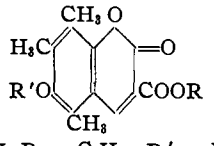
[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

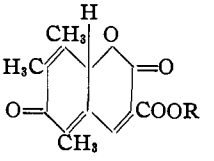
The Reaction between Duroquinone and Sodium Malonic Ester. III. Reduction Products[1]

BY LEE IRVIN SMITH AND RUSSELL O. DENYES

In 1926, Smith and Dobrovolny showed that 3-carbethoxy-5,7,8-trimethyl-6-hydroxycoumarin (I) was produced when duroquinone reacted with sodium malonic ester in benzene solution.[2]



I, R = C_2H_5; R′ = H
II, R = R′ = H
V, R = C_2H_5; R′ = Ac

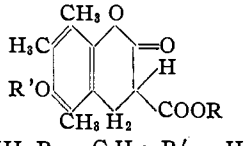


IA, R = C_2H_5
IIA, R = H

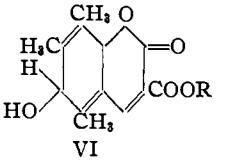
This product resulted from the union of one molecule of each of the reactants, accompanied by the loss of a molecule of alcohol and two hydrogen atoms. The substance and all of its derivatives which contained the free hydroxyl group were bright yellow; those derivatives in which the hydroxyl group was acetylated or methylated were colorless. Because of the loss of color which resulted when the hydroxyl group was fixed by methylation or acetylation, the primary product was originally given the tautomeric formula IA, although it was recognized at the time that all of the chemical properties of the substance could be better interpreted on the basis of formula I, and it was proved that the methylated products were certainly derived from structure I.[2,3]

In order to obtain more information regarding the location of the "mobile" hydrogen atom, it was decided to study the reduction of the primary product I or IA and the acid (II or IIA) derived from it by hydrolysis.

Catalytic reduction of the ester I gave a colorless dihydro derivative (III), m.p. 104–105°, which formed a monoacetate IV, m.p. 116–117°. Catalytic reduction of the acetyl derivative (V) of the ester I also gave the same monoacetyl derivative melting at 116–117°. The formation of the same acetyl derivative, regardless of whether reduction preceded or followed acetylation, was in itself evidence that the original ester had the structure I rather than IA, and this evidence definitely excluded any structure for the reduction product, such as VI, in which the quinoid ring of IA had been attacked.

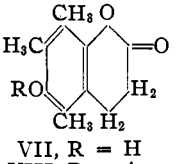


III, R = C_2H_5; R′ = H
IV, R = C_2H_5; R′ = Ac

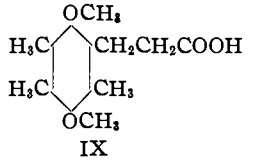


VI

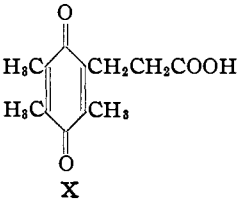
The dihydro derivative III, when hydrolyzed, gave a substance (VII) $C_{12}H_{14}O_3$, soluble in alkali, but insoluble in bicarbonate, and this same substance VII was obtained by catalytic reduction



VII, R = H
VIII, R = Ac



IX



X

of the acid II. Substance VII formed a monoacetyl derivative (VIII), and it gave a dimethoxy acid (IX) when subjected to the action of dimethyl sulfate and alkali. The action of sodium methoxide upon a benzene solution of VII produced a yellow quinone acid (X) which readily could be reduced to VII by zinc and acetic acid. The quinone acid (X) was then synthesized from pseudocumene; it was identical with the product obtained from VII and was converted into VII on reduction. The product obtained from the acid (II) by catalytic reduction was therefore proved to be the 6-hydroxy-3,4-dihydrocoumarin (VII), while the product obtained by catalytic reduction of the ester (I), and which gave VII on hydrolysis, must have been 6-hydroxy-3-carbethoxy-3,4-dihydrocoumarin (III).

When the ester I was reduced by zinc and acetic acid, three products were formed. One of these

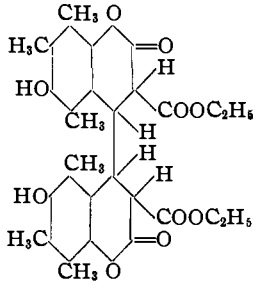
(1) Abstracted from a thesis by Russell O. Denyes, presented to the Graduate Faculty of the University of Minnesota, in partial fulfilment of the requirements for the degree of Doctor of Philosophy, October, 1935.

