[CONTRIBUTION FROM THE LEDERLE LABORATORIES]

The Antihemorrhagic Activity of Sulfonated Derivatives of 2-Methylnaphthalene

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The strongly antihemorrhagic product obtained by dissolving 2-methyl-1,4-naphthoquinone in sodium bisulfite has been formulated as the sodium salt of 2-methyl-1,4-naphthohydroquinone-3-sulfonic acid.[1] In contrast, other derivatives of the quinone known to be substituted in the 3-position show only a relatively slight antihemorrhagic activity and the apparently anomalous potency of the bisulfite solution led us to examine this product with a view to establishing the structure of the active component.

By warming 2-methyl-1,4-naphthoquinone with concentrated, aqueous sodium bisulfite, a solution was obtained which readily yielded a crystalline sodium salt when cooled to 0°. Acetone precipitated the product practically quantitatively, leaving in solution a material which, because of its relatively high solubility, could not be purified satisfactorily. After conversion to the potassium salts, however, the mixture gave a readily purified salt (representing about 20% of the original bisulfite product) which, with benzylthiuronium chloride, yielded a benzylthiuronium salt isomeric with that prepared from the readily crystallized sodium salt. Both benzylthiuronium salts were obtained also from the readily separated potassium salts formed by adding 2-methyl-1,4-naphthoquinone to concentrated, aqueous potassium bisulfite.

On cooling the solution of the potassium salts, a practically pure crystalline potassium salt was deposited and more of the same salt was precipitated from solution by dilution with acetone. Whereas this potassium salt was strongly antihemorrhagic, the isomeric salt obtained from the concentrated aqueous acetone solution (constituting no less than 25% of the original bisulfite product) was less than one-tenth as potent. An aqueous solution of the active salt was only slowly acted upon by potassium dichromate and gave 2-methyl-1,4-naphthoquinone as the product. The less active salt, however, was rapidly oxidized to a yellow crystalline salt from which the original salt was regenerated by reduction with sodium dithionite, as established through comparison of the benzylthiuronium compounds prepared from the respective potassium salts. These facile interconversions indicated that the oxidized and original salts were related as a quinone to the hydroquinone and that structure determinations could be restricted to one of the compounds.

With chlorosulfonic acid, the diacetate of 2-methyl-1,4-naphthohydroquinone was converted to a sulfonic acid and oxidized, as the sodium salt, to a yellow quinone sodium sulfonate which, through the potassium and benzylthiuronium salts, was shown identical with the quinone sulfonate formed by oxidation of the slightly active hydroquinone potassium sulfonate and the original, crude potassium bisulfite product. The conversion of the sodium sulfonate of 2-methyl-1,4-naphthohydroquinone diacetate to the quinone potassium sulfonate and of the latter to phthalic acid indicated that the substituted quinone was 2-methyl-1,4-naphthoquinone-3-potassium sulfonate. Confirmation of this structure was obtained in the transformation of the sodium sulfonate of 2-methyl-1,4-naphthohydroquinone diacetate with nitric acid to a nitroquinone, the conversion of the atter to phthalic acid by vigorous oxidation and, by catalytic reduction, to an amino-methylnaphthohydroquinone which, through the corresponding aminoquinone, was converted to phthiocol. These conversions established the nitroquinone as 2-methyl-3-nitro-1,4-naphthoquinone,[2] the parent sulfonic acid as the diacetate of 2-methyl-1,4-naphthohydroquinone-3-sodium sulfonate and the related quinone sulfonate as 2-methyl-1,4-naphthoquinone-3-potassium sulfonate.

The quinone sulfonate was formed in a yield of only 15% on oxidation of the solution obtained

(1) Moore, THIS JOURNAL, **63**, 2049 (1941).

(2) Substantiation for this structure was also afforded through the following series of transformations and comparisons of appropriate compounds. The monoacetate of 2-methyl-1,4-naphthohydroquinone, prepared in a 65% yield by partial deacetylation of the diacetate with aqueous ammonia, was coupled in glacial acetic acid with diazotized *p*-nitraniline and gave an azo derivative which, on vigorous oxidation, yielded phthalic acid and, on reduction and acetylation, was converted to a triacetate that readily acetylated to the corresponding tetraacetate and on oxidative hydrolysis gave phthiocol. The triacetate, identified by the aforementioned transformations as that of 2-methyl-3-amino-1,4-naphthohydroquinone, was identical with that obtained by acetylation of the aminohydroquinone produced by catalytic reduction of the nitroquinone and the latter, accordingly, was established as 2-methyl-3-nitro-1,4-naphthoquinone.

by heating 2-methyl-1,4-naphthoquinone with potassium bisulfite at 70–90° for ten minutes, but 70% of the quinone was recovered as the sulfonate from a solution heated thirty-five hours at 100°. Whereas the corresponding, slightly active 2-methyl-1,4-naphthohydroquinone-3-potassium sulfonate was oxidized quantitatively by potassium ferricyanide (Expt. 1, Table I), the strongly antihemorrhagic isomeric compound was untouched. That the latter compound was con-

TABLE I

TITRATION RESULTS

The applicability of the titrimetric methods was shown by experiments 1 and 2 a, b and c. The experimental procedure is described in the following notes which refer to the experiment of the corresponding number in the table.

