99% or better) was purchased from New England Nuclear Corp. (Boston, Mass.). The Zectran was commercial material recrystallized in hexane to a constant melting point. Elemental analyses were performed by the Microchemical Analytical Laboratory, Department of Chemistry, University of California, Berkeley. Melting points (uncorrected) were determined on a Fisher-Johns melting point apparatus.

***N*-Acetyl-4-nitro-3,5-xylyl-*N*-methylcarbamate (III).** To a small vessel containing 0.1 mmole of acetic anhydride-1-^{14}C (0.5 mc. of ^{14}C) was added 20.6 mg. (0.092 mmole) of 4-nitro-3,5-xylyl *N*-methylcarbamate (II), (Dow Chemical Co., Walnut Creek, Calif.), and 10 μl. of a 100-to-1 (by volume) acetic acid–concentrated sulfuric acid solution. The vessel was protected with a calcium chloride tube and the mixture was heated in an oil bath maintained at 130° to 140° C. for 2.5 hours.

To simplify the purification process, any unreacted carbamate was converted to product by heating the reaction mixture an additional 2 hours with 25 μl. (0.26 mmole) of added, unlabeled acetic anhydride. The reaction mixture was then cooled, dissolved in water, made basic with solid sodium bicarbonate, and extracted with methylene

chloride, Drying and evaporation of the solution gave a yellow solid which was reduced without further purification. The nonradioactive product was recrystallized from benzene and ether to give crystals melting at 96° to 97° C. Anal.: Theory C: 54.13, H: 5.30; Found C: 54.84, H: 5.57.

N-**Acetyl Zectran (I).** The acetylated nitrocarbamate was transferred to a microhydrogenator (Siggia, 1954) with absolute methanol (15 to 20 ml.), and 50 mg. of sodium acetate, 75 mg. of 8.7% palladium chloride on Norit, and 50 μl. of 37% formalin were added. The mixture was hydrogenated at 25° C. and atmospheric pressure until absorption of hydrogen essentially stopped. The mixture was filtered and evaporated. The solid remaining was purified by preparative thin-layer chromatography on silica gel G using ether-hexane (1 to 2) solvent. The silica gel of the middle of the band corresponding to approximately one quarter of the radioactive band detected by a Tracerlab 4π Scanner was removed and extracted with methylene chloride. The solvent was evaporated using a nitrogen stream to give *N*-acetyl Zectran.

The *N*-acetyl-1-^{14}C Zectran isolated was identical to a known sample of *N*-acetyl Zectran by thin-layer cochromatography silica gel G in at least two solvent systems—e.g., ether-hexane, 1 to 2; ether-hexane-ethanol, 70:20:3. The specific activity of the *N*-acetyl-2-^{14}C Zectran made under these conditions was approximately 2.0 mc. per mmole. A material of higher specific activity can be made by avoiding the dilution step with unlabeled acetic anhydride. The Zectran and acetyl Zectran in the final products then are separated by the preparative thin-layer chromatography on silica gel G plate using ether-hexane (1 to 2, R_f of Zectran = 0.1, R_f of acetyl Zectran = 0.33).

Over-all yield based on radioactivity was 6%. Radioactive purity determined by thin-layer chromatography resolved with ethyl ether–hexane (1 to 2) or ether-hexane (4 to 1) and measured with a Tracerlab 4π scanner was 98% or better based on peak area measurements. Chemical purity was established by chromogenic detection on the thin-layer chromatography plate using standard reagents (Krishna *et al.*, 1962).

Acetylation of Zectran with Acetyl Chloride. In a 100-ml. round-bottomed flask, 4.4 grams (0.02 mole) of Zectran, 5 ml. (0.07 mmole) of acetyl chloride, 5 ml. (0.04 mmole) of dimethylaniline (mono-free), and 50 ml. of xylene were heated under reflux for 20 hours. The mixture was added to ice water and was made basic with sodium bicarbonate. Extraction with methylene chloride, drying with anhydrous sodium sulfate, and then distillation gave a colorless oil (b.p. 110° to 140° C./0.25 mm. of Hg). Separation with thin-layer chromatography (silica gel G with ethyl ether–hexane 1 to 2) showed two components: *N*-acetyl Zectran (R_f = 0.33) and a component (R_f = 0.42) that constituted about a third of the total product.

LITERATURE CITED

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