Synthesis of Radiolabeled 1,1-Bis(*p*-chlorophenyl)-2-nitropropane

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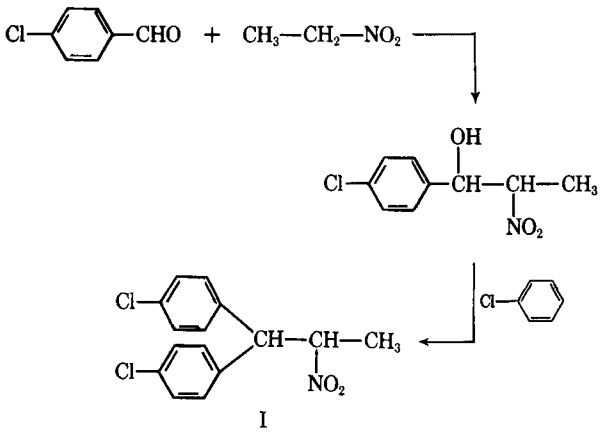
Abstract □ Radiolabeled 1,1-bis(*p*-chlorophenyl)-2-nitropropane was synthesized, and its purity was established by TLC. Autoradiograms of labeled and unlabeled products were compared for authenticity. The specific activity was determined.

Keyphrases □ 1,1-Bis(*p*-chlorophenyl)-2-nitropropane—synthesis, specific activity □ ^{14}C-Labeling—specific activity, 1,1-bis(*p*-chlorophenyl-2-nitropropane □ Liquid scintillation spectrometry—specific activity □ TLC—analysis

The primary objectives of this work were to devise a practical synthesis and to establish the purity of radiolabeled 1,1-bis(*p*-chlorophenyl)-2-nitropropane (I) for the purpose of subjecting the compound to distribution and metabolic studies in its use as an insecticide. The preparation of I is basically an adaptation of the method described by Hass *et al.* (1):



I

Scheme I

The desired compound (I) was obtained in 32.5% yield. Purity of the product was established by TLC, using two solvent systems and a commercial adsorbent.[1] Autoradiograms of the labeled product were compared to an authentic, unlabeled sample. The pure product had a specific activity of 13.85 ± 0.11 μc./mmole.

EXPERIMENTAL

1-*p*-Chlorophenyl-2-nitro-1-propanol—Fifty-six grams (0.4 mole) of *p*-chlorobenzaldehyde was stirred mechanically for 3 hr. with 200 ml. of water containing 50.0 g. (0.475 mole) sodium bisulfite. Thirty-three grams (0.44 mole) nitroethane, previously dissolved in 80 ml. of 6.25 *N* NaOH at 8–13°, was added in small portions to the stirred, thick, white suspension of bisulfite addition product. As the reaction proceeded, the magma dissolved and a flocculent precipitate formed. Stirring was continued for 18 hr. after the last addition of the alkaline nitroethane solution.

The reaction mixture was transferred to a separator, the yellow organic layer which settled to the bottom was removed, and the milky aqueous layer was extracted three times with 25-ml. portions of ether to remove additional organic material. The combined organic substances were extracted with 50-ml. portions of 10% aqueous sodium bisulfite solution until all unreacted *p*-chlorobenzaldehyde had been removed. The ethereal solution was dried with anhydrous sodium sulfate for 2 hr. and filtered; the solvent was removed under reduced pressure to give 70.0 g. of light-yellow oil. Fractionation of the crude product gave 31.0 g. (36.1%) of highly viscous yellow oil which distilled at 145–147° at 0.2 mm.

