[CONTRIBUTION FROM THE RHEUMATIC FEVER RESEARCH INSTITUTE, NORTHWESTERN UNIVERSITY MEDICAL SCHOOL]

The Characterization of Two Methylimidazoleacetic Acids as Radioactive Histamine Metabolites

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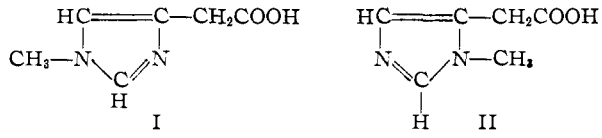
The injection of large amounts of low isotopic histamine has led to the isolation of three metabolites from the urine of rats and mice. Two of these, imidazoleacetic acid and ribosylimidazoleacetic acid, have been characterized. In this paper the third metabolite is shown to be a mixture of 1-methylimidazole-4-acetic acid and 1-methylimidazole-5-acetic acid in a ratio of approximately 75–25%.

The urine of rats and mice injected with large amounts of low isotopic histamine has yielded numerous radioactive metabolites. The first to be isolated was imidazoleacetic acid.[2] The second to be characterized was ribosylimidazoleacetic acid.[3]

A third radioactive metabolite was isolated in very small amount from the urine of mice injected with large amounts of low isotopic histamine. It was isolated as the hydrochloride by column chromatography procedures on silica gel, powdered cellulose and Dowex-50 columns. The elementary composition agreed closely with that of an N-methylimidazoleacetic acid, of which two isomers, 1-methylimidazole-4-acetic acid (I) and 1-methylimidazole-5-acetic acid (II), are possible.



I II

The two acids, both of which have previously been reported as the picrates,[4] were synthesized, and converted to the hydrochlorides, melting at 204–206 and 207–208°, respectively.

An attempt to characterize the metabolite by mixed melting point studies gave ambiguous results, so the metabolite was compared with the known acids by paper chromatography.

Developed papers sprayed with Pauly reagent[5] gave no colored spots, but under certain conditions, dilute solutions of brom cresol green gave well-defined blue spots; the R_f values found in *n*-butanol(2)–pyridine(2)–water(1), in isopropyl alcohol(2)–pyridine(2)–water(1), and in isopropyl alcohol(3)–pyridine(2)–water(1) were 0.23, 0.39 and 0.27 for the 1–4 acid I, and 0.19, 0.30 and 0.18 for the 1–5 acid II, respectively. No spots were found when amounts of acid less than 20 γ were run.

The unknown metabolite, when run in the three solvent systems against the acids I and II in 20 to 50 γ amounts showed only one spot with an R_f identical in each system with the 1–4 acid. An increase in the amount run to 100–200 γ, however, brought out a second, weaker spot identical in all systems with the 1–5 acid. A comparative run of 200 γ of the metabolite with a mixture of 150 γ of the 1–4 acid and 50 γ of the 1–5 acid gave pairs of spots which were indistinguishable by visual comparison. In a similar run against 180 γ of I with 20 γ of II, no spot was found for II after developing, indicating that the metabolite mixture contains more than 10% of II. Most satisfactory agreement of the chromatograms was found with a mixture of 75% of I and 25% of II.

These metabolites were isolated from the urine of animals given histamine in doses considerably greater than physiological, but the results lend support to the finding[6] that methylation is an important step in the metabolism of minute amounts of histamine. In studies on the injection of physiological amounts of high isotopic histamine, Schayer[7] found three peaks of radioactivity on paper chromatograms. The peak of highest R_f was found to be due to unchanged histamine and acetylhistamine. The peak of lowest R_f, peak 1, has been shown[8] to be due to the ribosylimidazoleacetic acid. No free imidazoleacetic acid can be detected under these conditions, as contrasted with the large amounts formed when large doses of histamine are used.

Studies are now being made to correlate Schayer's peak 2 metabolite with I and II.

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Experimental

Isolation of the Metabolite Mixture.—A 1.5-l. batch of the urine of mice which had been injected subcutaneously with 5 mg. per day of C^{14} histamine (1000 c.p.m.) labeled in position 2 of the imidazole ring, was made ammoniacal, filtered and extracted repeatedly with *n*-butanol to remove the major portion of unchanged histamine. The residual solution was evaporated to dryness *in vacuo* and the residue extracted with boiling methanol. Evaporation of the methanol gave 28 g. of material with a total of 250,000 c.p.m. This was dissolved in a minimum amount of water and passed through a silica gel column with ethanol, 1:1 ethanol–methanol, and methanol. The major portion of the free imidazoleacetic acid is eluted first as a broad weak band of radioactivity, followed by another broad band containing most of the other metabolites. The second band was isolated and placed on a cellulose powder column and eluted with 95% ethanol–5% concd. NH_4OH, 135 fractions of 10

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ml. each being collected. Four peaks of activity were found. The second peak, fractions 35–40, 16,000 c.p.m., was fractionated on a Dowex-50 column by the procedure of Hirs, Moore and Stein.[9] Ammonium formate buffers at *p*H 2.21, 2.80 and 3.92 failed to elute radioactive material, but activity was eluted at *p*H 5.75 using ammonium acetate buffer. The buffer salts were removed, and the metabolite recrystallized from alcohol–ether as the hydrochloride. A total of 16 mg. was obtained, melting at 196–198°, and having approximately 300 c.p.m. per mg.

