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3-Hydroxymethyl-*s*-triazolo[3,4-*a*]phthalazine, a Novel Urinary Hydralazine Metabolite in Man

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The elucidation of the structure of a new major metabolic product of hydralazine, 3-hydroxymethyl-*s*-triazolo[3,4-*a*]-phthalazine, is described. The structures of several other previously described metabolites of the drug, phthalazone, *s*-triazolo[3,4-*a*]phthalazine, and 3-methyl-*s*-triazolo[3,4-*a*]phthalazine, are confirmed. A metabolic pathway of hydralazine is also proposed.

The formation of antibodies to hydralazine (1) and/or its metabolites may play an important role in the pathogenesis of drug-induced systemic lupus erythematosus. Furthermore, the occurrence of this syndrome in patients on hydralazine therapy has been correlated with its rate of hepatic acetylation.[1,2] However, the metabolic fate of hydralazine has not been completely described in man or even laboratory animals. Our laboratories[3–5] and others[6] have shown that certain hydralazine metabolites are derived of the *s*-triazolo[3,4-*a*]phthalazine system. While these are unusual metabolites, their chemical structure is not conducive to the ready formation of covalently bound hapten–protein complexes capable of inducing production of antibodies and eventually the lupus syndrome. We wish here to report the discovery and structural elucidation of 3-hydroxymethyl-*s*-triazolo[3,4-*a*]phthalazine (**3**) as a human urinary metabolite of hydralazine. Among the known hydralazine metabolites **3** is the first one which possesses a functional group; the hydroxyl group may provide a handle by which a covalent bond to a protein could be formed. In addition, we confirmed the presence of *s*-triazolo[3,4-*a*]phthalazine (**5**) and phthalazone (**6**) as urinary metabolites of 1.[6]

Possible metabolic pathways giving rise to the production of **3** are shown in Scheme I. It arises very probably from metabolite **2** by enzymatic hydroxylation. An alternate pathway for the formation of **3** may possibly be conjugation of 1 with glycolic acid followed by ring closure. Attempts were made to prepare *s*-triazolo[3,4-*a*]phthalazine-3-carboxylic acid (**4**) by oxidation of **3**. The only product isolated was **5**. This finding suggests that **5** arises from **3** by further oxidation and subsequent decarboxylation. In a separate reaction, phthalazone was obtained by subjecting 1 to air in a slightly alkaline medium. This finding suggests that the appearance of **6** as a urinary metabolite of 1 can be due to its enzymatic and/or chemical oxidation.

The novel metabolite **3** is excreted only as a glucuronic acid conjugate. According to our estimates **3** seems to be a major urinary metabolite of 1 in man.

For this investigation we had 14 hypertensive patients available who were on hydralazine therapy ranging from 100 to 400 mg daily. Their 24-hr urine was collected and routinely checked by paper chromatography[3] for the presence of **2**. Every patient produced **2** which gave rise to a strong spot and could be detected without difficulties.[7]

After incubating the urine of these patients with β-glucuronidase, the combined chloroform extracts of the urine samples were subjected to silica gel preparative layer chromatography resulting in a number of bands. Inspection of the uv spectra of the constituents of these bands revealed the presence of compounds with the triazolo[3,4-*a*]phthalazine system in five of them.

An additional band contained **6**, a previously reported metabolite of 1.[6] A strong band with an R_f value of 0.35–0.40 in chloroform–methanol (15:1) and a blue fluorescence was identified as **3**, as follows. First, its uv spectrum was very similar[3] to that of **2**; in addition the spectrum did not show a red shift upon addition of base. This excludes presence of nuclear hydroxy groups. Second, its mass spectrum showed a molecular ion of 200 and its fragmentation[8] is very similar to that of **2**.[4] Third, its spectroscopic properties are identical with those of an authentic sample synthesized by us via a dehydrative cyclization reaction between 1 and glycolic acid (Figure 1).

