

Metabolism of Triphenylmethane and Triphenylcarbinol¹

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A study of the metabolism of triphenylmethane and triphenylcarbinol in the rabbit indicates that the major urinary metabolite of these two compounds is the corresponding glucuronide. When 1 gm of triphenylmethane is fed to rabbits, approximately 35% of the dose is excreted unchanged in the feces, 45-55% is excreted in the urine as a glucuronide, and a small amount is converted to 4-hydroxytriphenylmethane. Urinary excretion of triphenylcarbinol as the glucuronide accounts for approximately 43% of a 1-gm dose fed to rabbits. Thirty-three % of the triphenylcarbinol is excreted unchanged in the feces.

Triphenylmethane appears as a chemical moiety in a large number of compounds of commercial importance, many of which have biological activity. This includes compounds such as halogenated hydrocarbon insecticides, a number of tranquilizing drugs, and the triphenylmethane dyes. The biological activity of these compounds may be due largely to substituted groups, but also of interest is the metabolic fate of the triphenylmethane portion of the molecule. Triphenylcarbinol also occurs as the nucleus of many similar compounds. The only report of the metabolism of these two compounds was by Miriam *et al.* (9). They observed that 27% of a 2-gm dose of triphenylmethane and 12-30% of a 2-5-gm dose of triphenylcarbinol fed to rabbits was excreted unchanged in the urine. In addition, approximately 30% of a 5-gm dose of triphenylcarbinol was excreted unchanged in the feces of rabbits fed this compound. No metabolic products were found, and the fate of the remainder of the compound is not known. It is unusual to find such large amounts of water insoluble compounds excreted unchanged in the urine. Many similar aromatic hydrocarbons are at

least partially hydroxylated and excreted either free or in a conjugated form (11).

MATERIALS AND METHODS

Purification of commercial samples of these compounds was accomplished by recrystallization from alcohol-water until a sharp melting point was obtained (triphenylmethane, 93°C; triphenylcarbinol, 163°). All melting-point values are uncorrected centigrade.

Groups of three male albino rabbits were used in each study. The animals were maintained on a constant diet of oats and cabbage during the experimental period. Compounds were emulsified in corn oil and administered by stomach tube.

SAMPLE COLLECTION

Forty-eight-hour urine samples, free of feces, were collected before feeding corn oil, after feeding corn oil, and after feeding corn oil containing 1-2 gm of the compound being investigated. The feces were also collected for a 48-hour period after the ingestion of the compound. The urine and feces were refrigerated as soon as possible after being passed. A small amount of toluene was added to the urine as a preservative.

ANALYTICAL METHODS

The excretion of phenolic compounds in the urine was measured by the method of Folin and Ciocalteu (6) by using phenol as a standard. Glucuronides were determined by the procedure

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of Dische (4). Ethereal sulfates were determined as barium sulfate according to the method of Folin (5). The amount of metabolite excreted by each rabbit during a 4-day control period was subtracted from the amount excreted during the 4-day period after feeding the compound to obtain the effect of the compound on the excretory pattern.

EXTRACTION OF FECES

The feces were exhaustively extracted with ethyl ether, the extract was evaporated to dryness, and the residue was saponified with alcoholic potassium hydroxide. Hydrocarbons were extracted from the residue with hot ethanol and recrystallized from ethanol-water.

ISOLATION OF METABOLITES FROM TRIPHENYLMETHANE URINE

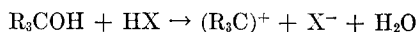
Attempts to isolate the glucuronides by solvent extraction (10) and by precipitation (8) were unsuccessful. Steam distillation of the urine (pH 7) gave a few milligrams of phenolic compound which crystallized as white needles. The residual urine was hydrolyzed for 3 hours with strong HCl and again steam-distilled. No phenolic compounds were found in the distillate. The hydrolyzed urine was then extracted with ethyl ether, the ether extract was evaporated to dryness, and the gummy residue was dissolved in pyridine and treated with benzoyl chloride. After dilution with ice water and removal of benzoic acid, the product was recrystallized from aqueous alcohol.

ISOLATION OF METABOLITES FROM TRIPHENYLCARBINOL URINE

The glucuronide was precipitated with basic lead acetate. A gum was obtained which had a

strong positive test for glucuronide and a strong yellow color in alcohol-HCl. Acid hydrolysis of the gum gave a phenolic compound. The glucuronide gum was methylated with diazomethane and then acetylated with acetic anhydride in pyridine. On dilution with ice-cold water, a yellow crystalline product was obtained. This was dissolved in ethanol, decolorized with activated charcoal, and reprecipitated by the addition of water.