1. The 2-methyl-1,4-naphthoquinone-3-potassium sulfonate (A) was reduced with hydrogen and platinum in 50 ml. of phthalate buffer (*p*H 4.0), the hydrogen was removed with nitrogen and the hydroquinone sulfonate titrated potentiometrically with 0.03 *N* $K_3Fe(CN)_6$.

2a. The sodium bisulfite addition compound (B) was heated at 100° in phosphate buffer (*p*H 5.9) in a sealed ampoule and an aliquot (2 ml.) of the solution transferred quickly to 50 ml. of deaerated phosphate buffer (*p*H 6.7) and titrated under nitrogen as before.

2b. An aliquot (2 ml.) of solution 2a was added to 60 ml. of acetate buffer (*p*H 4.6) containing 0.075 millimole of B and titration completed in nitrogen.

2c. An aliquot (2 ml.) of solution 2a was added to 60 ml. of phosphate buffer (*p*H 6.7) containing 0.0755 millimole of AH_2 prepared as described in 1.

3. Experiment was similar to 1, except that 2-methyl-1,4-naphthoquinone-3-sodium sulfonate (A) was used in phosphate buffer (*p*H 6.7) and 2,6-dichlorophenolindophenol (Eastman Kodak Co.) as the oxidizing agent.

4–7. Solutions of the sodium bisulfite addition compound (B) and sodium bisulfite (0.15 millimole per 2 cc. of solution) in phthalate buffer (*p*H 4, Expts. 4 and 5) and phosphate buffer (*p*H 6.7, Expts. 6 and 7) were heated at 100° in sealed ampoules, 2 ml. of the solutions were transferred to 50 ml. of deoxygenated phosphate buffer (*p*H 6.7) containing 3 ml. of distilled acetaldehyde and the titration completed under nitrogen. The bisulfite was added to eliminate the decomposition of the addition compound observed in the absence of this reagent; the acetaldehyde prevented reduction of the indophenol by the excess bisulfite.

8. A solution was prepared from the sodium bisulfite addition compound (B) and sodium bisulfite (0.3 millimole per 2 cc. of solution) in phosphate buffer (*p*H 6.3), an aliquot (1 ml.) of the solution was added to 25 ml. of phosphate–acetaldehyde buffer (*p*H 6.7) and titrated. An aliquot (1 ml.) of the original bisulfite solution was also submitted for bioassay (data in Table II).

9. An aliquot of solution 8 was heated at 100°, 1 ml. of the cooled solution was added to phosphate–acetaldehyde buffer (*p*H 6.7) as above and titrated. An aliquot (1 ml.) of the heated solution was submitted for bioassay (details in Table II). The quantities of quinone indicated refer to the original concentration of active salt (B) in solution prior to heating.

10–15. In the test of commercial samples of "2-methyl-1,4-naphthohydroquinone-3-sodium sulfonate," a sample (1 ml.) of the ampoule contents (the *p*H of the original ampoules is given in the table) was added to 5 ml. of phosphate–acetaldehyde buffer (*p*H 6.7) and titrated with 2,6-dichlorophenolindophenol. The 0.0116 millimole of AH_2 is equivalent to the "2 mg. of 2-methyl-1,4-naphthoquinone as the 2-methyl-1,4-naphthohydroquinone-3-sodium sulfonate" claimed as the concentration per ml. of the ampoule contents.

16–17. A sample (0.5 ml.) of the ampoule content was titrated as in Expts. 10–15 and an aliquot (0.5 ml.) was used for bioassay (data in Table II).