A strong band with an intense blue-purple fluorescence and an R_f value of 0.5–0.6 was rechromatographed on silica gel in acetone–cyclohexane (1:1). It separated into three new bands which were identified as **2**, **5**, and **6** in agreement with previous investigations.[6] Several of the other bands according to their uv spectra revealed the presence of additional compounds with the *s*-triazolo[3,4-*a*]phthalazine system. However, they are as yet unidentified because of their occurrence in very small amounts. (In order to attempt to identify them arrangements to get a larger group

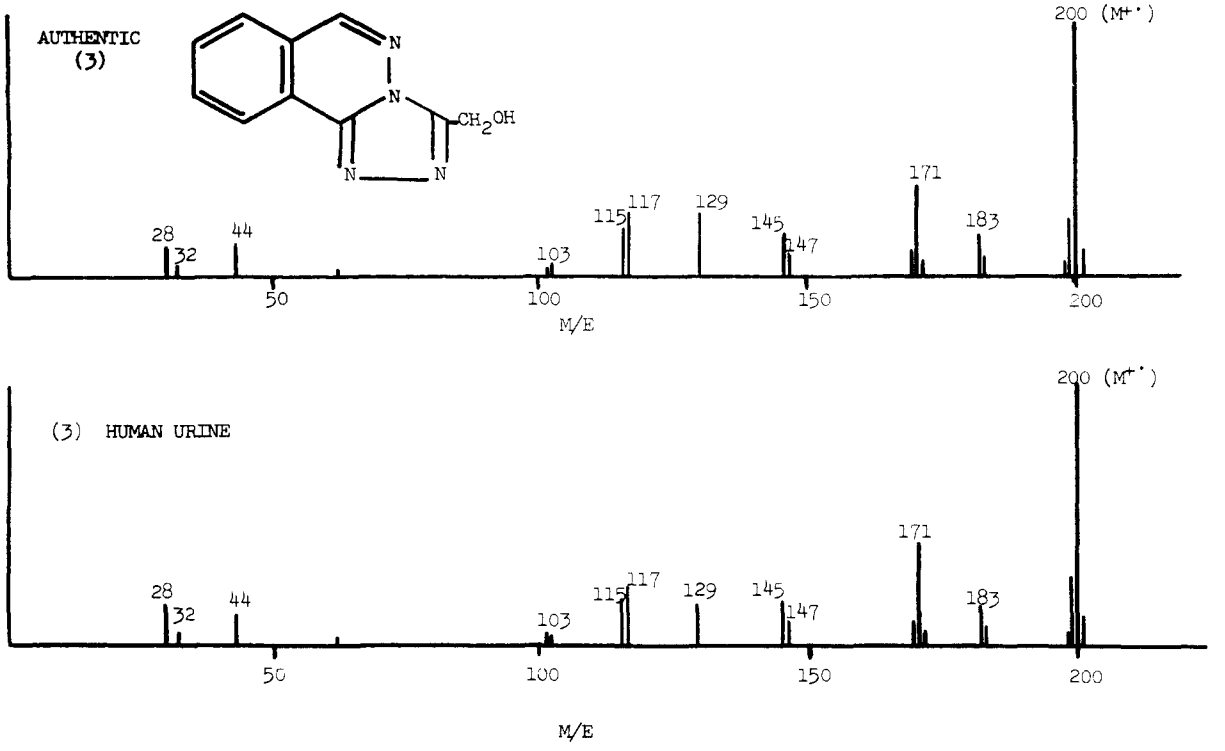


Figure 1.

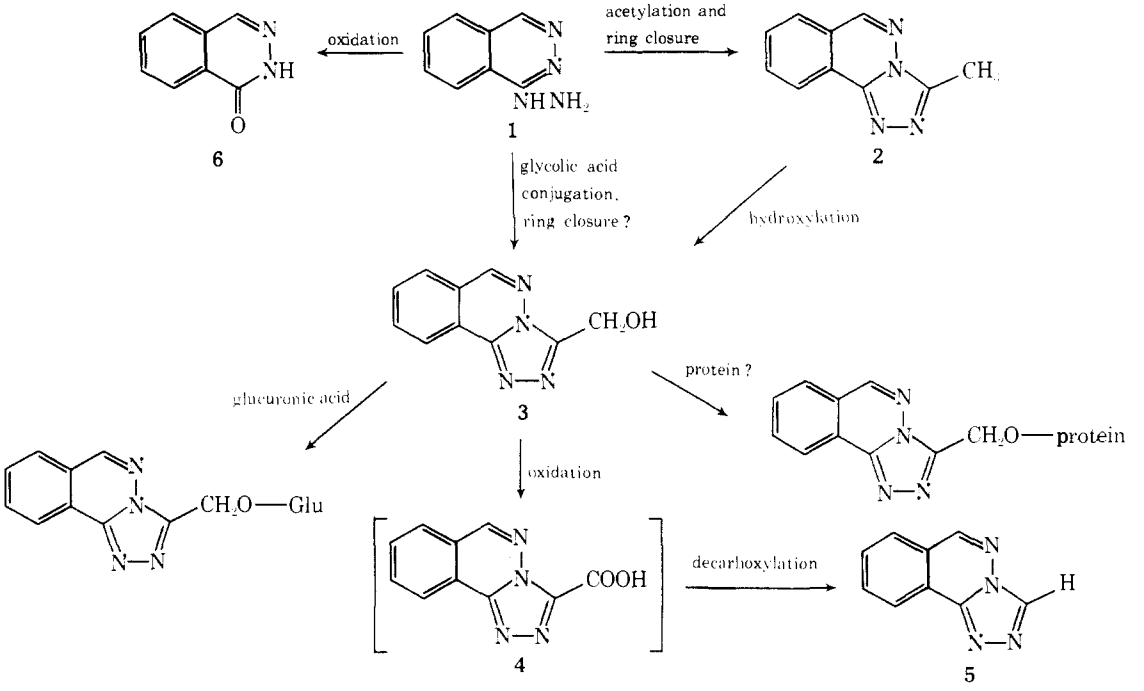
of hypertensive patients on hydralazine therapy have been made.)

