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EXPERIMENTS IN THE VERATROLE AND QUINOXALINE GROUPS

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As one branch of our work in the search for superior antimalarials (1, 2), we have had occasion to study the veratrole and quinoxaline groups, and have found them sufficiently interesting to justify additional exploration, because of the ease with which the 4,5-diamino- and 3,4,5-triamino-veratroles are prepared and the many reactions to which such vicinal polyamines are subject. Further, it is not improbable that prospecting in these fields may uncover compounds of biological or therapeutic significance. Attention is called particularly to recent work in the sulfaquinoxaline field (3–13).

Quinoxaline derivatives have also been studied by others with reference to possible antimalarial activity (14, 15) (S.N. 3078, 5038, 5043, 6593, 7749, 8002, 10080, 10081, 10084, 10085, etc.), but without discovering as yet any very promising compounds.

The antibacterial activity of some quinoxaline derivatives has been explored by O. Schales, S. Schales, and Friedman (16), and by McIlvain (17).

In the veratrole group, some experiments were conducted on the hydrolysis of 4,5-dinitroveratrole (I) by 40% hydrobromic acid, and the products isolated were the corresponding dinitroguaiacol (II) and dinitropyrocatechol (III). The former was identified by its m.p. (172–173°), the analysis of its scarlet sodium salt, and the conversion of this salt into 4,5-dinitroveratrole (I) by the action of methyl sulfate. The identity of the dinitropyrocatechol was established by its analysis, the analysis of its steel-blue disodium salt, and the methylation of the last named to the dinitroveratrole (I).

The 4,5-dinitropyrocatechol (III) was reduced to the diaminopyrocatechol (IV) by sodium hydrosulfite in aqueous solution. As Hoehn (18) has shown, the free (IV) oxidizes rapidly in the air to the diamino-*o*-quinone. No attempt was made, therefore, to isolate a pure (IV), but the reduction product of the dinitropyrocatechol was converted directly into the dihydroxyquinoxaline (VII) by condensation with glyoxal.

For the reduction of the 4,5-dinitroveratrole (I), the action of iron and hydrochloric acid upon its alcoholic solution was found more satisfactory than the use of tin and hydrochloric acid (1).

This diamine (VIII) was condensed with glyoxal to the corresponding quinoxaline (IX). The latter was very stable to nitrating agents. The product usually obtained was a mixture of the mononitro derivative (X) and of the corresponding guaiacol type (XI). No dinitro derivative was isolated in any of the experiments. Although no pure mononitro derivative was obtained, the product was sufficiently pure to yield the corresponding monoamino derivative on reduction. As this monoamine (XII) was identical with the monoamine

which resulted when 3,4,5-triaminoveratrole was condensed with glyoxal, the nitro group in (X) must be in position 8, and the structure of the aminodimethoxyquinoxaline must be (XII).

To contribute to the solution of the engrossing problem of the dependence of physiological action upon chemical constitution, a sulfanilamido (XIII) and a *beta*-diethylaminoethyl derivative (XIII) of the 8-amino-6,7-dimethoxyquinoxaline were prepared and tested but, as noted beyond, neither showed any promise of therapeutic usefulness.

4,5-Dinitroguaiacol was similarly reduced to the diamine (V), and the latter condensed with glyoxal to the methoxyhydroxyquinoxaline (VI). This latter compound was also separated as one of the products in the attempted conversion of 8-amino-6,7-dimethoxyquinoxaline (XII) into the corresponding 8-hydroxy-6,7-dimethoxyquinoxaline by diazotization. In another series of experiments, 8-amino-6,7-dimethoxyquinoxaline was diazotized in the presence of fairly strong sulfuric acid and treated with potassium iodide. The product was an unidentified crystalline iodo derivative, decomposing around 210°, insoluble in boiling water, readily soluble in cold dilute sodium carbonate solution, and which formed methoxyhydroxyquinoxaline (VI) when treated with an aliphatic amine in *n*-butanol solution.

In another group of experiments, the aminodimethoxyquinoxaline was converted into the diazoacetate which was decomposed by heating with copper powder, and on working up yielded the methoxyhydroxyquinoxaline (VI), and a crystalline product, m.p. 163–164°, which gave (VI) when digested with alkali.