Expt.	Sample millimoles A	B	AH_2	*p*H	Heated at 100°, min.	Oxidizing agent reduced, millimoles $K_3Fe(CN)_6$	2,6-Dichlorophenolindophenol	% AH_2 titrated	% Conversion B → AH_2	Microgram quinone injected	Average clotting time, min.
1	0.069	0.150		4.0		0.131		95			
2a		.141		5.9	240	.198			70		
2b		.216		5.9		.195			69		
2c		.141	0.0755	5.9		.349		100.6			
3	0.151	.170		6.7			0.152	100.5			
4		.122		4.0	15		.0114		9.4		
5		.122		4.0	30		.0216		17.8		
6		.122		6.7	15		.0420		35		
7		.122		6.7	30		.0773		63		
8		.0265		6.3			.0			3.2	10.8
										16.0	11.3
9		.0265		6.3	120		.0209		79	8.8	17.0
										2.5	>40
	Hykinone Serial										
10	007A076		0.0116	5.0			.0103	89			
11	007A076		.0116	5.0			.0093	80			
12	007A076		.0116	5.0			.0098	84			
13	009A026		.0116	5.2			.0095	82			
14	105A072		.0116	2.5			.00088	7.6			
15	109A013		.0116	2.3			.00103	8.9			
16	105A076		.0058	2.5			.00029	5.0		3.2	9.5
17	007A076		.0058	5.2			.0050	86		3.2	>40

verted to the hydroquinone sulfonate in heated solutions was shown by the increase in titrable material in solution (Expts. 2a, b and c) and the correspondence of the potentials with those for the system

2-Methyl-1,4-naphthoquinone-3-sulfonate + 2e + 2H $\rightleftarrows$ 2-Methyl-1,4-naphthohydroquinone-3-sulfonate[3]

In view of these results it is evident that the slightly active 2-methyl-1,4-naphthohydroquinone-3-potassium sulfonate is formed, largely if not exclusively, by rearrangement of the isomeric addition compound of the quinone and the bisulfite and that the latter primarily formed compound constitutes the principal component of the medically useful bisulfite solutions.

Titration with the more convenient 2,6-dichlorophenolindophenol as oxidizing agent (the applicability of which was shown in Expt. 3) showed that the conversion to the hydroquinone sulfonate was decreased with increase in acidity of the solution (Expts. 4–7). With the increase in the concentration of the hydroquinone sulfonate the antihemorrhagic activity of the solution correspondingly declined (Expts. 8 and 9). Similar relationships obtained for the commercially available solutions of 2-methyl-1,4-naphthoquinone in sodium bisulfite ("Hykinone," Expts. 10–17) and, accordingly, caution in the preparation and use of the bisulfite solutions intended for chemotherapeutic use is indicated.

Experimental

Preparation of Bisulfite Compounds (A) Sodium Salts.—The bisulfite solutions were warmed on a steam-bath and shaken with 2-methyl-1,4-naphthoquinone until solution was complete. In a typical experiment, 17.2 g. of the quinone dissolved, during five minutes, in 25 cc. of warm water containing 12 g. of sodium bisulfite. At 0° the solution deposited a solid which, washed with 40 cc. of acetone, gave 10.8 g. of a white, crystalline salt; the filtrate, diluted with 260 cc. of acetone and cooled to 5° for fifteen to twenty hours, gave 9.8 g. more of the same strongly antihemorrhagic salt (Table II). *Anal.* Calcd. for $C_{11}H_9SO_5Na$: Na, 8.3. Found: Na, 8.6.

The aqueous acetone, concentrated *in vacuo* to a volume of 25 cc. and treated with a solution of 10 g. of potassium chloride in 35 cc. of water, deposited, during one hour at 0°, a solid which was washed with cold 10% potassium chloride and yielded 5.2 g. of a crude potassium salt from which, after recrystallization from 8 cc. of water containing a trace of sodium dithionite, the slightly active (Table II) 2-methyl-1,4-naphthohydroquinone-3-potassium sulfonate (4.3 g.) was obtained. *Anal.* Calcd. for $C_{11}H_9SO_5K$: K, 13.4. Found: K, 13.1.

(3) A potentiometric study of this system will be presented in a subsequent communication.

(B) Potassium Salts.—A solution of 10.5 g. of anhydrous potassium carbonate in 50 cc. of water was saturated with sulfur dioxide at room temperature. 17.2 g. of the quinone was added at 70–90°, the clarified solution was concentrated *in vacuo* to a volume of about 35 cc. and, during one hour at 0°, deposited a solid which, washed with 40 cc. of acetone and recrystallized from 15 cc. of water, gave 5.2 g. of the highly active potassium salt. *Anal.* Calcd. for $C_{11}H_9O_5SK$: K, 13.4. Found: K, 13.0.

