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Intestinal Bacterial Reduction of 4,4′-Dihydroxystilbene to 4,4′-Dihydroxybibenzyl

Keyphrases □ 4,4′-Dihydroxystilbene—intestinal bacterial reduction to 4,4′-dihydroxybibenzyl □ 4,4′-Dihydroxybibenzyl—intestinal bacterial reduction from 4,4′-dihydroxystilbene □ Metabolism—*trans*-stilbene, intestinal reduction of 4,4′-dihydroxystilbene

To the Editor:

We previously reported the metabolic reduction of the ethylenic double bond in *trans*-stilbene to yield 4,4′-dihydroxybibenzyl to the extent of 2.1% in rabbit urine and 10.6% in guinea pig urine (1, 2). Continued studies in our laboratories have shown that rats administered *trans*-α-^{14}C-stilbene intramuscularly in peanut oil excreted 4.1% of the dose as the bibenzyl compound in urine. These rat studies also have indicated that 40% of the original activity as compared to only 2.3% for rabbits is eliminated over 19 days in the feces. 4,4′-Dihydroxybibenzyl was detected by cochromatography with a reference compound in TLC Systems I and II[1]. It was the major metabolite in rat feces in a yield of 12.4% of the dose.

These species differences, which are consistent with reports (3, 4) that polarity and molecular weight determine the extent of biliary excretion in a given species, led us to postulate that biliary excretion and subsequent intestinal bacterial action are responsible for the reduction of the 4,4′-dihydroxy metabolite of *trans*-stilbene to 4,4′-dihydroxybibenzyl. That is, the increased yield of the bibenzyl compound in the rat could be due initially to an increased biliary excretion of either 4,4′-dihydroxystilbene *per se* or, more significantly, its glucuronide conjugate. It is of interest in this regard that enterohepatic circulation has been reported for the related compound, diethylstilbestrol, with extensive excretion into rat bile as the monoglucuronide followed by hydrolysis and reabsorption of diethylstilbestrol (5–7).

Recently, Scheline (8) also postulated that 4,4′-dihydroxybibenzyl is produced through reduction by intestinal microflora. His conclusion was based upon a decreased yield of the bibenzyl compound in urine of rats treated with the antibiotic neomycin sulfate or by bile duct ligation prior to the administration of *trans*-stilbene. Our studies confirm this hypothesis by direct incubation of dihydroxystilbene in an intestinal microflora extract.

Incubation studies were with 4,4′-dihydroxy-α-^{14}C-stilbene obtained by TLC (System I) of the phenolic fraction derived from an ether extract of enzyme[2]-hydrolyzed urine of rabbits administered *trans*-α-^{14}C-stilbene. The dihydroxy compound, which cochromatographed with reference material in TLC Systems I and II, was eluted from silica gel with methanol. This extract was concentrated, a few drops of polysorbate 80 were added, and the remaining methanol was removed before the sample was diluted to 1 ml with 0.1 *M* phosphate buffer (pH 7.4). An aliquot (0.1 ml = 4300 dpm) of the polysorbate 80 suspension was added to 1 ml of the incubation medium previously described (9, 10). The intestinal bacterial extract for this mixture was obtained from the intestinal and cecal contents of male Sprague–Dawley rats, 250–300 g. After incubation at 37° for 24 hr under nitrogen, the mixture was quenched with 1 *N* HCl (1 ml) and continuously extracted with ether for 48 hr. Controls were prepared in the same manner except that the intestinal bacterial extract was autoclaved for 17 min at 121° before addition to the sample tubes containing substrate.

Methanol solutions of the ether extracts of the control and test samples were investigated for transformations by TLC System I. Reference compounds cospotted with the methanol solution were visualized by quenching of the fluorescent TLC plates, while radioactive components were located using a radiochromatogram scanner[3]. Quantitation was by liquid scintillation counting of 0.64-cm (0.25-in.) strips from the TLC plates.

The TLC results from sample incubations indicated a yield of 14% of 4,4′-dihydroxybibenzyl at R_f 0.40, but there was no indication of the reduced product with TLC of the control incubation. Thus, these incubation studies confirm the reduction by intestinal microflora of 4,4′-dihydroxystilbene and, together with reports (9, 11) of the reduction of cinnamic acids to phenylpropionic acids, establish the conjugated ethylenic bond as among the moieties (12) reduced by intestinal microflora.

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[1] TLC systems employed were: I, toluene–piperidine (5:2); and II, benzene–methanol (9:1). TLC plates, 5 × 20 cm and 0.25-mm layer thickness, were precoated with silica gel F-254 (Brinkmann Instruments Co.).

[2] Hydrolysis was at 37° for 72 hr with 1000 units of β-glucuronidase–aryl sulfatase (Sigma Chemical Co.) per ml of urine.

[3] Packard radiochromatogram scanner model 7201, Packard Instrument Co.

