Immunological Studies of Poisonous Anacardiaceae: Effect of Vehicle on Absorption of 3-*n*-Pentadecylcatechol and Its Diacetate Ester Derivative after Oral Feeding in Rats

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Abstract □ Tritium-labeled 3-*n*-pentadecylcatechol and its diacetate ester were fed to Sprague-Dawley rats. Both compounds were dissolved in ethanol and in corn oil vehicles and administered by gavage. The rats were placed in metabolic cages, and the urine and feces were collected. The radioactivity excreted in the urine and feces was determined by liquid scintillation counting, and the percentage of the administered dose was utilized as a measure of absorption. While there was no difference between the absorption of either compound, absorption was affected by the vehicle. Approximately 30% of the administered radioactivity appeared in the urine when ethanol was the vehicle, but about half that amount (14%) was excreted in the urine when the compounds were dissolved in corn oil. A subsequent bile cannulation study showed that the balance of the radioactivity found in the feces was not a result of biliary excretion. The majority of the activity recovered from urine and feces was eliminated within 48 hr after dosing. These data indicate that oil is a poor vehicle for GI absorption of urushiol components.

Keyphrases □ Absorption, GI—3-*n*-pentadecylcatechol and its diacetate derivative, effect of vehicle, rats □ 3-*n*-Pentadecylcatechol—poison ivy component, effect of vehicle on GI absorption, rats □ Urushiol—poison ivy component of 3-*n*-pentadecylcatechol, effect of vehicle on GI absorption, rats □ Poison ivy—3-*n*-pentadecylcatechol component of urushiol, effect of vehicle on GI absorption, rats

Three poisonous species of the family Anacardiaceae [*viz.*, *Toxicodendron radicans* (poison ivy), *T. diversilobum* (poison oak), and *T. vernix* (poison sumac)] are collectively responsible for most allergic contact dermatitis in the United States. Because of the ubiquity of these plants, ~80% of the U.S. population exhibit some allergic response to them, and 50% of the population is clinically allergic (sensitivity to 2 μg of urushiol or less) (1).

BACKGROUND

The allergenic constituents of these species commonly are referred to as urushiols and consist of dihydric phenols with either a 15- or 17-carbon side chain in the third ring position, which may or may not contain 1–3 double bonds (2, 3). The administration of plant extracts to desensitize humans has been practiced clinically since the 1920's. Urushiols are insoluble in water and usually are dissolved in corn, olive, or almond oil (4). Urushiols are extreme irritants, and injection or oral administration of these substances to sensitive humans in doses sufficient to produce prophylaxis requires extreme care (5, 6).

Toxicity can be eliminated by methylation of the ring hydroxyl groups (7, 8). Experiments in this laboratory showed that esterification of the urushiol hydroxyl groups markedly reduced their toxicity. However, these ester derivatives desensitize sensitive guinea pigs and produce immunological tolerance in naive guinea pigs when administered orally or intravenously (8).

The toxicities of the free *versus* esterified urushiols were compared in this laboratory. In mice, the LD_{50} of free poison oak urushiol *via* the intraperitoneal route was 73.75 mg/kg while that of the esterified urushiol was 324.70 mg/kg. Oral doses of the free urushiol in corn oil required to produce lethality were >600 mg/kg, while oral doses of 1600 mg/kg of the esterified urushiol in corn oil produced no lethality. From these studies, it appeared that corn oil prevented urushiol absorption and protected mice from its toxic effects. This study was undertaken to determine the effect of the vehicle on the absorption of the urushiol component, 3-*n*-pentadecylcatechol (I), and its diacetate derivative (II) after oral administration to rats.

EXPERIMENTAL

3-*n*-Pentadecylcatechol (I)—Poison ivy urushiol was isolated by ethanolic extraction of fresh leaves, berries, and green stems of *T. radicans*[1] using a reported extraction procedure (2). The urushiol extract was partially purified (60% pure) as previously described (3). Aliquots of the purified urushiol oil were reduced to I by catalytic hydrogenation, using 5% palladium-on-carbon, and the mixture containing I was purified further by passage over a dry silica gel 60 column with chloroform as the eluting solvent.

The eluting fractions were tested for I using 1% ethanolic ferric chloride; fractions containing I were combined, and the solvent was evaporated *in vacuo*. The residue containing I was purified further by passage over a polyamide column[2] with 90% ethanol in water as the eluting solvent. Fractions containing I were combined, and the solvent was evaporated. Further purification of I was accomplished by repeated crystallization of the residue in hexane. Colorless needle crystals of I resulted, mp 58–59°. The identity of I was confirmed by GLC and GLC–mass spectral analysis using previously described procedures (2, 9).