(2) Smith and Dobrovolny, THIS JOURNAL, **48**, 1693 (1926).

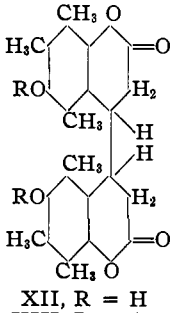
(3) Smith, *ibid.*, **56**, 472 (1934).

was the dihydro ester III (m. p. and mixed m. p. 104–105°; m. p. and mixed m. p. of the acetyl derivative IV, 116–117°). The second product was a dihydroxy diester (XI), m. p. 212–213°, which had the composition $C_{30}H_{34}O_{10}$; the third product was a dihydroxy compound (XII), m. p. 290–292°, which had the composition $C_{24}H_{26}O_6$. Reduction of the acid II by zinc and acetic acid gave two products, one of which was the dihydrocoumarin VII, m. p. and mixed m. p., 173–174°. The other product was the substance XII, m. p. 290–292°, which was also formed by reduction of the ester I.

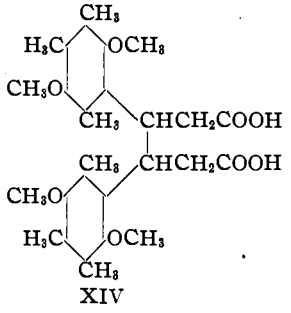
Substance XI, when hydrolyzed, lost two molecules of carbon dioxide and gave XII. Substances XI and XII were therefore most likely related in the same manner as III and VII, that is, XI was a dicarbethoxy derivative of XII. Substance XII formed a diacetyl derivative (XIII), and when subjected to the action of dimethyl sulfate and alkali, XII gave a tetramethoxy diacid (XIV). When XII was oxidized in alkaline solution, it gave a yellow diquinone diacid $C_{24}H_{26}O_8$ (XV) which could be converted into XII by reduction.



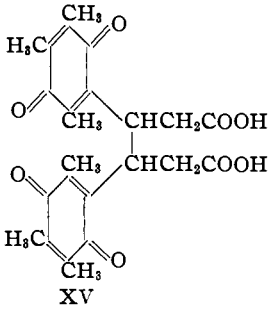
XI



XII, R = H
XIII, R = Ac



XIV



XV

Attempts were made to convert XIV into a cyclopentanone derivative, and thus to prove definitely that the bond connecting the two carbon chains was β to the two carboxyl groups and that XIV was a derivative of adipic acid. These experiments were not successful, but although definite proof was not obtained, it is hardly likely, in view of the results of Harries[4] and of many others upon the dimolecular reduction products of α,β-unsaturated esters and ketones, that the union of the two residues would occur elsewhere than in the β-position. Moreover, it is likely that those dimolecular products obtained by reduction of coumarins and described in the literature[5] as having the 3,3′ structure, in reality have the 4,4′ structure.

The structure of the reduction products, and the relationships among them, present a more consistent picture when interpreted in terms of formulas I and II for the ester and the acid, respectively, than is the case when IA and IIA are used. Not a single chemical property of the ester or acid is known, the interpretation of which requires formulas IA and IIA. It seems safe to say, then, that the reaction between duroquinone and sodium malonic ester leads to a 6-hydroxy-3-carbethoxycoumarin derivative. In spite of the fact that these substances are deeply colored (yellow) as long as the hydroxyl group is free, the cause of the color must be sought elsewhere than in a tautomerism as pictured by formulas I and IA. Most coumarins are colorless; a few are pale yellow, notably those which have the hydroxyl group in the 6-position and a carboxyl group in the 3- or 4-position. Of the colored coumarins, 6-hydroxy-4-carboxycoumarin, and its methyl derivative, 6-methoxy-4-carboxycoumarin, are yellow.[6] In the case of this methyl ether the color could not be due to any tautomerism to a quinoid form, for the hydroxyl is fixed by methylation.

The reaction between completely methylated quinones and sodium enolates of various sorts appears to be a promising method for synthesis of the 6-hydroxy-5,7,8-trimethylcoumarins substituted in the 3-position by such groups as carbethoxyl, acyl, cyano, etc. Work already in progress in this Laboratory indicates the reaction to be a general one, and has given some insight into the mechanism of the reaction.

