Contaminants in Commercial Dapsone

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Abstract □ Three contaminants commonly found in commercial dapsone were identified as 2,4′-sulfonylbis(benzeneamine), 4-(phenylsulfonyl)benzeneamine, and 4-(4′-chlorophenylsulfonyl)benzeneamine. The identities were based on the spectral analysis and unambiguous synthesis of each compound. Solvent-free, pure dapsone was prepared by recrystallization from chloroform.

Keyphrases □ Dapsone—commercial samples, contaminants identified □ Contaminants—identified in commercial samples of dapsone □ Antibacterial agents—dapsone, contaminants identified in commercial samples

Dapsone, 4,4′-sulfonylbis(benzeneamine), has long been used in chemotherapy. Its activity against streptococci (1), *Mycobacterium avium* (2), and *M. tuberculosis* (3) was first observed in the late 1930's. Since 1942, dapsone has been used primarily in the treatment of human leprosy (4), but it is also useful in the treatment of mycetoma (*Nocardia brasiliensis*) (5), actinomycosis (6), dermatitis herpetiformis (Duhring's disease) (7), and pyoderma gangrenosum (8).

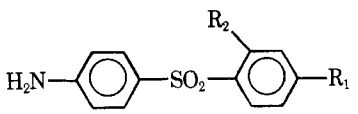
DISCUSSION

Although the antimalarial activity of dapsone was demonstrated in 1941 (9), it was not used in the treatment of malaria for the next 20 years because chloroquine was more effective. However, with the emergence of malaria strains resistant to chloroquine and other commonly used antimalarial drugs (10, 11), interest in dapsone as an antimalarial agent was revived (12, 13). Until recently, dapsone has been used as a daily prophylactic against falciparum malaria (14).

Considering the current interest in drug purity, commercial dapsone samples were examined by TLC; three contaminants[1] were consistently present. They were identified as 2,4′-sulfonylbis(benzeneamine) (I, R_f 0.62), 4-(phenylsulfonyl)benzeneamine (II, R_f 0.73), and 4-(4′-chlorophenylsulfonyl)benzeneamine (III, R_f 0.77). (The R_f of dapsone was 0.42.) As estimated by TLC, these contaminants constituted 2–9% of the samples. This paper describes the isolation and identification of these contaminants.

Recrystallization of commercial dapsone samples from chloroform enriched the subject contaminants in the mother liquor; through chromatography on silica gel, the various contaminants were separated. The isolated individual contaminants shared certain common chemical and spectral characteristics that are also common to dapsone. All four compounds responded positively to yield a similar color when sprayed with a diazotization reagent[2], suggesting the presence of an anilino group.

The IR spectra showed strong bands near 7.8 and 8.8 μm, indicative of a sulfone group in each molecule. The PMR spectra showed resonances only in the aryl region, apart from those due to exchangeable protons (*i.e.*, OH and NH_2) (Figs. 1–4). The spectra for I, II, and III (Figs. 2, 3, and 4, respectively) clearly showed a pair of doublets near δ 6.6 and 7.6 ppm with coupling constants of 8.8 ± 0.1 Hz. These characteristics are similar to those found in the spectrum for dapsone (Fig. 1) and are indicative of a 4-aminobenzenesulfonyl group, which is common to all four compounds.



I: R_1 = H, R_2 = NH_2
II: R_1 = R_2 = H
III: R_1 = Cl, R_2 = H

On the basis of mass spectral and elemental data, the molecular weight of I is 248, consistent with the empirical formula $C_{12}H_{12}N_2O_2S$. In addition to signals assigned the 4-aminobenzenesulfonyl group, the PMR spectrum of I (Fig. 2) showed four other protons in the aryl region. Analysis of the coupling constants (J = 1.8, 7.0, and 8.3 Hz) for the one-proton multiplet centered at δ 7.23 ppm suggested that this proton is coupled to two *ortho*- and one *meta*-protons. This arrangement is a 1,2-disubstituted benzene, which is substantiated by analysis of the three remaining aryl protons. The PMR spectrum of I also showed two broad overlapping singlets at δ 6.00 and 6.06 ppm, each representing two protons with both singlets exchangeable with deuterium oxide. These characteristics are consistent with those of two primary amino groups. Based on this evidence, I probably is 2,4′-sulfonylbis(benzeneamine).

