Characterization of Impurities in Sulfasalazine

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Abstract ☐ The chemical structures of four impurities isolated from sulfasalazine were determined. Three impurities are the by-products of the reaction process in drug synthesis, *i.e.*, during diazotization of sulfapyridine and during coupling with salicylic acid. Only one contaminant was identified as a starting material, sulfapyridine, in the drug synthesis. The four impurities were characterized as 2-[[*p*-(2-pyridylsulfamoyl)phenyl]azo]hydroxybenzene (I), 3-[[*p*-(2-pyridylsulfamoyl)phenyl]azo]salicylic acid (II), 5-[[*p*-[4-(2-pyridylanilino)]-*N*-phenyl]azo]salicylic acid (III), and sulfapyridine (IV). Compounds I–III are novel molecules, and IV is the precursor of sulfasalazine. The isolation of the impurities was accomplished by TLC and liquid extraction procedures. The methods used to characterize the impurities were a combination of IR, UV, and NMR spectroscopy, mass spectrometry, and TLC. For I and III, comparisons also were made with the synthesized materials to supplement the evaluation.

Keyphrases ☐ Sulfasalazine—various impurities isolated and characterized ☐ Impurities, various—isolated and characterized in sulfasalazine ☐ Antibacterials—sulfasalazine, various impurities isolated and characterized

The nature and number of impurities associated with the synthesis of sulfasalazine NF (1), 5-[[*p*-(2-pyridylsulfamoyl)phenyl]azo]salicylic acid, are of considerable interest to the regulatory agencies, the USP–NF, and the manufacturers of the drug substance. The drug product is used for treatment of arthritis (2) and ulcerative colitis (3).

Sulfasalazine is prepared by diazotizing sulfapyridine and coupling the diazotized intermediate with salicylic acid (4, 5). The common analytical procedures used for sulfasalazine are titration with titanium trichloride (6), polarography (7), spectrophotometry (6), and high-speed liquid chromatography (8). All procedures are specific for pure sulfasalazine; but in the presence of unknown impurities, these methods are questionable. Therefore, knowledge of the impurities is essential to interpret analytical results.

A recent quantitative analysis (9) of sulfasalazine utilized TLC and spectrophotometry, a modified version of which is official in NF XIV (1). Reference was also made (9) to the number of impurities (10–12) associated with sulfasalazine; however, none of them was identified except sulfapyridine by TLC.

A column chromatographic method was developed (10) to separate the impurities, and three impurities were characterized partially: a polymer, an undiazotized sulfamide, and a positional isomer of sulfasalazine. In spite of early attempts to gain information on the impurities, there is no conclusive evidence as to their identity and structure.

The purposes of this study were to isolate the impurities, to elucidate their structures by mass spectrometry, and to explain the similarities and differences of their structures with respect to sulfasalazine. A combination of other methods (UV, NMR, and IR spectroscopy and TLC) and comparison with authentic synthetic materials also were used.

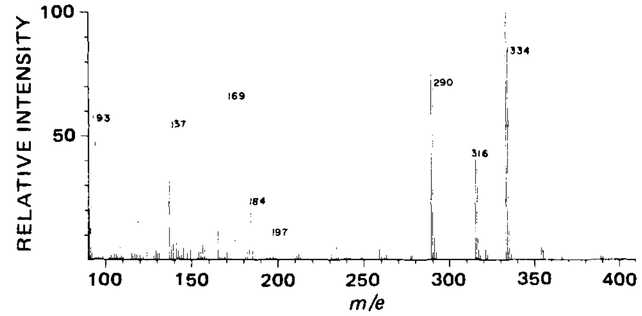

Figure 1—*Electron-impact mass spectrum of sulfasalazine.*

EXPERIMENTAL

Materials and Equipment—All chemicals were reagent grade. Silica gel[1] and aluminum oxide[2] TLC plates (0.250 and 0.500 mm) were prepared in this laboratory. IR spectra were obtained on a spectrophotometer[3] using potassium bromide pellets. UV spectra were determined on a recording spectrophotometer[4]. Mass spectra[5] were obtained at various temperatures using electron impact at 70 ev and chemical ionization with isobutane or methane gas. NMR spectra[6] also were obtained.