Triphenylcarbinol in ethanol and in the presence of a mineral acid forms an intense yellow color believed to be due to the formation of ionizable halo-chromic salts (7):



This reaction was of value in detecting triphenylcarbinol in solution as well as providing a means of determining the presence of the free hydroxy group on the methane carbon atom in isolated metabolites.

RESULTS AND DISCUSSION

TRIPHENYLMETHANE

Analytical Data

Ethereal sulfate and phenol excretion were not significantly increased in the urine of animals fed triphenylmethane. Increased glucuronide excretion (Table I) accounted for 450–550 mg of each gram of triphenylmethane fed if one assumes a 1:1 conjugation. The average excretion represents 52% of the 1-gm dose.

Fecal Excretion

Twenty-five to 35% of the 1 gm dose of triphenylmethane was excreted unchanged in the feces. This compound was identified by mixed melting point and by its character-

TABLE I
GLUCURONIDE EXCRETION BY RABBITS AFTER THE INGESTION OF TRIPHENYLMETHANE AND
TRIPHENYLCARBINOL

Compound fed—1 gm	Glucuronide excretion			Equivalent weight of compound fed (mg)	% of dose
	4-day total before compound (mg)	4-day total after compound (mg)	Increased excretion (mg)		
Triphenylmethane	325	754	429	535	53
	403	780	377	472	47
	441	891	450	562	69
Average:				523	52
Triphenylcarbinol	201	560	359	450	45
	170	449	279	349	35
	187	470	283	354	35
Average:				384	38