According to the mass spectral and elemental results, the molecular weight of II is 233, which is in agreement with the empirical formula $C_{12}H_{11}N_2O_2S$. The PMR spectrum of II showed a broad singlet of two exchangeable protons at δ 6.14 ppm, a doublet of two protons at δ 6.63 (J = 8.9 Hz) ppm, a multiplet of five protons centered near δ 7.55 ppm, and a multiplet of two protons centered near δ 7.84 ppm. After subtraction of the 4-aminobenzenesulfonyl doublet from the δ 7.55-ppm multiplet that is complementary to the δ 6.63-ppm doublet, the remaining three protons in the δ 7.55-ppm group and the two protons in the δ 7.84-ppm group closely resemble the pattern of a benzenesulfonyl. This observation suggests that II is 4-(phenylsulfonyl)benzeneamine.

The elemental and mass spectral data for III are consistent with a molecular weight of 267 and the empirical formula $C_{12}H_{10}ClN_2O_2S$. The PMR spectrum for III (Fig. 4) showed only two exchangeable protons, appearing as a broad singlet at δ 6.20 ppm, and four doublets in the aryl region in agreement with two 1,4-disubstituted benzenes. Data gathered for III are consistent with those for 4-(4′-chlorophenylsulfonyl)benzeneamine.

For validation of the structures proposed for I–III, these three compounds were prepared by unambiguous syntheses. Compound I was prepared by treating 4-acetamidobenzenesulfinic acid with 2-bromonitrobenzene (15) to give 4-(2′-nitrophenylsulfonyl)benzeneacetamide (IV). Reduction (16, 17) of IV gave 4-(2′-aminophenylsulfonyl)benzeneacetamide (V), which, upon hydrolysis, yielded I.

Compound II was prepared in high yield by diazotization of I with sodium nitrite and sulfuric acid followed by heating (18). Compound III was prepared by the method of Richter and Frey (19), in which 4-acetamidobenzenesulfonyl chloride was treated with chlorobenzene in a

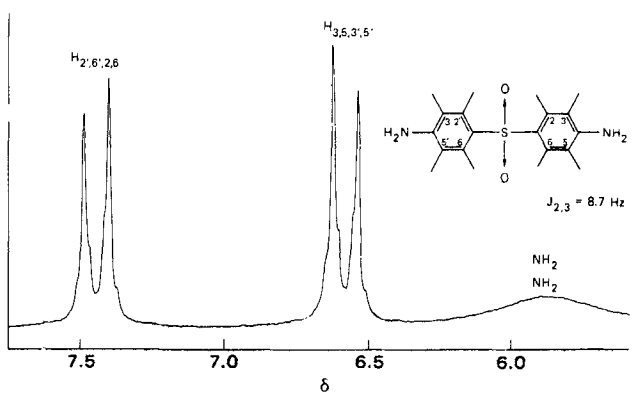


Figure 1—*PMR spectrum of dapsone.*

[1] Usually two trace impurities were found in addition to these three contaminants. Because of their very low concentrations—estimated at ≪0.1%—the trace impurities were not isolated and characterized. Dr. Robert Lofberg (personal communication), Walter Reed Army Institute of Research, identified one impurity as 4-(4′-hydroxyphenylsulfonyl)benzeneamine.

[2] The three contaminants and dapsone were separated on a silica plate. When sprayed with 1 *N* HCl, 2% $NaNO_2$, 3% sulfamic acid (all aqueous solutions), and 1% *N*-(1-naphthyl)ethylenediamine dihydrochloride in propylene glycol–water (1:2 v/v) in the order given, all four compounds responded positively to give pink spots.

