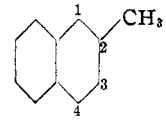
[CONTRIBUTION FROM THE ABBOTT LABORATORIES]

The Antihemorrhagic Activity of Sulfonated Derivatives of 2-Methylnaphthalene

BY M. B. MOORE

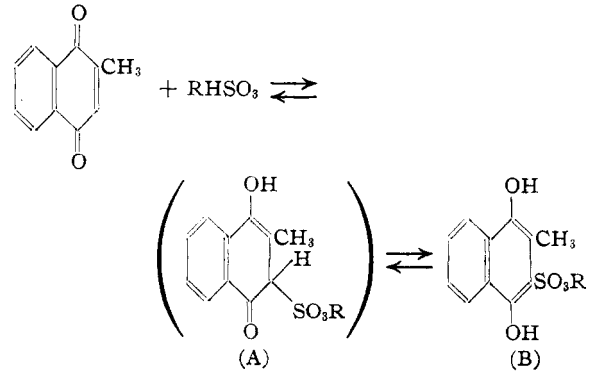
Since the discovery of the high antihemorrhagic (Vitamin K) activity of 2-methyl-1,4-naphthoquinone and of the corresponding hydroquinone, many related compounds have been prepared and tested. From the literature it appears that maximal activity is attained in the 2-methyl-1,4-naphthoquinone structure or its hydroquinone or quinhydrone.[1] The methyl group in the 2-position is of prime importance. Unsubstituted 1,4-naphthoquinone possesses only slight activity, of the order of a thousandth of that of the 2-methyl derivative, and the activity of 2-ethyl-1,4-naphthoquinone is of approximately the same low order.[2,3]

The substituents in the 1- and 4-positions necessary for maximum activity are not sharply limited. The quinone and hydroquinone possess approximately equal antihemorrhagic properties, as does 4-amino-2-methyl-1-naphthol[4]; and the activity of 1-amino-2-methyl-4-hydroxynaphthalene is only slightlv less.[4]

Maintaining the convention  for numbering, it may be said that the substitution of various groups at position 3 may or may not lessen the activity (on a molar basis), but is not known to enhance it in any case. Thus the natural K vitamins are no more active on a molar basis than 2-methyl-1,4-naphthoquinone. On the other hand, many groups in the 3-position may materially decrease the antihemorrhagic activity of the molecule: 2,3-dimethyl-1,4-naphthoquinone, 2-methyl-3-hydroxy-1,4-naphthoquinone (phthiocol) and 2-methyl-3-amino-1,4-naphthoquinone are much less active than the parent compound.[2,5,6] The 2,3-oxide of 2-methyl-1,4-naphthoquinone is somewhat less active than 2-methyl-1,4-naphthoquinone.[7] As an example of a different type, 2-methyl-3-dimethylaminoethylamino-1,4-naphthoquinone hydrochloride was prepared and tested in our laboratories and found to have an antihemorrhagic activity in aqueous solution approaching that of 2-methyl-1,4-naphthoquinone on a molar basis. The instability of this compound seems to prohibit its use as a practical antihemorrhagic substance.

We were led to make a study of various sulfonic acid salts by the early observation that 2-methyl-1,4-naphthoquinone is readily dissolved by warming with bisulfite solutions. For this purpose, one mole of bisulfite is sufficient, but an excess, up to two moles, is desirable to prevent oxidation by the air if the hydroquinone is desired. Such a solution possesses antihemorrhagic activity equivalent to that of the 2-methyl-1,4-naphthoquinone contained therein. These solutions are low in toxicity and do not have the irritating properties of the free quinone. Their stability is likewise very satisfactory. The data on pharmacology and stability will be reported elsewhere by Dr. R. K. Richards and Mr. F. J. Kirchmeyer.