Isolation and Analyses of Impurities—The impurities in sulfasalazine were selectively separated and isolated from TLC preparative plates. The drug in dimethylformamide was spotted on the plate, the solvent was evaporated using forced air, and the plate was developed in different solvents. The separated zones containing impurity components were scraped from the plate, eluted with the same solvent, and filtered. The solvent was evaporated, and the residue was dried at 90° for 2 hr. The separated spots also were visualized (red-yellow color) by spraying the plate with ferric chloride solution (1 g in 50 ml of 10% HCl) and heating at 100° for a few minutes.

Impurity component I was isolated from a silica gel HR plate using chloroform–acetone–acetic acid (90:5:5) (R_f 0.83). This impurity traveled ahead of sulfasalazine. This yellow material (I) was compared by TLC with the synthesized compound 2-[[*p*-(2-pyridylsulfamoyl)phenyl]azo]-hydroxybenzene, a by-product obtained from the reaction of diazotized sulfapyridine and phenol. In addition, impurity I was extracted into ether from a sodium bicarbonate solution of a commercial sample of sulfasalazine. IR, NMR, UV, and mass spectra were obtained on this impurity.

Impurity component II was isolated from an aluminum oxide plate,

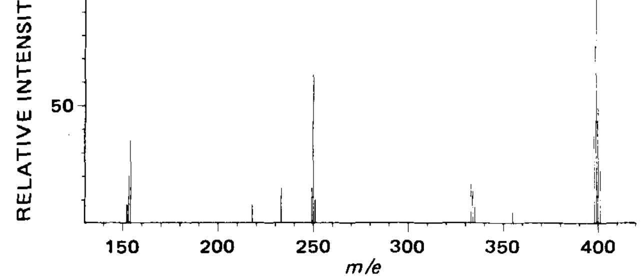

Figure 2—*Chemical-ionization mass spectrum of sulfasalazine.*

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[1] EM. Laboratories.

[2] Brinkmann Instruments.

[3] Perkin-Elmer model 221.

[4] Cary model 140.

[5] MS30 AEI mass spectrometer with DS30 data system, RMU6D mass spectrometer, and Finnigan 3300 mass spectrometer.

[6] Varian model T-60.