ACKNOWLEDGMENTS

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EXPERIMENTAL

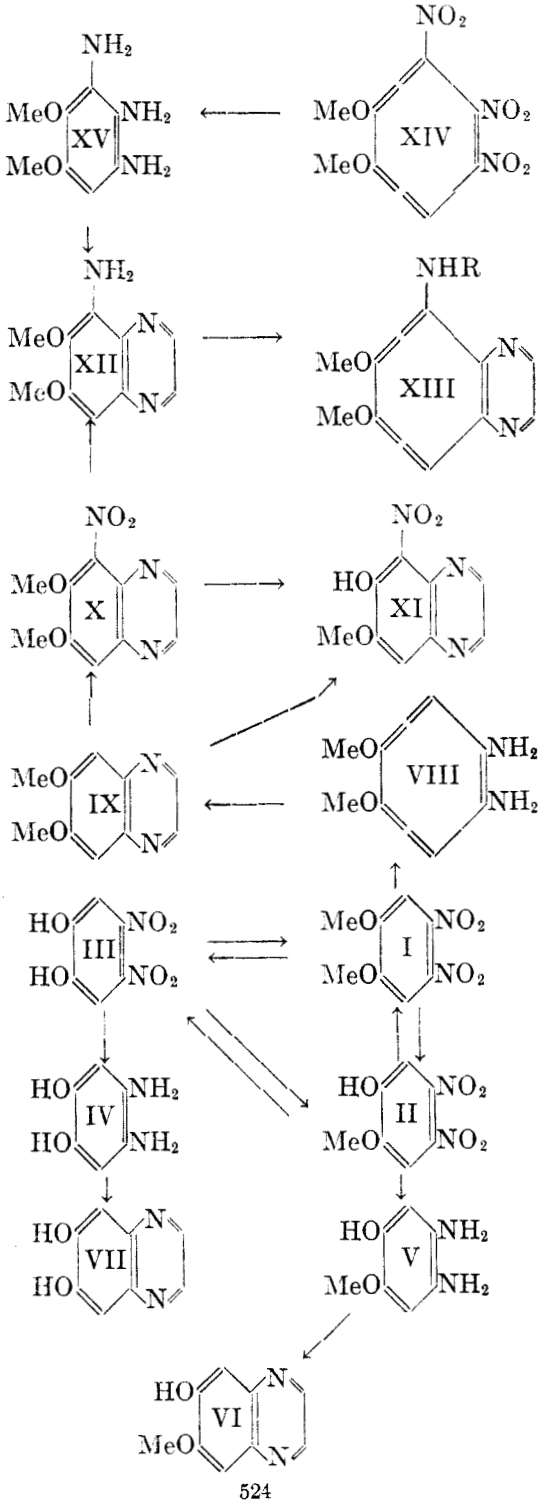
Unless otherwise specified, all m.p.'s recorded have been corrected for thermometer stem exposure.

DMQx. In what follows, this stands for *dimethoxyquinoxaline*, and *Qx* for quinoxaline.

4,5-Dinitroveratrole (I). The following method of preparation is considered an improvement upon that described by Frisch and Bogert (1).

To 192 cc. of concentrated nitric acid (sp. gr. 1.42), at 0–3°, 69 g. of veratrole was added dropwise, with stirring, during at least 80 mins. After continuing this stirring for another 5 mins., the temperature was permitted to rise to 3–5°, 105 cc. of concentrated sulfuric acid run in slowly (1 hr.), and the mixture stirred for 15 mins. longer at the same temperature. The pale yellow crystalline mixture was warmed gradually (20 mins.) to 54–55°, kept there for 10 mins., and the temperature then raised (5 mins.) to 58–60° and held at that point for 10 mins. There resulted a thin canary slurry which, in 5 mins., was cooled to 23–25°, poured slowly into a mixture of 200 g. of ice and 200 cc. of water, and the mixture then diluted with water to a volume of 2500 cc. The product was a pale yellow liquid, with a deposit of yellow

FLOW SHEET


NO2
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sandy crystals. After occasional stirring for 30 mins., this was filtered, the solid washed repeatedly with water until the last wash was neutral, and dried at 50–52° for at least 16 hrs.: m.p. 126–127°; yield, 94–96%. Frisch and Bogert (1) found the m.p. 127–128° for their product.

Further purification by recrystallization from alcohol, in the presence of Norit SG Neutral and Dicalite Superaid, gave fine pale yellow needles, m.p. 130–131°.

The pure compound was difficultly soluble in water or *n*-heptane, cold or hot. In methanol, it was moderately soluble hot, but only slightly soluble cold. In cold benzene it was moderately soluble, but dissolved easily when the temperature was raised. Its solubility in 100 cc. of alcohol was approximately 0.8 g. at 23°, and 6 g. at 75°; in 100 cc. of *n*-butanol, 1 g. at 23°, and 33 g. at 110°.