Experimental Section

Ultraviolet spectra were recorded with a Pye-Unicam (Series SP-8000) ultraviolet spectrometer. Infrared spectra were taken with a Perkin-Elmer IR-337 infrared spectrometer. A Varian T-60 NMR spectrometer was used to record nuclear magnetic resonance spectra. Mass spectra were obtained with an LKB-9000 gas chromatograph–mass spectrometer. The columns used were either 1% Dexsil or SE-30. Finally, all preparative layer chromatograms were made from silica gel (PF-254 and 366). Paper chromatograms were run with Whatman No. 1 paper.

(A) Identification of Metabolites. The 24-hr urine specimens were obtained from a total of 14 hypertensive patients; each sample was separated into two parts. One portion was adjusted to pH 5.0 and continuously extracted with chloroform for 48 hr. The other portion was also adjusted to pH 5.0, buffered with acetate, and incubated with β-glucuronidase at 37° for 24 hr, followed by continuous extraction with chloroform for 48 hr. The two extracts from each patient were subjected to paper chromatography (propanol–ammonia, 2:1). The ultraviolet spectrum of the material eluted from the chromatograms of the enzymatically treated extracts revealed the presence of a metabolite with a typical triazolo[3,4-*a*]phthalazine chromophore that was not found in the untreated extracts. Since this material was present in the urinary extracts of all the patients, the extracts from the enzyme-treated urine were

Scheme I. Metabolic Pathway of Hydralazine



combined and streaked on silica gel (PF 254 and 366 plates). The new metabolite, a combination band, containing three additional metabolites, as well as other material resulted. The band with R_f of ~0.4 was scraped and eluted with chloroform–methanol (1:1). The extract was evaporated to dryness. The mass spectrum of this residue showed peaks at *m/e* 200 (M^+), 183, 171, 147, 145, 129, 117, and 115. This spectrum was identical in every respect with that of authentic 3-hydroxymethyl-*s*-triazolo[3,4-*a*]phthalazine.

The combination band with R_f value 0.5 was also scraped; it was eluted with acetone. The acetone extract was concentrated and streaked on another silica gel plate and chromatographed with acetone–cyclohexane (1:1).[6] These bands appeared at R_f values 0.7, 0.8, and 0.9. These bands were scraped off individually and eluted with acetone. Evaporation of the acetone extracts provided the metabolites **2**, **5**, and **6** as confirmed by their mass spectra as follows: metabolite **2** (*m/e*) 183 (M^+), 169, 145, 117, 115; metabolite **5** (*m/e*) 170 (M^+), 129, 117, 115; and metabolite **6** (*m/e*) 146 (M^+), 115, and 90.

(B) **Chemical Studies.** (1) **Synthesis of 3.** Hydralazine, 4.0 g (0.022 mol), in 15 ml of 70% glycolic acid was refluxed for 24 hr. The cooled solution was washed with 250 ml of chloroform into a separatory funnel and the excess acid neutralized with saturated sodium bicarbonate solution. The liquids were separated from a solid by filtration and gave 2.4 g of product. The chloroform and water were separated, the chloroform was dried (K_2CO_3) and stripped off, and an additional 1.85 g of product precipitated upon addition of 50 ml of toluene: total yield 4.25 g (94%). Recrystallization from toluene gave mp 208–209°: ir spectrum (KBr) 3200 (OH), 2925, 2850 (CH), 1620, 1520, 1460 cm^{-1} (Ar); NMR spectrum (Me_2SO-d_6) singlet δ 9.0 (1 H), multiplet 8.7–7.7 (4 H), singlet 5.0 (2 H); uv spectrum (MeOH) sh 235, 240, 247, 264, 273 nm. Anal. ($C_{10}H_8N_4O$) C, H, N.

