Of particular interest are the experiments of du Vigneaud who has reported that in young rats the methionine methyl may be transferred to choline and to creatine[5] and that the choline[6] and betaine,[19] but not the creatine,[16] methyls may be transferred to homocystine.

Acknowledgments.—We wish to acknowledge financial assistance from the Theelin Fund administered by the Committee on Grants for Research of St. Louis University. We are also indebted to Merck and Co., Inc., for a supply of choline chloride and to Parke, Davis and Co., Inc., for a supply of Natola.

Summary

1. Hemorrhagic degeneration is the result of a dietary deficiency of choline and of the labile methyl supply.

2. Betaine, like methionine, contributes to the labile methyl supply of the body and may be substituted for choline. The effectiveness of betaine corresponds to the utilization of only one of the three methyl groups.

3. Creatine does not contribute to but does spare the labile methyl supply.

4. Cysteine, homocystine and glutathione, but not taurine, increase the severity of hemorrhagic degeneration.

ST. LOUIS, MISSOURI RECEIVED NOVEMBER 1, 1940

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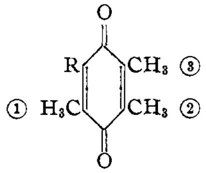
(19) Chandler and du Vigneaud, *J. Biol. Chem.*, **135**, 223 (1940).

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

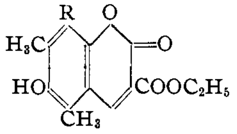
The Reaction between Quinones and Metallic Enolates. XIII. Trimethylethylquinone and Sodium Malonic Ester[1]

BY LEE IRVIN SMITH AND J. W. OPIE[2]

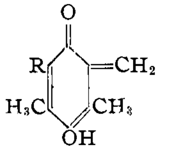
Trimethylbromoquinone (I) reacts with sodium malonic ester to produce 3-carbethoxy-6-hydroxy-5,7-dimethyl-8-bromocoumarin (II).[3] This coumarin is the only product of the reaction; the bromine atom is unaffected by the enolate, but it does exert a strong directing influence upon the formation of the pentad-enol system (III) from I, since of the three methyl groups, the one meta to the bromine atom reacts exclusively. In the previous paper[1] it was shown that while no product resulting from attack of sodium malonic ester upon the bromine atoms of dibromo-*m*-xyloquinone could be isolated, the reaction leading to the coumarin (IV) was difficult to control and, moreover, the coumarin IV differed from all its analogs previously studied in the great ease with which the heterocyclic ring could be opened. In dibromo-*m*-xyloquinone, there is no methyl group meta to either of the bromine atoms, and it appeared, when the results obtained with this quinone were compared those obtained with the monobromoquinone I, that the nature as well as the orientation of the substituents in the quinones exert a pronounced effect not only upon the course of the reaction, but also upon the ease with which it occurs and upon the stability of the heterocyclic ring in the coumarin formed.



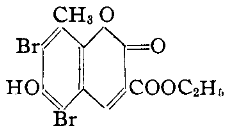
I, R = Br
V, R = C_2H_5



II, R = Br



III, R = Br



IV

In order to explore these effects further, a study has been made of the reaction between trimethylethylquinone (V) and sodium malonic ester. Having shown that tetraethylquinone was inert toward the enolate, it was assumed that the ethyl group of V would not react, and that the reaction with sodium malonic ester would be confined to the methyl groups. Each of the three methyl groups thus became a potential point of entry for the malonic ester residue, and three isomeric coumarins could result, depending upon the direction and magnitude of the orienting effect of the ethyl

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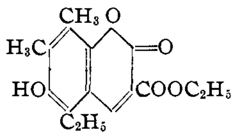
[1] Paper XII, THIS JOURNAL, **63**, 612 (1941).

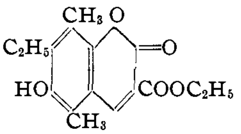
[2] Abstracted from a thesis by Joseph W. Opie, presented to the Graduate Faculty of the University of Minnesota, in partial fulfillment of the requirements for the Ph.D. degree, July, 1940.

[3] Smith and Johnson, THIS JOURNAL, **59**, 673 (1937).

