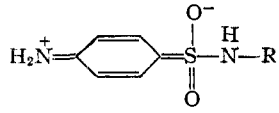
[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

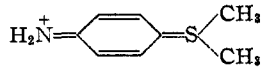
# p-Aminophenyldimethylsulfonium β-Naphthalenesulfonate and Antibacterial Activity[1]

BY PHILIP E. WILCOX,[2] JOHN H. OWEN[3] AND MARK A. STAHMANN

Kumler and Daniels[4] have proposed that the anti-*p*-aminobenzoic acid activity of sulfonamides is essentially due to the contribution of the resonant form



to the structure of these compounds. Accordingly it might be expected that a compound such as a *p*-aminophenyldimethylsulfonium salt would exhibit activity of the sulfonamide type since the positive sulfur should readily accept an electron pair from the ring. This would result in the resonant form



which would make a large contribution to the structure of the molecule. Furthermore, from the steric point of view, the *p*-aminophenyldimethylsulfonium cation resembles *p*-aminobenzoic acid and might possess anti-*p*-aminobenzoic acid activity in accordance with the general theory of metabolic antagonists.

Another theory of sulfonamide activity, which has been proposed by Bell and Roblin,[5] and Klotz,[6] correlates the activity of these compounds with their respective acid dissociation constants. From this point of view, it would not be expected that *p*-aminophenyldimethylsulfonium salts would show activity of the sulfonamide type.

In any case, sulfonium compounds are of interest entirely apart from their possible relation to sulfonamides since certain sulfonium compounds do have remarkable physiological activities.[7,8]

Although a great many variations of the sulfonamide structure have been studied, derivatives in which the sulfur appears in the form of a sulfonium group have not been reported. This communication describes the preparation and preliminary microbiological assay of such a compound, *p*-aminophenyldimethylsulfonium β-naphthalenesulfonate. The results furnish supplementary data on the question of the chemical structures which are essential for antibacterial activity of the sulfonamide type.

Many sulfonium compounds are unstable because of the ease with which they undergo dismutation and dealkylation or dearylation. Therefore, it was decided that the synthesis would have the greatest chance for success if the final step was the catalytic reduction under mild conditions of a stable *p*-nitrophenyldimethylsulfonium salt. Preliminary experiments on the hydrogenation of *p*-nitrophenyldimethylsulfonium methyl sulfate with palladium on activated carbon showed that the compound must be reduced in acid solution in order to prevent excessive reduction with the absorption of more than three molar equivalents of hydrogen. On the other hand, in acid solution never more than 80% of the theoretical amount of hydrogen was absorbed even though the catalyst was still active. This reduced solution proved to be quite unstable. The rather insoluble picrate could be isolated if picric acid was added immediately, but attempts to isolate other salts more suitable for antibacterial testing gave only mixtures or oils.

It was found, however, that a more stable solution could be obtained if the reduction was carried out in methanol–water in the presence of an excess of β-naphthalenesulfonic acid. When ether was added to this solution, the desired *p*-aminophenyldimethylsulfonium β-naphthalenesulfonate separated as long needles. Since the β-naphthalenesulfonate anion proved to have no activity in the microbiological tests, this salt was satisfactory for testing.

The structure assigned to the product, namely, *p*-aminophenyldimethylsulfonium β-naphthalenesulfonate, is based on the elementary analysis, the method of synthesis, the fact that the compound is quite soluble in polar solvents, but insoluble in non-polar solvents, and the fact that it gives a strong positive test for a free, primary amino group with glutaconic aldehyde. The instability of some of the sulfonium salts and the greater stability of the same cation in other salts is in conformity with the observations of other investigators, particularly those of Ray and Levine.[9]

*p*-Aminophenyldimethylsulfonium β-naphthalenesulfonate was tested for anti-*p*-aminobenzoic acid activity against *Staphylococcus aureus* H in a medium which has been described by McIlwain.[10] The compounds involved in the tests were dissolved in sterile water and added aseptically to the sterile medium just before inoculation. Each

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(4) Kumler and Daniels, THIS JOURNAL, **65**, 2190 (1943).

(5) Bell and Roblin, *ibid.*, **64**, 2905 (1942).

(6) Klotz, *ibid.*, **66**, 459 (1944).

(7) Kuhn, Bielig and Dann, *Ber.*, **73B**, 1080 (1940).

(8) Gilman and Philips, *Science*, **103**, 409 (1946).