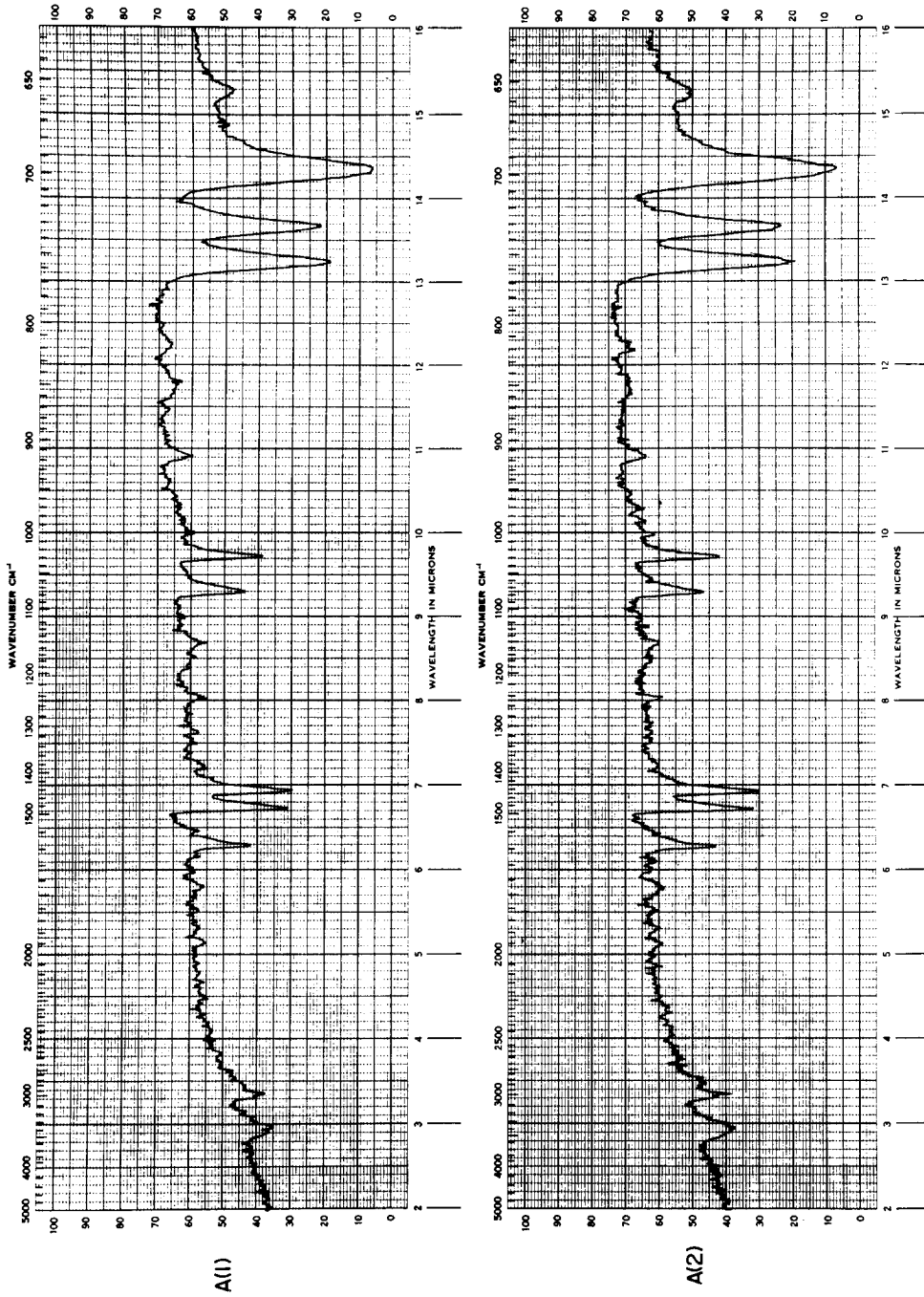


Fig. 1. A (1) Infrared spectrum of compound isolated from feces; A (2) infrared spectrum of pure triphenylmethane (Eastman, recrystallized).