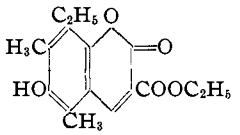
group. Thus coumarins VI, VII and VIII, respectively, would be formed by reaction at the methyl groups numbered 1, 2 and 3 in V.



VI



VII



VIII

Since, moreover, the ethyl group is not as highly polar as a bromine atom, it was considered quite probable that reaction of the quinone V would not be confined exclusively to any one of the methyl groups, as it was in the case of quinone I, but that a mixture of any two, or even all three of the coumarins VI, VII and VIII might result.

When sodium malonic ester was added to the quinone V in benzene,[4] there was formed, in excellent yield, an insoluble red sodium derivative. After removal of this sodium derivative, the filtrate, when cooled, deposited the hydroquinone corresponding to V. When the filtrate from the red salt was steam distilled, up to 40% of the starting material V could be recovered from the distillate. These results showed that the quinone V, like duroquinone, reacted in such a manner that half of the quinone was reduced to hydroquinone and that one mole of the quinone theoretically should give half a mole of coumarin and half a mole of hydroquinone. Upon this basis, the yield of red sodium derivative was practically quantitative, a result quite in contrast with that shown by dibromo-*m*-xyloquinone.[1]

The red sodium derivative, when suspended in ethanol and subjected to the action of hydrochloric acid, gave a yellow coumarin in over 90% yield. This coumarin, after crystallization from ethanol, melted at 148–165° and gave analytical values agreeing closely with the formula $C_{16}H_{18}O_5$ (VI, VII or VIII). Hydrolysis of this coumarin ester with hydrochloric acid[5] led to a coumarin acid which melted at 192–210°. Neither crystallization of this acid from acetic acid, nor precipitation of it from its solution in sodium carbonate, changed its melting point. Acetylation of the coumarin ester gave no solid acetate, but resulted in an oil which could not be crystallized.

The broad range in the melting points of both the ester and the acid, and the fact that the ester gave excellent analytical values in spite of its poor melting point, indicated that the substance was a mixture of at least two coumarins. When the ester (m. p. 148–165°) was dissolved in benzene and chromatographed on calcium carbonate, a narrow dark brown zone formed at the top of the tube, but most of the material remained unadsorbed. The benzene filtrate, on evaporation, gave material which melted at 172–179°. Substitution of aluminum oxide for calcium carbonate led to a definite yellow layer in the lower part of the tube, and from this zone an ester melting at 181–182° was obtained.

As the ester was fairly insoluble in ether, experiments were performed to determine whether or not the mixture could be separated by fractional solution in this solvent. No difference was found between the dissolved and undissolved portions of the substance, but it was found that repeated crystallization of the substance from ether–petroleum ether ultimately gave a product which melted at 183–184°, while very slow recrystallization from alcohol ultimately gave a product melting at 185–188°. The mother liquors from ether–petroleum ether solutions, on standing in a refrigerator for two months, deposited material which melted at 153–166°. This material was washed several times with ether and the ether was evaporated. The residue, after crystallization from petroleum ether, melted at 150–152° and had the composition $C_{16}H_{18}O_5$.

Hydrolysis of the 152° ester gave an acid $C_{14}H_{14}O_5$ which melted at 230–232°, and acetylation of the ester gave an acetate $C_{18}H_{20}O_6$ which melted at 151–153°, but which, when mixed with the 152° ester, melted at 125–130°. Hydrolysis of the 185° ester gave an acid which melted at 237–238°, but acetylation of this ester produced an acetate having the wide melting point range of 141–160° and it was not possible to obtain a purer product by recrystallization.

Thus chromatographic adsorption, combined with partial solution and crystallization, led to two coumarin esters with fairly narrow melting point ranges: A, melting at 185–188°, constituting about a third of the crude coumarin ester, and B, melting at 150–152°, constituting a much smaller part of the crude coumarin ester. Mix-

[4] Smith and Dobrovolny, THIS JOURNAL, **48**, 1693 (1926).