Experimental Part

For the preparation of the large amounts of duroquinone necessary for this research, the procedures of Smith and

(4) Harries, *Ann.*, **296**, 295 (1897).

(5) (a) Zwenger, *Ann. Suppl.*, **8**, 32 (1872); (b) Dyson, *J. Chem. Soc.*, **51**, 68 (1887); (c) Fries and Fickewirth, *Ann.*, **362**, 30 (1908); (d) Simonis and Peters, *Ber.*, **41**, 833 (1908); (e) v. Pechmann and Cohen, *ibid.*, **17**, 2135 (1884); (f) Michael, *Am. Chem. J.*, **5**, 436 (1883).

(6) Biginelli, *Gazz. chim. ital.*, **24**, II, 491 (1894); *Ber.*, **28**, IV, 115 (1895).

Dobrovolny[7] have been modified. and the yields of intermediates have been improved.

Dinitrodurene.—Sulfuric acid (100 cc.), fuming nitric acid (32 g., d. 1.5) and chloroform (200 cc.) were stirred vigorously and cooled (below 10° throughout) while *pure* solid durene (27 g.) was added a little at a time. The layers were separated and the sulfuric acid was extracted twice with chloroform. The combined chloroform solutions were washed free from acid with carbonate, dried over calcium chloride, and the solvent evaporated until the nitro compound began to crystallize. Hot alcohol (300 cc.) was added and the mixture was cooled. The solid was filtered, the filtrate was evaporated to dryness and the residue taken up in chloroform (40 cc.). This solution was treated with hot alcohol (120 cc.) as before. The solid weighed 41 g. (92%) and melted at 211–212°.

Duroquinone.—The stannichloride of diaminodurene (60 g.)[7] was suspended in a solution of ferric chloride (180 g.) in water (90 cc.) and hydrochloric acid (12 cc.), and the suspension set aside at room temperature for thirty-six hours. The mixture was steam distilled and the quinone in the distillate was recrystallized from alcohol; yield 25.8 g. (95%); m. p. 111–112°. The ester I was prepared by the method of Smith and Dobrovolny;[2] yield 47% in 0.2 molar runs; m. p. 183–184°.

Catalytic Reductions

The ester I (5 g.) was dissolved in alcohol (200 cc.) and a platinum oxide catalyst (0.02 g.) was added. The solution was shaken under 3 atm. pressure of hydrogen. When the yellow color of the solution disappeared (*ca.* one hour), the solution was filtered and the filtrate evaporated on the steam-bath. The residue was dissolved in aqueous alcohol at 60° and the solution, when cooled, deposited 4 g. (80%) of white **3-carbethoxy-5,7,8-trimethyl-6-hydroxy-3,4-dihydrocoumarin** (III); m. p. 104–105°.

Anal.[8] Calcd. for $C_{15}H_{18}O_5$: C, 64.7; H, 6.5. Found: C, 64.7, 64.6; H, 6.5, 6.6.

Acetyl Derivative (IV).—Acetylation of III (0.2 g.) with acetic anhydride (4 g.) and fused sodium acetate (0.2 g.) gave **3-carbethoxy-5,7,8-trimethyl-6-acetoxy-3,4-dihydrocoumarin** (IV), melting point 116–117° after crystallization from aqueous alcohol.

Anal. Calcd. for $C_{17}H_{20}O_6$: C, 63.75; H, 6.25. Found: C, 63.6, 63.7; H, 6.2, 6.0.

The acetyl derivative V, m. p. 176–177°[2] (p. 1705) (0.2 g.) dissolved in ethyl alcohol (50 cc.) and reduced as above, also gave IV, m. p. and mixed m. p., 116–117°.

5,7,8 - Trimethyl - 6 - hydroxy - 3,4 - dihydrocoumarin (VII).—The ester III (5 g.) was dissolved in acetone (10 cc.) and boiled for two hours with hydrochloric acid (80 cc., 20%). The solid, dried and crystallized from a mixture of chloroform and petroleum ether, was colorless and weighed 3.5 g. (95%). It melted at 173–174°.

Anal. Calcd. for $C_{12}H_{14}O_3$: C, 69.9; H, 6.8. Found: C, 69.6, 70.0; H, 6.5, 6.7.

