DISCUSSION

The low order of toxicity of potassium perrhenate (1) and the high order of toxicity of rhenium trichloride (2) have been confirmed and extended to determination of their intraperitoneal LD_{50}'s. The rhenium compounds tested showed no real irritating properties on the eyes or skin of animals and no effects on isolated intestine. This is a striking contrast to observations on other rare elements (8–10). The chemical composition of the rhenium compounds, their solubility, and their rate of decomposition determines their lethality to animals. Rhenium trichloride, which decomposes readily with the liberation of hydrochloric acid, was 10 times more toxic than potassium perrhenate.

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Keyphrases

Potassium perrhenate—pharmacology, toxicology
Rhenium trichloride—pharmacology, toxicology
LD_{50}—potassium perrhenate, rhenium trichloride
Eye irritation
Skin irritation
Ileum, isolated–effect on tonus, contractility

Photodisintegration Studies of ^{14}C-Carboxyl 2,3,5-Triiodobenzoic Acid

By R. H. JARBOE, JR., J. B. DATA, and J. E. CHRISTIAN

^{14}C-Carboxyl labeled 2,3,5-triiodobenzoic acid was examined at various intervals of time during exposure to ultraviolet irradiation. Photodisintegration occurred and the amount of degradation increased in quantity with increased duration of exposure. 2,5- and 3,5-diiodobenzoic acid were identified as two products resulting from the irradiation. Other unidentified products are also present.

IN CERTAIN PLANTS 2,3,5-triiodobenzoic acid (TIBA) has pronounced hormonal effects on the flowers, fruits, yields, metabolism, and translocation of natural plant constituents (1–8). Because these plants serve as a source of food to man or indirectly by first serving as food for animals which ultimately constitute man's diet, the question naturally arises as to the safety of this material to man. Furthermore, when TIBA is used on plants, it could be questioned whether the photodisintegration products from TIBA might be a health hazard. If sunlight causes photochemical deterioration either before or after absorption in the plant, the products of degradation could be translocated throughout the plant and eventually, directly or indirectly, become incorporated in foods.

EXPERIMENTAL

Materials—Glass chromatographic plates, 20 × 20 cm., utilizing silica[1] of 250 μ thickness, were used. A plastic preservative for thin-layer chromatograms, Brinkmann Instruments Co., Westbury, L. I., N. Y. was used.[2] The ultraviolet source (Ultra-Violet Products, Inc., South Pasadena, Calif., long-wave UV model S1 3660) was equipped with a long wavelength filter to approximate normal sunlight. The samples were counted in a Packard model 3003 Tri-Carb liquid scintillation spectrometer. The solvent system used was composed of a ratio of 10 parts of petroleum ether (b.p. 30–60°) and 1 part of propionic acid. Solvents and chemicals used in this study were of reagent grade or their equivalent.

Purification of TIBA-^{14}C—TIBA labeled in the carboxyl position with ^{14}C (9) was purified utilizing thick-layer chromatographic techniques prior to the photodisintegration studies. The purification procedure consisted of pipeting an alcoholic solution of approximately 93% TIBA-^{14}C onto previously prepared 1-mm. silica plates and air dried. The plates were developed in a solvent system of petroleum ether–propionic acid in the ratio of 10 parts of petroleum ether (b.p. 30–60°) and 1 part of propionic acid and subsequently exposed to photographic film (Eastman Kodak No Screen medical X-ray film). The resultant autoradiogram was used to identify TIBA-^{14}C. The TIBA-containing silica was scraped from the plate and placed in a Soxhlet extractor and extracted with anhydrous ether for a period of 72 hr. The Soxhlet extractor was wrapped with black paper to protect the contents from exposure to light. After the extraction period the ethereal solution was then concentrated to dryness under reduced pressure without heat in the dark.

Photochemical Degradation of TIBA-^{14}C—The TIBA-^{14}C used for the preparation of 30 ml. of a 2.1 × 10^{-2} % aqueous suspension was the same as that used in a study conducted on field grown soybeans by Spitznagle (9). About 5 ml. of the suspension was

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[1] Adsorbosil-1, Applied Science Laboratories, Inc., State College, Pa.
[2] Neaton

placed in a quartz cell used in spectrophotometric analyses work, and the solution exposed continuously to the ultraviolet source at a distance of 20 cm. for 8 weeks. The cell was equipped with a plastic lid which was sealed by vacuum grease to prevent atmospheric effects.

