Identity of a Major Chromogen in *p*-Aminosalicylic Acid and Sodium *p*-Aminosalicylate Dosage Forms

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Abstract □ A major chromogen found in *p*-aminosalicylic acid and sodium *p*-aminosalicylate dosage forms was identified to be 3,3′-dihydroxyazoxybenzene. This compound could be produced by oxidation or sunlight irradiation of *m*-aminophenol, a common impurity of *p*-aminosalicylic acid and sodium *p*-aminosalicylate. Methods of synthesis for 3,3′-dihydroxyazoxybenzene are described.

Keyphrases □ *p*-Aminosalicylic acid, sodium *p*-aminosalicylate dosage form—identification of major chromogen, 3,3′-dihydroxyazoxybenzene □ 3,3′-Dihydroxyazoxybenzene—identification as a major chromogen in *p*-aminosalicylic acid, sodium *p*-aminosalicylate dosage form, synthesis, photosynthesis □ Sodium *p*-aminosalicylate, *p*-aminosalicylic acid dosage form—identification of major chromogen, 3,3′-dihydroxyazoxybenzene

Decarboxylation of *p*-aminosalicylic acid into *m*-aminophenol and carbon dioxide was extensively studied (1–4). It seems generally agreed that *m*-aminophenol did not have a significant role in the adverse reactions of *p*-aminosalicylic acid but that the chromogenic factors probably did[1].

Although discoloration of aged tablets of *p*-aminosalicylic acid and its sodium salt has been a common phenomenon, chromogenic substances have not been identified in the past. In this study, experiments were designed to determine the source of the major chromogen occurring in the commercially available tablets of *p*-aminosalicylic acid, *p*-aminosalicylic acid–isoniazid, and sodium *p*-aminosalicylate. The identity of a major chromogen is described in this report.

EXPERIMENTAL[2]

Materials—All chemicals and solvents were of reagent grade. The following were used: *m*-aminophenol[3]; hydrogen peroxide, 30% solution[4]; zinc dust[5]; ethyl acetate[5]; ethanol[5]; chloroform[5]; methylene dichloride[5]; acetone[5]; and Eastman chromagram sheet silica gel 6061.

Synthesis of 3,3′-Dihydroxyazoxybenzene—Three grams of *m*-aminophenol was dissolved in a mixture of 50 ml. of hydrogen peroxide and 100 ml. of distilled water. The reaction was allowed to take place overnight at room temperature (about 25°). The dark-colored crude product was then filtered and collected (yield 1.2 g.). The crude product was dissolved in 50 ml. of hot ethyl acetate and filtered. The filtrate was transferred into a chromatographic column (1.2 × 26 cm.) packed with a slurry of aluminum oxide containing 20 g. dispersed in 100 ml. of ethyl acetate. The column was eluted with the same solvent. A cherry-red eluate was obtained. Evaporation of the eluate under reduced pressure yielded a dark residue. Recrystallization of the product in 70% ethanol gave rosettelike crystals in dark cherry-red (yield 98 mg.). The product was insoluble in dilute mineral acids and sodium bicarbonate solution but soluble in sodium carbonate or fixed alkaline solutions (a typical solubility property of phenols). The compound could be decolorized by zinc dust reduction in dilute hydrochloric acid.

Table I—R_f Values of 3,3′-Dihydroxyazoxybenzene

Samples[a]	Solvent Systems[b] I	II	III	IV	V	VI
R	0.15	0.25	0.30	0.48	0.52	0.70
A	0.15	0.25	0.30	0.48	0.52	0.70
B	0.15	0.25	0.30	0.48	0.52	0.70
C	0.15	0.25	0.30	0.48	0.52	0.70
D	—	—	—	—	—	—

[a] R = 3,3′-dihydroxyazoxybenzene; A = effervescent *p*-aminosalicylic acid tablet; B = buffered *p*-aminosalicylic acid–isoniazid tablet; C = sugar-coated sodium *p*-aminosalicylate tablet; D = sodium *p*-aminosalicylate USP; three lots of each tablet were tested. [b] I = chloroform–ethyl acetate (5:1); II = chloroform–ethyl acetate (3:1); III = chloroform–ethyl acetate (2:1); IV = chloroform–ethyl acetate (1:1); V = methylene dichloride–acetone (6:1); and VI = methylene dichloride–acetone (2:1).

