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Isolation, Identification, and Synthesis of the Major Sulpiride Metabolite in Primates

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Received March 27, 1981, from the *Corporate Health & Safety Department, Rohm and Haas Co., Bristol, PA 19007;* the [‡]*Adria Laboratories, Inc., Columbus, OH 43216;* and the [§]*Rohm and Haas Company, Research Laboratories, Spring House, PA 19477.* Accepted for publication January 6, 1982.

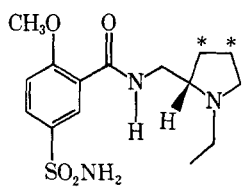
Abstract □ The major metabolite of sulpiride, *N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-sulfamoyl-2-anisamide (I), in the monkey is *N*-[(1-ethyl-5-oxo-2-pyrrolidinyl)methyl]-5-sulfamoyl-2-anisamide (II). It is also a metabolite in other laboratory animal species and possibly at very low levels in humans. Treatment of the urine from a monkey dosed orally with ^{14}C-I by dry column chromatography and high-pressure liquid chromatography (HPLC) produced the major metabolite in pure form. Characterization of the purified ^{14}C-radiolabeled metabolite by proton NMR, TLC, HPLC, and chemical ionization mass spectroscopy, along with subsequent comparison of a synthetically prepared sample, gave unequivocal structural confirmation.

Keyphrases □ Sulpiride—isolation, identification, and synthesis of major metabolites, monkeys □ High-pressure liquid chromatography—analysis, major sulpiride metabolites, monkeys □ Metabolites—sulpiride, isolation, identification, and synthesis, monkeys

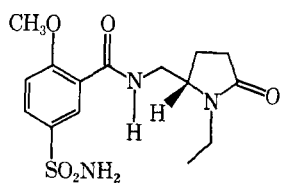
Sulpiride (I) is a structurally unique antipsychotic drug. Studies utilizing ^{14}C-labeled I indicated that, while this drug is metabolized to a very small extent in humans, the monkey produces a major metabolite which accounts for 10–30% of a single dose (1). Column chromatography on a strong cation exchange resin, with dilute acid, rapidly eluted this major metabolite, whereas I was retained. This behavior suggested that the metabolite was rendered less basic than the parent drug by a metabolic change on the pyrrolidine ring.

There are several model chemical compounds that possess a pyrrolidinyl moiety either as a fused five-membered ring or as the saturated heterocyclic structure analogous to I. Among these are mazindol (III), prolintane (IV), and tremorine (V).

One of the major biotransformations of these chemical models (Structures II, III–V) in analogous animal species is the oxidation of the alpha position of the five-membered ring to the lactam structures (VI, VII, and VIII). These metabolic changes suggest that I would be similarly biotransformed. It has been reported (2) that the metabolite oxytremorine (VI) is physiologically active and provided



I



II

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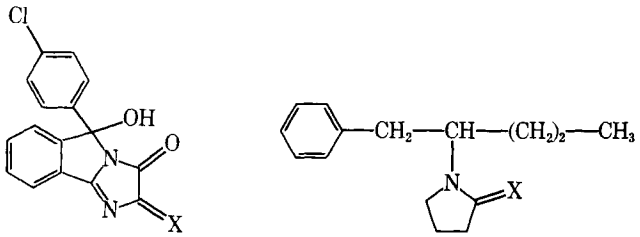
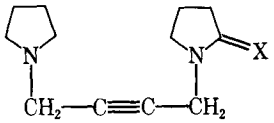


III: X = 2H; VI: X = O

IV: X = 2H; VII: X = O



V: X = 2H; VIII: X = O

further impetus for identification, synthesis, and biological evaluation of this suspected metabolite.

