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Identification of an Imidazolinium Salt, the Major Product from Reaction of Benzathine with Iodine

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Abstract □ The isolation and identification of an imidazolinium salt are described. Unambiguous determination of structure was accomplished by independent synthesis. The isolated product interferes in the iodometric assay of the benzathine salts of penicillin.

Keyphrases □ Imidazolinium salts—analysis, product from reaction of benzathine with iodine □ Penicillin V benzathine—analysis, iodometric assay, interference by imidazolinium salt reaction product □ Iodometric assays—penicillin V benzathine, interference by imidazolinium salt

A recent report (1) described the interference of benzathine (I) in the official iodometric assay of penicillin V benzathine as presently described in the "Code of Federal Regulations" (CFR) (2). A high-performance liquid chromatographic (HPLC) method (3) of analysis for formulations containing this drug was suggested subsequently.

The present article describes the isolation and identification of an imidazolinium salt (II) as the product from the reaction of iodine with benzathine.

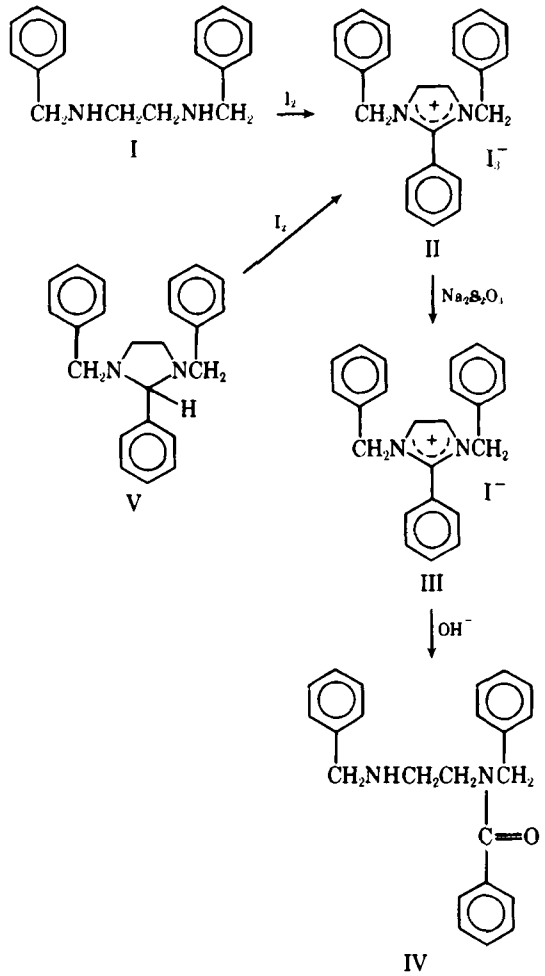
EXPERIMENTAL[1]

1,3-Dibenzyl-2-phenylimidazolinium Triiodide (II)—To 4.0 g of I in 5 ml of methanol at room temperature were added 5-ml aliquots of a methanolic iodine solution (2.5 g in 125 ml); the color was allowed to discharge after each addition. When a reddish color persisted, addition of methanolic iodine was continued until the precipitation of a red product was complete. The red solid was removed by filtration, and recrystallization from methanol–acetone yielded 2.1 g of II as deep-red needles, mp 163.5–165° [lit. (4) mp 165–166°]; NMR (acetone-d_6): δ 4.22 (s, 4, H_2C-4, H_2C-5), 4.68 (s, 4, H_2C-phenyl), 7.42 (s, 10, C-phenyl), and 7.85 (s, 5, phenyl-C-2).

Anal.—Calc. for $C_{23}H_{23}I_3N_2$ (mol. wt. 708.15): C, 39.01; H, 3.27; I, 53.76; N, 3.96. Found: C, 39.18; H, 3.36; I, 53.69; N, 4.05.

Conversion of II to *N*-Benzoyl-*N,N*-dibenzylethylenediamine (IV)—To a slurry of 1.303 g of II in 20 ml of methanol was added a saturated methanolic solution of sodium thiosulfate until a colorless solution was obtained. The solution was evaporated to dryness, and the residue was dissolved in chloroform and evaporated to dryness to give 934 mg of III as a pale-yellow foam; IR (film): 3030, 2915 (C–H), 1590 (C=N⁺<), 1250 (C–N), 1580, 1450, 750, and 700 (aromatic) cm^{-1}; NMR ($CDCl_3$): δ 4.17 (s, 4, H_2C-4, H_2C-5), 4.62 (s, 4, H_2C-phenyl), 7.37 (s, 10, C-phenyl), and 7.60–8.13 (m, 5, phenyl-C-2).

To 200 mg of III in 3 ml of methanol was added 3 ml of 3 *N* NaOH, and the solution was heated at 40° for 1.5 hr. Extraction with chloroform



Scheme I

[1] NMR spectra were obtained using Bruker WP-80 and Varian A-60A instruments. IR spectra were obtained on a Beckman IR-20 spectrophotometer. All melting points are uncorrected.

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yielded 137 mg of IV as a colorless oil; IR (film): 3310 (N–H), 3050, 3020, 1600, 750, 700 (aromatic), 2910, 2810 (C–H), and 1635 (C=O) cm^{-1}; NMR ($CDCl_3$): δ 1.34 (s, 1, N–H, exchangeable), 2.66–2.94 (m, 2, C–CH_2–NH), 3.25–3.78 [m, 4, C–CH_2–N–C(–)=O and NH–CH_2-phenyl], 4.66 (s, 2, phenyl-CH_2–N–C=O), and 7.13–7.50 (m, 15, aromatic).