[5] Smith and Denyes, *ibid.*, **58**, 304 (1936).

tures of A and B melted at intermediate temperatures. The wide difference in melting points between A and B appeared to be unusual, but not impossible, for isomers so closely related as are VI, VII and VIII. Each of the esters, on hydrolysis, gave acids with fairly sharp melting points (from A, 237–238°; from B, 230–232°), but only B gave an acetate which had a narrow melting point range (151–153°).

The separation of the crude coumarin ester into the components A and B was extremely tedious and time consuming, and the amounts of the two components obtained were not sufficient for degradative studies. Consequently, in order to settle the question as to the nature of A and B, the three coumarin esters VI, VII and VIII were synthesized.[6] 3-Carboxy-6-hydroxy-5-ethyl-7,8-dimethylcoumarin melted at 223–224° and its ethyl ester (VI) melted at 178–179.5°. 3-Carboxy-6-hydroxy-7-ethyl-5,8-dimethyl coumarin melted at 250°, and its ethyl ester (VII) melted at 199–201°. 3-Carboxy-6-hydroxy-8-ethyl-5,7-dimethylcoumarin melted at 232–234°, and its ethyl ester (VIII) melted at 173–174.5°.

The method of thermal analysis, using the pure compounds, shows that neither A nor B is a pure substance. Further, the data indicate that B is a mixture of all three esters, for the melting points of mixtures of B and each of the three pure esters lie above 152°, suggesting that as the concentration of any one of the pure components is increased, the melting point of the mixture with B tends to shift toward that of the added component. The fact that a mixture of the three pure esters melts at 153–160°, that is, at about the same temperature as any of the binary mixtures containing B, further indicates that B is composed of all three of the esters. A, on the other hand, appears to be a mixture composed only of VI and VII, for all of the binary mixtures of these three substances melt at 180–185°, while there is a great depression produced in the melting point of A, VI or VII when any of these substances is mixed with B or with VIII—*i. e.*, there is no VIII in A.

Hence the reaction between trimethylethylquinone (V) and sodium malonic ester leads to a mixture containing all three of the coumarin esters possible by attack of the reagent upon a methyl group of the quinone. This mixture cannot be separated into the pure components, but it can be separated into two fractions, one of which is an inseparable mixture of two of the coumarin esters (A = VI + VII) while the other (B) is an inseparable mixture of all three coumarin esters It follows, therefore, that the ethyl group in this quinone does not possess in this reaction, the strong directing effect shown by the bromine atom in trimethylbromoquinone I.

That the esters VI, VII and VIII, differing only in the position of very similar alkyl groups in positions 5, 7 and 8, should prove to be inseparable is—while disappointing—not particularly surprising and it has been observed before in cases in which a reaction produces isomers so closely related as are the esters VI, VII and VIII.[7]

Experimental Part[8]

3,6-Dinitro-1,2,4,5-tetraethylbenzene.—The hydrocarbon (3 g.)[9] was vigorously shaken with sulfuric acid (75 cc.) until solution was complete. A mixture of fuming nitric acid (20 cc., d. 1.5) and sulfuric acid (15 cc.) was added in portions and the mixture was vigorously shaken. After the addition was complete, the solution was warmed (70–80°) for a few moments, during which oxides of nitrogen were evolved. The solid nitro compound separated during the reaction. The mixture was poured over ice (300 g.) and the solid was removed and crystallized from ethanol. The product formed long white needles (2.15 g., 56%) which melted at 149–151°. Smith and Harris,[10] who prepared this compound by nitration of hexaethylbenzene, report it to melt at 143–145°.

3,6-Diamino-1,2,4,5-tetraethylbenzene Stannichloride.—The above dinitro compound (3.2 g.) was dissolved in boiling acetic acid (60 cc.) in a large flask. The flame was removed, and a hot solution of stannous chloride dihydrate (20 g.) in hydrochloric acid (100 cc.) was added all at once. After the initial very vigorous reaction subsided, the mixture was refluxed for thirty minutes and then was cooled in an ice-salt-bath. The stannichloride was filtered off and washed successively with cold hydrochloric acid, water, alcohol and ether. The product (7.78 g., 98%) could be crystallized from water.

