Synthesis of Compounds Related to Metabolites of Trimetazidine in Rabbits

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Abstract □ Synthetic methods of trimetazidine metabolites in rabbits are described. One of the metabolites, 3-hydroxy-2,4-dimethoxybenzylpiperazine, is a new compound and its chemical structure is established.

Keyphrases □ Trimetazidine metabolites, in rabbits—synthesis of related compounds □ 3-Hydroxy-2,4-dimethoxybenzylpiperazine—synthesis, structure determination □ NMR spectroscopy—structure determination □ IR spectrophotometry—structure determination

In a previous paper (1), absorption, excretion, and distribution of trimetazidine [1-(2,3,4-trimethoxybenzyl) piperazine dihydrochloride] in animals were investigated. The present work was performed to synthesize several compounds related to trimetazidine metabolites in rabbits. The compounds synthesized are shown here.

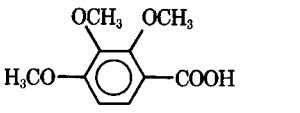
3-Hydroxy-2,4-dimethoxybenzylpiperazine is a new compound, and the chemical structure was established in the present work.

EXPERIMENTAL

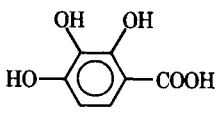
2,3,4-Trimethoxybenzoic acid, 2,3,4-trihydroxybenzoic acid, 2,3,4-trimethoxytoluene, 2,3,4-trihydroxytoluene, 1,3-dimethylpyrogallol, 2-ethyl-1,3-dimethylpyrogallol, 3-ethoxy-2,4-dimethoxy benzaldehyde, and 3-ethoxy-2,4-dimethoxybenzoic acid were synthesized by methods described in the literature (cited in Table I).

1-(2,3,4-Trimethoxybenzyl)-N^4-acetylpiperazine—To 10 g. of trimetazidine, a mixture of 20 ml. of acetic anhydride and 50 ml. of acetic acid was added and the reaction mixture was refluxed on a steam bath for 15 min. After cooling, 10% sodium carbonate solution was added to make the reaction mixture alkaline. The alkaline solution was extracted with ether, and the ethereal solution was evaporated on a steam bath. The residue was distilled *in vacuo*. Three grams of a colorless oily fraction, boiling at 175–185°/5 mm. Hg, was obtained.

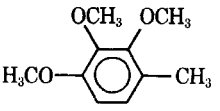
Anal.—Calc. for $C_{16}H_{24}N_2O_4$: C, 62.34; H, 7.79; N, 9.09. Found: C, 62.40; H, 7.81; N, 8.87.



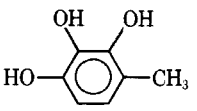
2,3,4-trimethoxybenzoic acid



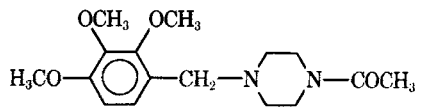
2,3,4-trihydroxybenzoic acid



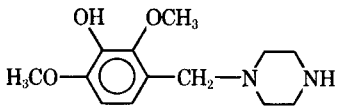
2,3,4-trimethoxytoluene



2,3,4-trihydroxytoluene



1-(2,3,4-trimethoxybenzyl)-N^4-acetylpiperazine



3-hydroxy-2,4-dimethoxybenzylpiperazine

1-(Monohydroxydimethoxybenzyl)piperazine Dihydrochloride—A mixture of 10 g. of trimetazidine dihydrochloride and 24 g. of anhydrous aluminum chloride in 250 ml. of dried nitrobenzene was stirred at 50–55° for 7 hr. After cooling, the reaction mixture was poured into ice water and the nitrobenzene layer was removed. The water layer was filtered, and the filtrate was treated with 96 g. of sodium bicarbonate and 52 g. of ethylenediaminetetraacetic acid to make the medium alkaline. This mixture was extracted with 2 l. of chloroform, and the chloroform was washed with water and dried with anhydrous sodium sulfate. After the solvent was removed from the chloroform layer, the residue was dissolved in carbon tetrachloride. Hydrochloric acid gas was conducted into the carbon tetrachloride solution under cooling, and the precipitate that separated out was recrystallized from a mixed solvent of methanol and ether and then methanol. Colorless crystals (1.4 g.,