Anal. Calcd. for $C_6H_9N_2O_2Cl$: C, 40.81; H, 5.14; N, 15.86; Cl, 20.08. Found: C, 40.98; H, 5.31; N, 15.59; Cl, 20.19.

Preparation of N-Methylimidazoleacetic Acid Hydrochlorides (I and II).—1-Methyl-5-carbomethoxyimidazole[10] was converted to 1-methyl-5-cyanomethylimidazole picrate by the method of Jones and McLaughlin.[11] The picrate was converted to the free base, and hydrolyzed by the method of Pyman,[4] the 1-methylimidazole-5-acetic acid being isolated as the picrate, m.p. 180–181°. The same picrate, together with the picrate of 1-methylimidazole-4-acetic acid, m.p. 189–190°, was also prepared by the methylation of cyanomethylimidazole by Pyman's procedure,[4] followed by fractional crystallization of the methylcyanomethylimidazole picrates.

The picrates were converted to the hydrochlorides by passage of their aqueous solutions through a column of Dowex-1 in the chloride form, evaporation of the eluate to dryness, and recrystallization from ethanol–ether.

1-Methylimidazole-5-acetic acid hydrochloride melted at 204–206°.

Anal. Calcd. for $C_6H_9N_2O_2Cl$: Cl, 20.08. Found: Cl, 20.18.

1-Methylimidazole-4-acetic acid hydrochloride melted at 207–208°.

Anal. Calcd. for $C_6H_9N_2O_2Cl$: Cl, 20.08. Found: Cl, 20.04.

Paper Chromatography of the Metabolite.—All the paper chromatograms of the metabolite were run at room temperature by the ascending procedure on Schleicher & Schüll No. 604 paper. Spots of I and II, or mixtures of the two, as controls, were placed on the paper as 1% solutions. The paper was run overnight in the desired solvent, dried at room temperature in the hood for 0.5 hour, sprayed with a 0.05% solution of brom cresol green in alcohol, and redried at room temperature for 0.5 hour.

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10-Hydroxymorphine[1]

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Cold chromic acid oxidation has been applied to several additional alkaloids of the morphine group. Δ^7-Desoxycodeine and neopine were converted to the corresponding 10-hydroxy compounds, while thebaine gave 14-hydroxycodeinone. Heroin under these conditions was uneffected, but O^3-allylmorphine was successfully oxidized, and after ether cleavage with sodium and liquid ammonia gave 10-hydroxymorphine. The *pK*'s and partition coefficients of morphine and 10-hydroxymorphine are compared.

The oxidation of codeine and a number of its derivatives[2,3] by cold chromic acid led to a series of compounds all of which were hydroxylated at the 10-position. Interest in these 10-hydroxy compounds stems from their possible occurrence as metabolic products of the morphine alkaloids and also from their possible biological activity, since introduction of the 10-hydroxyl group causes marked changes in the *pK* and solubility of these compounds. For these reasons, we have been interested in applying this hydroxylation reaction to further compounds in the morphine series, in particular to morphine.

The present report is concerned with the application of this oxidation to morphine, thebaine, neopine and Δ^7-desoxycodeine. The procedure was the same as that used previously and appears to be quite general. It consists in slowly adding a chromic acid–sulfuric acid solution to a cold solution of the alkaloid in dilute sulfuric acid. Quantities are adjusted so that there is very little further oxidation to the ketone, and the resulting mixtures of starting material and 10-hydroxy compound usually are separated through the increased water solubility of the latter.

Before this oxidation procedure could be applied to morphine, it was necessary to find a suitable protecting group for the phenolic hydroxyl in order to prevent oxidation of the aromatic ring. In addition to being stable to acid and oxidation, the conditions of the reaction, such a group subsequently must be removable under mild conditions since the hydroxylated molecule would now contain a secondary benzilic alcohol, a secondary allylic alcohol and an alicyclic double bond.

Cleavage of the 3-methyl ether with pyridine hydrochloride has been shown[4] to proceed in reasonable yield without affecting the allylic alcohol. To test whether the benzylic hydroxyl would survive this treatment, 10-hydroxy-Δ^7-desoxycodeine was heated with pyridine hydrochloride. However, in this case the conditions were apparently too drastic and led to a chlorine-containing, resinous product.

Protection was then sought through use of diacetylmorphine (heroin). Although heroin was stable to the conditions of acidity used in the oxidation, it was also stable to oxidation, and the only product isolated was heroin in 85% recovery. This indicated that esterification of the phenolic hydroxyl had deactivated the benzylic position and that an ether-type protecting group was required. Formation of benzyl ethers has frequently been used for this purpose since the benzyl group can be removed

(1) Supported by a grant from the National Institutes of Health, Bethesda, Maryland.

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