EXPERIMENTAL

Isolation of the Metabolite by Dry Column Chromatography—Urine from a male monkey (*Macaca mulatta*) which had been dosed orally with [^{14}C]3,4-pyrrolidine sulpiride (I) was dissolved in Solvent System I [1-butanol-acetic acid–water (2:2:1)] and placed on a nylon column (25-mm flat width × 280-mm flat length) packed with dry silica gel[1] to a height of 250 mm. When the elution was complete, the column was divided into 11 segments (25 mm each), excluding the upper and lower portions of the column which were discarded. The segments were slurried with absolute methanol (25 ml) and 10-μl aliquot portions were collected, diluted with 10 ml of scintillation cocktail[2], and counted in liquid scintillation counter. The results are plotted in Fig. 1. Fractions from segments II–V were slurried with methanol, filtered (0.5-μm filter), and concentrated under dry nitrogen. The residue was dissolved in Solvent System V [5 ml of propanol–NH_4OH (9:1)] and eluted with the same size column used for Solvent System I. When the elution was complete, the column was treated as above; the data are plotted in Fig. 2. Fractions 6–8 were diluted further, filtered, and concentrated under dry nitrogen. The residue was extracted with methylene chloride (25 ml), and two aliquot portions (50 μl) were removed and counted. The results showed that 239 μg of crude metabolite had been isolated.

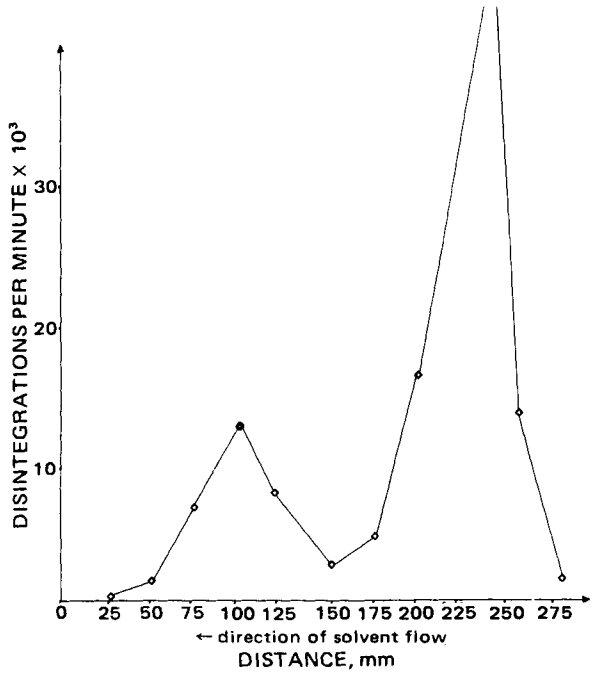


Figure 1—*Radiolabeled sulpiride and the metabolite.*

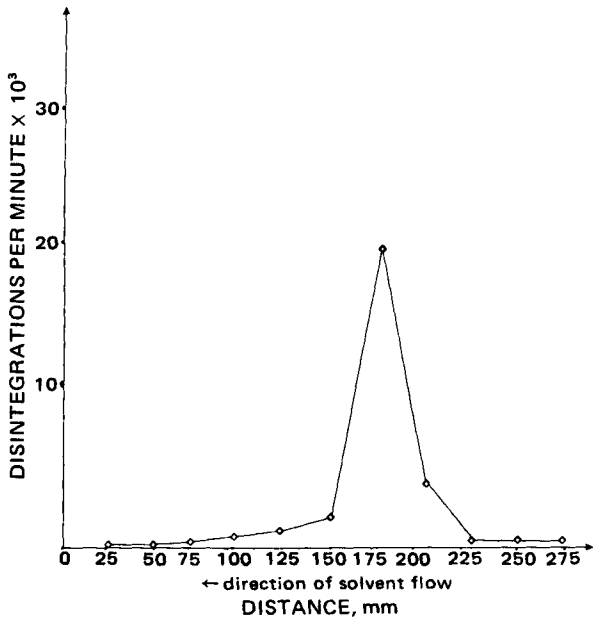


Figure 2—*Radiolabeled metabolite.*

Purification of the Metabolite by HPLC—The methylene chloride solution containing ~239 μg of radioactive material was concentrated to ~1.2 ml under nitrogen and filtered through a 0.5-μm filter. The filtered solution was loaded into a high-pressure liquid chromatographic (HPLC) injection assembly and the solvent overflow was collected and counted; it contained no detectable radioactivity. At zero time, the sample was injected on a preparative HPLC column[3] and the program initiated. A 50-min concave program from 100% water to 100% acetonitrile at 2.8 ml/min and with UV detection at 290 nm was used. The fractions were collected at 5-min intervals or when necessary by elution of a peak. Aliquots of up to 0.5 ml were taken from each vial for liquid scintillation counting. After four runs, it was determined by UV absorption and liquid scintillation counting that three vials (Fig. 3) contained ~77, 17, and 7 μg, respectively, of radioactive material. The vial with the highest radioactivity was concentrated under nitrogen and examined by ^{1}H-NMR.