The filtrate, after dilution with 280 cc. of acetone and separation of the precipitated, active salt, was concentrated *in vacuo* to a volume of 20 cc., treated with 40 cc. of water containing a trace of sodium dithionite and 5 g. of potassium chloride and deposited a solid (8.5 g.) which, when washed with cold 10% potassium chloride and recrystallized from 20 cc. of water containing a trace of sodium dithionite, gave 6.2 g. of pure 2-methyl-1,4-naphthohydroquinone-3-potassium sulfonate (*Anal.* Calcd. for $C_{11}H_9SO_5K$: K, 13.4. Found: K, 13.2) having only slight antihemorrhagic activity (Table II).

S-Benzylthiuronium Salts (A) from Active Salts.—A solution of the potassium salt (0.54 g.) in 5 cc. of water, treated at 0° with a solution of 0.4 g. of benzylthiuronium chloride in 5 cc. of cold water, gave a gummy precipitate that crystallized when triturated (yield crude dry salt 0.68 g.) and was purified by recrystallization from ethyl methyl ketone; m. p. 127–129° with decomposition.

Likewise the benzylthiuronium salt (*Anal.* Calcd. for $C_{19}H_{20}O_5N_2S_2$: C, 54.3; H, 4.8; N, 6.7; S, 15.2. Found: C, 54.2; H, 4.7; N, 6.5; S, 15.6) prepared from the active sodium salt melted at 127–129° (with decomposition) and the melting point of a mixture of the respective salts was not depressed.

(B) From the Slightly Active Sulfonates.—Under the same conditions the slightly active and relatively insoluble 2-methyl-1,4-naphthohydroquinone-3-potassium sulfonate separated from the potassium bisulfite solution gave a benzylthiuronium salt (85% yield) melting at 138–139° after crystallization from a 30% ethanol solution. *Anal.* Calcd. for $C_{19}H_{20}O_5N_2S_2$: C, 54.3; H, 4.8; N, 6.7. Found: C, 54.6; H, 5.0; N, 7.1. An identical salt was obtained from the potassium salt prepared from the easily soluble sodium salt of low antihemorrhagic activity and, also, by direct conversion of the latter, impure salt. The aqueous acetone solution of the crude sodium salt, isolated as described above, was evaporated to dryness *in vacuo*, the residue, dissolved in 10 cc. of water, gave no precipitate when diluted with 20 cc. of saturated sodium chloride and, after dilution with 75 cc. of water, was treated at 0° with 7 g. of benzylthiuronium chloride in 50 cc. of water. The resulting pasty solid, which could not be purified, was filtered off and the filtrate, treated with a solution of 2 g. of benzylthiuronium chloride in 20 cc. of water, gave a crystalline salt from which, after recrystallization from 30% ethanol, 1.6 g. of the pure benzylthiuronium salt (m. p., 138–139° with decomposition) was obtained. *Anal.* Calcd. for $C_{19}H_{20}O_5N_2S_2$: C, 54.3; H, 4.8; N, 6.7; S. 15.2. Found: C, 54.3; H, 4.8; N, 7.0; S, 15.6.

Oxidation of 2-Methyl-1,4-naphthohydroquinone-3-potassium Sulfonate (A) Isolated Pure Salt.—A solution of 1 g. of the pure salt in 5 cc. of warm water was treated with a solution of 0.4 g. of potassium dichromate and 0.37

cc. of concentrated sulfuric acid in 3 cc. of water, 0.7 g. of potassium chloride in 2 cc. of water was added, the mixture was cooled to 0°, the crude quinone sulfonate (0.77 g.) was filtered off, washed with ice-water and acetone and purified by crystallization from water. *Anal.* Calcd. for $C_{11}H_7SO_5K$: K, 13.5. Found: K, 13.6. The quinone sulfonate gave a yellow, crystalline benzylthiuronium salt; m. p. 156–157° after crystallization from 50% ethanol. *Anal.* Calcd. for $C_{19}H_{18}O_5N_2S_2$: C, 54.5; H, 4.4; N, 6.7. Found: C, 54.3; H, 4.4; N, 6.8.

(B) Salt Dissolved in Bisulfite Solution (a) Previous to Heating.—The bisulfite solution (prepared by adding 35 g. of 2-methyl-1,4-naphthoquinone at 70–90° to a solution of 21 g. of potassium carbonate in 400 cc. of water saturated with sulfur dioxide) was boiled with 4 cc. of concentrated sulfuric acid for fifteen minutes, treated with a solution of 24 g. of potassium dichromate and 18 cc. of concentrated sulfuric acid in 70 cc. of water at 25°, a solution of 42 g. of potassium chloride in 100 cc. of water was added, the crude product (8.5 g.) was filtered off, washed with ice-water and acetone and, after crystallization from 10 cc. of water, gave 5.5 g. of pure 2-methyl-1,4-naphthoquinone-3-potassium sulfonate. *Anal.* Calcd. for $C_{11}H_7SO_5K$: K, 13.5. Found: K, 13.6. The benzylthiuronium salt melted at 156–157° (with decomposition) and was identical with that obtained from the quinone sulfonate prepared by oxidation of the corresponding, pure hydroquinone sulfonate.