The acetyl derivative (VIII), crystallized from chloroform–petroleum ether, was white and melted at 147–148°.

Anal. Calcd. for $C_{14}H_{16}O_4$: C, 67.7; H, 6.5. Found: C, 67.3; H, 6.6.

The acid II was prepared by dissolving the ester I (5.5 g.) in acetone (50 cc.) and refluxing the solution for three hours with hydrochloric acid (200 cc., 20%). The dried solid weighed 4.8 g. (96%) and melted at 256–258° (dec.). This was a much better method of conducting the hydrolysis than that previously used, in which 80% sulfuric acid was employed[2] (p. 1703).

The acid II (0.2 g.) was dissolved in alcohol (50 cc.), and reduced catalytically as described above.

The product was VII, m. p. and mixed m. p., 173–174°; m. p. and mixed m. p. of the acetyl derivative (VIII) prepared from this product, 147–148°.

β-(2,5-Dimethoxy-3,4,6-trimethyl)-phenyl Propionic Acid (IX).—The dihydrocoumarin (1 g.) was dissolved in dry methyl alcohol (20 cc.) and dimethyl sulfate (8 g.). To the hot solution was added slowly a solution of potassium hydroxide (10 g.) in methyl alcohol (50 cc.). Most of the alcohol was then evaporated, water was added and the cooled solution extracted with ether. The aqueous layer was acidified and extracted with ether, the ether solution dried and evaporated. The residual oil crystallized when cooled. When recrystallized three times from chloroform–petroleum ether, it was white and melted at 132–133°.

Anal. Calcd. for $C_{14}H_{20}O_4$: C, 66.66; H, 7.9; OCH_3 (two), 24.8. Found: C, 66.5; H, 8.0; OCH_3, 25.4, 25.2.

The amide was prepared and when recrystallized from alcohol it melted at 188–189°.

Anal. Calcd. for $C_{14}H_{21}O_3N$: C, 66.9; H, 8.4. Found C, 67.0; H, 7.9.

Trimethyl-(β-carboxy-ethyl)-*p*-benzoquinone; β-(2,4,5-Trimethyl-*p*-benzoquinone)-propionic Acid (X).—The dihydrocoumarin, formula VII, (0.5 g.) was dissolved in benzene (10 cc.) and refluxed for thirty minutes with 10 cc. of a solution prepared from sodium (0.7 g.), methyl alcohol (15 cc.) and water (5 cc.). The reaction mixture was diluted with water, the benzene removed and the aqueous layer extracted with ether. The aqueous layer was then acidified and again extracted with ether. This ether solution was dried and the solvent evaporated. There remained a yellow oil which solidified when cooled. Several crystallizations from chloroform–petroleum ether gave a yellow solid which melted at 113–114°.

Anal. Calcd. for $C_{12}H_{14}O_4$: C, 64.86; H, 6.3. Found: C, 64.9; H, 6.2.

Reduction of the quinone acid (0.1 g.) in acetic acid (6 cc.) and water (1.5 cc.) by zinc dust (0.5 g.) gave the dihydrocoumarin VII, m. p. and mixed m. p., 172–173°.

Synthesis of the Quinone Acid, X

2,4,5-Trimethylcinnamic Acid.—Durylic aldehyde (30 g.), fused potassium acetate (15 g.) and acetic anhydride (30 g.) were refluxed (170–180°) for eight hours in an atmosphere of nitrogen. The reaction mixture was poured into water and steam distilled. Durylic aldehyde (10 g.) was recovered from the distillate. The residue from the steam distillation was made alkaline with sodium carbonate and the solution was boiled with norite and

(7) "Organic Syntheses," John Wiley and Sons, Inc., New York, N. Y., 1930, Vol. X, p. 40.

(8) F. J. Dobrovolny, Ph.D. Thesis, University of Minnesota 1925, p. 53.

filtered. The tar and norite were extracted with boiling carbonate solution, and the combined carbonate solutions were acidified. The acid was crystallized from dilute alcohol. The yield was 15 g. (58% corrected for the recovered aldehyde); m. p. 154–155°. Smith and Tawney[9] obtained 1.6 g. of this acid from 3.5 g. of durylic aldehyde *via* duralmalonic ester. In the hope of improving the yield, the acid was also synthesized from durylic aldehyde (5 g.), malonic acid (5 g.) and pyridine (5 g.) according to the method of Kuhn[10] but the yield was only 2 g. (30%) of acid melting at 149–151°.