Tetraethyl-*p*-benzoquinone.—The above stannichloride (7.8 g.) was added to a solution of ferric chloride (20 g.) in water (75 cc.). After standing for twenty-four hours, the mixture was steam distilled and the distillate was extracted with ether. Removal of the ether left 2.24 g. (90%, based upon the nitro compound) of quinone which melted at 60–62°.[11]

Tetraethylquinone and Enolates of Malonic Ester.—A. Ethyl malonate (1 cc.) was added to powdered sodium (0.15 g.) suspended in benzene (50 cc.) and the mixture was heated until the metal had reacted completely. The quinone (0.5 g.) in benzene (15 cc.) was added and the mixture was refluxed for ten days. The mixture became

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(6) Smith and Opie, THIS JOURNAL, **63**, 937 (1941).

(7) For example, Huender, *Rec. trav. chim.*, **34**, 25 (1915); Smith and Horner, THIS JOURNAL, **62**, 1349 (1940).

(8) Microanalyses by E. E. Hardy and E. E. Renfrew.

(9) Smith and Guss, THIS JOURNAL, **62**, 2625 (1940).

(10) Smith and Harris, *ibid.*, **57**, 1289 (1935).

(11) Smith and Harris, ref. 10, p. 1292, give the m. p. as 56–58°.

very dark, but no precipitate formed. Acidification followed by steam distillation gave tetraethylquinone (0.4 g., 80% recovery) and no other product could be isolated. B. Ethyl malonate (1 cc.) was added to a solution of sodium (0.156 g.) in dry ethanol (10 cc.). The quinone (0.5 g.) in dry ethanol (5 cc.) was added and the solution was warmed for several hours on the steam-bath. A succession of colors developed during the mixing of the reagents and the subsequent heating—first red, then green, followed by brown and finally olive green, and a small amount of brown solid separated. This was removed, washed, with alcohol, then suspended in water and acidified. Only a very few flakes of solid material separated. The original alcoholic filtrate from this material was acidified and steam distilled. The quinone (m. p. 59–62°, 0.36 g., 72%) was the only product obtained although a small amount of uncrystallizable oil remained in the distilling flask. C. This experiment was repeated, using potassium instead of sodium. The results were the same; over 70% of the quinone was recovered and no other product could be isolated. D. Experiment A was repeated, substituting potassium for the sodium. Again the only product isolated was unchanged quinone.

Trimethylethyl-*p*-benzoquinone (V).—5-Acetopseudocumene[12] was reduced to **5-ethylpseudocumene** by the method of Smith and Kiess.[13] The hydrocarbon (8.5 g.) was added 1 cc. at a time to a vigorously stirred and well cooled (0°) solution of potassium nitrate (20 g.) in sulfuric acid (280 cc.). The reaction mixture was poured over ice and the solid **3,6-dinitro-1,2,4,5-tetraethylbenzene** (7.0 g., 50%) was removed and crystallized from methanol, when it melted at 85–87°. Smith and Kiess[14] obtained this nitro compound (m. p. 87–88°) in 37% yield, but the method given here is decidedly superior to theirs. The use of a great excess of sulfuric acid and of potassium nitrate, and nitration as rapidly as possible, are essential for the success of the preparation. The dinitro compound was converted into trimethylethylquinone via the diamine stannichloride (which is appreciably soluble and must not be washed with large volumes of solvents) by the procedure of Smith and Kiess[14] except that the stannichloride was not converted into the diamine but was oxidized directly to the quinone. The quinone was steam distilled from the reaction mixture, and isolated from the distillate by ether extraction. Removal of the ether left the quinone as yellow needles which melted at 43–45°.[15]

Ethylpseudocumohydroquinone was prepared by boiling a solution of the quinone (2 g.) in acetic acid (10 cc.) and water (7 cc.) with zinc (20 mesh, 2 g.) until the mixture was colorless (thirty minutes). Boiling water (10 cc.) was added and the solution was at once decanted from the zinc. The zinc was washed with hot water (10 cc.) and the liquid was decanted into the main solution. The hydroquinone separated from the cooled solution in practically quantitative yield. After crystallization from benzene, it melted at 169–170°.