(b) Heated Solutions.—After thirty-five hours at 100°, the bisulfite solution (prepared as usual from 17.5 g. of 2-methyl-1,4-naphthoquinone, 10.5 g. of potassium carbonate, 200 cc. of water and sulfur dioxide) was treated with 2 cc. of concentrated sulfuric acid, a solution of 15 g. of potassium dichromate and 12 cc. of concentrated sulfuric acid in 45 cc. of water, 21 g. of potassium chloride in 50 cc. of water was added and the crude product, treated as before, gave 20.7 g. of 2-methyl-1,4-naphthoquinone-3-potassium sulfonate. The benzylthiuronium salt melted at 156–157° (with decomposition) and was identical with that derived from the quinone-sulfonate prepared by oxidation of pure 2-methyl-1,4-naphthohydroquinone-3-potassium sulfonate.

Reactions of the Potassium Sulfonate (A) Reduction.—A suspension of 1.9 g. of the quinone sulfonate in 10 cc. of water, reduced at 45° with 1.3 g. of sodium dithionite, yielded 1.85 g. of the hydroquinone sulfonate which gave a benzylthiuronium salt (m. p. 138–139°, with decomposition), identical with that obtained from the 2-methyl-1,4-naphthohydroquinone-3-potassium sulfonate isolated directly from the bisulfite solution as previously described.

(B) Oxidation.—Two grams of the quinone sulfonate, oxidized with 6 g. of potassium permanganate in alkaline solution, gave 0.8 g. of phthalic acid, m. p. 194–196°, which was converted to the anhydride, m. p. 128–130°, and did not depress the melting point of an authentic sample.

Oxidation of 2-Methyl-1,4-naphthohydroquinone-3-sodium Sulfonate (A) Isolated Salt.—The crude sodium salt, converted to the pure potassium salt (0.5 g.) as previously described and oxidized with 0.2 g. of potassium dichromate and 0.2 cc. of concentrated sulfuric acid in 5 cc. of water, gave 0.39 g. of 2-methyl-1,4-naphthoquinone-3-potassium sulfonate; the yellow benzylthiuronium salt, m. p. 155–156° (with decomposition), was identical with that prepared from the quinone sulfonate obtained on oxidation of pure 2-methyl-1,4-naphthohydroquinone-3-potassium sulfonate.

(B) In Bisulfite Solution.—A solution, prepared from 91.5 g. of sodium bisulfite, 105 g. of 2-methyl-1,4-naphthoquinone and 1.2 liters of water at 95°, was boiled with 12 cc. of concentrated sulfuric acid for fifteen minutes, a solution of sodium dichromate (72 g.) and concentrated sulfuric acid (54 cc.) in 210 cc. of water was added at 25°, followed by 350 g. of sodium chloride and, after twenty hours at 5°, a crude product was filtered off which, after two crystallizations from water, gave 13 g. of 2-methyl-1,4-naphthoquinone-3-sodium sulfonate. *Anal.* Calcd. for $C_{11}H_7SO_5Na$: Na, 8.4; S, 11.7. Found: Na, 8.3; S, 11.9. The benzylthiuronium salt melted at 156–157° (with decomposition) and was identical with the corresponding salt previously described.

Reduction of the Sodium Sulfonate.—A solution of 1.37 g. of the sodium salt in 50 cc. of water was reduced catalytically (with platinum oxide catalyst; absorption stopped at one mole), the solution was stabilized with 0.5 g. of sodium bisulfite, catalyst was filtered off and washed with 20 cc. of water and 20.5 cc. of the filtrate gave, after appropriate treatment, 0.37 g. of a benzylthiuronium salt, m. p. 138–139°, which was identical with the salt obtained directly from the crude 2-methyl-1,4-naphthohydroquinone-3-sodium sulfonate isolated as previously described.

In a bioassay, the stabilized sodium sulfonate solution showed only slight antihemorrhagic activity at a level of 50 micrograms (Table II).