2,4,5-Trimethylhydrocinnamic acid[9] was prepared in 98% yield by catalytic reduction of the unsaturated acid (5 g.); m. p. 95–96°.

Methyl Ester.—The acid (10 g.) was refluxed for four hours with methyl alcohol (100 cc.) and sulfuric acid (10 cc.). The product, an oil which boiled at 125–127° under 4 mm., weighed 9 g. (90%).

Anal. Calcd. for $C_{13}H_{18}O_2$: C, 75.7; H, 8.8. Found: C, 76.5; H, 8.8.

2,4,5-Trimethyl-3,6-dinitrohydrocinnamic Acid.—Trimethylhydrocinnamic acid (4 g.) was dissolved in chloroform (25 cc.). The solution was stirred with sulfuric acid (20 cc.) and kept at 30–40° while fuming nitric acid (3 cc., d. 1.52) was slowly added. The chloroform layer was separated and extracted with sodium carbonate. The acid layer was poured onto ice and the carbonate extraction added to it. The solid was removed and crystallized several times from alcohol. It was white and melted at 206–207°.

Anal. Calcd. for $C_{12}H_{14}O_6N_2$: C, 51.07; H, 5.0. Found: C, 50.9; H, 5.1.

The amide was white and melted at 215–216°.

Anal. Calcd. for $C_{12}H_{15}O_5N_3$: C, 51.23; H, 5.37. Found: C, 51.35; H, 5.5.

The methyl ester was prepared from the acid and diazomethane. It was crystallized from chloroform–petroleum ether, was white and melted at 164.5–165°.

Anal. Calcd. for $C_{13}H_{16}O_6N_2$: C, 52.65; H, 5.56. Found: C, 52.7; H, 5.44.

This dinitro ester was also formed by slowly stirring the methyl ester of trimethylhydrocinnamic acid (7.5 g.) into a cold (10°) mixture of sulfuric acid (50 cc.) fuming nitric acid (10 cc., d. 1.52), and chloroform (50 cc.). The layers were separated, the chloroform was evaporated and the acid layer poured onto ice. The combined solids were crystallized from chloroform–petroleum ether. The product weighed 8.5 g. (80%) and the m. p. and mixed m. p. was 164–165°.

5,7,8-Trimethyl-6-amino-3,4-dihydrocarbostyril was obtained by reducing either the dinitro acid or its methyl ester. The dinitro acid (1 g.) dissolved in acetic acid (10 cc.) and boiled for fifteen minutes with a solution of stannous chloride (7 g.) in hydrochloric acid (8 cc.). The solution was made strongly alkaline, the precipitate was removed, washed with water, dried and extracted with chloroform. The chloroform solution was concentrated, petroleum ether was added and the product crystallized by cooling. Recrystallization from chloroform–petroleum ether gave a pale yellow solid which melted at 223–224°.

Anal. Calcd. for $C_{12}H_{16}ON_2$: C, 70.6; H, 7.85. Found: C, 70.5; H, 7.98.

A similar reduction of the methyl ester (8.5 g.) gave 5 g. (85%) of the aminodihydrocarbostyril, m. p. and mixed m. p., 223–224°.

Acetyl Derivative.—Recrystallized from methyl alcohol, this was white and melted at 320°.

Anal. Calcd. for $C_{14}H_{18}O_2N_2$: C, 68.30; H, 7.37. Found: C, 68.45; H, 7.5.

The Quinone Acid (X).—The aminodihydrocarbostyril (1.5 g.) was dissolved in hydrochloric acid (2 cc.) and a warm solution of ferric chloride (24 g.) in water (12 cc.) was added. The mixture was allowed to stand, with occasional shaking, for five days. It was then extracted with ether, the ether washed with water and dried over anhydrous sodium sulfate. The ether was evaporated and the residue crystallized from chloroform–petroleum ether. The yellow crystals, which weighed 1 g. (56%), melted at 113–114°, and the mixed m. p. with the quinone acid X obtained by oxidation of VII was also 113–114°. Reduction of this quinone acid gave VII, m. p. and mixed m. p., 173–174°; m. p. and mixed m. p. of the acetyl derivative, 147–148°.