TLC studies of the compound in various solvent systems (Table I) showed only one distinct spot under visible or UV light, m.p. about 210° dec.; visible spectrum: $\lambda_{max.}$ 525 nm. (ethanol); IR spectrum (KBr): $\bar{\nu}$ (max.) 3250 cm.$^{-1}$ (aromatic C—H stretching), 1430, 1500, 1550 cm.$^{-1}$ (aromatic C=C in-plane vibrations), 1600 cm.$^{-1}$ (N=N stretching), 1280 cm.$^{-1}$ (N→O stretching), 1210, 1380 cm.$^{-1}$ (O—H bending), and 3620 cm.$^{-1}$ (O—H stretching).

Anal.—Calc. for $C_{12}H_{10}N_2O_3$: C, 62.60; H, 4.34; N, 12.16. Found: C, 62.85; H, 4.38; N, 12.06.

Photosynthesis of 3,3′-Dihydroxyazoxybenzene—Three grams of *m*-aminophenol was dissolved in 200 ml. of ethanol in a stoppered flask. The sample solution was then subjected to intermittent sunlight irradiation in a temperature range of 16–27°. After 96 hr. of irradiation, the sample solution was evaporated to dryness under reduced pressure. To remove unreacted *m*-aminophenol from the residue, the crude product was dissolved in 120 ml. of ethyl acetate and extracted with ten 50-ml. portions of dilute hydrochloric acid solution followed by three 50-ml. portions of water in a 250-ml. separator. The ethyl acetate layer was then transferred into a chromatographic column (1.2 × 26 cm.) packed with a slurry containing 20 g. of aluminum oxide dispersed in 100 ml. of ethyl acetate. The column was eluted with the same solvent. A cherry-red eluate was obtained, which was then evaporated to dryness under reduced pressure. Recrystallization of the product from 70% ethanol gave rosettelike dark cherry-red crystals (yield 52 mg.).

TLC studies of the product in various solvent systems (Table I) showed only one distinct spot when visualized under visible or UV light. Visible and IR spectra of the compound were identical to those of 3,3′-dihydroxyazoxybenzene obtained in the preceding synthesis.

Identification of 3,3′-Dihydroxyazoxybenzene in *p*-Aminosalicylic Acid and Sodium *p*-Aminosalicylate Dosage Forms—Tablets of effervescent *p*-aminosalicylic acid, buffered *p*-aminosalicylic acid–isoniazid, and sodium *p*-aminosalicylate were tested. Sample powder equivalent to 3–4 g. of *p*-aminosalicylic acid was dissolved in 100 ml. of water. The solution was filtered, and the filtrate was extracted with two 25-ml. portions of ethyl acetate. The combined ethyl acetate layer was washed with three 25-ml. portions of water. Evaporation of the ethyl acetate layer under reduced pressure yielded a brown residue. A similar procedure was also applied to the reference material of sodium *p*-aminosalicylate. Symbols A, B, C, and D were designated to represent residues obtained from effervescent *p*-aminosalicylic acid tablets, *p*-aminosalicylic acid–isoniazid tablets, sodium *p*-aminosalicylate tablets, and sodium *p*-aminosalicylate USP, respectively.

[1] Private communication from Dr. L. M. Lueck, Parke-Davis & Co., Detroit, Mich.
[2] A Bausch & Lomb 505 recording spectrophotometer and a Beckman IR-8 recording spectrophotometer were used.
[3] Eastman Organic Chemicals.
[4] Anachemia Chemicals Ltd.
[5] Fisher.

The residues were dissolved in a very small amount of methanol for TLC studies. An amount of 0.5 mcg. of 3,3′-dihydroxyazoxybenzene dissolved in methanol was used for spotting. For Samples A, B, C, and D, sufficient amounts to make the spot visually distinct were applied. Thin-layer plates were developed in various solvent systems, and the spots were located by visible or UV light. The results are given in Table I.

RESULTS AND DISCUSSION

Preliminary studies indicated that air oxidation of an aqueous solution of *m*-aminophenol was much faster than that of *p*-aminosalicylic acid and its sodium salt. It is thus postulated that some of the chromogens which occurred in aged *p*-aminosalicylic acid dosage forms might be contributed from oxidation of *m*-aminophenol, a common impurity of *p*-aminosalicylic acid and its sodium salt (5, 6).

