

### A STUDY OF THE MOLISCH TEST FOR CARBOHYDRATES<sup>1</sup>

Russell S. Waters and Arthur M. Pardee  
University of South Dakota

Definite proof of the mechanism of the Molisch reaction and of the structure of the red-violet colored compound formed during the reaction would be valuable because it might lead to the discovery of a method to use the Molisch reaction for quantitative determinations of carbohydrates.

The first attempt to explain the reaction was made by Brederick, in 1931 and 1932, at the University of Liepzig.<sup>2</sup> Beginning with 5-hydroxymethyl-furfural and alpha-naphthol, he synthesized a colorless compound, which when analyzed, showed an empirical formula of  $C_{26}H_{20}O_4$ . When this compound was treated with concentrated sulfuric acid, he obtained the dyestuff  $C_{26}H_{18}O_7S$ . And when the mixture was heated, he obtained another dyestuff  $C_{26}H_{18}O_{10}S_2$ .

This led him to the conclusion that the reaction occurred in three steps. First, the carbohydrate was dehydrated to furfural or a substituted furfural, which under the influence of concentrated sulfuric acid, coupled with two molecules of alpha-naphthol giving the intermediate colorless compound shown in Figure 1.

Then he believed this compound was oxidized to the quinoidal structure and sulfonated in the position ortho to the remaining phenol group as shown in Figure 2. Further heating would sulfonate the 7 position.

Brederick's reasoning is logical but his proof is not definite. The purpose of this study was to show what position or positions the sulfonic acid groups occupied, assuming that some sort of coupling did take place. To do this, as many as could be obtained of the alpha-naphthol sulfonic acids were separately mixed with furfural and treated with a dehydrating agent to see which one would give the Molisch

1. Condensed from the thesis submitted by Waters in partial fulfillment of the requirements for the degree of master of arts.

2. Ber., 65, 1110 (1932).

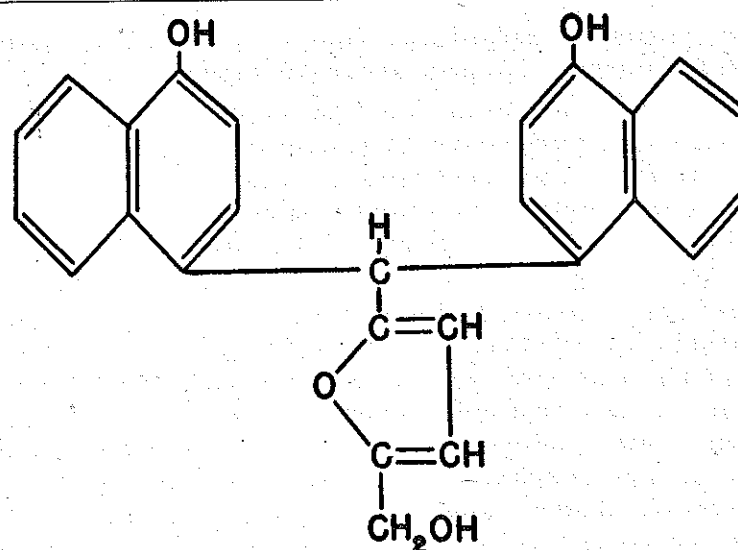


Fig. 1. Structure of the intermediate colorless compound as postulated by Brederick.

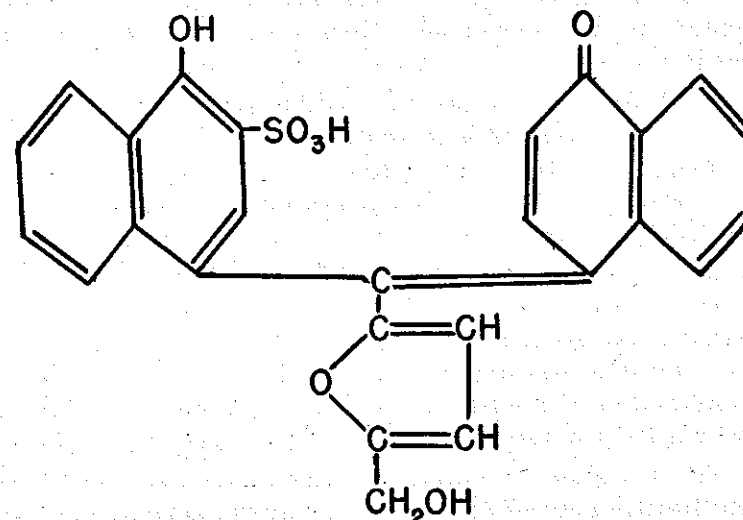


Fig. 2. Structure of the final dyestuff as postulated by Brederick.

test. Syrupy phosphoric acid was used instead of concentrated sulfuric acid to prevent further sulfonation.