(9) Ray and Levine, *J. Org. Chem.*, **2**, 267 (1937).

(10) McIlwain, *Brit. J. Exptl. Path.*, **23**, 95 (1942).

tube was inoculated with one drop of a 24-hour culture which had been diluted thirty times. The *p*H of the medium was 6.9 and, after twenty-four hours of incubation at 37°, the growth was determined by measuring the turbidity with an Evelyn colorimeter. For comparison, sulfanilamide was tested in parallel with the sulfonium salt. The activity of each compound was also tested in the presence of *p*-aminobenzoic acid at concentrations of 10 micrograms and 200 micrograms per milliliter.

TABLE I

THE ANTIBACTERIAL ACTIVITY OF *p*-AMINOPHENYLDIMETHYLSULFONIUM β-NAPHTHALENESULFONATE (I), SULFANILAMIDE (II), AND THE EFFECT OF *p*-AMINOBENZOIC ACID

| Compound I or II | *p*-Aminobenzoic acid micrograms/ml. | Bacterial growth, expresesd in Evelyn units in the presence of I or II at the following concentrations, and *p*-aminobenzoic acid Micrograms per ml. 1000 | 500 | 250 | 125 | 62.5 | 31.7 | 0 |
|---|---|---|---|---|---|---|---|---|
| I | 0 | 0 | 0 | 5 | 11 | 25 | 28 | 30 |
| I | 10 | 0 | 4 | 13 | 15 | 22 | 24 | 32 |
| I | 200 | 0 | 13 | 18 | 25 | 25 | 26 | 29 |
| II | 0 | 0 | 0 | 0 | 1 | 1 | 12 | 30 |
| II | 10 | 8 | 12 | 19 | 23 | 26 | 28 | 29 |
| II | 200 | 8 | 15 | 22 | 25 | 26 | 28 | 30 |

The results of representative tests are presented in Table I. This table shows that sulfanilamide produced almost complete inhibition of growth at a concentration of 62.5 micrograms per ml., and that the addition of either 10 or 200 micrograms of *p*-aminobenzoic acid per ml. allowed almost full growth at 250 micrograms and only partial growth at 1000 micrograms of sulfanilamide per ml. *p*-Aminophenyldimethylsulfonium β-naphthalenesulfonate produced complete inhibition of growth at 500 micrograms per ml. and partial growth at 125 micrograms per ml. The addition of *p*-aminobenzoic acid allowed almost full growth at 125 and partial growth at 500 micrograms per ml. Sodium β-naphthalenesulfonate showed no antibacterial action at a concentration of 2000 micrograms per ml.

These results show that the *p*-aminophenyldimethylsulfonium ion has a low order of antibacterial activity and that this activity is slightly reduced by *p*-aminobenzoic acid. Since it is probable that this ion possesses a resonant form with a quinoidal structure and a positive nitrogen, its low activity indicates that such a resonant form is not sufficient for high action of the sulfonamide type. Apparently, for high anti-*p*-aminobenzoic acid activity, it is necessary to have a negative group comparable to the carboxylate group in the position para to the amino group. However, it must be recognized that the cationic nature of the sulfonium ion may prevent the compound from reaching the points where it could act against a bacterium.

Although sulfonium compounds with a long alkyl chain have high antibacterial activity, the aryl sulfonium group in itself has but a low order of antibacterial activity.[7] In this connection, Freedlander and French[11] have recently shown that methyldiphenylsulfonium nitrate shows no antibacterial activity against *E. coli*, *Staph. aureus*, *B. proteus*, or the tubercle bacillus. Our results would suggest that the introduction of a para amino group into an aromatic sulfonium salt increases the antibacterial activity.

## Experimental

**Di-*p*-nitrophenyl Disulfide.**—This compound was prepared according to the method described for the ortho analog by Bogert and Stull.[12]

***p*-Nitrophenylmethyl Sulfide.**—The disulfide was converted into *p*-nitrophenylmethyl sulfide, according to Brand,[13] in a yield of 61% by the reduction of di-*p*-nitrophenyl disulfide with alkaline sodium sulfide and subsequent direct methylation with methyl sulfate. The product melted sharply at 72.0 to 72.5° in agreement with the literature.

