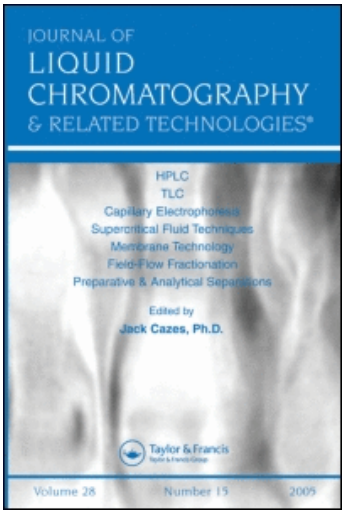

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Liquid Chromatography of Oxidized Sulfanilamide Derivatives

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LIQUID CHROMATOGRAPHY OF OXIDIZED SULFANILAMIDE DERIVATIVES

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ABSTRACT

Chromatographic behavior on reverse phase HPLC is reported for a group of N^4- and other oxidized sulfanilamide derivatives of biological interest. 4-Hydroxylaminobenzenesulfonamide decomposes in aqueous solution in a pH-dependent manner. At room temperature at pH 10, it converts almost quantitatively to azoxybenzene-4,4'-disulfonamide and 4-nitrobenzenesulfonamide.

INTRODUCTION

The past decade has seen a resurgence in the use of sulfonamide drugs for therapy of certain urinary and respiratory tract infections. This has been accompanied by an increasing incidence of allergic reactions some of which are serious (1). For studies in our laboratory directed towards assessing the immunological status of sulfonamide drug allergy, a series of oxidized derivatives of sulfanilamide, 1, was required as prospective antigens. These included 4-hydroxylaminobenzenesulfonamide, 2, 3-hydroxysulfanilamide, 5, and N^4-glycolylsulfanilamide, 6, known metab-

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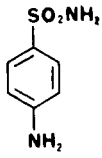
olites of sulfanilamide (2-5), and azoxybenzene-4,4'-disulfonamide, 4, (6), azobenzene-4,4'-disulfonamide, 8, (6), and 4-nitrobenzenesulfonamide, 3, required for reference. Structures are shown.



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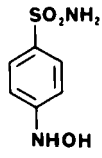

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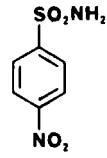

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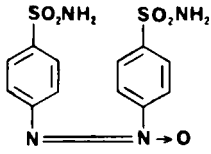

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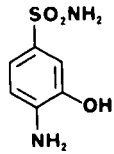

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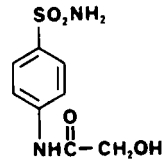

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Although prepared according to routes available in the literature, in our hands many of these compounds were difficult to characterize. Some melted with decomposition at high temperatures, and often elemental analyses also were not definitive. In addition several appeared to decompose upon recrystallization or storage.

We therefore undertook to examine these compounds by high performance liquid chromatography (HPLC). This paper reports chromatographic constants for these compounds under reverse phase conditions. Observations determined by HPLC are also reported on the decomposition of compd 2 in aqueous solution.

MATERIALS AND METHODS

Chemicals

Sulfanilamide was purchased from Matheson, Coleman and Bell, South Plainfield, NJ; sulfanilic acid from Fisher Scientific, Pittsburgh, Pa; p-nitrobenzenesulfonamide from Aldrich, Milwaukee,

WI. 3-Hydroxysulfanilamide (4), 4-hydroxylaminobenzenesulfonamide (7) and N^4-glycolylsulfanilamide (5) were synthesized as reported.

Azobenzene-4,4'-sulfonamide (6) and azoxybenzene-4,4'-disulfonamide (6) were prepared as described except that for analysis the latter was recrystallized from DMSO-H_2O at room temp. The material analyzed as a solvate with DMSO.

Anal. Calcd. for $C_{12}H_{12}N_4O_5S_2 \cdot C_2H_6SO$: C, 38.7; H,4.18; N,12.9; S, 22.1. Found: C, 38.8; H, 4.20; N, 12.7; S, 22.4.

Mass spectral analysis confirmed solvation and molecular weight. DMSO (M/Z 78.0) was released at a probe temp near 60°C. Higher molecular weight species appeared within 240°-350°C. Parent peak M/Z 356.0; calcd. for $C_{12}H_{12}N_4O_5S_2$: 356.0.

Water was deionized and then distilled through glass. MeOH was HPLC grade (Burdich and Jackson Laboratories, Muskegon, MI); acetic acid was Baker Reagent.

Instrumentation

HPLC was performed with a Model 6000A solvent delivery system (Waters Associates, Milford, MA), U6K injector (Waters), Holochrome detection system (Gilson Electronics, Middleton, WI) and Model 6051 recorder (Gilford, Oberlin, OH).

UV/viz spectra were recorded on an LKB Ultraspec 4050 spectrometer with a wavelength scan/multiwavelength program operated on an Apple II Plus computer.

Mass spectral analysis was performed with a Hewlett-Packard 5988A GC-MS system with a 70 eV beam. Compd 2 was introduced directly into the spectrometer via the direct insertion probe. Probe temperatures started at 30°C and increased at 30°/min up to 350°C.