Identification—*NMR Analysis of the Metabolite*—The sulpiride metabolite sample which had been purified by dry column chromatography and HPLC was dried in a conical vial. The vial was placed under a nitrogen blanket (water free), and 6 μl of dimethyl sulfoxide[4] was added

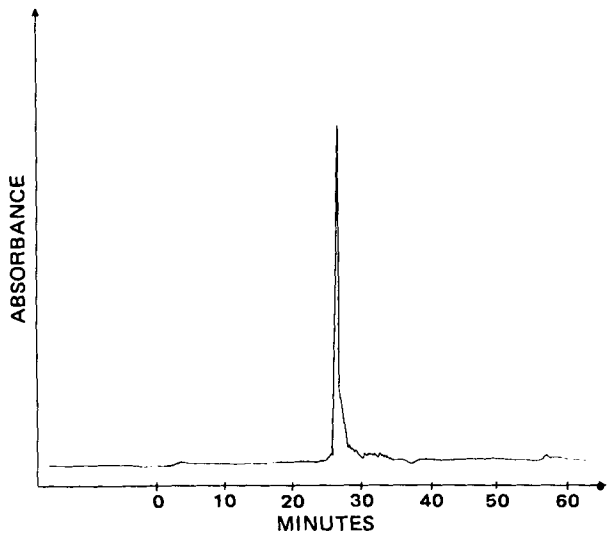


Figure 3—*Isolation of the radiolabeled metabolite by HPLC.*

[1] Grace, 60–200 mesh.
[2] Hydromix.
[3] Varian Micropak C-H.
[4] 100% deuterated, Merck.

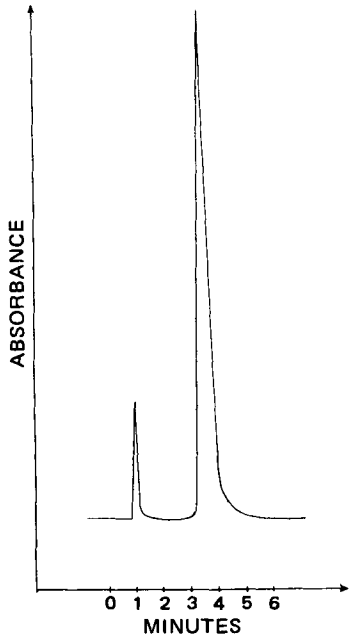
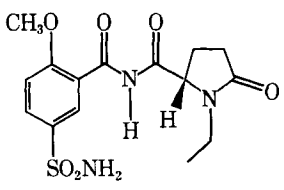


Figure 4—*Comparison of the elution times of radiolabeled metabolite and internal standard.*

to the vial. When the material had dissolved, the solution was transferred *via* capillary action to a 1-mm glass tube and the ends sealed with corks. The tube was removed from the nitrogen blanket and sealed immediately with a torch. The 100 MHz ^{1}H-NMR spectrum was obtained using Fourier transform spectroscopy. The time average of 4872 free induction decays (collected with a 1-sec acquisition time) yielded the following spectrum: δ 0.98 (triplet, J = 7 Hz, CH_3), 1.75–2.26 (multiplet, $2CH_2$), 2.87–3.59 (multiplet, CH, $2CH_2$), 3.88 (singlet, CH_3), 7.21 (singlet, NH_2), and 7.29–8.31 (multiplet, 3 aromatic). Analysis of this spectrum compared to that of sulpiride suggested that the metabolite had the structure of II. The ^{1}H-NMR spectrum of synthetic II was obtained at a later date in a similar manner and was essentially identical to that of the metabolite.