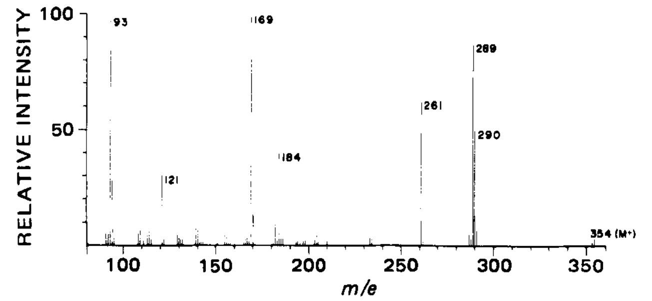


Figure 3—*Electron-impact mass spectrum of I.*

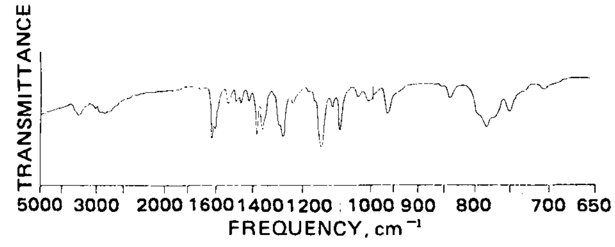


Figure 4—*IR spectrum of I.*

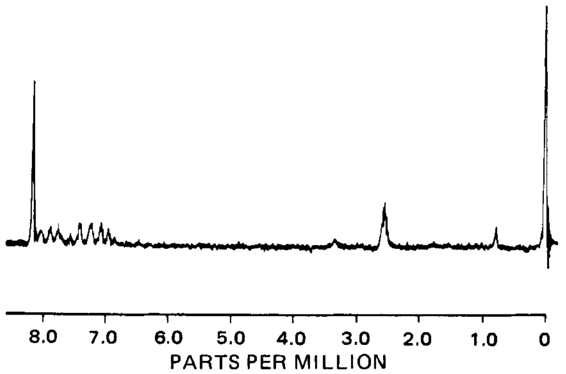


Figure 5—*The 60-MHz spectrum of I.*

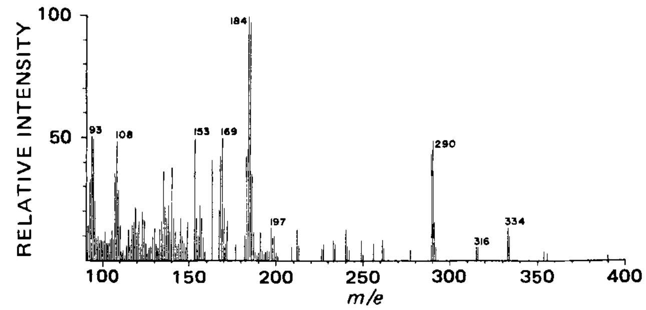


Figure 6—*Electron-impact mass spectrum of II.*

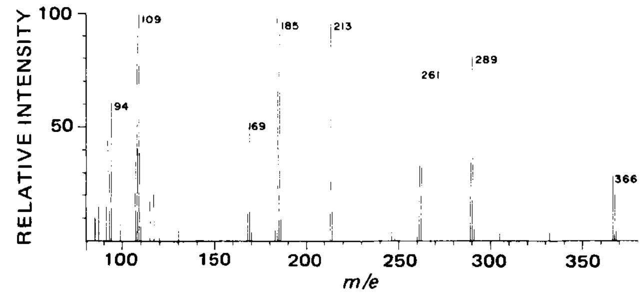
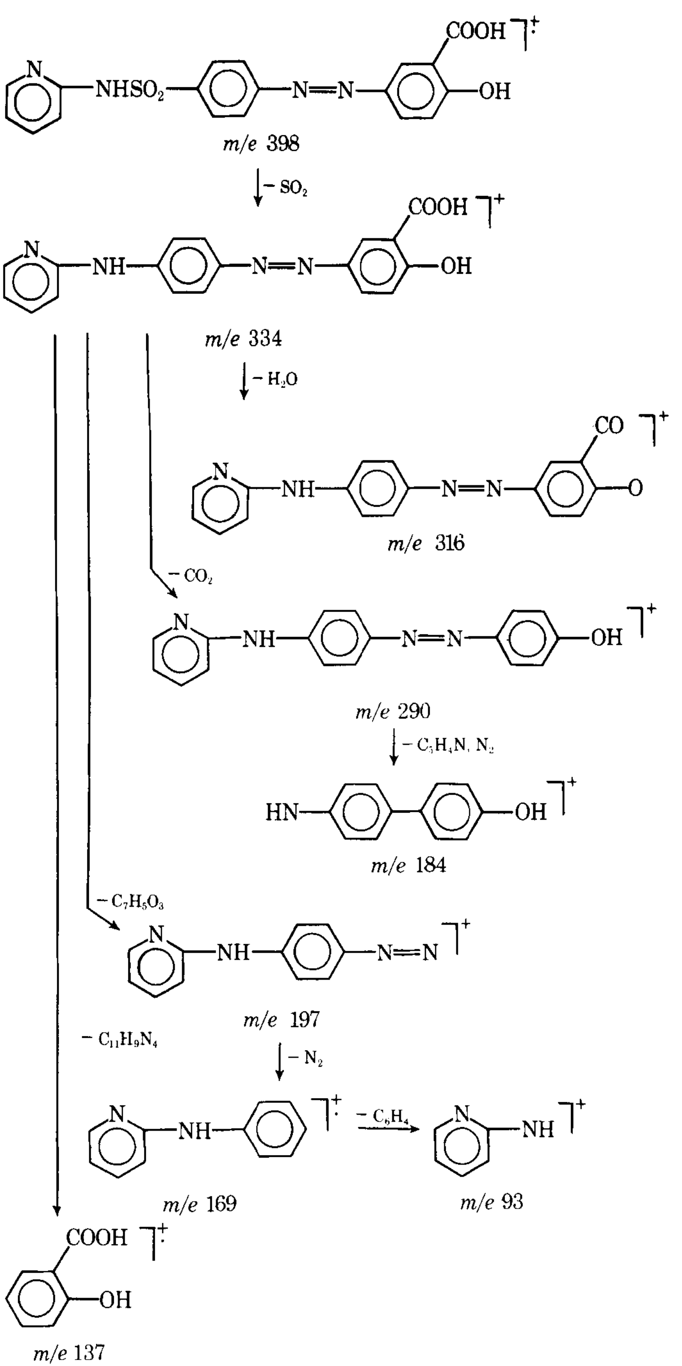