Anal. Calcd. for $C_{11}H_{16}O_2$: C, 73.33; H, 8.88. Found: C, 73.58; H, 9.13.

The **diacetate,** prepared in the usual way and crystallized from ligroin, melted at 136–136.5°.

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.18; H, 7.57. Found: C, 68.38; H, 7.65.

Trimethylethylquinone and the Sodium Enolate of Malonic Ester.—Ethyl malonate (19.2 g., 0.12 mole) was added to a suspension of powdered sodium (2.77 g., 0.12 mole) in dry, thiophene-free benzene (100 cc.) and the mixture was refluxed until all of the metal had reacted. The quinone (10 g., 0.06 mole) in benzene (50 cc.) was added and the mixture was refluxed. After a few minutes, a red color developed and after four hours a red solid began to deposit as a crust on the wall of the flask. The flask was shaken vigorously from time to time in order to break up this crust. Refluxing was continued for twenty-four hours, after which the mixture was cooled, the red sodium derivative was filtered and the adhering benzene was removed under reduced pressure. The red solid was triturated in a mortar successively with water, alcohol and ether. After drying, the solid weighed 10.4 g. (60.8%). The cooled benzene filtrates deposited white needles (1.13 g., equivalent to 11.16% of the starting material) of trimethylethylhydroquinone (m. p. 169–170°). After removing this, hydrochloric acid was added and the benzene solution was steam distilled. The benzene layer in the distillate was separated and the solvent, as well as the excess ethyl malonate, were removed under reduced pressure. There remained 1.75 g. of trimethylethylquinone (17.50% of the starting material). Thus 89.46% of the quinone used was accounted for.

3 - Carbethoxy - 6 - hydroxy - bz - dimethylethylcoumarin (VI + VII + VIII).—The red sodium derivative (3.7 g., 0.012 mole) was suspended in dry ethanol and hydrochloric acid (5 cc.) was added. The red color immediately gave way to the yellow color of the coumarin, and a white precipitate of sodium chloride formed. Water (100 cc.) was added and the coumarin ester (3.1 g., 90%) was removed and dried. It melted at 148–165°.

Anal. Calcd. for $C_{16}H_{18}O_5$: C, 66.24; H, 6.20. Found: C, 66.14, 66.52; H, 6.41, 5.97.

The 185° Material (A).—1. From alcohol. The crude coumarin mixture (3.1 g.) was dissolved in alcohol (20 cc.) and the hot solution was filtered through a plug of glass wool. After standing for thirty minutes, the solution had cooled to 25° and a fluffy yellow precipitate (1.5 g., m. p. 177–180°) had formed. This was removed and dissolved in 25 cc. of hot 1:1 absolute ethanol and ligroin. The solution was cooled slowly to 25°, the yellow solid (0.7 g., m. p. 185–186°) was removed. The filtrate, when cooled to 0°, deposited a second crop (0.35 g.) which melted at 181–184°. These two solids showed no depression in m. p. when mixed, hence the two were combined (total 1.05 g.) to form the product A.

Anal. Calcd. for $C_{16}H_{18}O_5$: C, 66.24; H, 6.20. Found: C, 65.81, 65.68; H, 6.26, 6.32.

Further concentration of any of the filtrates from this material led to deposition of lower melting solids, but these melted over a very wide range (153–160°, 150–165°) and no improvement in the melting point value resulted when the solids were recrystallized repeatedly.

2. From ether-petroleum ether. The crude coumarin

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[12] Smith and Guss, THIS JOURNAL, **59**, 804 (1937).

[13] Smith and Kiess, *ibid.*, **61**, 284 (1939).

[14] Smith and Kiess, *ibid.*, **61**, 993 (1939).

[15] Smith and Kiess, ref. 14, p. 994, give the m. p. as 43°.

ester was crystallized once by dissolving it in hot alcohol and cooling the solution to 0°. The product melted over a wide range starting at 147° and extending to above 170°. This material was shaken with ether, filtered and petroleum ether was added to the filtrate. The solution was concentrated until solid appeared in the hot solution, when enough ether was added to redissolve the solid. The resulting solution was allowed to cool slowly to 25° when it deposited material which melted at 178–180°. When recrystallized again from ether–ligroin, this material melted at 182.5–184°. It was possible by repeated ether extraction of the crude coumarin to obtain more of the substance melting at 184° but with every subsequent extraction the melting points of the extracted materials became progressively lower until finally only material melting at 140–150° was obtained. Further crystallization of these low melting products did not give any substances with higher melting points.