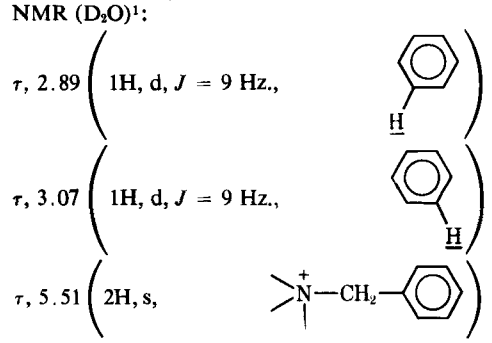
Table I—Syntheses of Several Compounds

Compound	Yield, %	Melting Point	Formula	Anal., % Calc.	Anal., % Found	References
2,3,4-Trimethoxybenzoic acid		98–100°				(2–6)
2,3,4-Trihydroxybenzoic acid	22	198–201°	$C_7H_6O_5$	C, 49.41 H, 3.53	C, 49.60 H, 3.44	(7, 8)
2,3,4-Trimethoxytoluene	52	B.p. 97–98° /1 mm. Hg	$C_{10}H_{14}O_3$	C, 65.93 H, 7.69	C, 65.82 H, 7.91	(9)
2,3,4-Trihydroxytoluene	14	94–97°	$C_7H_8O_3$	C, 60.00 H, 5.71	C, 60.18 H, 5.85	(7, 8)
1,3-Dimethylpyrogallol	23	B.p. 133–134°/17 mm. Hg				(10)
2-Ethyl-1,3-dimethylpyrogallol	88	B.p. 98–99° /16 mm. Hg				(8)
3-Ethoxy-2,4-dimethoxybenzaldehyde	61	Not purified				(8)
2,4-Dinitrophenylhydrazone of 3-ethoxy-2,4-dimethoxybenzaldehyde		170°	$C_{17}H_{18}N_4O_7$	C, 52.30 H, 4.65 N, 14.36	C, 52.36 H, 4.60 N, 14.46	(8)
3-Ethoxy-2,4-dimethoxybenzoic acid	14	91–92°	$C_{11}H_{14}O_5$	C, 58.39 H, 6.24	C, 58.55 H, 6.34	(8)

14.6% in theoretical amount), melting at 240–245° dec., were obtained.

Anal.—Calc. for $C_{13}H_{22}Cl_2N_2O_3$: C, 48.01; H, 6.82; Cl, 21.80; N, 8.61. Found: C, 48.13; H, 7.00; Cl, 21.94; N, 8.56.

NMR (D_2O)[1]:



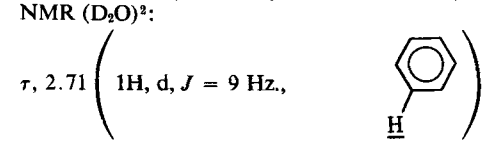
τ, 6.05 (3H, s, —OCH_3)
τ, 6.08 (3H, s, —OCH_3)
τ, 6.32 (8H, s, 4z —CH_2—)

NMR(dimethyl sulfoxide)[1]:, τ, −0.04 (1H, broad s, —OH). IR cm.$^{-1}$: δ_{C-H} 820 (KBr); $\nu_{C=C}$ 1620 (KBr); ν_{O-H} 3400 (KBr).

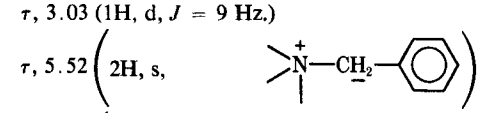
Ethylated Compound of 1-(Monohydroxydimethoxybenzyl)piperazine Dihydrochloride—Ethylsulfate (3.9 ml.) and then 12.7 ml. of 10% sodium hydroxide solution were added dropwise successively to a mixture of 0.7 g. of sodium hydroxide and 2.85 g. of the compound, 1-(monohydroxydimethoxybenzyl)piperazine, in 7.7 ml. of 10% sodium hydroxide solution, under refluxing and stirring. The reaction was continued for an additional hour under refluxing and stirring. After 2.5 ml. of diethylsulfate and then 3.9 ml. of 20% sodium hydroxide solution were added dropwise to the reaction mixture, stirring and refluxing were still continued for 20 min. After cooling, the reaction mixture was extracted with chloroform, and the chloroform layer was washed with water and dried with anhydrous sodium sulfate. The residual oily substance, after evaporation of chloroform, was dissolved in methanol; hydrochloric acid gas was conducted into this solution. The crystalline substance that separated out after the addition of a small amount of ether to the methanol solution was recrystallized from a mixed solvent of methanol and ether. A colorless crystal (0.6 g., 18.0% in theoretical amount), melting at 211–213° dec., was obtained.

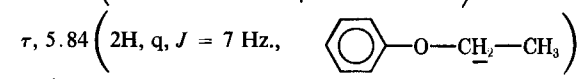
Anal.—Calc. for $C_{17}H_{30}Cl_2N_2O_3$: C, 53.54; H, 7.93; Cl, 18.59; N, 7.35. Found: C, 53.45; H, 8.02; Cl, 18.79; N, 7.22.

NMR (D_2O)[2]:



τ, 3.03 (1H, d, J = 9 Hz.)