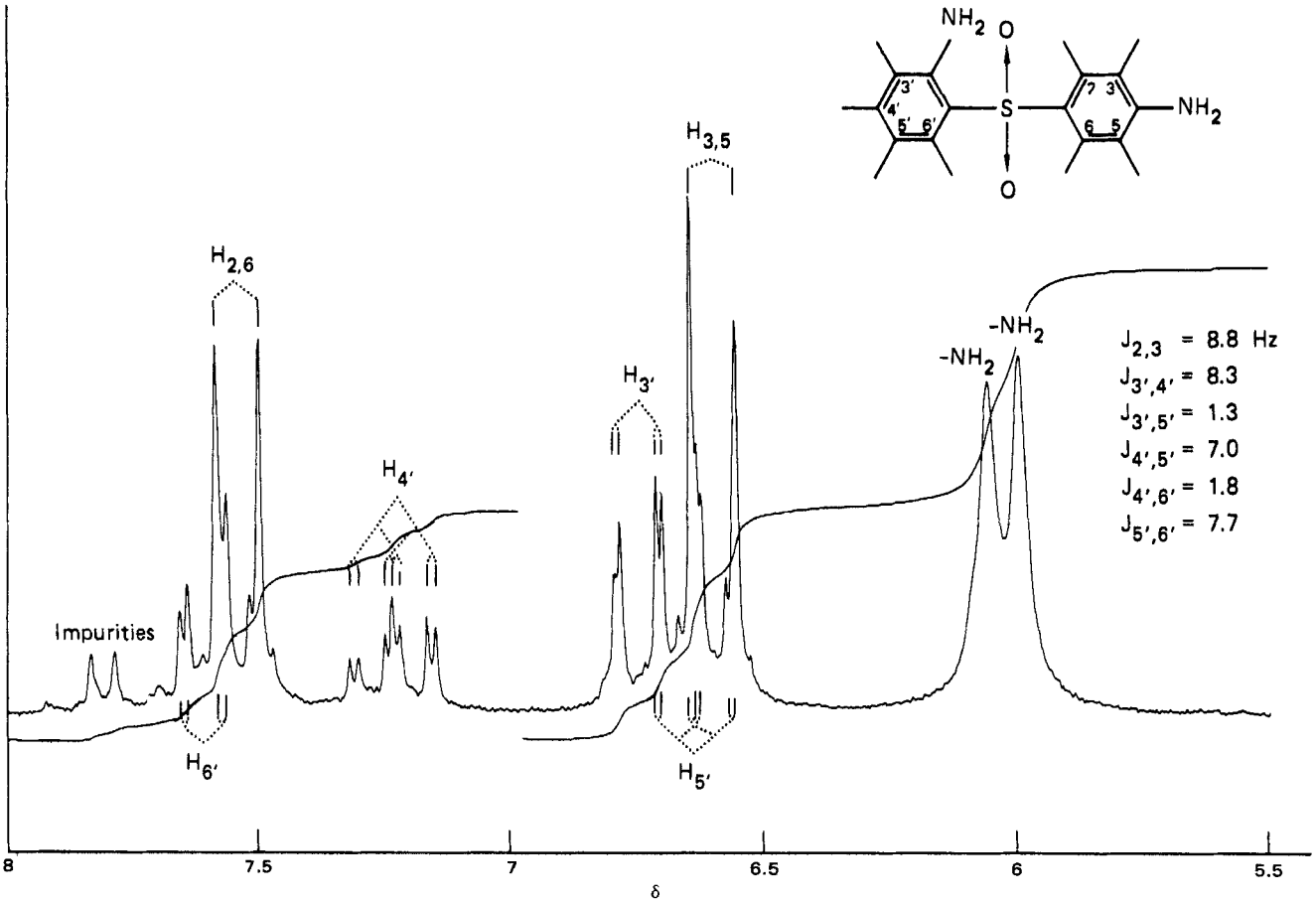


Figure 2—*PMR spectrum of contaminant I.*

Friedel–Crafts sulfonation followed by hydrolysis of the acetyl group.