3. By chromatographic adsorption. Chromatographic adsorption on calcium carbonate or alumina (Brockmann or Baker) was of little value as a means of separating the coumarin isomers, although this method was very efficient in removing tarry impurities present in the crude coumarin mixture. On calcium carbonate, a brown layer at the top of the column was held tenaciously, but practically all of the coumarin was unadsorbed. Evaporation of the filtrate gave material which melted at 172–179°. When the coumarins were chromatographed from benzene onto Brockmann alumina, zones were formed as follows: a small brown zone, followed by a rather extensive pink and yellow zone and finally a pure yellow zone at the bottom. Only the last gave any material on elution (alcohol), and from this there was obtained material melting at 181–182° after one recrystallization from absolute ethanol.

Coumarin Acid from A.—A solution of A (0.5 g., m. p. 179–180°) in acetone (40 cc.) was refluxed for ninety minutes with hydrochloric acid (40 cc.). The coumarin acid (m. p. 214–222°) was removed and crystallized from acetic acid. It then melted at 237–238°.

The 152° Material (B).—The coumarin mixture (obtained by decomposition of 10 g. of the red sodium derivative) was crystallized once by the ether–ligroin method, giving 3.53 g. of material melting at 178–180°. The filtrates when set aside in a refrigerator for two months deposited material which melted at 153–166°. This substance was washed with ether and the solutions, when evaporated, left material which melted at 150–152°. The residue from the ether washing was dissolved in a larger quantity of ether and the solution was poured through a tube containing alumina. The filtrate was evaporated and the residue, after crystallization from ether–petroleum ether, melted at 150–152°.

Anal. Calcd. for $C_{16}H_{18}O_5$: C, 66.24; H, 6.20. Found: C, 65.95; H, 6.24.

Acetate.—The 152° material (0.5 g.) was dissolved in acetic anhydride (10 cc.). A drop of sulfuric acid was added and the solution was refluxed for fifteen minutes, then poured over ice. Excess ammonium hydroxide was added and the solid was removed and crystallized from dilute acetic acid (50%). The acetate melted at 151–152°. When mixed with B (m. p. 150–152°) it melted at 126–130°.

Anal. Calcd. for $C_{18}H_{20}O_6$: C, 65.04; H, 6.02. Found: C, 64.88; H, 6.02.

Coumarin Acid from B.—The 152° material B (0.5 g.) was dissolved in acetone (40 cc.), hydrochloric acid (40 cc.) was added and the mixture was refluxed for ninety minutes. The acid, removed from the chilled mixture and crystallized from dilute acetic acid, melted at 230–232°.

Anal. Calcd. for $C_{14}H_{14}O_5$: C, 64.20; H, 5.43. Found: C, 63.93; H, 5.41.

Summary

1. Trimethylethylquinone has been condensed with sodium malonic ester. The initial product, a red sodium derivative, is formed in excellent yields.

2. The red sodium derivative, when decomposed by acids, gives a mixture of coumarin esters. This mixture was separated into two parts, A, melting at 185°, and B, melting at 150–152°, by fractional solution and fractional crystallization.

3. Both A and B have been shown to be inseparable mixtures. B contains all three of the possible coumarin esters, VI, VII and VIII derivable from trimethylethylquinone by reaction at a methyl group, while A contains but two of the coumarin esters, VI and VII.

4. The ethyl group in trimethylethylquinone does not exert any pronounced orienting effect upon the reaction between the quinone and sodium malonic ester, such as is shown by the bromine atom in trimethylbromoquinone.

5. Tetraethylquinone did not react with sodium or potassium malonic esters under any of the conditions tried.

MINNEAPOLIS, MINNESOTA

RECEIVED DECEMBER 11, 1940