Figure 7—*Electron-impact mass spectrum of III.*



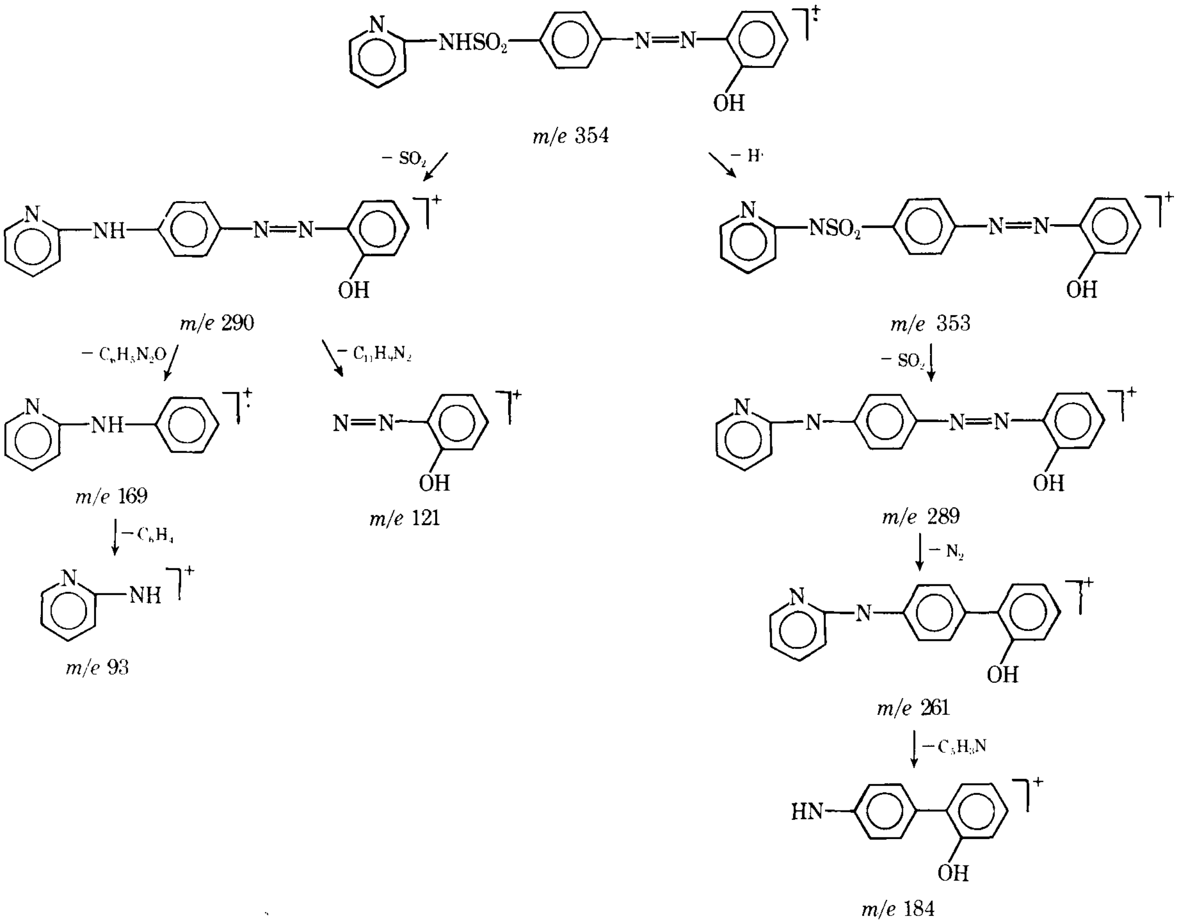
Scheme I

traveling behind sulfasalazine, using chloroform–methyl ethyl ketone–formic acid (70:15:10) (R_f 0.48). Its mass spectrum was obtained.

Impurity component III was isolated from a silica gel plate, traveling below II, using chloroform–methyl ethyl ketone–formic acid (30:50:20) (R_f 0.45). Compound III was also compared with an authentic material synthesized as follows. About 1 g of sulfapyridine was cautiously heated in a beaker until a dark mass resulted and the evolution of sulfur dioxide subsided. The dark mass was triturated with 30 ml of chloroform to extract the soluble fraction. The chloroform was evaporated, and the residue obtained was chromatographed on a TLC plate (silica gel HR) using ether as the solvent system.

Three yellow zones (A, B, and C) were separated on the plate and eluted with ether. The residue from each zone was diazotized and coupled with salicylic acid, and the resultant product was dried at 90° for 1 hr. TLC, IR, and mass spectra of the synthesized material from zone A (origin) was compared with impurity component III. Material isolated from zone A was also analyzed by mass spectrometry.