1,1-Bis(*p*-chlorophenyl)-2-nitropropane-^{14}C—To 25 ml. of 2% fuming sulfuric acid, maintained at 10° and mechanically stirred, was added 57 mg. (0.50 mmole) of chlorobenzene-^{14}C (2.98 mc./mmole)[2] and 3.8 ml. of unlabeled chlorobenzene used to rinse the shipping vial. When the mixture had reached bath temperature, 8.78 g. (0.041 mole) of 1-*p*-chlorophenyl-2-nitro-1-propanol was added dropwise. The cooling bath was removed; the reaction mixture was stirred for an additional 1.5 hr., poured onto 100 g. cracked ice, and allowed to stand for 2 hr. The product was extracted exhaustively with ether, and the combined ether extracts were washed successively with 50-ml. portions of 5% sodium bicarbonate. 10% sodium bisulfite, and water. The ether was removed under reduced pressure, the oily residue was steam distilled, and the product remaining in the distillation flask was dissolved in ether.

The ethereal solution was dried with anhydrous sodium sulfate for 2 hr. and filtered. The ether was removed under reduced pressure, and the viscous oil was poured onto a watch glass to solidify overnight. About 2 ml. absolute ethanol was added to the original flask to dissolve residual material. The alcoholic solution and the solidified material were combined, crystallized from hot ethanol, and twice recrystallized from ethanol to obtain 4.1276 g. (32.5%) of a white, crystalline product.

TLC—Microscope slides coated with commercial adsorbent were placed vertically in individual, small, square, amber bottles containing 10 ml. of the desired developing solvent. The bottles were capped and slides were equilibrated overnight. Five microliters of the sample, in methanol, was pipeted onto each slide about 1 cm. above the solvent level. The slides were developed for about 10 min., air dried, exposed to UV radiation to locate I, and sprayed with a plastic preservative if subsequent autoradiography was intended. Repeated runs with authentic samples of I gave an R_f value of 0.48 in hexane–ethyl acetate (9:1) and 0.30 in hexane–acetic acid (9:1).

Autoradiography—For autoradiography, separate solutions of the authentic unlabeled product and of the labeled product containing 10 mg./ml. were prepared in methanol. Five-microliter samples of the authentic and labeled products were chromatographed in parallel on 20 × 20-cm. commercial adsorbent thin-layer plates, using both of the solvent systems described previously. In both solvent systems, UV irradiation of the plates disclosed identical R_f values for authentic and unlabeled samples. In neither solvent was there evidence of impurities. The plates were sprayed with plastic preservative to prevent sloughing, exposed for 6 days to film, developed, and fixed.[3] For both solvent systems, a single radioactive

[1] Adsorbosil P-1, Allied Science Laboratories Inc., State College, Pa.

[2] Uniformly labeled.

[3] Eastman No Screen medical X-ray film. Developed and fixed in Eastman Kodak liquid developer and liquid fixer, respectively.

spot, corresponding to the position of the authentic unlabeled sample, was obtained. There was no tailing in the hexane–ethyl acetate system, but there was a small amount of tailing in the hexane–acetic acid system.

Determination of Specific Activity—A 10 mg./ml. solution of 1,1-bis(*p*-chlorophenyl)-2-nitropropane-^{14}C in methanol was used to determine the specific activity of the compound.

Five samples were prepared by pipeting 100 μl. of the solution into five scintillation vials containing a liquid scintillation cocktail composed of 0.14% PPO (2,5-diphenyloxazole) and 0.01% dimethyl POPOP [1,4-bis(4-methyl-5-phenyl-2-oxazolyl)benzene] in equal volumes each of toluene and 2-ethoxyethanol. These samples were cooled in a Packard TRI CARB liquid-scintillation spectrometer, model 3003, for 0.5 hr. The window width of 50–100° was chosen. Utilizing an internal standard of toluene-^{14}C (toluene-^{14}C, 5.01×10^5 dis./min./ml., R-21 Tracerlab), the observed counts per minute were converted to disintegrations per minute and corrected for quenching and counter efficiency. A specific activity of 13.85 ± 0.11 μc./mmole was obtained.

REFERENCE

(1) H. B. Hass, M. B. Neher, and R. T. Blickenstaff, *Ind. Eng. Chem.*, **43**, 2875(1951).