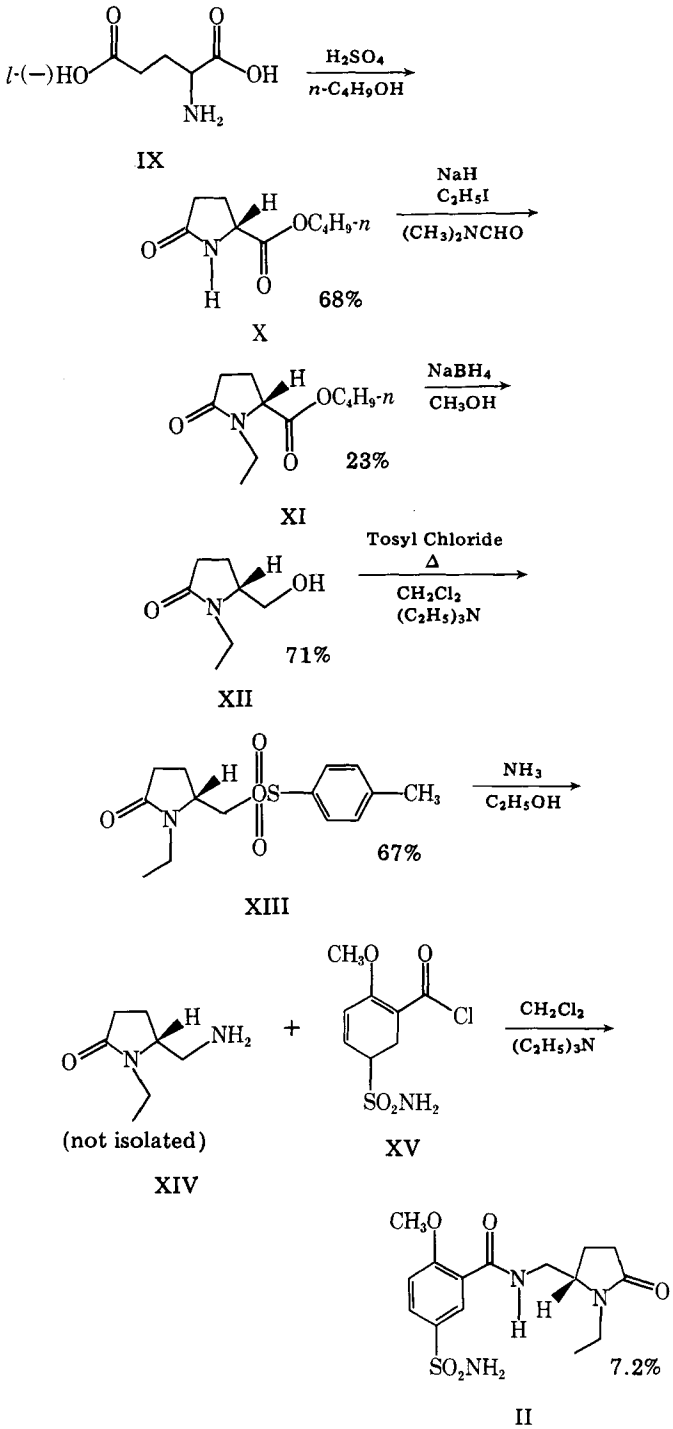
Chemical Ionization Mass Spectral Analysis of the Metabolite and the Synthetically Prepared Material—The data for the purified metabolite sample were of good quality, probably because of the higher purity. The methane chemical ionization mass spectrum shows the protonated molecular ion of the α-pyrrolidinone derivative at mass spectrum 356 and also association ion peaks for $(M+, C_2H_5)^+$ at 384 and $(M+, C_3H_5)^+$ at 396. However, a second component was also observed with peaks at 370 and 398, implying a molecular weight of 369. The amount of this component cannot be estimated from the chemical ionization mass spectroscopic data, but is probably <10% of II, since it was not readily observed in the ^{1}H-NMR data. This second component was probably a derivative of sulpiride which was oxidized at two positions on XVI.
A mass spectral scan of synthetic II showed a multitude of ions and could not be readily interpreted. Evidently the impurities in the preparation were masking the major component, perhaps due to higher volatility or stability.