Impurity components IV and V (R_f 0.40 and 0.35, respectively) traveled below III in the same TLC system. Their mass spectra were obtained.



Scheme II

RESULTS AND DISCUSSION

The isolation and identification of four impurities associated with sulfasalazine were accomplished by TLC and mass spectral analyses. Chemical-ionization and electron-impact mass spectra were obtained to determine molecular ions and to follow the mass fragmentation pattern of the components. It was evident from the electron-impact mass spectra that the impurity components and sulfasalazine were unstable since no measurable molecular ion was detected. The electron-impact and chemical-ionization mass spectra of sulfasalazine isolated from an alumina TLC plate (R_f 0.76) in a pure form are shown in Figs. 1 and 2. The mass fragmentation pattern is shown in Scheme I.

Impurity I, isolated by TLC, was a yellow material characterized as 2-[[*p*-(2-pyridylsulfamoyl)phenyl]azo]hydroxybenzene. The electron-impact mass spectrum of I is shown in Fig. 3 with the molecular ion *m/e* 354. The mass fragmentation pattern is shown in Scheme II. The synthesized material, a by-product of the phenol reaction with diazotized sulfapyridine, and the ether-soluble material extracted from sodium bicarbonate solution of sulfasalazine showed the same TLC characteristics (R_f 0.85), UV spectrum (in methanol, λ_{max} 243 and 326 nm), and principal IR bands (2825, 1620, 1600, 1380, 1355, 1270, 1135, 1082, 962, 784, and 750 cm^{-1}) (Fig. 4). The NMR spectrum (Fig. 5) of I in dimethyl sulfoxide showed a multiplet (6.9–8.2 ppm) characteristic of substituted aromatic rings. No carboxylic proton was detected.

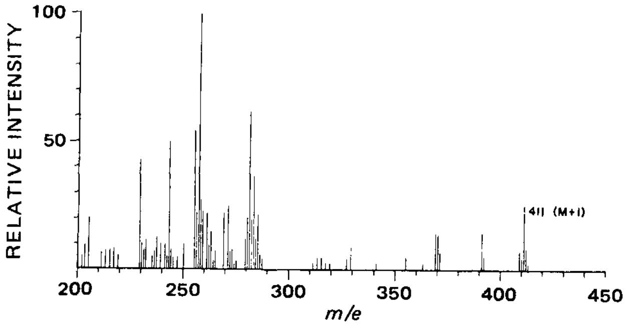
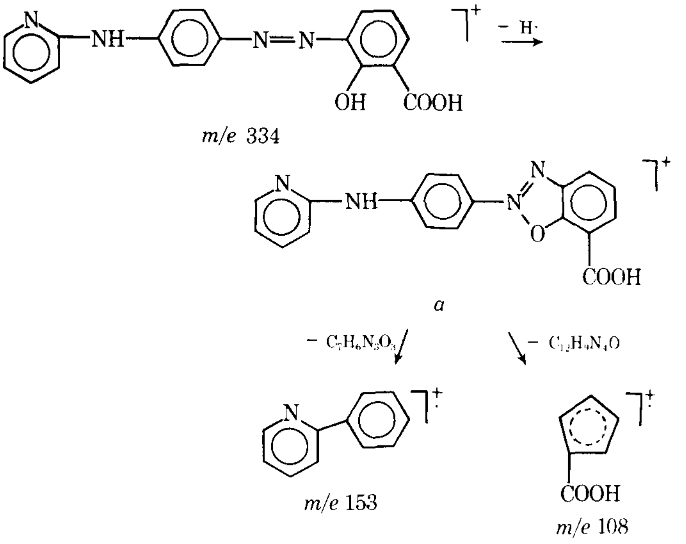
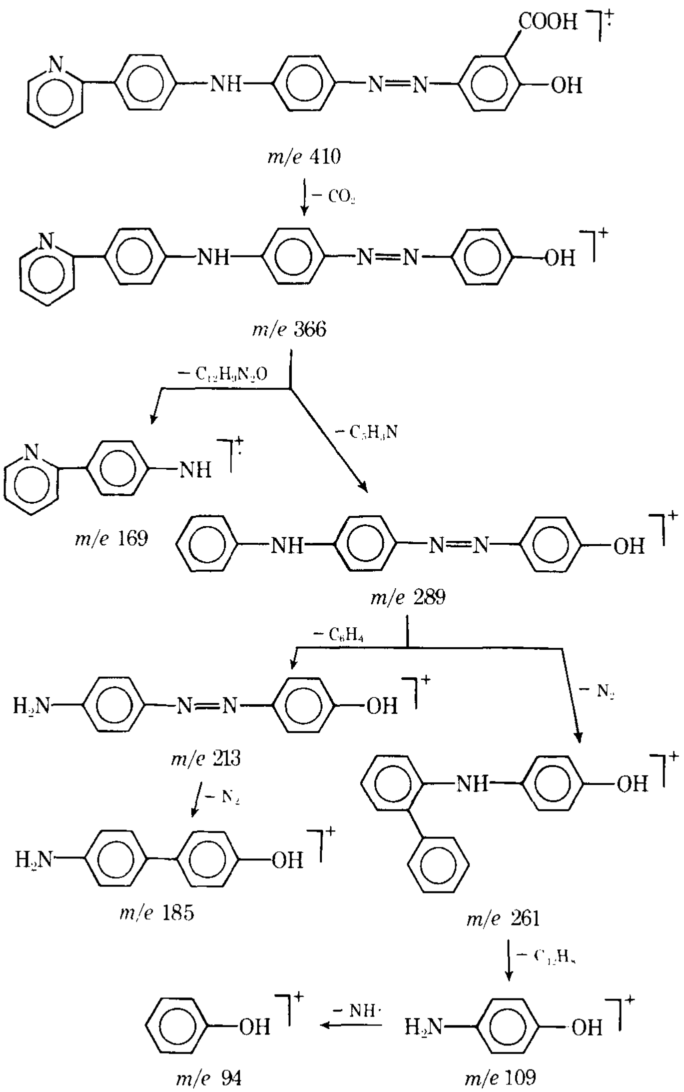