The sodium salts of the alpha-naphthol 2-, 4-, and 5-sulfonic acids were bought and purified by recrystallization. The 6-, 7-, and 8-isomers were prepared in the laboratory also in the form of their sodium salts. Attempts to make the alpha-naphthol-3-sulfonic acid was not successful.

The alpha-naphthol 6- and 7-sulfonic acids (sodium salts) were prepared by the well known Bucherer reaction. In making the 6-sulfonate, 50 grams of alpha-naphthylamine-6-sulfonic acid were heated on the steam bath with 400 grams of 40% aqueous sodium bisulfite and 200 ml. of water. The heating was continued for 45 minutes after solution was complete. The solution was then made basic to phenolphthalein, with 6 N NaOH and boiled until no more ammonia was given off (about five hours). The precipitated sodium salt of alpha-naphthol-6-sulfonic acid was filtered off and recrystallized from an acid solution.

The preparation of the 7-sulfonate was the same as for the 6- except that the first reaction required three hours instead of one hour and the intermediate bisulfite addition product was not as soluble. More water was added to dissolve it.

The properties of the two products were very similar. They were very soluble in water but insoluble in an acid solution. They both gave violet colors when mixed with  $\text{FeCl}_3$  solution. They decomposed on heating, giving off strong disagreeable odors.

It was found that alpha-naphthol-8-sulfonic acid could not be made by the Bucherer reaction without the use of an autoclave so attention was turned to the diazotization reaction. However, during the final hydrolysis of the reaction, the sulfonic acid seemed to be converted to its anhydride, in which form it was isolated in a high degree of purity.

Fifty grams of alpha-naphthylamine-8-sulfonic acid were dissolved in 800 ml. of 50% sulfuric acid by heating. The solution was cooled to 0° C. and held at that temperature while sodium nitrite solution (20 grams in 50 ml.

water) was added slowly (45 minutes) until a test with starch iodide paper showed the presence of excess nitrous acid.

After standing overnight, the solution was refluxed vigorously for one and one-half hours to hydrolyze the diazonium salt. A precipitate was formed which was identified as the anhydride of alpha-naphthol-8-sulfonic acid (naphthosulton). It was extracted with chloroform and recrystallized from benzene. This precipitate was unexpected but proved to be very advantageous since it was easily purified and easily hydrolyzed in 3 N sodium hydroxide to give a pure product of sodium alpha-naphthol-8-sulfonate.

The final product was identified by the transient deep green color produced when it was treated with ferric chloride solution. It was not nearly as soluble as the other isomers.

All of the alpha-naphthol sulfonic acids (sodium salts) which were used were white needles when crystallized from water. They were all insoluble in organic solvents, slightly soluble in alcohol and all except the 8-sulfonic acid were very soluble in water. They all gave characteristic color tests with ferric chloride. The 2-, 4-, and 5-sulfonates gave transient blue colors, the 6- and 7- gave violet colors and the 8- gave a transient deep green color.

Each one of the sodium alpha-naphthol sulfonates was treated with furfural and phosphoric acid in the following manner and any color changes were noted. Two drops of a 20% solution of the sulfonate were added to 5 ml. of furfural and this mixture was shaken with 3 ml. of 85% phosphoric acid in a test tube.

A summary of the colors obtained in these tests appears in Table I. The depth of color was found to be dependent on the concentration of furfural. A 1% solution of furfural in ethanol was found to give best results. In some cases, higher concentrations produced charring.

In the regular Molisch test a reddish-violet color is formed. Similar colors were obtained with both the 2- and 4-naphthol sulfonic acids. It is possible then that in the

Molisch test either one or both of these two sulfonic acids are formed. If the 2-acid is formed, coupling probably takes place in the 4 position as Bredereck suggested. If the 4-acid is formed, the coupling probably occurs at the 2 position.

TABLE I.

A summary of the results obtained by mixing 2 drops of a sodium alpha-naphthol sulfonate, 5 ml. of furfural and 3 ml. of 85% phosphoric acid

Position of sulfonate group	Color
2	red-violet
4	red-violet
5	blue
6	yellow
7	yellow
8	blue

## BIBLIOGRAPHY

- Bredereck, Hellmut, **Ber.** **64 B**, 2856-2859 (1931).  
Bredereck, Hellmut, **Ber.** **65 B**, 1110-1113 (1932).  
Bucherer, Hans, **J. prakt. Chem.**, (2) **69**, 49 (1904) and  
(2) **70**, 345 (1904).  
Molisch, Hans, **Monatsh.**, **VII**, 198 (1896).