The Diacetate of 2-Methyl-1,4-naphthohydroquinone-3-sodium Sulfonate.—A solution of 10 g. of 2-methyl-1,4-naphthohydroquinone diacetate in 50 cc. of chloroform was treated with 2.3 cc. of chlorosulfonic acid and, after twenty hours at room temperature, with cold water. After neutralization, the product, precipitated with sodium chloride and washed with 15% sodium chloride solution, was dissolved in acetone and the residue obtained from the filtered, evaporated solution was triturated with ether; yield 9.1 g. The salt prepared similarly and crystallized from a methanol–isopropyl alcohol solution melted at 148–150° with decomposition. *Anal.* Calcd. for $C_{15}H_{13}SO_8Na$: Na, 6.4. Found: Na, 6.4.

Oxidation of the Hydroquinone Diacetate.—A solution of 2 g. of the sodium sulfonate in 10 cc. of 50% acetic acid was heated for ten minutes at 80–100° with 0.8 g. of chromium trioxide in 2 cc. of water, 5 g. of potassium chloride in 20 cc. of water was added, the precipitated quinone-sulfonate (0.9 g.) was filtered off, washed with ice-water and acetone and recrystallized from water. *Anal.* Calcd. for $C_{11}H_7SO_5K$: K, 13.5. Found: K, 13.5. The benzylthiuronium salt melted at 154–155° and was identical with that derived from pure 2-methyl-1,4-naphthoquinone-3-potassium sulfonate.

2-Methyl-3-nitro-1,4-naphthoquinone.—To 4 cc. of concentrated nitric acid and 12 cc. of water was added, in small portions and during five minutes, 10 g. of 2-methyl-1,4-naphthohydroquinone diacetate-3-sodium sulfonate. At about 50° the gummy mixture solidified, the solid was thoroughly washed with water, precipitated from a hot

solution in acetic acid by dilution with water and finally was purified by crystallization from an acetic acid–methanol mixture; yield 2.8 g.; m. p. 124.5–125.8°. *Anal.* Calcd. for $C_{11}H_7NO_4$: C, 60.8; H, 3.2; N, 6.5. Found: C, 60.5; H, 3.4; N, 6.5. From the mother liquor 0.8 g. more of the nitroquinone was obtained by dilution with water.

Oxidation of the nitroquinone (1 g.) with 4 g. of potassium permanganate in 80 cc. of hot water and 2 cc. of 10% alkali gave 0.6 g. of phthalic acid, m. p. 185–190°, which was converted to the anhydride, m. p. 128.5–130.5°, and did not depress the melting point of an authentic sample.

Reduction of 2-Methyl-3-nitro-1,4-naphthoquinone. (A) Product Isolated as the Hydrochloride.—A mixture of 3 g. of the nitroquinone, 0.3 g. of platinum oxide and 30 cc. of acetic acid was hydrogenated in the usual way and the reduction stopped with the absorption of 4 moles of hydrogen. The solution was filtered into 45 cc. of concentrated hydrochloric acid containing 0.1 g. of stannous chloride, the precipitated solid was filtered off at 0°, washed with acetone, a hot, filtered solution of the crude solid (2.3 g.) in 20 cc. of water containing 0.5 cc. of concentrated hydrochloric acid and 0.05 g. of stannous chloride was diluted with concentrated hydrochloric acid and, when cooled, yielded the pure hydrochloride, m. p. 205–207° (with decomposition). *Anal.* Calcd. for $C_{11}H_{12}O_2NCl$: C, 58.5; H, 5.4; N, 6.2. Found: C, 58.6; H, 5.6; N, 6.3.

(B) Product Isolated as the Triacetate.—In a similar, subsequent reduction, using 5 g. of the nitroquinone, the reduced product in 50 cc. of acetic acid was heated with 50 cc. of acetic anhydride and 2.5 g. of anhydrous sodium acetate at 80–100° for ten minutes, the triacetate was precipitated from the filtered solution by addition of water and purified by crystallization from an acetic acid–ethanol solution; yield 3.6 g.; m. p. 214–215°. *Anal.* Calcd. for $C_{17}H_{17}O_5N$: C, 64.8; H, 5.4; N, 4.4. Found: C, 64.9; H, 6.0; N, 4.7. Concentration of the mother liquor yielded an additional 1.1 g. of the triacetate; m. p. 212–213°.

The triacetate (0.5 g.), boiled with 5 cc. of acetic anhydride and 0.5 g. of anhydrous sodium acetate, gave the tetraacetate (0.5 g., m. p. 172–174°) which, recrystallized from a mixture of benzene and heptane, melted at 173–174.5°. *Anal.* Calcd. for $C_{19}H_{19}O_8N$: C, 63.8; H, 5.4; N, 3.9. Found: C, 63.9; H, 5.7; N, 4.1.