τ, 6.01 (3H, s, —OCH_3)
τ, 6.07 (3H, s, —OCH_3)
τ, 6.32 (8H, s, 4x —CH_2—)
τ, 6.62 (2H, q, J = 7 Hz., >N^+—CH_2—CH_3)
τ, 8.61 (6H, t, J = 7 Hz., >N^+—CH_2—CH_3 and —O—CH_2—CH_3)

IR cm.$^{-1}$: δ_{C-H} 830 (KBr); $\nu_{C=C}$ 1615 (KBr).

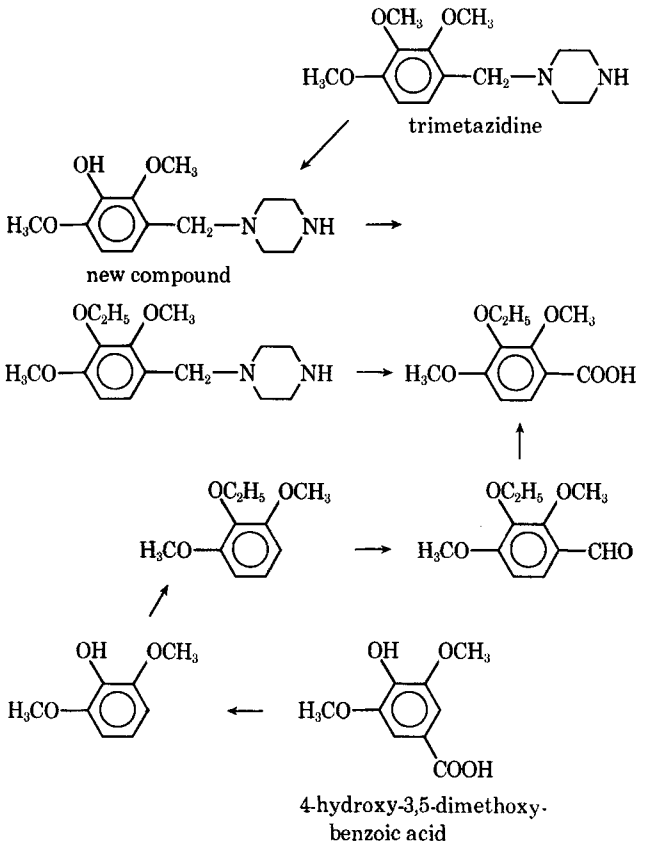
Potassium Permanganate Oxidation Product of Ethylated 1-(Monohydroxydimethoxybenzyl)piperazine Dihydrochloride—A mixture of 1.2 g. of potassium permanganate and 0.2 g. of the compound, ethylated compound of 1-(monohydroxydimethoxybenzyl)-piperazine, in 20 ml. of water was stirred at 85–90° until the color of potassium permanganate solution disappeared. Manganese dioxide that precipitated was dissolved into the reaction mixture by the introduction of sulfur dioxide gas; this reaction mixture was extracted with ether. An acidic component was extracted with saturated sodium bicarbonate solution from the ethereal solution after washing with water. The water layer was extracted with ether after acidification with hydrochloric acid, and this ethereal solution was washed with water and dried with anhydrous sodium sulfate. Crystals, after evaporation of the ether, were recrystallized from petroleum ether (b.p. 60–70°). A colorless crystal (16 mg., 13.5% in theoretical amount), melting at 89°, was obtained and coincided with the compound 3-ethoxy-2,4-dimethoxybenzoic acid in its melting point and IR spectrum.

[1] Internal standard: tetramethylsilane.
[2] Internal standard: dioctyl sodium sulfosuccinate.

RESULTS AND DISCUSSION

The compounds, 2,3,4-trimethoxybenzoic acid, 2,3,4-trihydroxybenzoic acid, 2,3,4-trimethoxytoluene, 2,3,4-trihydroxytoluene, 1-(2,3,4-trimethoxybenzyl)-N^4-acetylpiperazine, and 3-hydroxy-2,4-dimethoxybenzylpiperazine, were used as the authentic samples for identification of trimetazidine metabolites in rabbit urine.

One of the trimetazidine monohydroxydimethoxybenzyl piperazine metabolites (3-hydroxy-2,4-dimethoxybenzylpiperazine) is a new compound and was obtained from demethylation of trimetazidine with anhydrous aluminum chloride. The chemical structure of this new compound was established as shown in Scheme I.



Scheme I

The results of instrumental analysis of the new compound reveal the following facts:

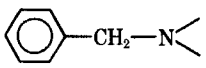
1. *AB*-type couplings at τ 2.89 and 3.07 were observed in the NMR spectrum of the new compound. J_{AB} = 9 Hz. in the NMR (D_2O) existed in the range of 5–10 Hz. in *J*-value, corresponding to 2H adjoined with each other. The signals of τ 6.05 and 6.08 came from 3H and showed the existence of two methoxy groups on a benzene skeleton

2. Absorptions corresponding to $\nu_{C=C}$ at 1620 cm.$^{-1}$ and δ_{C-H} at 820 cm.$^{-1}$ were recognized in the IR spectrum of the new compound.