Reductions by Zinc and Acetic Acid

The ester I (12.5 g.) was dissolved in acetic acid (500 cc.), zinc (10 g.) was added and the mixture was refluxed. From time to time a little more zinc was added, and refluxing was continued until the yellow color disappeared. The excess zinc was removed, the solution was diluted to two liters with water, cooled and extracted with chloroform. The chloroform solution was dried over sodium sulfate and the solvent was evaporated on the steam-bath. Benzene (100 cc.) was added to the residual oil and the mixture was warmed. The white solid (A) which separated was removed and the filtrate was concentrated and cooled. The white solid (B) which separated at this point was removed and the benzene was evaporated from the filtrate. The residual oil was dissolved in warm alcohol, and sufficient water was added to produce incipient crystallization. The cooled solution deposited an oily solid (C, 3.0 g.), which was removed. The filtrate from C was evaporated and the residual oil extracted with petroleum ether. This solution, when concentrated and cooled, deposited a white solid (D, m. p. 85–95°). Evaporation of the filtrate from D left an oil (1.0 g.) which could not be crystallized.

Recrystallization of A from acetic acid gave XII (0.5 g.) m. p. 290–292°. B was dissolved in benzene (200 cc.), the solution concentrated until crystallization began, and cooled. The white crystalline product (m. p. 160–162°), recrystallized first from chloroform–petroleum ether, and then from aqueous alcohol, yielded XI (2.0 g.), m. p. 212–213°. The oily solid C, recrystallized from aqueous alcohol, gave XI, m. p. 212–213° (1.0 g.), and I, m. p. 183–184° (1.5 g.). Recrystallization of D (m. p. 85–95°) first from chloroform–petroleum ether and then from aqueous alcohol, gave III (3.5 g.), m. p. 104–105°. The total yield of pure products from the reduction of 12.5 g. of I was 8.5

(9) Smith and Tawney, THIS JOURNAL, **56**, 2169 (1934).
(10) Kuhn, *Ber.*, **63**, 2164 (1930).

g., which consisted of XII, 0.5 g.; XI, 3.0 g.; III, 3.5 g.; recovered I, 1.5 g.

The acid II (5.0 g., m. p. 260°), dissolved in acetic acid (500 cc.) was refluxed with zinc dust (10 g.) until the yellow color disappeared. The solution was diluted with water (250 cc.), and hydrochloric acid was added to dissolve the excess zinc. The clear solution was then diluted to two liters with water, cooled and the white solid removed (E). The filtrates were extracted with chloroform, the chloroform layer dried over sodium sulfate and the solvent evaporated, leaving a brown solid (F). E, recrystallized from acetic acid, gave XII (2.0 g.), m. p. 290–292°. F was dissolved in chloroform, the solution was boiled with charcoal and Fuller's earth until colorless, filtered and petroleum ether added to the filtrate. The resulting solid, recrystallized from chloroform–petroleum ether, gave VII (1.0 g.), m. p. and mixed m. p., 172–174°; m. p. and mixed m. p. of the acetyl derivative (VIII), 147–148°.

3,3'-Dicarbethoxy-6,6'-dihydroxy-5,5',7,7',8,8'-hexamethyl-3,3',4,4'-tetrahydro-4,4'-dicoumarinyl (XI), crystallized from aqueous alcohol, was white and melted at 212–213° (dec.).

Anal. Calcd. for $C_{30}H_{34}O_{10}$: C, 64.98; H, 6.18; mol. wt., 554. Found: C, 65.11; H, 6.04; mol. wt. (Rast), 490.

The diacetate, recrystallized from alcohol, was a white solid, m. p. 218–219°.

Anal. Calcd. for $C_{34}H_{38}O_{12}$: C, 63.95; H, 5.95. Found: C, 64.06; H, 5.90.

Hydrolysis of XI (0.5 g.) by refluxing it with 50% sulfuric acid (30 cc.) and alcohol (5 cc.) for one hour, gave XII, m. p. and mixed m. p., 292° (dec.); m. p. and mixed m. p. of the diacetate, 296–298°.

6,6'-Dihydroxy-5,5',7,7',8,8'-hexamethyl-3,3',4,4'-tetrahydro-4,4'-dicoumarinyl (XII) was formed either by reduction of I or II by zinc and acetic acid, or by hydrolysis of XI. Crystallized from acetic acid, it was white and melted at 290–292° (dec.).