Pentadecylcatechol Acetate (II)—Crystalline I was dissolved in acetic anhydride and pyridine and was stirred at room temperature overnight. The reaction mixture then was poured over crushed ice and extracted with chloroform. The chloroform extract was washed successively with chilled dilute sulfuric acid, water, saturated sodium bicarbonate, and again with water. The chloroform solution was dried (sodium sulfate), and the solvent was evaporated, yielding II. GLC–mass spectral analysis showed quantitative conversion to the diacetate derivative.

3-*n*-Pentadeca-8,11,14-trienylcatechol (Triolefinic Congener)—The triolefinic congener of I was isolated from purified poison ivy urushiol extract. Urushiol (>60% pure) was converted to its diacetate derivative with acetic anhydride and pyridine. The urushiol acetate was passed over a dry silica gel[3] column impregnated with silver nitrate with 1% methanol in chloroform as the eluting solvent. The polarity of the eluting solvent was increased gradually to 4% methanol in chloroform, and fractions containing the mono-, di-, or triolefinic congener acetates were pooled separately based on their TLC similarities on silver nitrate-impregnated silica gel plates. The solvent was evaporated from the pool containing the triolefinic congener acetate, and the allergenically active triolefinic congener was regenerated by basic hydrolysis using 10% sodium carbonate in dioxane–water (4:1). The reaction mixture then was acidified, and the free congener was determined by GLC and GLC–mass spectral analysis of the trimethylsilyl derivative (9).

Radiolabeled Material—Fifty milligrams of I containing 50 μg of the triolefinic congener was tritium reduced[4]. The specific activity was 10.6 mCi/mg of I.

[1] *T. radicans* (L.) Kuntze (Anacardiaceae) was collected near the University of Mississippi campus in Oxford, Miss., by M. A. ElSohly in 1975. The plant samples were authenticated by Dr. M. W. Quimby. Voucher species are stored in the drug plant herbarium at the School of Pharmacy, University of Mississippi, University, MS 38677.

[2] MN-polyamide SC, <0.07-mm particle size, Brinkmann Instruments, Westbury, N.Y.

[3] Silica gel 60, 70–230 mesh, MCB Manufacturing Chemicals, Cincinnati, Ohio.

[4] New England Nuclear, Boston, Mass.

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Table I—Cumulative Percentage of Orally Administered Tritiated Pentadecylcatechol (I) or Its Diacetate Derivative (II) Excreted in Urine

Hours Postdose	Tritiated I in Ethanol	Tritiated I in Oil	Tritiated II in Ethanol	Tritiated II in Oil
6	7.35 ± 2.84	4.41 ± 1.13	8.27 ± 3.57	3.63 ± 2.21
24	21.53 ± 5.65	11.49 ± 1.32	24.41 ± 2.06	11.90 ± 3.57
48	25.02 ± 7.04	13.07 ± 0.90	28.40 ± 1.66	13.17 ± 3.84
72	25.82 ± 7.33	13.55 ± 0.91	29.92 ± 1.87	13.71 ± 3.97
96	26.55 ± 7.71	13.97 ± 0.99	30.74 ± 1.94	14.11 ± 4.09
120	28.33 ± 7.37	14.22 ± 1.02	31.50 ± 1.84	14.40 ± 4.16

To prepare the labeled acetate derivative, the tritium-labeled I was converted to the acetate derivative using the procedure described earlier. Labeled materials were purified by preparative silica gel TLC to a purity of >90% prior to use.

Ethanol and Corn Oil Solutions of I and II—Aliquots of tritiated I or tritiated II in benzene were evaporated to dryness, and unlabeled I and II crystals were added as carrier. Ethanol and corn oil were the vehicles used to prepare solutions containing 2 mg of I/ml or the equivalent of 2 mg of I as the acetate per milliliter. Specific activities of prepared solutions were determined by liquid scintillation counting using internal standardization.

Animals—Female Sprague–Dawley rats, 250–300 g, were obtained from an in-house breeding colony. They were fasted 12 hr prior to intragastric administration of the radiolabeled solutions.

Urine and Fecal Excretion—Four animals each were administered 1-mg po doses of tritiated I or tritiated II in either corn oil or ethanol. After dosing, animals were housed in individual metabolism cages, and urine and feces were collected at 6, 24, 48, 72, 96, and 120 hr. The urine volumes were measured, and aliquots were mixed with 15 ml of scintillation cocktail[5]. After drying in a desiccator, the feces were weighed, frozen in liquid nitrogen, pulverized, and uniformly mixed. Aliquots of 80–120 mg were weighed accurately and then combusted in a sample oxidizer[6]; the resulting tritiated water was incorporated into 15 ml of liquid scintillation cocktail[7].