3. In the NMR (dimethyl sulfoxide) spectrum of the new compound, the signal of τ −0.04, which disappeared in the NMR (D_2O), was detected.

4. The IR absorption of ν_{O-H} at 3400 cm.$^{-1}$, which disappeared when the compound was ethylated, was detected.

These facts support an existence of a phenol group in the new compound. It is reasonable to consider that the signal of τ 5.51 comes from 2H, is not coupling, and is shifted differing from that of τ at about 6.7 in usual absorption due to:



Also, it can be interpreted that a benzyl skeleton having two methoxys, one phenolic hydroxy, and two hydrogens adjoined with each other is bound with piperazine at the position of one nitrogen atom, N^+, in the piperazine skeleton. The signal of τ 6.32 due to 8H was observed as a sharp singlet in the NMR spectrum of the new compound.

The chemical structure of the new compound was established also from the IR spectrum of the compound; that is, the potassium permanganate oxidation product of ethylated 1-(monohydroxydimethoxybenzyl)piperazine coincided with that of the compound 3-ethoxy-2,4-dimethoxybenzoic acid synthesized separately, and no depression in a mixed melting-point determination was observed.

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Color Reaction of Thyroxine and Its Derivatives with Nitrous Acid

S. E. SAHEB and M. A. SANASSIAN

Abstract □ The 3′,5′-diiodo-4′-hydroxydiphenylether system in thyroxine was shown to be indispensable for the success of the color reaction with nitrous acid. This reaction was also successful with sodium hypochlorite and chlorine gas. The products were separated, and evidence is presented to show that this reaction involves an oxidation of the 3′- and/or 5′-position to iodoso or iodoxy derivatives while the amino acid side chain remains intact.

Keyphrases □ Thyroxine, derivatives—chemical nature of color reaction with nitrous acid □ 3′,5′-Diiodo-4′-hydroxydiphenylether system—role in color formation of thyroxine and nitrous acid

Thyroxine (I), when treated with nitrous acid, is reported to give a characteristic red color, even in a concentration of 1 in 40,000 (1). This standard procedure is described in various pharmacopeias (2) for the identification of thyroxine. Roche and Michel (3) reported on the analytical aspect of this reaction and suggested its use as a quantitative method for the determination of thyroxine. The absorption of the color formed obeyed Beer–Lambert's law within a certain concentration range, 100–300 mcg. This paper reports on the chemical nature of this reaction, which has not been investigated previously.

EXPERIMENTAL

Thyroxine (I), tetraiodothyropropionic acid (II), 3,5,3′-triiodo- and 3,5-diiodothyronine, 3,5-di- and 3-iodotyrosine, and tyrosine were used[1]. The purity of the commercially available thyroxine was checked by UV, TLC, and IR and was found acceptable. However, C, H analyses purveyed by any manufacturer were beyond the accepted limits. It was used as such without further purification. Both the D- and L-forms were used whenever available without any difference in results. Spectrophotometric readings were taken on Perkin-Elmer 202 (UV, visible) and 237 (grating IR) instruments with KBr pellets. Silica gel G was used[2].

Color Formation (1, 3)—To a solution of 5 mg. of thyroxine or its derivatives in 5 ml. of 95% acidic ethanol (enough hydrochloric acid to bring the solid in solution), a freshly prepared $NaNO_2$ (1%) solution was added dropwise until the color of the starch iodide paper became blue. The yellow color produced was intensified on boiling. When the cooled solution was made alkaline (pH 9) with ammonia, an intense red color developed, which became yellow on acidification (pH 1.5). Similarly, when the acidic solution of thyroxine was treated with NaOCl, it turned green, then yellow upon heating, and red with dilute alkali. With Cl_2 gas, the solution remained colorless until made alkaline; then it became red, and it turned yellow with acids. When the procedure was applied to Compound VI, molecular iodine and *p*-methoxyphenol were obtained (*cf.*, 4). With 3,5,3′-triiodo- and 3,5-diiodothyronine, 3,5-di- and 3-iodotyrosine, and tyrosine, the reaction mixture acquired a yellow color but did not turn red with base (*cf.*, 3).

Preparation of *N,O*-Diacetylthyroxine[3] (IV)—One gram of the sodium salt of thyroxine or its analogs was dissolved in 150 ml. of freshly distilled, cold acetic anhydride. The solution was left to stand overnight at room temperature. The solvent was evaporated to dryness under vacuum and the white amorphous residue, A, was

[1] Fluka-Buchs, Switzerland.

[2] E. Merck.

[3] This method was communicated by G. Hagen of the Veteran's Administration Hospital, St. Louis, Mo.