***p*-Nitrophenyldimethylsulfonium Methyl Sulfate and Picrate.**—*p*-Nitrophenylmethyl sulfide was methylated with methyl sulfate according to the method of Brand and Stallmann.[14] The product was recrystallized once from hot methanol and again by the addition of ethyl ether to a methanol solution to give a yield of 84%, m. p. 157.0 to 158.5° (dec.). The picrate was produced in a yield of 95% by the addition of a saturated solution of sodium picrate to an aqueous solution, m. p. 135° to 136° as compared to 137° as reported by Baker and Moffett.[15]

***p*-Aminophenyldimethylsulfonium Picrate.**—Palladium catalyst on activated carbon, prepared according to Hartung,[16] was used for the hydrogenation of *p*-nitrophenyldimethylsulfonium methyl sulfate (3.0 g., 10 millimoles) dissolved in 100 ml. of 90% methanol which contained 20 millimoles of sulfuric acid. The hydrogenation stopped abruptly after 24 millimoles of hydrogen had been absorbed (80% of theory). When this solution is allowed to stand, it became yellow and no crystalline sulfonium salt could be isolated. However, when 3.0 g. (13 millimoles) of picric acid in 100 ml. of water was added immediately to the filtered solution, 3.2 g. (79% yield) of orange prisms separated, m. p. 152 to 153°.

*Anal.* Calcd. for $C_8H_{12}NS \cdot C_6H_2N_3O_7 \cdot H_2O$: C, 42.0; H, 4.0; N, 14.0; S, 8.0. Found: C, 42.3, 42.3; H, 3.8, 3.7; N, 14.1, 14.1; S, 7.9.

When a methanol solution of this picrate was refluxed for a few hours, long needles of some different, much less soluble picrate separated. This derived compound melted at 165.0 to 165.5° and had an elementary analysis (C, 43.6; H, 3.1; N, 14.5) which indicated that the conversion may have been a reduction.

***p*-Aminophenyldimethylsulfonium β-Naphthalenesulfonate.**—*p*-Nitrophenyldimethylsulfonium methyl sulfate (4.25 g., 14 millimoles) and β-naphthalenesulfonic acid (3.60 g., 17 millimoles) were dissolved in 75 ml. of methanol containing 5% water, and the solution was hydrogenated with palladium catalyst on activated carbon. A total of 30 millimoles of hydrogen was absorbed or 72% of theory. The slightly yellow solution was filtered and 100 ml. of ethyl ether was added immediately. After cooling at 0° for two hours, 3.9 g. (68% yield) of nearly colorless needles of the monohydrate were collected. The product was recrystallized by dissolving it in 120 ml. of absolute methanol, removing the insoluble material by filtration, adding 250 ml. of 30–60° ligroin, and cooling at 0°. The final yield of material was 3.1 g. or 54%, m. p.

(11) Freedlander and French, *Proc. Soc. Expll. Biol. Med.*, **63**, 319 (1946).

(12) Bogert and Stull, "Organic Syntheses," Col. Vol. I, John Wiley and Sons, Inc,. New York, N. Y., 1941, p. 220.

(13) Brand, *Ber.*, **42**, 3463 (1909).

(14) Brand and Stallmann, *ibid.*, **54**, 1578 (1921).

(15) Baker and Moffett, *J. Chem. Soc.*, 1722 (1930).

(16) Hartung, THIS JOURNAL, **50**, 3370 (1928).

130° (dec.). The product was dried at 80° for four hours for analysis.

*Anal.* Calcd. for $C_8H_{12}NS \cdot C_{10}H_7O_3S \cdot H_2O$: C, 56.9; H, 5.5; N, 3.7; S, 16.9. Found: C, 56.9, 56.7; H, 5.6, 5.8; N, 3.6, 3.7; S, 16.7.

Further recrystallization did not raise the melting point, but seemed to cause deterioration. The salt was soluble in water, methanol, ethanol, and acetic acid, but was insoluble in ether, chloroform, and benzene. Aqueous base causes the salt to decompose with the formation of an orange color and water insoluble material.

**Test for Free Primary Aromatic Amino Group.**—Both the *p*-aminophenyldimethylsulfonium picrate and β-naphthalenesulfonate yielded a color characteristic of substances containing a primary aromatic amino group when treated with 4-pyridylpyridinium chloride hydrochloride.[17] On the other hand, the second picrate derived from the initial picrate by heating in methanol gave a completely negative test, indicating that the primary amino group had been destroyed.

(17) Feigl, "Qualitative Analysis by Means of Spot Tests," Nordemann Publishing Company, New York, N. Y., 1937, p. 283.