HPLC Analysis of the Metabolite—A high-pressure liquid chromatograph equipped with a valve-loop injector (10 μl), an amino column[5], and a variable wavelength detector set at 240 nm was used. The mobile phase was prepared by adding 15 ml of concentrated ammonium hydroxide to a 400-ml mixture of 97.5 acetonitrile–2.5% water. This mixture was pumped through the column at 1 ml/min. Using this system I eluted at 3.3 min, with an internal standard of *p*-toluenesulfonamide eluting at 1.0 min (Fig. 4).



XVI

[5] Varian Micropak-NH_2.



Scheme I—Synthetic route to II.

A solution of 1.8 mg of II in 5 ml of acetonitrile was prepared. A 10-μl aliquot was injected and a retention time of 1.9 min measured (Fig. 5A). A sample of the radioactive metabolite was dissolved in 1 ml of acetonitrile, and a 5-μl aliquot was injected. The retention time by UV absorption and collected radioactivity was found to be 1.9 min (Fig. 5B), identical to that of the synthetic material. The two chromatograms are shown in Fig. 5.

Synthesis of Metabolite II—*Butyl-5-oxopyroglutamate (X)*—Scheme I shows the synthesis of II: A 2-liter flask containing *l*(−)-glutamic acid (IX) (147 g, 1.0 mole) and 1-butanol (625.0 g, 8.45 moles) was stirred at room temperature. The suspension was treated with concentrated sulfuric acid (125.0 g, 1.27 moles) in portions and gave a clear solution. The reaction was heated at reflux with the removal of water for 25 hr and the pH adjusted to 4.5 with 1 *N* Na_2CO_3 and concentrated *in vacuo*. The viscous oil obtained was stirred with benzene (500 ml) while adjusting the pH to 9.5 with 2 *N* NaOH. The organic layer was separated,

Table I—Elemental Analysis of Compounds II, X-XIV

Compound Number	Empirical Formula	mp/bp°	Elemental analyses: (Calc) Found			
			C	H	N	S
X	$C_9H_{15}NO_3$	162–3, 1.7 mm	58.74	8.56	8.53	—
			(58.36)	(8.16)	(7.55)	—
XI	$C_{11}H_{19}NO_3$	135–6, 0.75 mm	63.25	9.63	7.05	—
			(61.95)	(8.98)	(6.56)	—
XII	$C_7H_{13}NO_2$	159, 3.5 mm	58.26	9.38	9.72	—
			(58.72)	(9.15)	(9.77)	—
XIII	$C_{14}H_{19}NO_4S$	84–85	56.65	6.51	4.99	10.76
			(56.54)	(6.44)	(4.70)	(10.78)
XIV	$C_7H_{14}N_2O$	Oil	59.53	9.79	—	—
			(59.13)	(9.92)	—	—
II	$C_{15}H_{21}N_3O_5S$	227–228	48.70	5.83	10.98	11.30
			(50.69)	(5.95)	(11.82)	(9.02)

and the aqueous layer was further extracted with benzene (2 × 250 ml). The organic layers were combined, washed with saturated sodium chloride solution, and concentrated to give a lightly colored oil. The oil was distilled (160–165°, 1.7 mm) to give 126.0 g (68%) of a colorless product.

Butyl-N-ethyl-5-oxopyroglutamate (XI)—A 500-ml flask equipped with a mechanical stirrer, reflux condenser, thermometer, and funnel was used. Compound X (37.0 g, 0.2 mole) was dissolved in dimethylformamide (200 ml, previously dried over 4-Å molecular sieves for 24 hr). Sodium hydride (9.5 g of 50% dispersion, 0.2 mole) was added in portions, the reaction exothermed to 50°, and the solution became amber. The reaction was maintained at 50° for 1 hr and then cooled. Ethyl iodide (31.2 g, 0.2 mole) was then added dropwise at a rate sufficient to maintain a temperature of 50°. After the addition was complete, the reaction was heated at 50° for 2 hr and stirred to room temperature overnight. The reaction mixture was poured into water (1000 ml), extracted with carbon tetrachloride (3 × 600 ml), dried over magnesium sulfate, filtered, and concentrated to give 35.5 g of crude product. The crude material was distilled (136°, 0.75 mm) to give 18.7 g (44%) of pure product.