Action of hydrobromic acid upon 4,5-dinitroveratrole. When 5 g. of 4,5-dinitroveratrole was refluxed for 11 hours with 50 cc. of 40% hydrobromic acid, it yielded two alkali-soluble products, one of which (a) was freely soluble in water, while the other one (b) was not. The crude product (b) was converted into a solution of the sodium salt in order to eliminate a little insoluble unchanged dinitroveratrole. After reprecipitation with dilute hydrochloric acid and recrystallization from 50% ethanol, in the presence of active carbon and a filter-aid, it was obtained in pale yellow hair-like crystals, m.p. 172–173°, in agreement with the m.p. recorded in the literature (19) for *4,5-dinitroguaiacol*; yield, 1.15 g. Its identity was further established by analysis of its sodium salt, and remethylation of the latter to *dinitroveratrole* (m.p. 130°).

Anal. Calc'd for $C_7H_3N_2NaO_6$: Na, 9.76. Found: Na, 9.88.

Dinitroguaiacol (II) was only slightly soluble in hot water or *n*-heptane, but quite soluble in alcohol or ether. It crystallized well from toluene or 50% alcohol, and was not volatile with steam.

Its scarlet sodium salt was salted out by excess of strong aqueous sodium hydroxide, sodium carbonate, or brine, and was but slightly soluble in absolute alcohol. The ammonium salt was more easily soluble, and was reprecipitated in orange crystals by 15 *N* ammonium hydroxide solution.

4,5-Dinitropyrocatechol (III). The 4,5-dinitropyrocatechol recovered by ether extraction from the hydrobromic acid filtrate and washings in the preparation of the dinitroguaiacol, after purification formed canary sandy crystals, melting with decomposition at 166.5–167.5°; yield, 1.12 g. A mixture of benzene and nitroethane proved to be the best medium for its crystallization. The product gave a positive nitrogen, but negative halogen and negative Liebermann nitroso test. It was soluble in water (to a yellow solution), ethanol, *n*-butanol, acetone, nitroethane, ether, or dichloroethyl ether; slightly soluble in boiling benzene, xylenes, heptane, or *o*-dichlorobenzene; fairly hygroscopic, not volatile with steam, and stable to hot dilute silver nitrate solution. Its aqueous solution was slightly acid to Congo and became purple when made alkaline. By acidification and extraction with ether, the (III) was recovered.

Anal. of this product, still slightly impure, gave the following results:

Calc'd for $C_6H_4N_2O_6$: C, 36.0; H, 2.0

Found: C, 35.6; H, 1.93.

Attempted determination of nitrogen gave such erratic results as to be worthless.

The anhydrous disodium salt was quite soluble in 80% alcohol, but only slightly in absolute alcohol. It was prepared with absolute alcoholic sodium hydroxide and was isolated in dark steel-blue crystals, which exploded violently at elevated temperatures.

Anal. Calc'd for $C_6H_2N_2Na_2O_6$: Na, 18.75. Found: Na, 18.75, 18.95.

Under 6 cc. of dry toluene, 0.225 g. of the dry disodium salt was pulverized, 0.5 cc. of freshly distilled methyl sulfate added, and the mixture refluxed for 16 hours. After chilling, 3 cc. of water was added, followed by 3 cc. of 5 *N* ammonium hydroxide. The purpose of the latter was not only to destroy excess of the methyl sulfate, but also to retain in the aqueous layer any unchanged dinitropyrocatechol and intermediate dinitroguaiacol.

The flask was stoppered, well shaken, some Filter-Cel added, and the mixture filtered with suction through crepe paper into a small separatory funnel, flushing with a small quantity of toluene and dilute ammonium hydroxide. After 15 mins.' shaking the charge was allowed to stand for an hour, and separated into a pale yellow upper toluene layer and a lower blood-red aqueous one. The toluene layer was washed with a small amount of water, and acidification of these aqueous extracts yielded some *4,5-dinitroguaiacol* (m.p. 172–173°), as a fluffy yellow precipitate. From the toluene layer, *4,5-dinitroveratrole* was isolated (0.1 g.) which melted at 130° when crystallized from alcohol, and showed no change in this m.p. when mixed with an authentic sample of different origin.

4,5-Diaminopyrocatechol (IV) has been described by Hoehn (18), who obtained it by reducing 4-acetamino-5-nitrosopyrocatechol in alcoholic solution with stannous chloride, and found that the free base was very sensitive to the oxygen of the air, rapidly changing into the 4,5-diamino-*o*-quinone.

Attempts to reduce 4,5-dinitropyrocatechol, in aqueous solution with metallic iron or zinc; or, in alcoholic solution, by the action of hydrogen, in the presence of the Adams-Voorhees catalyst, yielded a very impure product.

Instead of trying to separate a pure diamine, however, it was found much more satisfactory to condense the reduction product of the dinitro coupound (III) directly with glyoxal, to the *6,7-DihydroxyQx* (*VII*), according to the following procedure: A solution of 14.5 g. of commercial 90% powdered sodium hydrosulfite in 80 cc. of water was chilled to 5°. To this, there was added rapidly a solution of 2 g. of 4,5-dinitropyrocatechol in 20 cc. of water, while the temperature was kept down by an ice-bath. After standing for 5 mins. longer at low temperature, the mixture was warmed gradually (5 mins.) to 65–67°, held there for 5 mins. (not much longer, or a red dye begins to form), and the clear yellow solution then chilled rapidly to 28–30° and 10 cc. of glacial acetic acid added.