Equivalence of the unambiguously synthesized materials to those isolated from commercial dapsone samples was established by IR, UV, PMR, TLC, GLC, and mixed melting-point comparisons.

The appearance of these impurities in the dapsone samples probably resulted from contaminations in the starting material. An established method for preparing dapsone is the coupling of 4-chloronitrobenzene with sodium 4-acetamidobenzenesulfonate, followed by reduction of the nitro group to the amine and hydrolysis of the acetamido group (20). The presence of 2-chloronitrobenzene, chlorobenzene, and 1,4-dichlorobenzene in the 4-chloronitrobenzene would have resulted in I, II, and III, respectively.

EXPERIMENTAL

IR spectra were determined as mineral oil mulls on a commercial spectrometer[3]. PMR spectra were recorded as dimethyl sulfoxide-d_6 solutions on a 100-MHz spectrometer[4]; chemical shifts were reported on a δ scale using an internal tetramethylsilane reference (δ 0.00 ppm). UV spectra were scanned as solutions in 95% ethanol on a recording spectrometer[5]. Melting points were obtained on a commercial apparatus[6]. TLC utilized silica plates developed with chloroform–ether–methanol (7:2:1 v/v).

GLC data were obtained for the trifluoroacetyl derivatives[7] on a gas chromatograph equipped with a flame-ionization detector. A 1.9-m × 2-mm i.d. glass column packed with 3% ultraphase SE-30 coated on 80–100-mesh Chromosorb W (HP)[8] was used. Temperatures were 250° (inlet), 200° (column), and 300° (detector); the carrier gas was nitrogen at 40 ml/min. All chemicals were reagent grade or the best quality available.

Recrystallization of Commercial Dapsone—Two grams of commercial dapsone[9] was dissolved in 500 ml of chloroform with reflux. The solution was filtered hot, and the filtrate was allowed to evaporate slowly to 200 ml on a steam bath. Then the solution was carefully set aside to allow crystal formation. The needles that formed were collected, washed with cold chloroform, and dried. From this material, 1.6 g of solvent-free dapsone was obtained. This material was chromatographically homogeneous, mp 177–178° [lit. (21) mp 175–176°][10].

The mother liquor from this recrystallization was evaporated to dryness, and the residue was used for isolation of the contaminants.

Isolation of I–III—The residue from the recrystallization mother liquor was redissolved in 10 ml of chloroform. The solution was applied onto 1-mm thick silica gel plates, which were developed in chloroform–ether–methanol (7:2:1 v/v). The resolved bands were located by 254-nm UV light and scraped from the plates. The respective bands from numerous plates were combined and extracted with methanol.

The respective methanol solutions were reduced to minimum volumes on a steam bath under a nitrogen stream, and the chromatographic-isolation procedure was repeated until all three contaminants isolated were chromatographically homogeneous. At this stage of purity, each contaminant was crystallized from hexane–methanol (10:1).

Compound I—mp 114–116° [lit. (22) mp 117°]; TLC R_f 0.62; GLC retention time, 3.0 min; *m/e* 248.

Anal.—Calc. for $C_{12}H_{12}N_2O_2S$: C, 58.04; H, 4.87; N, 11.28; S, 12.91. Found: C, 57.93; H, 4.94; N, 10.98; S, 12.71.

[3] Perkin-Elmer model 137.
[4] Varian HA-100.
[5] Cary model 15.
[6] Mel-Temp.
[7] Approximately 1 mg of each compound was treated with 0.3 ml of trifluoroacetic anhydride. The mixture was heated until a complete solution resulted, and excess trifluoroacetic anhydride and trifluoroacetic acid were evaporated with a stream of dried nitrogen. The residue was redissolved in 0.4 ml of benzene plus 1 drop of trifluoroacetic acid and then injected into the gas chromatograph (Micro-Tek 220).
[8] Johns-Manville, Celite Division, New York, N.Y.
[9] Lot 8L-2223 (typical of many Sterling Winthrop lots analyzed), Sterling Winthrop Research Institute, Rensselaer, N.Y.
[10] In this study, material recrystallized several times from 95% ethanol still contained the subject impurities.