N-Ethyl-5-hydroxymethyl-2-pyrrolidinone (XII)—A 250-ml flask equipped with a magnetic stirrer, reflux condenser, thermometer, and funnel was used. Compound XI (25.0 g, 0.12 mole) was added dropwise to a solution of absolute methanol (120 ml) containing sodium borohydride (11.4 g, 0.30 mole) at room temperature. The addition of the ester caused an exotherm and the reaction temperature was maintained below 50° with ice. When the addition was complete, the reaction was heated at reflux overnight. The reaction mixture was concentrated, the viscous oil slurried with a minimum amount of anhydrous ethanol, then chromatographed on a glass column (58 mm × 600 mm) which had been packed with slurried silica gel[1]. The product was eluted with ethanol, concentrated, and the oil obtained was extracted into benzene, dried over magnesium sulfate, filtered, and concentrated to give 12.2 g (71.2%) of pure product.

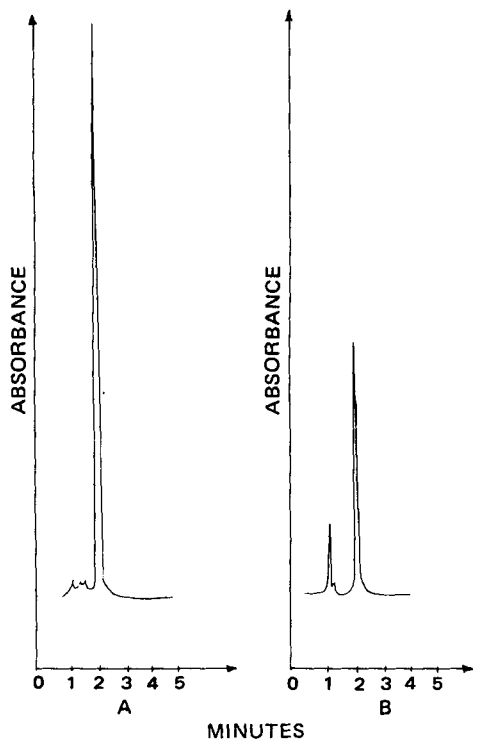


Figure 5—*Comparison of the elution times of the synthetic (A) and radiolabeled metabolite (B).*

N-Ethyl-5-tosylmethyl-2-pyrrolidinone (XIII)—A 100-ml flask equipped with a reflux condenser and drying tube was used. Compound XII (7.24 g, 50.77 mmoles), 4-toluenesulfonyl chloride (19.1 g, 100.0 mmoles), and triethylamine (10.1 g, 100.0 mmoles) were dissolved in methylene chloride (50 ml) and heated overnight. The rection mixture was poured into dilute sodium bicarbonate solution (10 ml of a saturated solution was diluted to 100 ml), the layers were separated, and the organic layer was extracted with 0.1 *N* HCl (2 × 100 ml), dried over magnesium sulfate, filtered, concentrated, and refrigerated, overnight. The tan solid obtained was stirred with heptane (5 × 100 ml), filtered, and air dried to give 10.15 g of product (mp 80–82°). The product was crystallized from ethyl acetate–heptane (1:1) (mp 84–85°, 67% yield).