Figure 8—*Chemical-ionization mass spectrum of III.*

Impurity II was a pink color, and its electron-impact mass spectrum is shown in Fig. 6. This impurity was characterized as an isomer of sulfasalazine, 3-[[*p*-(2-pyridylsulfamoyl)phenyl]azo]salicylic acid. The mass spectrum of II was similar to that of sulfasalazine, except for two additional peaks at *m/e* 108 and 153. In addition, a peak at *m/e* 137 is of small abundance as compared to sulfasalazine fragmentation. The occurrence of fragment ions *m/e* 153 and 108 and a low abundance of *m/e* 137 ion can be best explained by possible electron-impact-induced rearrangement of *m/e* 334 ion of II to generate an intermediate (*a*), which can fragment as shown in Scheme III.



Scheme III



Scheme IV

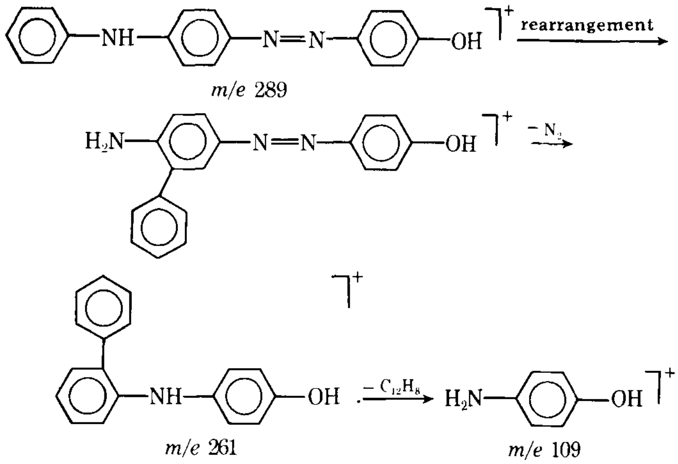
If an intermediate of type *a* through skeletal rearrangement can be postulated, then the cleavage of the azobenzene bond and the expulsion of carbon monoxide would lead to the formation of the five-membered ring species at *m/e* 108. This type of rearrangement would also explain the small abundance of the *m/e* 137 ion in II as compared to a relatively large abundent ion in sulfasalazine where a cyclic intermediate of type *a* is not possible. The UV spectrum of II in methanol was similar to that of the parent drug.