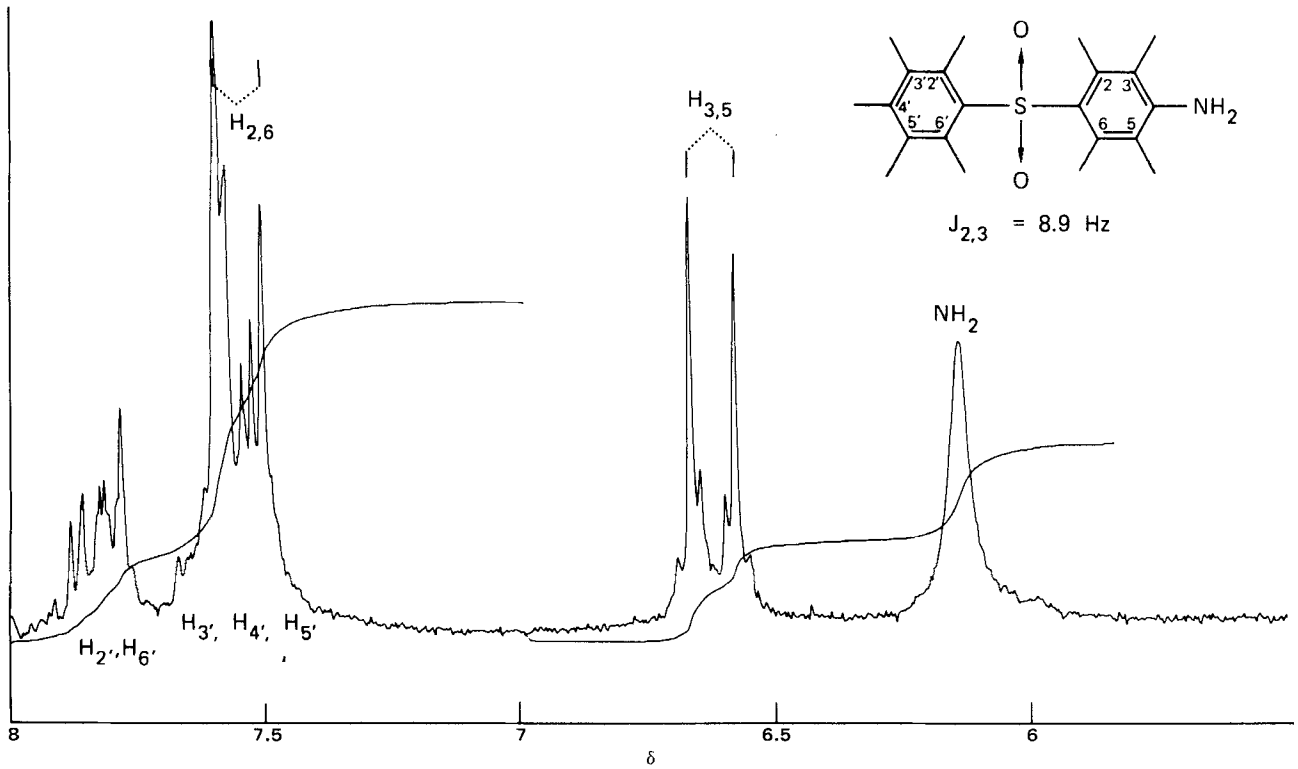


Figure 3—*PMR spectrum of contaminant II.*

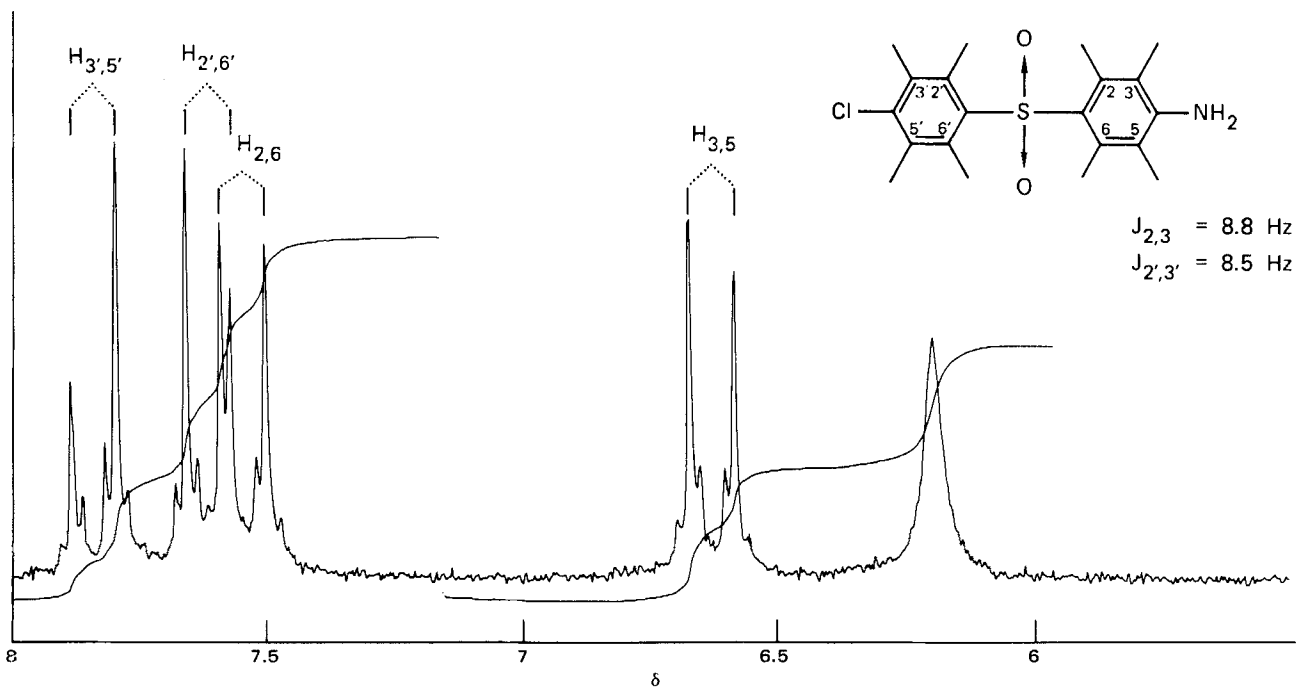


Figure 4—*PMR spectrum of contaminant III.*

Compound II—mp 167–170° [lit. (23) mp 172–173°]; TLC R_f 0.73; GLC retention time, 2.5 min; *m/e* 233.

Anal.—Calc. for $C_{12}H_{11}NO_2S$: C, 61.78; H, 4.75; N, 6.00; S, 13.72. Found: C, 61.50; H, 4.70; N, 5.97; S, 13.40.

Compound III—mp 186–188° [lit. (24) mp 187.8–188.4°]; TLC R_f 0.77; GLC retention time, 4.0 min; *m/e* 257.

Anal.—Calc. for $C_{12}H_{10}ClNO_2S$: C, 53.83; H, 3.76; Cl, 13.24; N, 5.23; S, 11.98. Found: C, 54.00; H, 3.80; Cl, 13.49; N, 5.36; S, 12.10.