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Development of Tolerance to Pentobarbital

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Abstract □ Tolerance to pentobarbital developed 4 hr. after the first injection and reached a peak at 17–22 hr., after which it decreased to nonsignificant levels by 48 hr. The duration and frequency of administration of pentobarbital affected the degree of development of tolerance to pentobarbital. PTI (Percentage Tolerance Index) decreased progressively with the increase in the number of injections. When four injections were given within the span of 28 hr., the animals showed a greater degree of tolerance on the third injection which was administered 24 hr. after the initial injection. Tolerance was also present on the fourth injection but to a lesser degree when compared with the third injection. The experimental data from this study suggest that tolerance to pentobarbital does develop and is the result of the pentobarbital stimulating its own metabolizing enzyme.

Keyphrases □ Pentobarbital—tolerance development, rats □ Drug tolerance—pentobarbital, rats □ Tolerance, pentobarbital—rats

Animals can be shown to develop tolerance to barbiturates, pentobarbital and thiopental (1). Gruber and Keyser found that dogs tend to become tolerant to a variety of barbiturates (2). As a criterion of tolerance, they used the reduction of the sleeping time elicited by the same dose after it had been repeated several times. According to Goodman and Gilman, tolerance to barbiturates has developed when, after repeated administration, a given dose produces a decreasing effect or, conversely, when increasingly larger doses must be given to obtain true barbiturate effects obtained with the original dose (3). Tatum *et al.* provide the classic definition of tolerance as "a phenomenon characterized by the fact that more drug must be used to produce equivalent effects" (4). Jaffe and Sharpless have shown that some degree of physical dependence can be produced in as little as 20 hr. after pentobarbital administration (5). Singh has also shown that tolerance to pentobarbital and thiopental is developed within 24 hr. (1). The purpose of this paper is to report that: (*a*) a certain time lapse occurs before the tolerance is developed, and (*b*) this developed tolerance reaches a peak and then declines.

EXPERIMENTAL

Female albino rats, random by breed (Caeserian Drive One) and weighing 125–175 g., were used. Pentobarbital sodium was dissolved in distilled water. The volume of each injection was kept constant, *i.e.*, 1 ml./kg. All injections of pentobarbital (25 mg./kg.) were given intraperitoneally. The sleeping time (difference between loss of righting reflex and regain in righting reflex in minutes) was determined.

Animals were divided into five major groups:

Group 1—Pentobarbital was administered to 34 animals at zero time and at an interval of 24 hr.

Group 2—Eighteen subgroups were composed of 10 to 15 animals each. In these subgroups, the first injection was given at zero time and then the second injection was given at intervals of 2, 3, 4, 7, 9, 13, 17, 18, 19, 20, 21, 21, 22.5, 22.5, 24, 48, 72, and 168 hr. Some experiments (21 and 22.5 hr.) were duplicated.

Group 3—Pentobarbital, 25 mg./kg., was administered daily to 10 animals at intervals of 24 hr. for 16 days.

Group 4—Three subgroups were comprised of 10 to 15 animals each. To each subgroup, pentobarbital, 25 mg./kg., was administered at intervals of 0, 2, 24, 26; 0, 3, 24, 27; and 0, 4, 24, 28 hr.

Group 5—Clinical signs, *i.e.*, water consumption, urine output, food consumption, and growth, were observed in animals that had developed tolerance to pentobarbital, 25 mg./kg. Each animal was housed separately in a metabolism cage, and initial observations on each animal were taken. Each animal was given two injections of pentobarbital after the initial observations. Then the animals were allowed to recover on the third and fourth day. Statistical methods used were those of Snedecor (6).

Because most work on the development of tolerance to barbiturates has been done on male rats, guinea pigs, mice, and dogs, in this project the authors decided to study tolerance to pentobarbital in female white rats. Percentage tolerance index (PTI) was computed as follows (1):

$$\frac{\text{hypnotic effect of first injection}}{\text{hypnotic effect of second injection}} \times 100$$

If PTI is unity, *i.e.*, 100%, it indicates no tolerance. PTI greater or less than unity indicates tolerance or cumulative effect. Before