Impurity III isolated from the TLC plate was characterized as 5-[[*p*-[4-(2-pyridylanilino)]-*N*-phenyl]azo]salicylic acid. The electron-impact and chemical-ionization mass spectra are shown in Figs. 7 and 8. The molecular ion was at *m/e* 410. The proposed mass fragmentation pattern is shown in Scheme IV, and the computer-produced data are listed in Table I. The difference in 12 mass units between sulfasalazine (*m/e* 398) and III (*m/e* 410) accounts for the replacement of SO_2 in the former with the disubstituted aromatic ring, C_6H_4. Furthermore, no sulfur was found in III by a standard microanalytical method.

Table I—Mass Fragmentation Ions of III

Calculated Mass	Measured Mass	Formula	*m/e*
—	—	$C_{24}H_{18}N_4O_3$	410[a]
366.1481	366.1335	$C_{23}H_{18}N_4O$	366
289.1215	289.1100	$C_{18}H_{15}N_3O$	298
261.1153	261.1129	$C_{18}H_{15}NO$	261
213.0901	213.0905	$C_{12}H_{11}N_3O$	213
185.0839	185.0898	$C_{12}H_{11}NO$	185
169.0765	169.0764	$C_{11}H_9N_2$	169
109.0527	109.0526	C_6H_7NO	109
94.0418	94.0432	C_6H_6O	94

[a] Molecular ion from chemical ionization.



Scheme V

The appearance of an intense peak at *m/e* 109 in III can be explained by the promoted rearrangement of the diphenylamine group in the *m/e* 289 ion by electron impact and the subsequent loss of N=N and a biphenyl group to give *p*-hydroxyaniline (Scheme V). A similar type of rearrangement was proposed (11) for diaryl ethers. The absence or very low intensity of the *m/e* 109 peak in sulfasalazine and in components I and II supports the presence of a diphenylamine group in III.

The chemical-ionization mass spectrum of product A, which resulted from heated sulfapyridine and was isolated by TLC (zone A, R_f 0.0), is shown in Fig. 9. This material had molecular ion *m/e* 261 and was characterized as 4-amino-4′-(2-pyridyl)diphenylamine. Zone A material, when

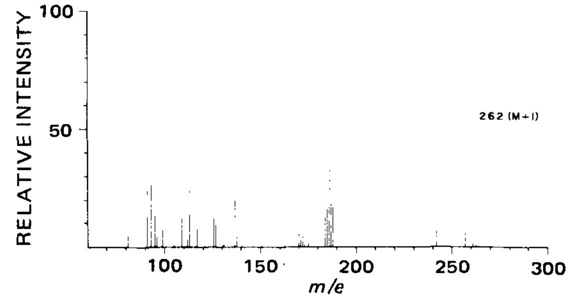


Figure 9—*Chemical-ionization mass spectrum of product A isolated from heated IV.*