Benzoylation of Benzathine to Give IV—To 960 mg of I in 3 ml of chloroform and 0.5 ml of triethylamine was added dropwise 550 mg of benzoyl chloride in 1 ml of chloroform. After 1.5 hr at room temperature, the solution was added to 20 ml of water, brought to pH 11 with sodium hydroxide, and extracted with chloroform. Workup afforded 1.5 g of white solid. A portion of the crude product was chromatographed by preparative TLC to yield 65 mg of IV.

1,3-Dibenzyl-2-phenyltetrahydroimidazole (V)—Equimolar (0.02 *M*) amounts of I and benzaldehyde with 150 mg of *p*-toluenesulfonic acid in 100 ml of benzene were condensed over 3 hr to yield a white solid, 4.1 g, mp 99–99.5° (hexane) [lit. (5) mp 99°].

Oxidation of V to II—To 1.235 g (3.76 mmoles) of V in 10 ml of benzene was added dropwise 956 mg (3.76 mmoles) of iodine in 100 ml of benzene. To the resulting solution was added, all at once, an equal aliquot of iodine in benzene. The solution was cooled, and the red solid, 2.8 g (90%), was collected. Recrystallization from methanol–acetone gave deep-red needles of II, mp 163.5–166°.

RESULTS AND DISCUSSION

The reaction of benzathine with iodine in methanol resulted in the isolation of one product; the NMR spectrum was consistent with Structure II. Further confirmation of Structure II was derived from the partial reduction of II to the iodide III, which, under basic conditions, yielded the ring-opened benzoylbenzathine (IV), analogous to other imidazolinium salts (6).

Unambiguous confirmation of Structure II resulted from the iodine oxidation of the tetrahydroimidazole (V) to II.

Product II probably results from a reaction sequence beginning with oxidation of benzathine to give an imine, which then hydrolyzes to give the benzaldehyde (Scheme I). The benzaldehyde is free to condense with intact benzathine to give V, which oxidizes with excess iodine to II.

Therefore, this reaction sequence leads to erroneously low results in the CFR iodometric assay as presently described for penicillin V benzathine. It also accounts for the fact that interference by benzathine in the iodometric assay is dependent on pH since the initial oxidation requires a free amino function.

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Capacity-Limited Gut Wall Metabolism of 5-Aminosalicylic Acid, a Therapeutically Active Metabolite of Sulfasalazine, in Rats

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Abstract □ The metabolic fate of 5-aminosalicylic acid (reported to be the active therapeutic moiety of sulfasalazine) was assessed in fasting rats as a function of dose (25–200 mg/kg) and administration route (oral, intraperitoneal, and intravenous). 5-Aminosalicylic acid is subject to both capacity-limited presystemic (apparently during first passage through the intestinal epithelium) and systemic acetylation. The possibility exists that 5-aminosalicylic acid also is acetylated presystemically after oral sulfasalazine administration to patients with inflammatory bowel disease. Any alteration in the absorption rate of this active metabolite from the colon could affect the time course of local anti-inflammatory activity if *N*-acetyl-5-aminosalicylic acid is inactive or less active than 5-aminosalicylic acid.

Keyphrases □ 5-Aminosalicylic acid—metabolism, gut wall, effect of dose and administration route □ Sulfasalazine metabolites—5-aminosalicylic acid, metabolism, gut wall, effect of dose and administration route □ Antibacterial agents—sulfasalazine, 5-aminosalicylic acid metabolite, metabolism, gut wall, effect of dose and administration route □ Intestinal epithelium—metabolism, 5-aminosalicylic acid, effect of dose and administration route

Sulfasalazine (salicylazosulfapyridine) is widely used for the treatment of ulcerative colitis and Crohn's disease (1). After oral administration to rats and humans, the drug is almost completely metabolized in the colon and cecum by bacterial azo reductases. The metabolic products sulfapyridine and 5-aminosalicylic acid are absorbed from the colon and are further metabolized to N^4-acetylsulfapyridine, to the *O*-glucuronide and *O*-sulfate conjugates of 5′-hydroxysulfapyridine and N^4-acetyl-5′-hydroxysulfapyridine, and to *N*-acetyl-5-aminosalicylic acid (2–6).

Several investigators (3, 5–8) suggested that sulfasalazine itself may not be an active therapeutic agent but may serve only as a means of delivering its metabolic products, 5-aminosalicylic acid (a possible anti-inflammatory agent) and sulfapyridine (an antibacterial agent), to the inflammation site in the colon where either or both of these agents exert the desired pharmacological effects. Recent studies in which sulfasalazine, 5-aminosalicylic acid, and sulfapyridine were administered rectally to patients with ulcerative colitis (9) and idiopathic proctitis (10) suggest that 5-aminosalicylic acid is the active therapeutic moiety of sulfasalazine and acts topically on the inflammed mucosa. Although this finding requires confirmation in a larger patient population and does not explain the mode of action of 5-aminosalicylic acid, it may be relevant that sulfasalazine and 5-aminosalicylic acid are inhibitors of prostaglandin synthesis (10–13) and that patients with active ulcerative colitis have increased fecal levels and colonic venous blood levels of prostaglandins (13). Prostaglandins are known to be involved in inflammation

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