This at first appears to be an instance in which substitution in the 3-position has little influence on antihemorrhagic activity. Assuming initial 1,4-addition to the conjugated system, the reaction may be formulated as follows



The reaction undoubtedly proceeds in this direction at least to some extent, as shown by the fact that oxidation and salting out by the method used by Fieser and Fieser[8] for potassium 1,4-naphthoquinone-2-sulfonate gives the expected potas-

(1) Dann, *Proc. Soc. Exptl. Biol. Med.*, **42**, 663 (1939).

(2) Fernholz, Ansbacher and MacPhillamy, THIS JOURNAL, **62**, 430-432 (1940).

(3) Dam, Glavind and Karrer, *Helv. Chim. Acta*, **23**, 224-233 (1940).

(4) Emmett, Kamm and Sharp, *J. Biol. Chem.*, **133**, 285-286 (1940).

(5) Fernholz and Ansbacher, *Science*, **90**, 215 (1939).

(6) Almquist and Klose, *Proc. Soc. Exptl. Biol. Med.*, **45**, 55-59 (1940).

(7) Fieser, Tishler, and Sampson, THIS JOURNAL, **62**, 1628-1629 (1940).

(8) Fieser and Fieser, *ibid.*, **57**, 491-494 (1935).

sium 2-methyl-1,4-naphthoquinone-3-sulfonate. Likewise, it was shown that 2,3-dimethyl-1,4-naphthoquinone is not dissolved by bisulfite, even when a large excess is used at boiling temperature. This may be explained by the lack of a hydrogen at position 3 which can migrate to the oxygen to form the more stable structure (B). However, the 3-sulfonate group with the quinone structure decreases the activity to approximately one-eightieth that of the hydroquinone compound as it occurs in solution. Considering the nearly equal activity of 2-methyl-1,4-naphthoquinone and 2-methyl-1,4-naphthohydroquinone, it appears probable that the high activity of compound (B) above is due to an equilibrium in the solution which cannot be set up when (B) has been oxidized to the corresponding quinone. Compounds of 2-methyl-1,4-naphthoquinone with other bisulfites, as benzylamine bisulfite, in aqueous solution possess the same high activity.

To show the effect of sulfonic acid groups in other positions in the molecule, 2-methylnaphthalene was sulfonated and the 1-, 6- and 8-sulfonic acid salts isolated in fairly pure condition for bioassay. In very high doses, the sodium salt of 2-methylnaphthalene-1-sulfonic acid showed some antihemorrhagic effect. At the same high levels, no activity whatever was observed from the 6- and 8-isomers. In view of the activity of the 1-isomer, it was considered that the sodium salt of 2-methyl-1-aminonaphthalene-4-sulfonic acid should possess even higher antihemorrhagic effect. However, this compound showed little or no more activity than sodium 2-methylnaphthalene-1-sulfonate. As an intermediate, 1-nitro-2-methylnaphthalene was obtained and was found approximately equal to the sodium 1-sulfonate.

TABLE I

Compound	Solvent	Anti-hemorrhagic activity, (%)
2-Methyl-1,4-naphthoquinone	Water	100
Sodium 2-methyl-1,4-naphthohydroquinone-3-sulfonate	Water	100
Potassium 2-methyl-1,4-naphthoquinone-3-sulfonate	Water	1.25
2 - Methyl - 3 - dimethylaminoethylamino - 1,4 - naphthoquinone hydrochloride	Water	25.0[a]
Benzylamine 2-methyl-1,4-naphthohydroquinone-3-sulfonate	Water	100
Sodium 2-methylnaphthalene-1-sulfonate	Water	0.0013
Sodium 2-methylnaphthalene-6-sulfonate	Water	.000
Sodium 2-methylnaphthalene-8-sulfonate	Water	.000
2-Methyl-1-nitronaphthalene	Oil	.0016
2-Methyl-1-amino-4-sulfonic acid	Water	.002

[a] Approximate value.

The bioassays were carried out by Flemintine Peirce Dann according to her previously published method.[1] The activities of the compounds are reported in Table I as percentages of that of 2-methyl-1,4-naphthoquinone on a molar basis, thus avoiding any confusion as to different Vitamin K "units."