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BOOKS

REVIEWS

Terpenoids and Steroids, Vol. 3. Senior Reporter, K. H. OVERTON. Specialist Periodical Reports, The Chemical Society, Burlington House, London WIV OBN, England, 1973. 527 pp. 15 × 22 cm. Price £12. (Orders should be addressed to The Publication Sales Officer, The Chemical Society, Blackhorse Road, Letchworth, Herts., SG6 IHN, England)

This is the third volume on terpenoids and steroids in a valuable series first published 3 years ago. The aim of the various series of *Specialist Periodical Reports* is to provide systematic, comprehensive, and critical review coverage of progress in the major areas of chemical research. The various series are being published annually or biennially on such topics as Amino Acids, Peptides, and Proteins; Alkaloids; Photochemistry; Foreign Compound Metabolism in Mammals; Carbohydrate Chemistry; and Biosynthesis.

This volume does not contain a subject index but is organized in a systematic manner which facilitates finding any information being sought. The six pages in the Table of Contents outline this reference in detail. The chapters are divided into many sections which are identified in boldface type in the text as well as in the Table of Contents. These sections are further divided into subsections. Chapter titles are found at the top of every second page of the text. There is an author index which is helpful to those following the research of a given individual.

This review is illustrated with drawings of over 2500 chemical structures. It is documented with 1800 references which are listed at the bottom of the first page of each chapter where used.

Part I, which covers the terpenoids, is divided into six chapters covering the usual chemical classes of terpenoids and a seventh chapter on biosynthesis of terpenoids and steroids. Pharmaceutical scientists will find many sections of special interest, *e.g.*, analytical methods, biological activity, cannabinoids, and diterpene alkaloids.

Part II, which covers steroids, is divided into two large chapters. The chapter on steroid properties and reactions is divided into sections based upon more common functional groups, a section on compounds of nitrogen and sulfur, and sections upon such important subjects as molecular rearrangements, stereochemistry and conformational analysis, functionalization of nonactivated positions, and photochemical reactions. The chapter on steroid synthesis includes sections on oestranes, androstanes, pregnanes, cholestanes, and total synthesis as well as sections of special interest to pharmaceutical scientists which include insect and plant hormones, alkaloids, sapogenins, bufodienolides, and cardenolides.

It is a credit to the eight reporters who wrote this volume that it is so well done. It meets the standards set by the Chemical Society for these "Reports." Although this volume includes a comprehensive review of chemical studies of terpenoids and steroids, it devotes no space to the isolation and structure determination of these substances from plant or animal sources. This was the only disappointment to the reviewer.

Everyone interested in the chemistry of terpenoids and/or steroids should have access to this volume and others in the series. It would be a great time saver. The cost will tend, however, to limit the distribution of this series to libraries and the more dedicated researchers and scholars in these fields.

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Reaction Mechanisms in Organic Analytical Chemistry. By KENNETH A. CONNERS. Wiley, New York, N.Y., 1973. xiii + 634 pp. 14.5 × 22.5 cm. Price $18.50.

Since the early 1950's, when chemical kinetics became a popular tool for studying drug stability, there has been a need for a suitable graduate level text covering drug-oriented physical organic chemistry and stressing kinetics and mechanisms in aqueous solutions. This problem has been made more acute by the trends of graduate level chemistry courses in these areas toward more sophisticated theoretical concerns at the expense of the subject matter most useful in pharmaceutical research and many other applied sciences. Some biochemistry departments have countered this trend by introducing in their programs new courses which make use of relatively recent books in the field of bioorganic mechanisms. While these are useful texts, they are not entirely appropriate for considering either stability or analysis of drugs.

Dr. Conners, a well-established author, has done an excellent job in providing a text on the kinetics and reactivity of organic functional groups in aqueous solutions for both the pharmaceutical and analytical chemist. Many examples used in the book are drugs. The book is well suited for self study or formal courses. There are 13 chapters and each chapter is followed by practice problems.

The book is unique in many ways. It presents the most comprehensive section on pH–rate profiles that I have seen in a text to date. In this treatment, errors which are commonly made in the literature are clearly pointed out.

In yet another section, a unique summary of reactions used in organic analysis is provided. The book is comprehensive, clearly and skillfully written, well referenced (more than 1000 citations), and effectively organized. The chapter titles are: Introduction, Chemical Equilibria, Reaction Rates, Extrathermodynamic Relationships, Selectivity, Sensitivity, Electrophilic Aromatic Substitution, Nucleophilic Aromatic Substitution, Nucleophilic Aliphatic Substitution, Addition to Carbon–Carbon Multiple Bonds, β-Elimination, Addition to Carbon–Heteroatom Multiple Bonds, and Acyl Transfer.

In summary, I would highly recommend this book to anyone interested in the reactivity, analysis, or aqueous stability of organic functional groups.

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