N-Ethyl-α-aminomethyl-2-pyrrolidinone (XIV)—A 300-ml stainless steel autoclave equipped with a pressure gauge (1 × 10^3 psi) was employed. Ethanol (200 proof, 100 ml) was cooled and saturated with ammonia for 1.5 hr. *N*-Ethyl-5-tosylmethyl-2-pyrrolidinone (XIII) (5.0 g, 16.9 mmoles) was added and the solution was stirred and heated at 80° overnight. The resulting amber solution was diluted with water (30 ml) and then the ethanol was removed *in vacuo*. The remaining aqueous solution was cooled and the pH adjusted to 14 with solid potassium hydroxide. The resulting alkaline solution was extracted with ethyl acetate (3 × 100 ml) and the aqueous solution was readjusted to pH 14 and further extracted with ethyl acetate (2 × 100 ml). The organic layers were combined and concentrated to a minimum volume and further extracted with 1 *N* HCl (30 ml). The acid solution was made alkaline to litmus, extracted with ethyl acetate (3 × 300 ml), dried over magnesium sulfate, filtered, and concentrated to give 220 mg of an amber oil (9.3% yield).

N-[(1-Ethyl-2-pyrrolidinyl)methyl-5-oxo]-5-sulfamoyl-2-anisamide (II)—A 100-ml single-neck flask equipped with a magnetic stirrer, reflux condenser, and drying tube was used. Thionyl chloride (50 ml, 0.68 mole) and 2-methoxy-5-sulfamoyl-benzoic acid (7.0 g, 26.6 mmole) were mixed, pyridine (4 drops) was added, and the reaction was heated at reflux overnight. The thionyl chloride was removed *in vacuo*, and benzene (2 × 50 ml) was added and removed *in vacuo* to remove the final traces of thionyl chloride.

α-Aminomethyl-*N*-ethyl-2-pyrrolidinone (XIV) (220 mg, 1.55 mmoles) and triethylamine (160 mg, 1.58 mmoles) were dissolved in 10 ml of chloroform in a 50-ml flask equipped with a magnetic stirrer and a reflux condenser, and cooled in ice. A chloroform solution (3.5 ml) containing 2-methoxy-5-sulfamoyl-benzoyl chloride (402 mg, 1.61 mmoles) was added dropwise (a slight exotherm was observed). The reaction was stirred to room temperature and then heated at reflux for 3.5 hr. The mixture was cooled, concentrated, and the resulting gummy black tar was triturated with water and allowed to stand. A gray solid formed, which was filtered and air dried, to yield 40 mg of the desired product. Further trituration of the solid with absolute methanol followed by filtering and air drying gave a gray solid (mp 228° with decomposition, 7.2%).

The melting points were obtained on a melting point apparatus[6] and are uncorrected. The elemental analyses for these compounds are given in Table I.

[6] Thomas–Hoover.

DISCUSSION

The major metabolite of sulpiride in monkeys was obtained by isolation from urine followed by chromatographic purification. The chromatogram in Fig. 3 represents the sample that was submitted for identification by ^{1}H-NMR, chemical ionization mass spectroscopy, and HPLC. Comparison for the ^{1}H-NMR spectra of the metabolite and sulpiride showed that some biotransformation had occurred to the pyrrolidine ring, where it appeared that alpha oxidation had taken place similar to the model compounds, VI–VIII.

This conclusion was further supported by the fact that sulpiride is retained on a cation exchange column, while metabolite II can be eluted with an acidic solvent system. The different chemical behavior of these two species was attributed to the differences in the basicity of the sulpiride pyrrolidine ring nitrogen, which was a tertiary amine prior to biotransformation and subsequently was converted into a cyclic amide of lesser basicity. The synthesis of compound II by an unambiguous route (Scheme I) and analysis by ^{1}H-NMR of the synthetic material gave unequivocal evidence that alpha oxidation had occurred. In addition, a sample of the radiolabeled metabolite and the synthetically prepared sample were compared by HPLC and found to have identical retention times (Fig. 5).

Previous attempts to obtain a mass spectrum on II by electron impact had failed, probably due to the presence of impurities. Subsequently, chemical ionization mass spectral data were obtained on the same sample that had been subjected to ^{1}H-NMR. The spectrum was of good quality and demonstrated that II has a molecular weight of 355. A compound of molecular weight 369 ($\simeq$10%) was also observed. Although its structure has not been identified, it has undergone oxidation at two methylene groups and may be the compound shown by XVI.