Twenty-five microliter samples were removed at 0, 1, 2, 4, 8, 12, 24, 48, and 72 hr. and at 1, 2, 3, 4, 5, 6, 7, and 8 weeks. These samples were spotted on thin-layer silica plates. After development in the petroleum ether–propionic acid solvent, the plates were air dried and sprayed with undiluted plastic preservative to prevent sloughing of the silica during the photographic exposure period. To prepare the autoradiogram the thin-layer plates were exposed for about 5 days to Eastman Kodak No Screen medical X-ray film and then developed and fixed in Eastman Kodak liquid developer and liquid fixer, respectively.

The radioactivity of each labeled compound, separated on the thin-layer plates, was quantitatively determined in the Packard Tri-Carb liquid scintillation spectrometer. The radioactive areas were scraped from the plates and placed into a counting vial which contained a scintillator fluid composed of 0.4% PPO (2,5-diphenyloxazole) and 0.01% dimethyl POPOP [1,4-bis(4-methyl-5-phenyl-2-oxazoyl)benzene] in an equal volume each of toluene and 2-ethoxyethanol. Because TIBA was proved to be soluble in the scintillator fluid, suspension gel was not needed. A preliminary determination of possible quenching indicated no appreciable change in the counting rate with the amounts of silica contained in the samples.

Figure 1 is a drawing of the autoradiogram of the thin-layer chromatogram showing the separated degradation products of TIBA-^{14}C. Identification of the compounds was accomplished using unlabeled standards of TIBA, 2,5-, and 3,5-diiodobenzoic acid and location with bromocresol green reagent (10).

Figures 2 and 3 show the quantitative relationships between labeled TIBA and the products resulting from the deterioration as the irradiation time is increased.

Results—The TIBA-^{14}C at zero time contained 2.15% impurity after the thick-layer chromatographic purification process. This situation results from either the impurity not being completely removed in the purification process or that some type of degradation occurs during the isolation

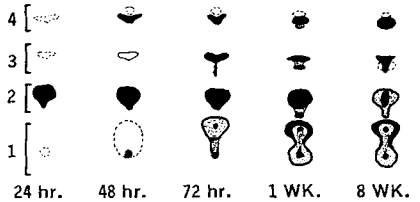

Fig. 1—Autoradiogram of the thin-layer chromatogram of TIBA-^{14}C and its products of photodisintegration at different time intervals. Key: spot 1, unidentified material at the origin; spot 2, TIBA-^{14}C; spot 3, 2,5-diiodobenzoic acid; spot 4, 3,5-diiodobenzoic acid. Unidentified impurities associated with these spots were not separated further nor identified. The spot with its associated impurities (represented by bracketed area) was removed, assayed, and calculated as one component.

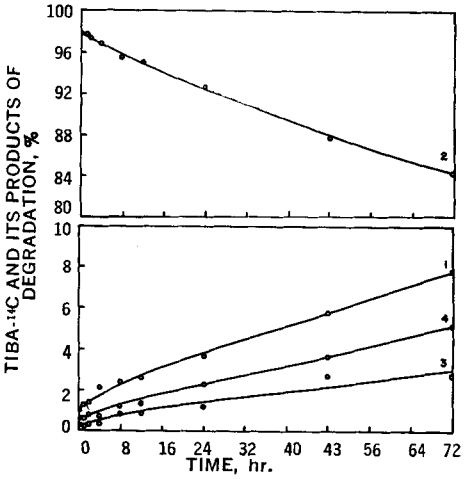

Fig. 2—Graphic relationship between the percentage of TIBA-^{14}C (curve 2) and the percentage of its products of degradation (curves 1, 3, and 4) at different hourly intervals. The curve number corresponds to the spot number in Fig. 1 where the nature of the products of degradation is explained.

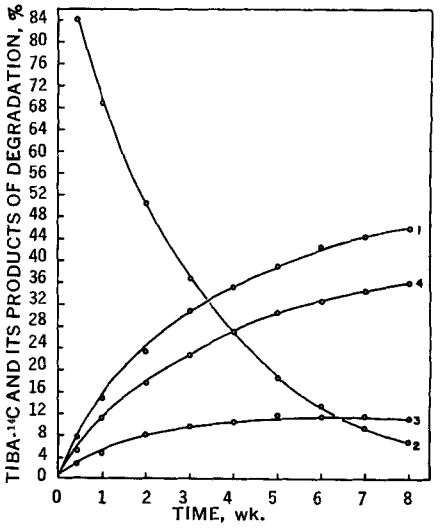

Fig. 3—Graphic relationship between the percentage of TIBA-^{14}C (curve 2) and the percentage of its products of degradation (curves 1, 3, and 4) at different weekly intervals. The curve number corresponds to the spot number in Fig. 1 where the nature of the products of degradation is explained.

which makes it difficult to obtain the desired product in absolutely pure form. Control samples of the TIBA-^{14}C kept in a light-resistant container showed no significant change at the end of 5 and 8 weeks.

