Plant	Location	Yield, g. per kg. (dry)
Nolina bigelovi (Torr.)	Goldroad, Ariz.	0.4
N. macrocarpo (Wats.)	Central Arizona	0.1
N. paraviflora (H. B. K.)	Tehuacan, Mexico	0.1
N. texana (Wats.)	Central Texas	0.2
Trillium sessile californicum	Ukiah, Calif.	0.1
Yucca arizonica (McKel.)	Sonoita, Ariz.	1.4
Y. thompsoniana	Big Bend, Texas	0.1

The identification of sitosterol was established by the analyses of the sterol and its acetate along with mixed melting point determinations on both. Sitosterol melted 133–136° and its acetate melted 123–126°. In all cases the analyses never varied more than three-tenths from the calculated carbon and hydrogen values.

Of particular interest are those *Magueys* used for the manufacture of an intoxicating liquor known as tequila. These include *M. mescal azul*, *M. mescal bermejo*, *M. mescal chato* and *M. mescal mano larga*. In the course of time the natives have singled out these species as the best sources for its manufacture. Although many *Magueys* contain steroidal sapogenins, all of these above contain sitosterol.

Summary

1. Some three hundred additional plants have been investigated for steroidal sapogenins.

2. From sixty-six plants thirteen new sapogenins have been isolated. Structures are suggested.

3. From one hundred and sixty-two plants, eight previously known sapogenins and sitosterol have been isolated.

STATE COLLEGE, PENNA. RECEIVED APRIL 28, 1943

[CONTRIBUTION FROM THE CHEMICAL LABORATORY, AMERICAN MEDICAL ASSOCIATION]

Water-Soluble Derivatives of Menadione[1]

BY AMEL R. MENOTTI(1a)

The antihemorrhagic activity of sulfonated derivatives of 2-methylnaphthalene has been the subject of publications by Moore[2] and Baker, Davies, McElroy and Carlson.[3] The results of the present investigation provide further proof that (a) the primary product formed from the interaction of sodium bisulfite with menadione (2-methyl-1,4-naphthoquinone) is a monobisulfite addition product, and (b) that this addition product rearranges under the influence of heat to form the sulfonated derivative, 2-methyl-1,4-naphthohydroquinone-3-sulfonate. These two products have been characterized, and methods are herein described for their identification in pharmaceutical preparations.

Menadione, when shaken with a saturated aqueous sodium bisulfite solution at room or slightly elevated temperature, yielded a clear, yellow solution which, on cooling to 0°, deposited a white, crystalline, trihydrated salt having the characteristics of a bisulfite addition product. If the solutions were heated at 90–100°, the quantity of bisulfite addition product obtained on cooling to 0° decreased with increase in heating time. After heating for twenty to thirty hours, no crystalline organic salt precipitated on cooling or on the addition of organic solvents. However, if saturated potassium chloride solution was added, the cooled solution deposited crystals of a potassium salt which exhibited properties of an organic sulfonate. The optical properties of the sulfonate and its behavior toward dilute alkalies, *o*-phenanthroline-ferrous complex solution and ethyl cyanoacetate, differed radically from the menadione bisulfite addition product. The sulfonate could be detected in the mother liquid, by precipitation with *o*-phenanthroline-ferrous complex, when the bisulfite addition product was made from warmed solutions. The greater solubility of the sodium salt of the sulfonate in contrast with that of the addition product made possible a complete separation on cooling the reaction mixture. The crystallized, purified menadione-sodium bisulfite addition product was converted to 2-methyl-1,4-naphthohydroquinone-3-sulfonate in 20–30% yield by solution in water and heating at 100° for twenty-four hours in a closed vessel. No bisulfite addition product

---

(1) The Council on Pharmacy and Chemistry of the American Medical Association has accepted the name "Menadione" as a non-proprietary designation for 2-methyl-1,4-naphthoquinone" (*J. A. M. A.*, **116**, 1054 (1941)), (1a) Present address, Cheplin Biological Laboratories, Syracuse, N. Y., and the name "Menadione Bisulfite as a non-proprietary designation for the water soluble monosodium bisulfite addition product of menadione" described in this paper (*J. A. M. A.*, **121**, 839 (1943)).

(2) M. B. Moore, THIS JOURNAL, **63**, 2049 (1941).

(3) B. R. Baker, T. H. Davies, L. McElroy and G. H. Carlson, *ibid.*, **64**, 1096 (1942).

could be recovered at the end of the heating period, but relatively large quantities of 2-methyl-1,4-naphthohydroquinone separated from the cooled solution, and the 2-methyl-1,4-naphthohydroquinone-3-potassium sulfonate could be obtained from the filtrate by the addition of concentrated potassium chloride solution.