All samples were dark-adapted overnight and then counted for 10 min or 1% (2σ) error in a liquid scintillation counter[8]. Absolute activity was calculated by the use of an external standard channel ratio quench correction curve. The radioactivity in both urine and feces was expressed as the percentage of the activity of the dose excreted for the sampling period and the cumulative percent of dose amounts. Average excretion values for each collection interval then were calculated for each group.

Biliary Excretion—When results of the oral dosing study indicated that most of the administered dose was excreted in the feces, another study was conducted to see if this result was due to biliary excretion of the labeled material or to a lack of absorption of the compound from the GI tract.

The bile ducts of four rats were cannulated. Animals were dosed orally with a freshly prepared solution containing the equivalent of 1 mg of I in the form of tritiated II in ethanol. The bile was collected, and the volume was measured at 6, 12, 24, and 30 hr. Aliquots of bile were mixed with scintillation cocktail[5] and measured for radioactivity.

RESULTS AND DISCUSSION

Urine and Fecal Excretion—Tritium-labeled I and II were dissolved in corn oil and in ethanol, and the percentage of the orally administered radioactivity appearing in the urine was taken as a measure of absorption in rats. The radioactivity appearing in the urine at 6, 24, 48, 72, 96, and 120 hr after oral dosing was determined and expressed as the cumulative percent excreted (Table I). Approximately 30% of the administered radioactivity appeared within 120 hr in the urine of rats dosed with the ethanol solutions. Only half that amount (14%) of the activity was excreted in the urine when these compounds were given in oil. In this study, the absorption patterns of the two compounds were similar in both vehicles. Seventy-five percent or more of the total activity excreted in the urine appeared within the first 24 hr regardless of the vehicle or compound. The rapid absorption of I also was noted by Godfrey *et al.* (10) from an orally administered glycerol–95% ethanol solution.

Table II—Cumulative Percentage of Orally Administered Tritiated Pentadecylcatechol (I) or Its Diacetate Derivative (II) Excreted in Feces

Hours Postdose	Tritiated I in Ethanol	Tritiated I in Oil	Tritiated II in Ethanol	Tritiated II in Oil
24	35.04 ± 8.94	61.31 ± 4.22	36.26 ± 8.08	74.65 ± 12.71
48	49.94 ± 1.74	69.42 ± 4.10	52.04 ± 5.10	79.33 ± 11.48
72	51.99 ± 2.18	70.45 ± 4.06	54.91 ± 5.73	81.51 ± 10.62
96	52.73 ± 2.41	70.83 ± 4.09	55.55 ± 5.75	81.78 ± 10.53
120	52.98 ± 2.46	71.03 ± 4.12	55.90 ± 5.77	81.91 ± 10.50

The radioactivity excreted in the feces at 24, 48, 72, 96, and 120 hr after oral dosing is expressed as cumulative percent excreted (Table II). The majority of the administered radioactivity of both compounds was eliminated in the feces. There was a greater percentage of tritiated I and II excreted in the feces of rats dosed with oil solution (71 and 82%, respectively) than in the feces of rats receiving the ethanol solution, apparently due to reduced absorption of these compounds from corn oil. As with the urinary excretion patterns, most of the activity eliminated in the feces (≥64%) appeared within the first 24 hr after dosing, and the bulk of the remaining material appeared during the second 24-hr period. The total recovery of the administered radioactivity within 120 hr ranged from 81 to 96%.

Biliary Excretion—Studies by Godfrey *et al.* (10) showed a persistence of I in the bile, liver, spleen, appendix, and body fluids, which was taken as evidence for enterohepatic circulation of I and its metabolites. Since the majority of administered radioactivity in the present study was eliminated in the feces within the first 24–48 hr, it was of interest to determine if some activity recovered in the feces might have been due to absorption followed by excretion into the feces. Thus, the bile ducts of rats were cannulated, and the animals were dosed with tritiated II in ethanol. The bile was collected at 6, 12, 24, and 30 hr, until negligible amounts of activity appeared in the bile.

Approximately 2% of the administered activity appeared in the bile every 6 hr for the first 24 hr for a cumulative total of 8.85%. After 30 hr, negligible amounts were observed in the bile. Since 8.85% of the administered radioactivity was excreted in the bile within the first 24 hr, it can be assumed from the previous experiment that a maximum of 15% of the activity appearing in the feces (64% of the administered doses) represents biliary-excreted activity.

The results of the present study indicate that oils are poor vehicles for administering urushiols or their water-insoluble derivatives. The investigation of other vehicles to improve the absorption of these compounds is in progress. Additional studies also are being conducted to determine the urinary and biliary metabolites of these compounds. Optimal absorption may significantly alter the cost of prophylaxis since less drug will be required to obtain therapeutic tissue levels. In addition, decreasing the amount of material eliminated in the feces might significantly reduce the toxic side effects, particularly perianal dermatitis.

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