Chromatography

Chromatography was performed on a reverse phase uBondapak C_{18} column (10 u particle size, 30 cm x 4 mm, Waters). The mobile phase consisted of 5-40% MeOH-1% acetic acid and is specified in the Table. Flow rate was 1 ml/min; AUF 0.1. Absorbancy was

TABLE

Elution Volumes of Sulfanilamide and Related N^4- and Other Oxidized Derivatives by Reverse Phase HPLC

COMPD	SYSTEM A	SYSTEM B[a] volume, ml	SYSTEM C
1. Sulfanilamide	4.9	4.1	3.4
2. 4-Hydroxylamino-benzenesulfonamide	4.3	3.4	3.2
3. 4-Nitrobenzene-sulfonamide	17.2	5.6	4.6
4. Azoxybenzene-4,4'-disulfonamide	-[b]	14.1	7.6
5. 3-Hydroxysulfanilamide	4.6		
6. 4-Glycolylsulfanilamide	7.4		
8. Azobenzene-4,4'-di-sulfonamide		18.4	

[a]System A was 5% MeOH-1% HOAc; System B, 30% MeOH-1% HOAc; System C, 40% MeOH-1% HOAc.
[b]Not eluted within 34 ml.

determined at 254 nm. Quantitation was carried out by calculation of the peak area. For the decomposition experiments, correction was made for the difference in absorption of each sulfonamide by relating it to sulfanilamide.

Stability Studies

An aqueous solution of Compd 2 (2 mg/ml) was adjusted with N NaOH to pH 10 and was stirred magnetically in a 10-ml beaker at 25°C. At the intervals specified in Fig 2, 1-ml samples were taken

and acidified with 0.5 ml of 40% MeOH-1% HOAc to terminate the reaction. Samples were then diluted to 10 ug/ml with MeOH followed by H_2O and were analyzed directly by HPLC.

PH-dependence of the decomposition of Compd 2 was determined by adjusting an aqueous solution (2 mg/ml) with N HCl or N NaOH to the pH values specified in Fig 3. Samples were allowed to stand at room temp for 1 hr and then were diluted and analyzed as described above.

Identification of Decomposition Products of Compd 2

Identification was based upon elution volumes on chromatography and co-chromatography in 2 solvent systems, and by comparison of the uv/viz absorption spectra of the isolated products with authentic samples. Decomposition products were isolated for spectroscopy by stirring a solution of 4 mg of Compd 2 in 2 ml at pH 10 for 1 hr. The mixture was diluted to 5 ml with 30% MeOH-1% HOAc. Compd 4 was collected by centifugation and washed with 30% MeOH. It was dried and dissolved in MeOH for spectroscopy. Compd 3 was isolated by chromatography of the centrifugate in System B. Fractions eluting within 5.5-6.4 ml were collected, combined and scanned. Upon re-examination on HPLC, isolated Compds 3 and 4 each chromatographed as a single peak with the expected retention volume.

RESULTS AND DISCUSSION

HPLC provided a rapid method for characterization of a series of N^4- and other oxidized derivatives of sulfanilamide. This procedure proved useful in monitoring the synthesis of these sulfonamides and assessing their purity and stability. A typical chromatogram is shown as Figure 1. The Table gives the elution volumes of these compounds on reverse phase liquid chromatography with 3 mobile phase systems.

Stability of 4-Hydroxylaminobenzenesulfonamide (2)

Previous attempts to purify Compd 2 by recrystallization from H_2O were unsatisfactory with respect to recovery and mp of the

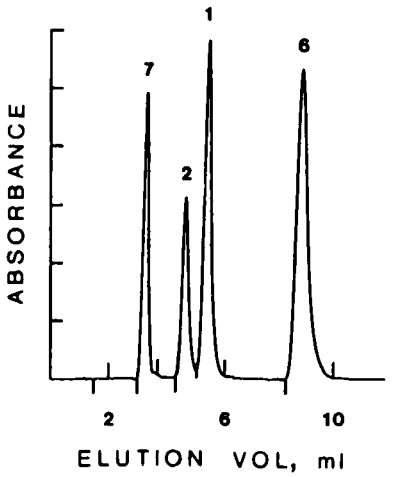

FIGURE 1

Chromatogram showing the separation in System A (see Table) of sulfanilic acid, 7, and Compds 2, 1, and 6, resp. Other conditions given under "Chromatography."

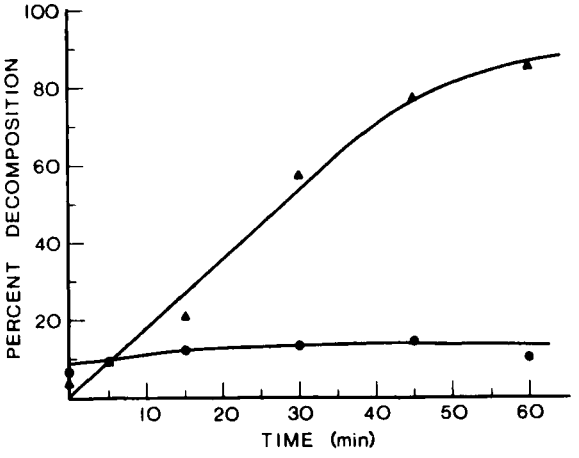

FIGURE 2

Rate of decomposition of 4-hydroxylaminobenzenesulfonamide in aqueous solution, pH 10, at +25°C. ▲—▲, formation of Compd 4; ●—●, formation of Compd 3. Chromatography was in System C (see Table). Other conditions given under "Chromatography" and "Stability Studies."