## Summary

A new compound, *p*-aminophenyldimethylsulfonium β-naphthalenesulfonate, has been synthesized. The picrate of the sulfonium cation has also been prepared.

Microbiological tests showed that the sulfonium sulfonate has a low order of antibacterial activity which is slightly reversed by *p*-aminobenzoic acid.

The activity of the sulfonium salts has been related to the problem of the mode of action of sulfonamides.

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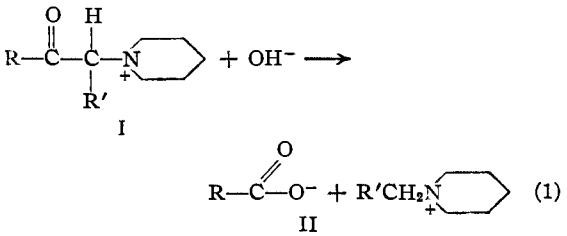
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

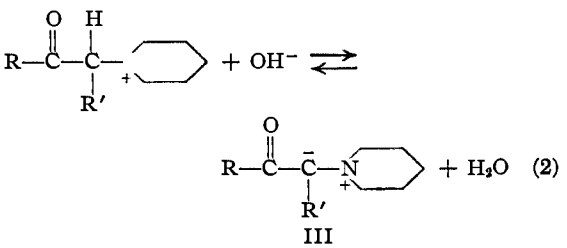
# Mechanism of the Alkaline Cleavage of β-Ketoalkylpyridinium Salts[1]

BY RALPH G. PEARSON AND ROBERT L. DILLON

The alkaline hydrolysis of β-ketoalkylpyridinium salts to give the corresponding acid and a simpler alkylpyridinium salt[2] according to equation (1) was studied in some detail by Kröhnke.[3]

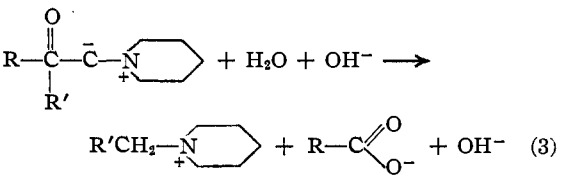


He showed by kinetic methods that in the presence of excess alkali the reaction was first order and that the stronger the acid, II, formed by the reaction, the greater the rate of hydrolysis. Furthermore, he showed[4] that the initial reaction of the cation, I, with alkali is a typical acid–base equilibrium in which the cation acts as an acid

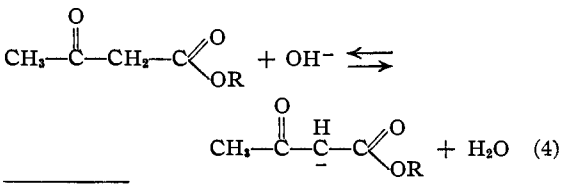


He proposed the name "enol-betaine" for the isolable compound III, writing its structure with the double bond between the two carbon atoms. The enol-betaines in general are highly colored, soluble in organic solvents and not stable in air.

The mechanism that he assumed for the cleavage included the rapid establishment of equilibrium (2) and the subsequent rate-determining reaction of the enol-betaine with excess hydroxide ion and water. If reaction (2) goes well to the right then pseudo first order kinetics will be obtained since hydroxide ion is not used up in (3). However, Kröhnke worked with only one set of concentrations for all of the compounds which he investigated and it can readily be shown that there are several other mechanisms which will turn out to be first order in excess alkali.



For example in the formally similar hydrolysis of acetoacetic ester by dilute alkali, Goldschmidt and Oslan[5] showed that the reaction was first order under a variety of conditions and the mechanism included an acid-base equilibrium and a rate-determining reaction between hydroxide ion and the unneutralized acetoacetic ester



(1) Based on the M.S. thesis of Robert L. Dillon.

(2) (a) Bamberger, *Ber.*, **20**, 3344 (1887); (b) Babcock, Nakamura and Fuson, THIS JOURNAL, **54**, 4407 (1932); (c) Babcock, and Fuson, *ibid.*, **55**, 2946 (1933).

(3) Kröhnke, *Ber.*, **70B**, 864 (1937).

(4) Kröhnke, *ibid.*, **66B**, 604 (1933); **68B**, 1177 (1935).

(5) Goldschmidt and Oslan, *ibid.*, **32**, 3390 (1899); **33**, 1140 (1900).