(2) **Oxidation of 3-Hydroxymethyl-*s*-triazolo[3,4-*a*]phthalazine.** The oxidation was attempted by three different methods in order to obtain *s*-triazolo[3,4-*a*]phthalazine-3-carboxylic acid.

(a) Permanganate. To 100 ml of distilled water in a 250-ml round-bottom flask was added 1 g (0.005 mol) of **3**. The pH of the solution was adjusted to 10.0 with Na_2CO_3 and a solution containing 0.80 g of $KMnO_4$ in 10 ml of distilled water was added. A brown precipitate of MnO_2 appeared immediately. The mixture was permitted to stir at room temperature for 2 hr. Excess permanganate was destroyed by addition of a small quantity of 2-propanol. The MnO_2 precipitate was filtered off.

The pH of the clear filtrate was adjusted to 3.0 with 10% sulfuric acid; no precipitate formed. The pH was readjusted to 10.0 and continuously extracted with chloroform, followed by evaporation to dryness. The solid residue was recrystallized from ethanol. The spectral properties indicate that this compound was *s*-triazolo[3,4-*a*]phthalazine: yield 0.5 g (59%); mass spectrum 170, 129, 117, and 115; NMR ($CDCl_3$) multiplet δ 7.80–8.51 (4 H), singlet 8.69 (1 H), singlet 8.82 (1 H); ir (KBr) 3090 (CH, Ar), 3040 (CH, Ar), 3000 (CH, aliphatic), 2950 (CH, aliphatic), 2900, 2220 cm^{-1}; uv spectrum (MeOH) [λ_{max} (log ϵ)] sh 232 (3.40), 237 (3.80), 243 (3.3), sh 262 (1.06), sh 272 (1.00), sh 282 nm (0.83).

(b) Molecular Oxygen. The alcohol (1 g) and 0.4 g of Pt black were suspended in 500 ml of distilled water in a 1000-ml round-bottom flask. The pH was adjusted to 6.7 with a phosphate buffer.[9] The mixture was warmed on a water bath and stirred vigorously. Oxygen gas was bubbled through two interconnected gas dispersion tubes into the reaction mixture for 6 hr. Only starting material was recovered.

(c) Dichromate. In a round-bottom flask, equipped with a mechanical stirrer, dropping funnel, and reflux condenser, was placed 1.0 g (0.005 mol) of the title compound. Potassium dichromate, 0.5 g (0.0017 mol) in 50 ml of water, was added. The flask was heated on a water bath while a mixture of 0.25 g of H_2SO_4 in 10 ml of water was added dropwise for a period of 1 hr. When complete, the reaction mixture was cooled and nearly neutralized with 50% NaOH and then completely neutralized with saturated sodium carbonate. The chromium hydroxide precipitate was filtered off and the clear filtrate was acidified with 10% H_2SO_4. No precipitate formed and the solution was readjusted to a pH of about 10 and extracted with chloroform. The spectral properties of the product obtained indicate it to be identical with **5** (vide supra).

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Preparation and Analgesic Activity of 3,6-Diacetylnormorphine and 6-Acetylnormorphine

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3,6-Diacetylnormorphine (norheroin) and 6-acetylnormorphine have been prepared in excellent yield through the 3,*N*-bis(*tert*-butoxycarbonyl) derivative of normorphine via acetylation and selective removal of protecting groups. This general procedure would be applicable to the preparation of various 3,6-diesters or 6-monoesters of normorphine. The analgesic potency of norheroin was found to be the same as that of 6-acetylnormorphine, about 0.05 that of heroin. The onset, peak, and duration of action of these compounds were nearly identical and comparable with morphine.

Only a few secondary amines show good in vivo analgesic potency. One of these, nordesomorphine, has an ED_{50} of 2.21[1] (compared with its parent, desomorphine, which has an ED_{50} of 0.09[1,2]). Normorphine and norcodeine do not display much in vivo analgesic activity when the common routes of administration are used,[3] presumably because they are too polar to pass through a "blood brain barrier", or their more rapid metabolism causes facile elimination from the animal.

Insofar as we are aware, only normorphine and norco-