Experimental Part

2-Methyl-1,4-naphthoquinone Compounds with Bisulfites.—These were usually prepared in aqueous solution by warming 2-methyl-1,4-naphthoquinone with one or two moles of the bisulfite in distilled water until solution resulted. Attempts to isolate the products in a crystalline condition were unsuccessful. Bisulfites of potassium, calcium, ethanolamine, diethylamine, dibutylamine, morpholine, diethylaminoethanol, benzylamine, and laurylamine, and tetramethylammonium bisulfite dissolved 2-methyl-1,4-naphthoquinone to give light-colored aqueous solutions. Aniline and ammonium bisulfites gave dark colored solutions. The amine bisulfites may be prepared and treated with 2-methyl-1,4-naphthoquinone in alcoholic solution.

Potassium 2-Methyl-1,4-naphthoquinone-3-sulfonate.—To a solution of 18 g. of pure potassium bisulfite in 200 cc. of water was added 17.5 g. of 2-methyl-1,4-naphthoquinone and the mixture stirred and gently warmed until solution resulted. The solution was filtered if necessary. It was then oxidized to the quinone and salted out by the method of Fieser and Fieser[8]; yield, 8.5 g. (30%). *Anal.* Calcd. for $C_{11}H_7O_5SK$: S, 11.04. Found: S, 10.68.

2-Methyl-3-β-dimethylaminoethylamino-1,4-naphthoquinone.—2-Methyl-1,4-naphthoquinone-2,3-oxide was prepared according to the directions of Madinaveitia.[9] To a suspension of 2.5 g. of the oxide in 12A absolute alcohol was added 1.5 cc. N,N-dimethylethylenediamine and the mixture shaken and allowed to stand overnight at room temperature. A beautiful dark red color developed and all dissolved. The alcohol and excess amine were removed by evaporation in vacuum and the residue dried in a vacuum desiccator. The final residue was a dark red glassy material which could be powdered. *Anal.* Calcd. for $C_{15}H_{18}O_2N_2$: N, 10.84. Found: N, 10.17. The product was not quite completely soluble in one equivalent of hydrochloric acid, and the insoluble material appears black. Upon standing, the aqueous solution of the hydrochloride gradually deposits a dark precipitate. A clear red solution of the hydrochloride was used for bioassays.

2-Methylnaphthalene Sulfonic Acids.—2-Methylnaphthalene was sulfonated under varying conditions which are reported to give mostly the 8-,[10] a mixture of 1- and 8-,[11]

(9) Madinaveitia, *Rev. acad. cienc. Madrid*, **31**, 617–647 (1934).

(10) Dziewónski and Wulffsohn, *Bull. intern. acad. polon. sci.*, Ser. A, 143–148 (1929); *C. A.*, **25**, 1515 (1931).

(11) Veselý and Páč, *Collection Czechoslov. Chem. Commun.*, **2**, 471–485 (1930); *C. A.*, **24**, 5296–5297 (1930).

and 6-sulfonic acids.[12] The barium salts were fractionally crystallized, converted to the sodium salts and the position of the sulfonic group proved by conversion to the acid chloride and to the amide. The products were bioassayed as the sodium salts.

1-Nitro-2-methylnaphthalene.—This compound was prepared by nitration of 2-methylnaphthalene by the method published by Fierz-David and Mannhart.[13] Recrystallized from methanol; m. p. 81–82°.

1-Amino-2-methylnaphthalene-4-sulfonic Acid.—The 1-nitro-2-methylnaphthalene was reduced, and the amine sulfate converted to the 1-amino-4-sulfonic acid as directed by Fierz-David and Mannhart.[13] The product was a fine, white light powder. *Anal.* Calcd. for $C_{11}H_{11}O_3NS$: N, 5.90. Found: N, 5.93.

The author wishes to express thanks to Mr. E. F. Shelberg for the microanalyses here reported, and to Mrs. Flemintine Peirce Dann for the bioassays.