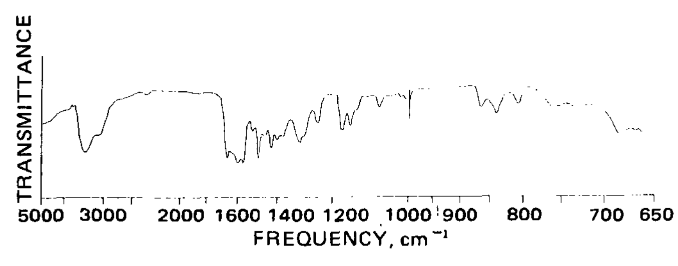


Figure 10—*IR spectrum of III.*

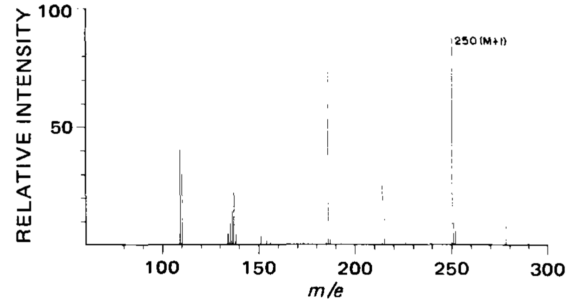


Figure 11—*Chemical-ionization mass spectrum of IV.*

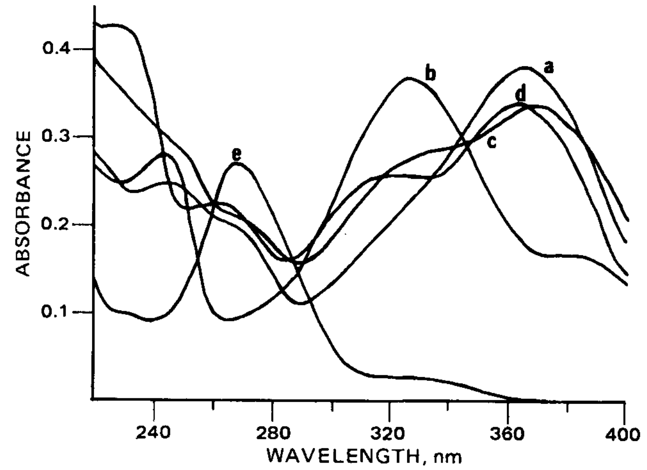
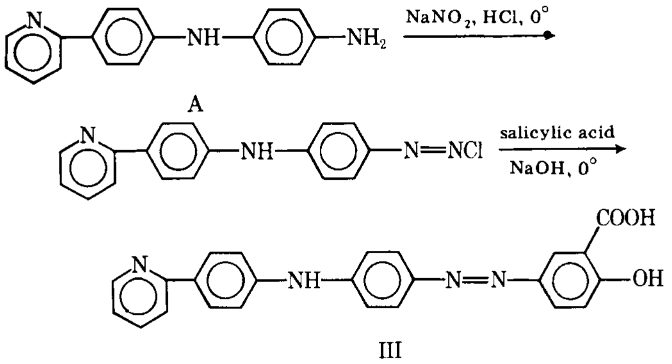


Figure 12—*UV spectra of sulfasalazine (a), I (b), II (c), III (d), and IV (e). Spectra were obtained in methanol* (10^{-5} M).

diazotized and coupled with salicylic acid, gave a product corresponding to impurity III. The sequence of synthetic steps is shown in Scheme VI. The synthesized material was found to be comparable with impurity III by TLC, mass spectrometry, and IR spectrophotometry (3400, 3100, 1660, 1595, 1485, 1429, 1308, 1249, 1168, 836, 758, and 683 cm^{-1}) (Fig. 10).

The mass spectrum of IV, isolated by TLC, is shown in Fig. 11 and was identified as sulfapyridine, *m/e* 249. The presence of sulfapyridine as an impurity in sulfasalazine was also confirmed by TLC. Impurity V, isolated from the TLC plate, was not fully characterized. The chemical-ionization mass spectrum showed a molecular ion, *m/e* 390.

Four lots of sulfasalazine from different manufacturers showed all impurities described; impurity III was the major one. The UV spectra of I–IV and sulfasalazine are shown in Fig. 12. Compounds I–III are the novel compounds, and their structures reveal some characteristic properties similar to sulfasalazine. Thus, the presence of these impurities in the drug will interfere in analytical methods such as UV spectroscopy, polarography, and nonaqueous titration. Identification of other impurities in sulfasalazine will be reported in the near future.



Scheme VI

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Solid-State Dispersions Employing Urethan

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Abstract ☐ The dissolution rates of a number of drug–urethan solid-state dispersion systems were studied. A marked enhancement of the initial dissolution rates of several poorly water-soluble drugs was found when they were incorporated into a urethan matrix by heat fusion. These differences were considerable when pure substances such as griseofulvin, hydrocortisone, chloramphenicol, and acetaminophen were compared to the urethan–drug solid dispersion. Physical mixtures of the medicinal agents with urethan also gave a marked increase in the amount of drug in solution, with the value in most cases being over one-half that of the solid-state dispersion. Data are given, comparing ultrafiltration with samples filtered through cotton, regarding drug content remaining in solution.

Keyphrases ☐ Solid-state dispersions—various drug–urethan systems, dissolution rates compared to pure drug substances and physical mixtures ☐ Dissolution rates—various drug–urethan solid-state dispersions, compared to pure drug substances and physical mixtures ☐ Urethan—solid-state dispersions with various drugs, dissolution rates compared to pure drug substances and physical mixtures

The application of relatively soluble and nontoxic substances as vehicles for difficultly soluble medicinal agents in the form of solid-state dispersions is well documented (1–7). Dialkylamides and polyethylene glycols were shown to increase absorption of steroids and griseofulvin, respectively (8, 9).

Drugs may be poorly soluble in GI fluids, leading to difficult and erratic absorption when administered orally. Absorption of insoluble medicaments is sometimes a function of the dissolution rate of these substances in aqueous solutions (10–12). Solid solutions or dispersions may function to increase dissolution rates by breaking