Isolation of the metabolite by column chromatography and purification by HPLC with subsequent identification by Fourier transform-NMR, chemical ionization mass spectroscopy, HPLC, and unambiguous chemical synthesis has shown that the structure of the major metabolite of sulpiride is II.

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Potential Radiosensitizing Agents III: 2-Nitro-4-Acetylimidazole Analogs

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Received November 2, 1981, from the *Department of Pharmacology, Tulane University School of Medicine, New Orleans, LA 70112.* Accepted for publication, December 31, 1981.

Abstract □ New analogs of 2-nitroimidazole have been synthesized in an effort to minimize the toxicity and increase selective sensitization of hypoxic mammalian cells toward lethal effects of ionizing radiation. 2-Nitro-4(5)-acetyl-5(4)-methylimidazole was synthesized from the corresponding 2-amino analog and then reacted with oxiranes to produce the corresponding 1-substituted 2-propanol and 3-methoxy-2-propanol derivatives. The biological results of radiosensitizing activity of these agents against Chinese hamster cells (V-79) indicated that the 3-methoxy-2-propanol derivative was a more effective radiosensitizer than misonidazole *in vitro*. Evaluation of the acute toxicity of these agents as determined by LD_{50} demonstrated no significant difference between these agents and misonidazole suggesting that the 3-methoxy-2-propanol analog may possess a therapeutic advantage over misonidazole.

Keyphrases □ Radiosensitizing agents, potential—2-nitro-4-acetylimidazole analogs □ 2-Nitro-4-acetylimidazole—analogs, potential radiosensitizing agents □ Analogs—2-nitro-4-acetylimidazole, potential radiosensitizing agents

In a continuing effort to develop new effective radiosensitizers to sensitize selectively the relatively resistant hypoxic tumor cells toward radiotherapy, an additional electron affinic acetyl function has been incorporated into the 2-nitroimidazole nucleus. A direct correlation between the sensitizing efficiency and electron affinity of the radiosensitizers has been demonstrated (1). Initially, a series of 2,4-dinitroimidazoles were synthesized in an effort to increase the electron affinity of the 2-nitroimidazole nucleus (2, 3). The 1-(2-hydroxy-3-methoxypropyl)-2,4-dinitroimidazole was found to be the most effective radiosensitizer of this series (4). However, the 2,4-dinitroimidazole derivatives were found to be generally more toxic than misonidazole (5), an agent currently under clinical trials for evaluation as a radiosensitizer. It was deemed desirable to study the effect of another electron affinic group other than the nitro function at the 4-position of the 2-nitroimidazole nucleus. Accordingly, 4-acetyl substituted 2-nitroimidazole analogs have been synthesized. This modification was thought to be of interest in view of the report that 1-methyl-2-nitroimidazole-5-carboxaldehyde sensitized the hypoxic Chinese hamster cells *in vitro* to ionizing radiation at much lower concentrations (25 μM) than misonidazole (1).

BACKGROUND

It is obvious that an aldehyde function is a metabolically unstable group, and perhaps in addition to high electron affinity, lack of an hydroxyl group in the side chain at the 1-position may have contributed toward enhanced cytotoxicity of this agent. Therefore, the synthesis of 4-acetyl analogs of 2-nitroimidazole with a 2-hydroxypropyl side chain at the 1-position was undertaken. The molecular design of agents described in this report was also related to the structure of metronidazole, a known but less potent radiosensitizer than misonidazole, in that the functional groups at 2- and 5-positions were reversed to provide a 2-nitro-5-methyl analog, since 2-nitroimidazoles have been reported to be more effective radiosensitizers (6).

The synthesis of the required intermediate 2-amino-4(5)-acetyl-5(4)-methylimidazole (III) was accomplished by the known mononuclear 1,2,4-oxadiazole imidazole rearrangement with minor modification of utilizing sodium methoxide in dimethylformamide rather than sodium ethoxide as a base (7). The starting material 3-amino-5-phenyl-1,2,4-oxadiazole, readily obtained from hydrolysis of the *N,O*-dibenzoyl hydroxyguanidine (8), was condensed with an equimolar amount of acetylacetone in anhydrous toluene in the presence of *p*-toluenesulfonic acid

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