It was observed that with increased time of exposure to the ultraviolet light the products resulting from the degradation increased in quantity and number since the solvent system used in the separation no longer sharply or completely separated the components of the mixture (compare the degree of resolution in Fig. 1 for the same spot at different time intervals of exposure). Impurities associated with the identified components were not separated but were counted as the entire spot (identified compound

plus its associated impurity). This was done for the purpose of evaluating the relationship between the concentration of TIBA-^{14}C and the products resulting from the photodisintegration. The unidentified compound(s) remaining at the origin were treated in an analogous manner.

The trace of unidentified impurities (spot 1) remaining at the origin increased in quantity to almost 8% of the total radioactivity at the end of 72 hr. of exposure and to 46% after 8 weeks. The quantity of TIBA-^{14}C (spot 2) decreased to 84% at the end of 72 hr. of exposure and to 7% at the end of 8 weeks. Spot 3 of the control (0 time) was identified as 2,5-diiodobenzoic acid. Spot 3 increased to 3% at the end of 72 hr. and 11% at the end of 8 weeks. *o*-Iodobenzoic acid, using the petroleum ether–propionic acid system for separation, has an R_f value very close to that of 2,5-diiodobenzoic acid and may be one of the impurities associated with it. Spot 4 in the control (0 time) was identified as 3,5-diiodobenzoic acid. This compound along with other products of photodisintegration associated with it increased to 5% at the end of 72 hr. and 36% at the end of 8 weeks. *m*-Iodobenzoic acid and also benzoic acid have R_f values using the above solvent system close to that of 3,5-diiodobenzoic acid and are probably impurities associated with the latter. At subsequent time intervals additional unidentified products from the degradation of TIBA-^{14}C became apparent.

CONCLUSIONS

2,3,5-Triiodobenzoic acid in a 2.1×10^{-2}% aqueous suspension is photochemically degraded by ultraviolet light. Ninety-three, 88, 84, 69, and 7% of TIBA are recovered from solution after 24, 48, 72 hr., 1 and 8 weeks of exposure, respectively. Two products of degradation were identified as 2,5- and 3,5-diiodobenzoic acid. From this study, it can be concluded that TIBA might be degraded in a like manner by sunlight after its application to plants.

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Keyphrases

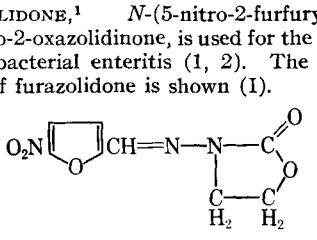
^{14}C-Carboxyl 2,3,5-triiodobenzoic acid
UV photodegradation of ^{14}C-carboxyl 2,3,5-triiodobenzoic acid
Degradation products of ^{14}C-carboxyl 2,3,5-triiodobenzoic acid
TLC separation
Liquid scintillation spectrometry—analysis

Method Specific for Determination of Furazolidone in Urine: Evidence for Drug-Related Metabolites

By R. D. HOLLIFIELD and JOHN D. CONKLIN

Furazolidone, *N*-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidinone, is a chemotherapeutic drug used orally for the treatment of bacterial enteritis. A new, more specific analytical method for the determination of furazolidone in urine is described. Utilizing this procedure, furazolidone was not detected in urine samples collected from dogs and humans following oral administration of the drug. Evidence is provided for the presence of drug-related metabolites.

FURAZOLIDONE,[1] *N*-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidinone, is used for the oral treatment of bacterial enteritis (1, 2). The structural formula of furazolidone is shown (I).



I

Previously, urinary concentrations of furazolidone were measured either by bioassay (1) or by the method of Nakamura and Inoue (3), which is based on the conversion of the drug to 5-nitrofurfural phenylhydrazone. A new analytical procedure, more specific for the determination of furazolidone in urine, is described in this report. Results obtained by this procedure and by the Nakamura and Inoue method are presented regarding furazolidone concentrations in dog and human urine following oral drug administration.

EXPERIMENTAL

Drug Administration—Micronized furazolidone (about 5 μ or less) in gelatin capsules was administered orally to unfasted, adult, male beagle dogs at 1.25 mg./Kg. q.i.d. at 4-hr. intervals (5 mg./Kg./day). Urine samples were then collected by catheterization at selected intervals. Furazolidone as a

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[1] Eaton Laboratories' trademark for furazolidone is Furoxone.