Anal. Calcd. for $C_{24}H_{26}O_6$: C, 70.25; H, 6.32; mol. wt., 410. Found: C, 69.96; H, 6.07; mol. wt. (Rast), 405.

The diacetate (XIII), crystallized from acetic acid or alcohol, was white and melted at 298° (dec.).

Anal. Calcd. for $C_{28}H_{30}O_8$: C, 68.02; H, 6.07. Found: C, 68.03; H, 5.86.

β,β'-Di-(2,5-dimethoxy-3,4,6-trimethylphenyl)-adipic Acid (XIV).—The dicoumarinyl XII (1 g.) was suspended in methyl alcohol (20 cc.) and dimethyl sulfate (8 g.). To the hot mixture was added slowly a hot solution of potassium hydroxide (10 g.) in methyl alcohol (50 cc.). The reaction mixture was diluted with water, most of the alcohol was boiled off and the aqueous solution was acidified. The precipitate was removed, the filtrate extracted with chloroform and the chloroform evaporated. The solids were combined and crystallized from acetic acid. The substance was white and melted at 309° (dec.). It was relatively insoluble in most solvents. The molecular weight could not be determined by the Rast method because the substance was too insoluble in camphor.

Anal. Calcd. for $C_{28}H_{38}O_8$: C, 66.89; H, 7.60. Found: C, 67.04; H, 7.64.

The dimethyl ester was obtained by the action of diazomethane upon a suspension of the acid in chloroform–ether. The solid remaining after evaporation of the solvents was extracted with chloroform, in which the acid was insoluble. The ester, crystallized from methyl alcohol, was white and melted at 217–218°.

Anal. Calcd. for $C_{30}H_{42}O_8$: C, 67.92; H, 7.90. Found: C, 68.17; H, 7.80.

β,β'-Di-(3,4,6-trimethylbenzoquinonyl-2,5)-adipic acid (XV) was obtained when the dicoumarinyl XII (2 g.) was dissolved in 3% sodium hydroxide (150 cc.) and the solution boiled for ten minutes with norite. The solution was filtered and the filtrate was acidified. A dark red oil separated which, when cooled, solidified to a red amorphous solid. This was removed and recrystallized from acetic acid. It formed a yellow solid, melting at 256–258° (dec.).

Anal. Calcd. for $C_{24}H_{26}O_8$: C, 65.16; H, 5.88; mol. wt., 444. Found: C, 65.30; H, 5.97; mol. wt. (Rast), 445.

The dimethyl ester was obtained by the action of diazomethane upon XV. It was a yellow solid melting at 293–294°.

Anal. Calcd. for $C_{26}H_{30}O_8$: C, 66.45; H, 6.39. Found: C, 66.40; H, 6.14.

Reduction, by zinc and acetic acid, of either the quinone acid XV or its dimethyl ester, led to the dicoumarinyl XII, m. p. and mixed m. p. 290–292°.

Pyrolysis of the Tetramethoxy Diacid XIV.—The action of acetic anhydride (10 cc.) at 300° upon the acid (1 g.) gave only a black insoluble material which was largely carbon. No ketone was obtained, nor was any of the starting material recovered. The experiment was repeated at 225–235° and the product was unchanged material. At 250–270°, the products were unchanged material and carbon.

Pyrolysis of the Thorium Salt of XIV.—The acid (0.5 g.) was dissolved in dilute sodium hydroxide and the solution was neutralized with hydrochloric acid using phenolphthalein as the indicator. Thorium chloride (0.5 g.) was added, and the solution evaporated to dryness. The residue was heated for one hour at 250° in an atmosphere of nitrogen. During the heating, an oil condensed on the flask above the surface of the salt. The reaction product was cooled and extracted with chloroform. The only pure substance obtained was a small amount of the acid, m. p. and mixed m. p., 309°.

Summary

1. The ester I, obtained by the action of sodium malonic ester on duroquinone, and the acid II obtained from the ester by hydrolysis, have been reduced by two methods.

2. Reduction of I and II catalytically gave only monomolecular products; reduction by zinc and acetic acid gave both monomolecular and dimolecular products.

3. All of the reduction products were dihydrocoumarin derivatives. No evidence was obtained

that the ester and acid reacted in the tautomeric forms IA and IIA.

4. The reaction between a completely methylated quinone and sodium enolates appears to be a general method for preparation of 6-hydroxy methylated coumarins with substituents in the 3-position.