Preparation of V—4-Acetamidobenzenesulfinic acid (2.6 g) was dissolved in 12 ml of methanol containing 1 g of sodium hydroxide. To this solution was added 20 ml of dimethylformamide containing 3.8 g of 2-bromonitrobenzene, and the resulting solution was refluxed (108°) for 1.5 hr. The methanol was distilled, and the remaining solution was refluxed (164°) for an additional 2.5 hr. After cooling, water was added to the dimethylformamide solution until precipitation was complete.

The precipitate was collected, washed with warm water followed by methanol, and air dried. The dried material consisted of 3.6 g of IV, mp 244–246°. Compound IV (2.0 g) was added to 60 ml of 6 *N* acetic acid (aqueous), and the mixture was heated to reflux. During the 1st hr of reflux, iron filings (1 g) were slowly added to the refluxing solution; after a total reflux time of 3 hr, the mixture was allowed to cool. After the insoluble material was removed by filtration, the filtrate was evaporated

to dryness, and the residue was extracted several times with a total of 300 ml of methanolic 0.1 *N* NaOH.

The insoluble matter was removed by filtration, and the filtrate was again evaporated to dryness. The residue was triturated several times with a total of 400 ml of water. The insoluble material was collected by filtration and redissolved in 30 ml of methanol, and the solution was filtered. The clear filtrate was diluted with 150 ml of water to cause the complete precipitation of V. The white precipitate was collected and dried; the material weighed 1.9 g (80% yield, mp 97–99°).

Compound I—Compound V (500 mg) and 100 ml of ethanol–concentrated hydrochloric acid (4:1 v/v) were refluxed for 2 hr. The mixture was filtered, and the pH of the filtrate was adjusted to ~7.0 with dilute sodium hydroxide. The ethanol was removed by evaporation, and the precipitate that resulted was collected and dried. The material weighed 440 mg (88% yield, mp 115–117°).

Anal.—Calc. for $C_{12}H_{12}N_2O_2S$: C, 58.04; H, 4.87; N, 11.28; S, 12.91. Found: C, 58.14; H, 4.87; N, 11.25; S, 12.88.

Compound II—Compound I (500 mg) was dissolved in 100 ml of ethanol–benzene (4:1 v/v) by warming on a steam bath. To this solution was added 0.5 ml of concentrated sulfuric acid, followed by 350 mg of sodium nitrite—in portions—as rapidly as the violent reaction would permit. After the reaction moderated, the solution was heated to reflux for 2 hr. The reaction was allowed to cool, and then the insoluble matter was removed by filtration.

The filtrate was neutralized with sodium hydroxide and evaporated to dryness. The residue was triturated twice with a total of 200 ml of water, and the insoluble matter was collected by filtration. The solid matter was exhaustively extracted with 20 ml of ethanol; the insolubles were removed by filtration. The ethanol filtrate was diluted with 80 ml of water to cause the complete precipitation of II, which was collected by filtration and dried. The material weighed 320 mg (64% yield, mp 169–172°).

Anal.—Calc. for $C_{12}H_{11}NO_2S$: C, 61.78; H, 4.75; N, 6.00; S, 13.72. Found: C, 62.04; H, 4.76; N, 5.81; S, 13.48.

Compound III—4-Acetamidobenzenesulfonyl chloride (1 g) and aluminum chloride (3 g) in 25 ml of chlorobenzene were stirred for 2 hr at 140°. The mixture was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in 50 ml of methanol; the insolubles were removed by filtration. Concentrated hydrochloric acid (10 ml) was added to the filtrate, and the resulting solution was refluxed for 2 hr.

The mixture was allowed to cool, and then the solution was filtered. The filtrate was neutralized with dilute sodium hydroxide and evaporated to dryness. The residue was triturated twice with a total volume of 20 ml of water. The water-insoluble matter was recrystallized from aqueous methanol to yield III, mp 187–189°.

Anal.—Calc. for $C_{12}H_{10}ClNO_2S$: C, 53.83; H, 3.76; Cl, 13.24; N, 5.23; S, 11.98. Found: C, 53.69; H, 3.83; Cl, 13.54; N, 5.32; S, 12.18.

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