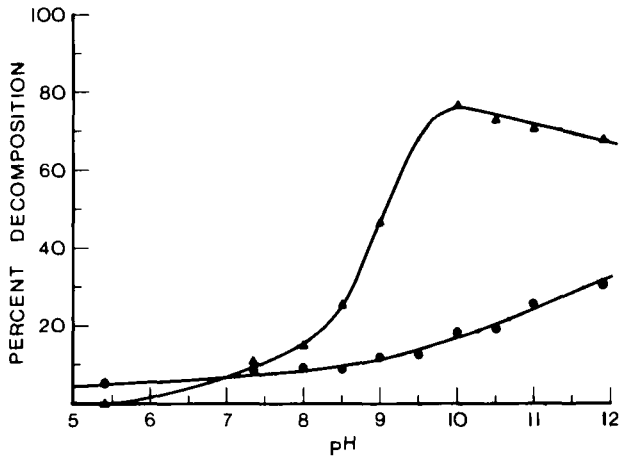

FIGURE 3

PH-dependence of the decomposition of Compd 2 in aqueous solution. ▲-▲, formation of Compd 4; ●-●, formation of Compd 3. Conditions as in Figure 2.

product. When an aqueous solution of Compd 2 was allowed to stand at pH 10 and then was examined by HPLC, extensive decomposition was evident. The major products were identified as Compds 3 and 4. After 1 hr approximately 85% of 4 and 10% of 3 had formed with less than 5% of the starting 2 + 1 present. Recovery was 93%. Figure 2 shows the rate of decomposition under this condition to Compds 3 and 4.

The decomposition of Compd 2 was markedly pH-dependent. Aqueous solutions were reasonably stable at pH 3 to 6, but underwent rapid change above pH 8 (Figure 3).

Analogy for the formation of Compds 3 and 4 exists in the oxidation by hydrogen peroxide or Caro's acid of aniline to β-phenylhydroxylamine, nitrosobenzene, azobenzene and azoxybenzene. The latter is a product of condensation of hydroxyamino and nitroso compounds (8). Nitro derivatives also have been observed to result from similar chemical oxidation of hydroxyamines of primary amines (8, 9).

Compd 2 is a product of microsomal metabolism of sulfanilamide in rat liver (2) that can elicit a positive skin test reaction in guinea pigs previously sensitized with sulfanilamide (7). The N-hydroxylation reaction apparently involved in the biosynthesis of Compd 2 from sulfanilamide represents a frequently seen initial step in the metabolism of primary aromatic amines (10). The marked lability of Compd 2 in aqueous solution that we encountered suggests that in biological studies with Compd 2, and probably other aromatic N-hydroxyamines of similar structure and reactivity, appropriate care is necessary when selecting conditions of pH and storage.

ACKNOWLEDGMENTS

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REFERENCES

1. Koch-Weser, J., Sidel, V.W., Dexter, M., Parish,C., Finer, D.C. and Kanarek, P., Adverse reactions to sulfisoxazole, sulfamethoxazole and nitrofurantoin, Arch. Inter. Med., 128, 399-401, 1971.

2. Thauer, R.K., Stoffler, G. and Uehleke, H., N-Hydroxyliering von sulfanilamid zu p-hydroxyaminobenzolsulfonamid durch levermikrosomen, Naun-Schmiedebergs Arch. Exp. Path. Pharmak., 252, 32-42, 1965.

3. Williams, R.T., The isolation and identification of 3-hydroxysulfanilamide as the oxidation product of sulfanilamide in the rabbit, Biochem. J., 40, 219-222, 1946.

4. Williams, R.T., The metabolism of sulfonamides. The detection of 3-hydroxysulfanilamide in the urine of patients treated with sulfanilamide, Biochem. J., 41, 1-8, 1947.

5. Fries, W., Kiese, M. and Lenk, W. Additional route in the metabolism of sulfanilamide, Xenobiotica, 1, 241-256, 1971.

6. Seikel, M.K., Oxidation products of sulfanilamide, J. Am. Chem. Soc., 62, 1214-1216, 1940.

7. Yamamoto, I. and Eyanagi, R., Positive skin reaction induced by 4,4'-azoxybenzenedisulfonamide in relationship to the sulfonamide allergy, Int. Arch. Allergy appl. Immunol., 54, 538-541, 1977.

8. Fuson, R.C., Reactions of Organic Compounds, John Wiley and Sons, New York, 1962, 239-240; 531.

9. Roberts, J.D. and Caserio, M.C., Modern Organic Chemistry, W.A. Benjamin, Inc., New York, 1967, 465-466.

10. Uehleke, H., N-Hydroxylation, Xenobiotica, 1, 327-338, 1971.