To effect mild oxidation of *m*-aminophenol, hydrogen peroxide was used as the oxidizing agent in this study. The reaction conditions chosen were room temperature and about neutral pH. A dark cherry-red compound was isolated and identified as 3,3′-dihydroxyazoxybenzene. Sunlight irradiation of *m*-aminophenol in ethanol also yielded the same azoxy compound.

Commercially available tablets of *p*-aminosalicylic acid, *p*-aminosalicylic acid–isoniazid, and sodium *p*-aminosalicylate were tested for the presence of this specific chromogen. TLC studies of samples in various solvent systems clearly indicated that 3,3′-dihydroxyazoxybenzene is a common chromogen which occurred in those samples being tested (Table I), although this compound was not detected in the reference material of sodium *p*-aminosalicylate.

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 23, 1971, from *65 High Park Avenue, No. 211, Toronto, Ontario, Canada.*

Accepted for publication August 31, 1971.

Microanalysis was conducted by the Microanalysis Laboratory Ltd., Toronto.

Inhibition of Nicotinate Phosphoribosyl Transferase by Nonsteroidal Anti-Inflammatory Drugs: A Possible Mechanism of Action

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Abstract □ When incubated with ^{14}C-nicotinic acid and phosphoryl-ribose-1-pyrophosphate, human platelet lysate incorporates radioactivity into nicotinic acid mononucleotide. Under these conditions, the apparent K_m of nicotinic acid for nicotinate phosphoribosyl transferase is 2.4×10^{-5} *M*. Along with 2-hydroxynicotinic acid ($K_i = 2.3 \times 10^{-4}$ *M*), the following nonsteroidal anti-inflammatory compounds competed reversibly with nicotinic acid for the enzyme: flufenamic acid ($K_i = 4.6 \times 10^{-5}$ *M*), mefenamic acid ($K_i = 7.6 \times 10^{-5}$ *M*), salicylic acid ($K_i = 1.6 \times 10^{-4}$ *M*), phenylbutazone ($K_i = 1.6 \times 10^{-4}$ *M*), and indomethacin ($K_i = 4.2 \times 10^{-4}$ *M*). Such inhibition may explain the decreases in nicotinamide adenine dinucleotide phosphate content of rat liver after administration of salicylate. Furthermore, suppression of nicotinamide adenine dinucleotide biosynthesis by anti-inflammatory drugs may restrain mucopolysaccharide biosynthesis and, thereby, reduce inflammatory responsiveness. Hence, this antagonism of niacin may be involved in the pharmacologic activity and, particularly, the toxicity of anti-inflammatory drugs.

Keyphrases □ Nicotinate phosphoribosyl transferase—inhibition by nonsteroidal anti-inflammatory drugs, mechanism of action, using radiolabeled nicotinic acid □ Human platelet lysate—evaluation of influence of nonsteroidal anti-inflammatory drugs on nicotinate phosphoribosyl transferase, mechanism of action □ Nicotinic acid, radiolabeled—used to evaluate influence of nonsteroidal anti-inflammatory drugs on nicotinate phosphoribosyl transferase

The human platelet incorporates 7-^{14}C-nicotinic acid into nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, and other compounds (1) consistent with intermediates of the pathway of Preiss and Handler (2, 3). Such incorporation is hindered by analogs of nicotinic acid, by salicylic acid, and by other anti-inflammatory drugs (4–6).

Data concerning the intact platelet suggested inhibition of nicotinamide adenine dinucleotide biosynthesis early in the pathway, probably at the step catalyzed by nicotinate phosphoribosyl transferase (5). To evaluate this possibility further, the influence of various nonsteroidal anti-inflammatory drugs on nicotinate phosphoribosyl transferase in human platelet lysate was studied.

METHODS

The following compounds were obtained from commercial sources: 7-^{14}C-nicotinic acid (sp. act. 59.1 mc./mmole)[1], phosphoryl-ribose-1-pyrophosphate[2], nicotinamide mononucleotide[3], salicylic

[1] Amersham Searle.
[2] Mann Research Lab.
[3] Sigma.