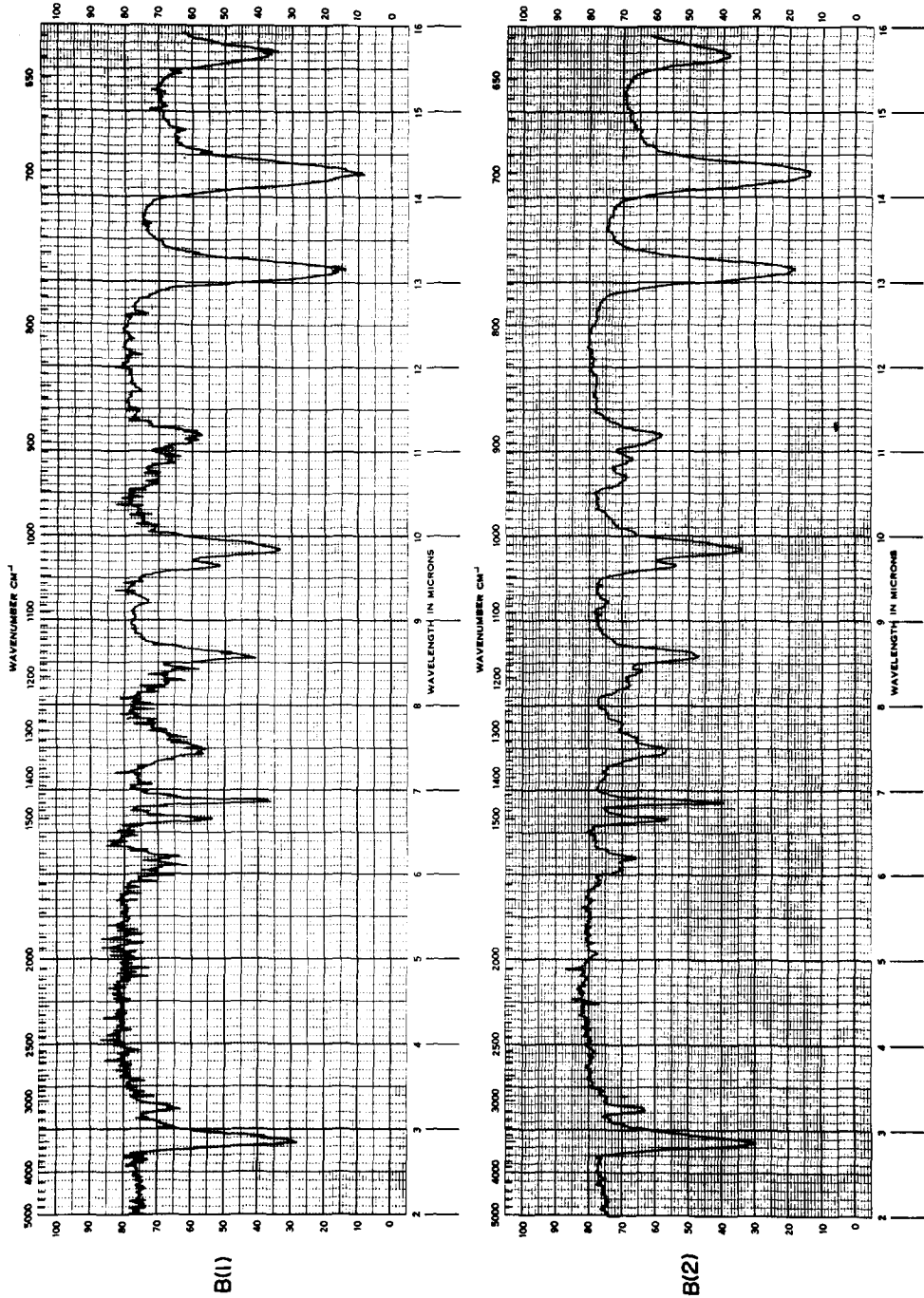


Fig. 2. B(1) Infrared spectrum of compound isolated from feces; B(2) infrared spectrum of pure triphenylcarbinol.

istic infrared spectrum. The spectra of the isolated compound and pure triphenylmethane are identical (Fig. 1). Due to the insoluble nature of this compound, it is probable that the fecal excretion represents compound not absorbed from the intestinal tract. However, one cannot rule out absorption and biliary excretion on the basis of the present data.

Urinary Metabolites

A few milligrams of phenolic compound isolated by steam distillation of the urine melted at 110°, which is characteristic of 4-hydroxytriphenylmethane (2,3).

The glucuronide, shown by analytical data to account for approximately 50% of the ingested 1-gm dose, could not be isolated and crystallized by conventional techniques. After hydrolysis of the glucuronide, a phenolic compound was released which did not readily crystallize. Elemental analysis of the crystalline benzoate derivative (m.p. 117°–118°) was consistent with the formation of the benzoate of hydroxytriphenylmethane (Found: C, 85.4; H, 5.6; O, 8.8; C₂₆H₂₀O₂ requires: C, 85.7; H, 5.5; O, 8.8). The position of the hydroxyl group of this phenolic compound is still in doubt. The conjugation with glucuronic acid has apparently not taken place on the methane carbon since hydrolysis of such a compound would yield triphenylcarbinol, which crystallizes readily. The benzoate of triphenylcarbinol melts at 165°–166° (1), which is not consistent with the m.p. of the benzoate of the hydrolysis product of the glucuronide.

The results of the present study indicate that the metabolism of 1 gm of triphenylmethane in rabbits proceeds as follows: 25–35% excreted unchanged in the feces, a trace as 4-hydroxytriphenylmethane, and 45–55% as the glucuronide in the urine.

TRIPHENYLCARBINOL

Analytical Data

As with triphenylmethane, the analytical data gave no indication of increased excretion of ethereal sulfates or phenolic compounds following the ingestion of 1 gm of triphenylcarbinol by rabbits. However, the glucuronide analysis (Table I) showed an

increased excretion sufficient to account for approximately 38% of the 1 gm dose of triphenylcarbinol if one assumes a 1:1 conjugation with glucuronic acid.

Fecal Excretion

Approximately 35% of the dose was isolated from the feces of rabbits fed 1 gm of compound. Identification of the triphenylcarbinol was confirmed by the mixed melting point and the identity of the infrared spectrum with that of the pure compound (Fig. 2).

Urinary Metabolites

Isolation from urine of the glucuronide of triphenylcarbinol as methyl (triphenylmethanol tri-*o*-acetyl glucosid) uronate is consistent with the elemental analysis (Found: C, 64.4; H, 5.3; C₃₂H₃₂O₁₁ requires: C, 64.8; H, 5.4). Since hydrolysis of the glucuronide gum gave a phenolic compound, it is apparent that the glucuronide is not conjugated at the methane carbon atom but is formed by an ether linkage to one of the benzene rings. Additional evidence for this linkage is the immediate positive color reaction of the conjugate in alcohol-HCl, which is indicative of the presence of the free hydroxyl group on the tertiary carbon atom of triphenylmethane.

Excretion of glucuronide (38%) in the urine and unchanged compound in the feces (33%) accounts for approximately 70% of the 1-gm dose of triphenylcarbinol.

In spite of exhaustive extraction of urine with ethyl acetate, ether, and benzene, we were not able to confirm the report by Miriam *et al.* (1927) of the urinary excretion of relatively large amounts of unchanged triphenylmethane and triphenylcarbinol.

It is apparent from the analytical data that the major metabolite of both triphenylmethane and triphenylcarbinol is the corresponding glucuronide. No evidence was obtained for glucuronide linkage with the methane carbon atom.

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