(12) Dziewónski, Schoenówna and Waldmann, *Ber.*, **58**, 1211–1218 (1925).

(13) Fierz-David and Mannhart, *Helv. Chim. Acta*, **20**, 1024–1040 (1937).

Summary

Compounds formed by the reaction of 2-methyl-1,4-naphthoquinone with various metallic or amine bisulfites have been found to be highly water-soluble and to possess a degree of Vitamin K activity equivalent to that of the 2-methyl-1,4-naphthoquinone contained therein.

Other sulfonated 2-methylnaphthalenes were prepared and tested for antihemorrhagic activity. The sulfonic acid group was found to be relatively inert from the antihemorrhagic viewpoint. In the 1-position only slight activity is conferred. This is likewise true of 1-amino-2-methylnaphthalene-4-sulfonic acid. In the 3-position in such an active molecule as 2-methyl-1,4-naphthoquinone the sulfonic group reduces the activity. 2-Methylnaphthalene sulfonated at the 6- or 8-position showed no antihemorrhagic activity at high levels.

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The Action of Hydrogen Peroxide in *t*-Butanol upon *d*-Glucal and Triacetyl-*d*-glucal in the Presence of Osmium Tetroxide

BY ROBERT C. HOCKETT, ALVA C. SAPP[1] AND SARAH R. MILLMAN[2]

Among the several methods available for conversion of an aldose sugar into its epimer, none is so simple and efficient as could be wished.[3] The procedure of Bergmann[4] in which, by the action of benzoperacid and subsequent hydrolysis, the elements of hydrogen peroxide are added to the double bond of a glycal, results in formation of a mixture of epimeric aldoses in which one isomer generally predominates to a high degree. If, therefore, the glycal be prepared originally from that epimer which is less abundant among the products of hydroxylation the series of transformation may represent an epimerization on a practical scale in this direction. In the reverse direction, the extremely small yield will usually render the procedure useless for preparative purposes. In several instances, the fortunate preponderance of a less easily available epimer over its abundant relative in such products, has made the method valuable in practice. Thus ribose has been prepared from arabinose,[5] talose from galactose[6] and various glycosyl-mannoses[7] from disaccharides containing glucose as the reducing part.

In all these cases the conversion was from a sugar possessing the *trans* configuration of the hydroxyl groups on carbons two and three into the epimer with the *cis* configuration in these positions[8]

(1) A preliminary study of this problem was submitted by Mr. Alva Charles Sapp as a thesis in partial fulfillment of the requirements for the degree of Master of Science in October, 1937.

(2) The study was extended by Miss Sarah Ruth Millman as part of a thesis which was submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in June, 1940. Miss Millman was Ellen H. Richards Fellow in Chemistry from 1935 to 1938. A paper including the present report was presented at the Boston Meeting of the American Chemical Society in September, 1939.

(3) Tollens–Elsner, "Kurzes Handbuch der Kohlenhydrate," Leipzig, 1935, p. 16.

(4) Bergmann and Schotte, *Ber.*, **54**, 440, 1564 (1921).

(5) Gehrke and Aichner, *Ber.*, **60**, 918 (1927); Austin, THIS JOURNAL, **56**, 1152 (1934); Karrer, *et al.*, *Helv. Chim. Acta*, **18**, 1435 (1935).

(6) Levene and Tipson, *J. Biol. Chem.*, **93**, 631 (1931); Komada, *Bull. Chem. Soc. Japan*, **7**, 211 (1932).

(7) Watters and Hudson, THIS JOURNAL, **52**, 3473 (1930); Evans and Dauben, *ibid.*, **60**, 886 (1938); Haworth, *et al.*, *J. Chem. Soc.*, 2336, 2644 (1930).

(8) Since the configuration of carbon one is labile on account of prototropic changes, no direct evidence has been obtained to show whether additions to the glycal double bond are of the "*cis*" or "*trans*" type.