2-Methyl-3-amino-1,4-naphthoquinone.—A suspension of 10 g. of 2-methyl-3-nitro-1,4-naphthoquinone in 50 cc. of acetic acid was reduced catalytically as previously described, the resulting solution was filtered into a solution of 24 g. of ferric chloride in 50 cc. of concentrated hydrochloric acid and 50 cc. of water, 50 cc. of water was added and the precipitated solid (7.5 g., m. p. 158–160°), recrystallized from ethanol, gave the pure red aminoquinone; m. p. 162–162.5°. *Anal.* Calcd. for $C_{11}H_9NO_2$: C, 70.6; H, 4.8; N, 7.5. Found: C, 69.8; H, 4.8; N, 7.7. Extraction of the original aqueous filtrate with chloroform yielded 0.3 g. of impure aminoquinone; m. p. 152–156°.

The aminoquinone (1 g.) was boiled fifteen minutes with 25 cc. of 10% sodium hydroxide, the filtered solution was acidified and yielded a crude product which, recrystallized from aqueous ethanol containing a few drops of acetic acid, gave 0.81 g. of phthiocol; m. p. 171–172°; no depression in melting point of a mixture with an authentic sample. *Anal.* Calcd. for $C_{11}H_8O_3$: C, 70.2; H, 4.3. Found: C, 70.2; H, 4.4.

2-Methyl-1,4-naphthohydroquinone Monoacetate.—A suspension of 50 g. of 2-methyl-1,4-naphthoquinone and 15 g. of anhydrous sodium acetate in 150 cc. of acetic anhydride was reductively acetylated, the filtered solution poured into water, the moist solid suspended in 450 cc. of methanol containing 22 cc. of 28% ammonia water and the mixture heated to 45° until solution was complete. After twenty hours at room temperature in an atmosphere of nitrogen, the solution was diluted with 2 liters of water, the mixture was extracted with chloroform, the washed extract was concentrated *in vacuo* to a small volume, the residue diluted with carbon tetrachloride and gave the crystalline monoacetate (42 g., m. p. 124.5–125.8°). *Anal.* Calcd. for $C_{13}H_{12}O_3$: C, 72.2; H, 5.6. Found: C, 72.4; H, 5.9.

2-Methyl-(3-*p*-nitrophenylazo)-1,4-naphthohydroquinone Monoacetate.—A filtered solution of diazotized *p*-nitraniline (prepared from 9.5 g. of the amine, 11.5 cc. of concentrated hydrochloric acid, 42 cc. of water and a solution of 5 g. of sodium nitrite in 10 cc. of water) was added to 14.7 g. of the hydroquinone monoacetate in 250 cc. of acetic acid at room temperature. After two hours, the red azo derivative was filtered off and thoroughly washed; yield 15.6 g.; m. p. 274–276° after crystallization from dioxane. *Anal.* Calcd. for $C_{19}H_{15}N_3O_5$: N, 11.5. Found: N, 11.9.

The azo compound (1 g.) was added (during eleven hours, using a Soxhlet extractor) to 7 g. of potassium permanganate in 300 cc. of acetone, the resulting mixture, after addition of 3 g. of potassium permanganate, was boiled ninety minutes longer and solvent was distilled. The residue was boiled ten minutes with 80 cc. of water, 2 cc. of 10% alkali and 4 g. of potassium permanganate, the mixture was acidified, boiled for one-half hour, manganese oxide was dissolved with sulfurous acid and the solution was extracted with chloroform. The aqueous solution, saturated with sodium chloride and extracted with ethyl acetate, gave 0.3 g. of phthalic acid, m. p. 185–190°, which was converted to the anhydride, m. p. 129–131°, and did not depress the melting point of a mixture with a pure, authentic sample.

The azo compound (2.4 g.) was reduced catalytically in 75 cc. of acetic acid, the resulting nearly colorless solution was heated to 100° for twenty minutes with 25 cc. of acetic anhydride and 2 g. of anhydrous sodium acetate, the filtered solution was diluted with 25 cc. of water and the mixture evaporated nearly to dryness. The residue was washed with water, extracted with 15 cc. of hot acetone, solvent was evaporated from the extract, the residue was crystallized from ethanol and the crude product (0.9 g., m. p. 210–211°), recrystallized from ethanol, gave pure 2-methyl-1,4-diacetoxy-3-acetaminonaphthalene, m. p. 213–214°; a mixture with the triacetate prepared from 2-methyl-3-nitro-1,4-naphthoquinone did not depress the melting point. *Anal.* Calcd. for $C_{17}H_{17}O_5N$: C, 64.8; H, 5.4; N, 4.4. Found: C, 65.0; H, 5.4; N, 4.6.