The sodium bisulfite addition product of menadione may be identified as follows

(1) Addition of dilute alkalies to an aqueous solution of the addition product results in the immediate precipitation of free menadione with recovery up to 95–98% by treatment with 10% ammonium hydroxide solution. Under similar conditions, purified 2-methyl-1,4-naphthohydroquinone-3-sodium or potassium sulfonate yields a reddish-orange color, but no menadione.

(2) Craven's color test[4] for menadione, dependent on the coupling reaction of ethyl cyanoacetate in alkaline solution with naphthoquinones (unsubstituted in the 2 or 3 positions), applies equally well to the menadione bisulfite addition product and provides proof that the 3 position is unsubstituted in the latter compound because 2,3 disubstituted naphthoquinones do not respond to this reaction. Pinder and Singer[5] have employed this reaction for the determination of menadione. The same procedure has been found satisfactory for the determination of the bisulfite addition product in pharmaceutical preparations. The sulfonic acid obtained from heated solutions does not react with ethyl cyanoacetate, but becomes dark orange colored in solution due to the presence of alkali.

(3) The optical properties serve to identify the product in crystalline form. Immersion in a liquid of appropriate refractive index may be used to determine roughly the amount of impurity present.

The 2-methyl-1,4-naphthohydroquinone-3-potassium or sodium sulfonate may be identified by

(1) Treatment of dilute, neutral or slightly acid, aqueous solution of 2-methyl-1,4-naphthohydroquinone-3-potassium sulfonate with a few drops of a 0.025 *M* solution[6] of *o*-phenanthroline-ferrous complex results in the immediate formation of a scarlet red precipitate (soluble in butyl alcohol). The sodium bisulfite product does not react with *o*-phenanthroline-ferrous complex.

(2) Solutions of the sulfonate yield a reddish color when treated with dilute solutions of alkalies but in contrast to the bisulfite addition product deposit no menadione.

(3) Optical data which provide a positive means of identification of the crystalline dihydrated potassium salt of the sulfonic acid are provided in the experimental part.

Experimental

Menadione Bisulfite Addition Product.—The clear yellow solution obtained after shaking 5 g. of menadione with a warm solution of sodium bisulfite in 16 ml. of water was kept overnight at 0°, then filtered and the white crystals washed with alcohol and dried; yield, 5 g. Recrystallization, by placing about 4 volumes of ethanol on a saturated aqueous solution and allowing slow diffusion, gave clear plates 1 cm. long which in the micro-melting point apparatus lost solvent at 100° and then became translucent and melted with decomposition at 154–157°. In a capillary, when introduced into the bath at 115°, the substance melted at approximately 126°. The benzylisothiourea salt[3] melted at 126.5–128.0°.

Anal. Calcd. for $C_{11}H_9O_5SNa \cdot 3H_2O$: C, 40.00; H, 4.58; Na, 6.96; $3H_2O$, 16.35. Found: C, 40.2; H, 4.5; Na, 7.4. Loss on drying at 80° in a vacuum over phosphorus pentoxide for one hour was 15.0%. The anhydrous material was extremely hygroscopic.

Between crossed nicols the crystals appeared as birefringent, rectangular plates, exhibiting negative elongation and parallel extinction. Conoscopic observation of crushed fragments indicated a positive biaxial interference pattern with $2V = 60 \pm 5°$. *Refractive indices.* $\alpha = 1.535$; $\beta = 1.565$; $\gamma = 1.630$ (all ± 0.002). Common views exhibited α and γ.

2-Methyl-1,4-naphthohydroquinone-3-potassium Sulfonate.—A solution of 17.2 g. of menadione and 12 g. of sodium bisulfite in 25 ml. of water, heated to 98° for twenty-two hours and then placed at 0° for two hours deposited no menadione sodium bisulfite addition product but an appreciable quantity of 2-methyl-1,4-naphthohydroquinone. The filtrate, after addition of 30 ml. of saturated potassium chloride solution, yielded, during twelve hours at 0°, 12 g. of white crystals which darkened on prolonged exposure to air when wet. The sulfonate, recrystallized from 10 ml. of hot water containing a trace of hydrosulfite, yielded a precipitate with *o*-phenanthroline-ferrous complex solution, did not give Craven's color test and gave no menadione on treatment with alkali. In the micromelting point apparatus the crystals lost solvent at 100° and melted with decomposition at 193–196°.

Anal. Calcd. for $C_{11}H_9O_5SK \cdot 2H_2O$: C, 40.23; H, 3.99; K, 11.91; $2H_2O$, 11.0. Found: C, 39.6; H, 4.2; K, 11.8; loss on drying one-half hour in vacuum over phosphorus pentoxide, 11.4%.