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[CONTRIBUTION FROM NORTHWESTERN UNIVERSITY MEDICAL SCHOOL]

Studies on Proteins in Liquid Ammonia. III. Reaction of Sodium in Liquid Ammonia with Proteins and Related Substances

BY R. G. ROBERTS AND C. O. MILLER

In the previous paper[1] of this series, we reported the results of our investigation of the reactions of certain amino acids, dipeptides, diketopiperazine and related substances with sodium in liquid ammonia. Some of these substances are reduced and all are acidic in liquid ammonia. We have made a similar study using several proteins.

Experimental

The analytical procedure was essentially the same as described in the previous paper. The proteins were dried by heating in an electric oven at 85° for forty-eight hours, followed by transfer to a vacuum desiccator containing concentrated sulfuric acid for an additional forty-eight hours. Drying experiments were carried out with casein (Pfanstiehl) in which the casein was dried by the following methods: (1) air drying by exposure to atmosphere at room temperature for three months, (2) drying in a vacuum desiccator over concentrated sulfuric acid for three months, (3) drying at 85° in an electric oven for forty-eight hours followed by drying in a vacuum desiccator for forty-eight hours, (4) drying in an electric oven at 85° for six weeks, (5) drying in an electric oven at 85° for six weeks followed by additional drying in a vacuum desiccator over concentrated sulfuric acid for six weeks, (6) drying for six weeks in an electric oven at 105° followed by additional drying for six weeks in a vacuum desiccator over concentrated sulfuric acid and (7) drying at 115° in an electric oven for six weeks followed by an additional six weeks in a vacuum desiccator over concentrated sulfuric acid. Effectiveness of drying was determined by the amount of hydrogen liberated by casein in liquid ammonia when excess sodium was added. Casein dried by methods 3, 4, 5, 6 and 7 gave the smallest volumes of hydrogen and in equivalent amounts within the limits of experimental error.

Drying experiments with silk fibroin, edestin (Pfanstiehl), egg albumin (Merck) and meat peptone (Armour) showed that either drying them for forty-eight hours in a vacuum desiccator over concentrated sulfuric acid or heating them in an electric oven at 75 to 115° for forty-eight hours removed all of the water, but that a combination of the two was more effective and gave minimum hydrogen liberation.

Whether this method of drying removes all of the water from the protein, we do not know. When traces of water are present in a protein, there is a brief period of rapid reaction with sodium in liquid ammonia, which subsides to a slow rate of reaction characteristic of proteins. If the proteins, after the drying treatment, still contain water, it must be bound water or water of constitution which cannot be removed without decomposition of the protein. While bound water would give slightly higher figures for hydrogen evolved, it will not alter the shape of the curves. Each curve will have a slightly higher position on the ordinate axis.

In general, proteins react with sodium in liquid ammonia to give hydrogen more slowly than either amino acids or dipeptides. The reaction of sodium with amino acids and dipeptides is complete in a few minutes, with diketopiperazine in about one hour, and with most proteins in two or three hours.

The drying of certain proteins presents some difficulties. When zein and gliadin, freshly precipitated from absolute alcohol, were kept in a vacuum desiccator over sulfuric acid for a few days, they were found to be quite soluble or dispersed in liquid ammonia. This is in agreement with a previous observation made by Taft[2] that zein and gliadin are soluble or dispersed in liquid ammonia. However, after drying zein and gliadin at 80° for forty-eight hours, they were found to be practically insoluble in liquid ammonia.

The data are given graphically in Fig. 1. Moles of hydrogen evolved are plotted against atomic weights of sodium used, the nitrogen content of the sample portions of proteins being the same in all cases. An excess of sodium was considered to have been used when the liquid ammonia solution remained blue for three hours. Sodium reacts with liquid ammonia very slowly. The quantity of hydrogen liberated when sodium reacts with a protein is corrected for this. The ratios of moles of sodium to gram atoms of nitrogen necessary to use to have an excess of sodium for each of the proteins were: edestin, 1.25; silk fibroin, 1.50; casein, 1.00; and egg albumin, 1.25. No attempt was made to determine the end-point exactly.

(1) C. O. Miller and R. G. Roberts, THIS JOURNAL, **56**, 935 (1934).

(2) Taft, *Trans. Kansas Acad. Sci.*, **32**, 38 (1929); *J. Phys. Chem.*, **34**, 2722 (1930).