The triacetate (0.5 g.) was boiled (thirty minutes) with 15 cc. of 10% alkali, through which a stream of air was

TABLE II

Supplement compound	Level, micrograms	No. chicks	% Clotting at <10 min.	% Clotting at <15 min.	Average clotting time, min.
None		10			>40
Quinone	1	12	42	58	13.4
Crystalline potassium bisulfite addition	5	10	10	90	10.8
	1.6	13	16	16	29.2
2-Me-1,4-naphthohydroquinone-3-potassium-sulfonate	50	13		8	36.4
	5	13	0		>40
None		8			>40
Quinone	1	10	100	100	7.6
Crystalline sodium bisulfite addition	5	9	78	89	8.7
	3.2	19	84	89	8.4
	1.6	22	27	59	17.0
None		9			>40
Quinone	1	13	61	85	10
2-Me-1,4-naphthohydroquinone-3-sodium-sulfonate	50	22	5	14	
	25	17	0	0	>40
None		10			>40
Quinone	1	9	89	100	7.1
Solution 8, Table I	3.2	8	25	100	10.8
Solution 9, Table I	16	11	64	82	11.3
	8.0	9	33	33	17.0
	2.0	9	11	22	>40
Solution 17, Table I	3.2	12	0	0	>40
None		8			>40
Quinone	1	12	92	100	6.5
Solution 16, Table 1	3.0	10	60	90	9.5

bubbled, the filtered solution was acidified and the precipitated phthiocol purified by sublimation *in vacuo;* yield, 0.07 g.; m. p. 170–172°; no depression in melting point of a mixture with an authentic sample.

Bioassays.—Day old chicks were depleted on the diet of Almquist and Stokstad[4] (modified in that the cod-liver oil was omitted from the diet) for a period of ten to twelve days, or until the clotting time exceeded thirty minutes as measured by the capillary tube method. Injections of the aqueous solutions (0.1 cc.) were made in the leg muscle and, after eighteen hours, the clotting time was ascertained by the capillary tube method. Chicks fed orally with 1 microgram of 2-methyl-1,4-naphthoquinone in Wesson oil were used as positive controls. The claimed concentration of active component in the "Hykinone" solution was used as a basis for the dilution of the solutions injected. The results are summarized in Table II.

(4) Almquist and Stokstad, *J. Nutrition*, **12**, 329 (1936).

Summary

1. The crystalline sodium and potassium bisulfite addition products of 2-methyl-1,4-naphthoquinone have been isolated.

2. The sodium and potassium salts of 2-methyl-1,4-naphthohydroquinone-3-sulfonic acid, isomeric with the corresponding, strongly antihemorrhagic bisulfite addition compounds, have been isolated and shown to have only slight biochemical activity.

3. The structure of the slightly antihemorrhagic salts was established by unequivocal synthesis.

4. Conversion of the primarily-formed bisulfite compounds to the corresponding salts of 2-methyl-1,4-naphthohydroquinone-3-sulfonic acid in heated solutions was established and correlated with the change in biochemical activity of the solution.

5. Precautions in the preparation and use of the bisulfite solutions for medicinal purposes are indicated.

PEARL RIVER, N. Y. RECEIVED FEBRUARY 19, 1942

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, UNIVERSITY OF NORTH CAROLINA]

The Constitution of Natural Tannins. VIII. Coloring Matters Derived from Anthracene-9-aldehyde

BY ALFRED RUSSELL AND W. B. HAPPOLDT, JR.[1]

Recently, Russell and Speck[2] have shown that coloring matters analogous to benzopyrilium salts but containing naphthalene nuclei can be prepared using the regular procedure—condensation of appropriate aldehydes and ketones by anhydrous hydrogen chloride in dry solvents. It has now been established that related compounds containing anthracene nuclei can be prepared through the condensation of 9-anthraldehyde with various hydroxy-, methoxy-, acetoxy- and benzoyloxy acetophenones. The condensations were effected either by the use of anhydrous hydrogen chloride, or using aqueous alcoholic alkali. With one exception, where a flavanone (Type Formula II) was obtained, the condensation products were chalcones (Type Formula I). To preserve simplicity of nomenclature, the new chalcones have been called 9-anthralacetophenones (*cf.* benzalacetophenones). Analogously, the single flavanone is a hydroxy-2-anthryl-9-benzopyrone. There is included also a single chalcone (III) derived from 9-anthraldehyde and methyl-β-naphthyl ketone.

(1) From a dissertation submitted to the Faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1941.

(2) Russell and Speck, THIS JOURNAL, **63**, 851 (1941).