Between crossed nicols the crystals appeared as birefringent, six-sided tablets, exhibiting parallel extinction and positive elongation. Conoscopic observation of crushed fragments indicated a positive biaxial pattern with $2V = 40 \pm 5°$. *Refractive indices.* $\alpha = 1.565$; $\beta =$

---

[4] R. Craven, *J. Chem. Soc.*, 1605 (1931).

[5] J. Pinder and J. Singer, *Analyst*, **65**, 7 (1940).

[6] G. H. Walden, Jr., L. P. Hammett and R. P. Chapman, THIS JOURNAL, **55**, 2650 (1933).

1.590; γ = 1.730 (all ±0.002). Common views exhibited α and γ.

2-Methyl-1,4-naphthohydroquinone Diacetate-3-potassium Sulfonate from Menadione Bisulfite Solutions.—1.8 g. of the potassium sulfonate, isolated as described above, was boiled with 10 ml. of acetic acid and 10 ml. of acetic anhydride for one-half hour. The reaction mixture, when concentrated under vacuum to approximately 10 ml., cooled and filtered, yielded 1.5 g. of white microcrystalline powder. The substance was extracted with 50 ml. of ethanol from a Soxhlet thimble and recrystallized from a saturated aqueous solution.

Properties: white, birefringent, elongated plates which exhibit refractive indices of 1.491 and 1.690 and an oblique extinction angle of 31°. Craven's color test was negative and no precipitation was observed with *o*-phenanthroline-ferrous complex. On the micromelting point stage the crystals melted indefinitely with decomposition above 205°.

Anal. Calcd. for $C_{15}H_{13}O_7SK$: C, 47.86; H, 3.48; K, 10.39. Found: C, 47.5; H, 3.5; K, 10.4.

2-Methyl-1,4-naphthohydroquinone Diacetate-3-potassium Sulfonate from 2-Methyl-1,4-naphthohydroquinone Diacetate and Chlorosulfonic Acid.—A solution of 2.4 ml. of chlorosulfonic acid in 10 ml. of chloroform was added to 10 g. of 2-methyl-1,4-naphthohydroquinone diacetate in 40 ml. of chloroform. After twelve hours at 25°, 2 g. of ice was added, the mixture neutralized to congo red with cold 6 *N* potassium hydroxide solution and the aqueous layer separated, diluted with 20 ml. of saturated potassium chloride solution and cooled. The 5 g. of white crystals obtained was fractionally recrystallized from hot water and gave as the first crop 2 g. of flat rectangular plates (subsequent crops contained mixtures of the mono and diacetate derivatives), highly birefringent and exhibiting negative elongation and parallel extinction between crossed nicols. Conoscopic observation of the crystals as formed indicated a biaxial interference figure with an optic axis in the field of view. No bisectrix views were found. *Refractive index.* β = 1.600. On the micromelting point stage the material lost solvent near 100° and melted with decomposition at 168–170°. This substance gave a precipitate with *o*-phenanthroline-ferrous complex; Craven's color test was negative, indicating a substituted 3 position, and analysis indicated the monoacetate of 2-methyl-1,4-naphthohydroquinone-3-potassium sulfonate.

Anal. Calcd. for $C_{13}H_{11}O_6SK \cdot H_2O$: C, 44.30; H, 3.72; K, 11.09; H_2O, 5.11. Found: C, 44.1; H, 3.7; K, 11.0; loss on drying at 78° in vacuum over phosphorus pentoxide for one hour, 4.83%.

The filtrate from the original reaction mixture was diluted with an additional 20 ml. of saturated potassium chloride solution and after twelve hours at 0° yielded 3 g. of white crystals (more of same on concentrating mother liquid), which were purified by extraction from a Soxhlet thimble with ethanol and recrystallized from hot water. The crystal structure, refractive indices, extinction angle, melting point and analytical data indicated complete identity with the 2-methyl-1,4-naphthohydroquinone diacetate-3-potassium sulfonate obtained above from heated menadione bisulfite solutions.

Summary

1. The chemical and optical properties of a crystalline, trihydrated sodium bisulfite addition product of menadione have been determined, and its conversion to 2-methyl-1,4-naphthohydroquinone-3-sulfonate is described.

2. The chemical and optical properties of a crystalline, dihydrated 2-methyl-1,4-naphthohydroquinone-3-potassium sulfonate isolated from heated bisulfite menadione solution have been determined.

3. Methods for the identification of these two products have been presented.

4. The 2-methyl-1,4-naphthohydroquinone diacetate-3-potassium sulfonate has been prepared by acetylation of the 2-methyl-1,4-naphthohydroquinone-3-sulfonate obtained from heated menadione-bisulfite solution and by sulfonation of 2-methyl-1,4-naphthohydroquinone diacetate.

CHICAGO, ILL. RECEIVED SEPTEMBER 29, 1942