Three g. of glyoxal bisulfite powder (Carbide and Carbon's $C_2H_2O_2 \cdot 2NaHSO_3 \cdot H_2O$, M.W. 284, glyoxal content 20%) was introduced in one lot, the mixture stirred for about 10 mins. at 28–30°, to aid solution, warmed (15 mins.) to 95–100°, and held at that temperature for an hour. After cooling to room temperature and standing for 2 hrs., the precipitate was filtered out, washed thoroughly with water and dried at 55° to constant weight; yield, 1.3 g., or 81%. Sublimed at 255° and 1 mm. pressure it was obtained as a yellow crystalline solid, which began to darken at 260° and finally charred without fusion. It was too sparingly soluble to be crystallized from any of the neutral solvents tried. It dissolved quite readily in dilute sodium carbonate or ammonium hydroxide to a clear yellow solution. Reprecipitated by dilute acetic acid, a very stable colloidal yellow suspension was formed, not coagulated by heating whereas acidification of the preheated solution reprecipitated the original compound in crystalline form. The sublimate was obviously a very weak base since, at room temperature, it was insoluble in and unchanged by *N* hydrochloric acid. With 3 *N* hydrochloric acid, it formed the violet crystalline *monohydrochloride*, which was only very slightly soluble (about 1% at 98°) in acid of this strength. Suspended in water, this salt was hydrolyzed to the insoluble yellow free base and the water became strongly acid to Congo paper.

The monohydrochloride, when dried for 2 hrs. at 56°, lost no weight.

Anal. Calc'd for $C_8H_6N_2O_2 \cdot HCl$: C, 48.4; H, 3.5; N, 14.1; Cl, 17.8.
Found: C, 48.1; H, 3.55; N, 14.1; Cl, 17.5.

4,5-Diaminoguaiacol (V) *and 6-Methoxy-7-hydroxyQx* (VI) *from 4,5-Dinitroguaiacol.* The dinitroguaiacol (2.14 g.) was reduced in alcoholic solution by iron and hydrochloric acid, and then condensed with glyoxal, essentially as described in the following for the preparation of the 6,7-DMQx (IX) from the diaminoveratrole.

The crude product was obtained in pale pinkish matted crystals, m.p. 236–237.5°; yield, 1.51 g. Recrystallized from 50% (by volume) ethanol, it formed silvery nacreous plates, m.p. 238–239°.

Anal. Calc'd for $C_9H_8N_2O_2$: C, 61.4; H, 4.55; N, 15.9.
Found: C, 60.72; H, 4.47; N, 15.85.

The *copper salt* was prepared by the addition of copper sulfate to an aqueous solution of the sodium salt. Warmed with 0.25 normal acetic acid, a clear solution was obtained, which deposited nacreous leaves of the initial compound (m.p. 238–239°) as it cooled. This copper salt dissolved readily in normal ammonium hydroxide solution at room temperature to a clear green solution, which precipitated copper sulfide with normal sodium sulfide, and with normal sodium hydroxide formed a blue cupric hydroxide jelly. Neither cupric nor lead acetates gave any precipitates with the initial compound in alcoholic solution.

The canary yellow *silver salt* was difficultly soluble even in warm dilute acetic acid. It was variable in composition, apparently indicating a changing proportion of neutral and basic salts.

This salt reacted readily with cold dilute sodium sulfide solution or warm dilute sodium hydroxide solution.

Neither the copper nor the silver salt gave any evidence of being a chelate compound.

Benzoyl derivative. Subjected to a Schotten-Baumann reaction with benzoyl chloride, this compound (VI) yielded pale tan nacreous leaves (from alcohol), m.p. 136.5–137.5°; soluble in ethanol, benzene, or hot heptane; difficultly soluble in water, or in cold heptane.

Anal. Calc'd for $C_{16}H_{12}N_2O_3$: C. 68.6; H, 4.3; N, 10.0.

Found: C. 68.64; H, 4.9; N, 10.02.

4,5-Diaminoveratrole (VIII) was prepared by Frisch and Bogert (1) by reducing the dinitro compound (I) with tin and hydrochloric acid. For the present investigation, we used iron and hydrochloric acid upon an alcoholic solution of the nitro compound, as described beyond in the case of the trinitroveratrole (XIV). Of the dinitro derivative, 11.4 g. was used; and of the trinitro, 9.1 g., the rest of the charge remaining practically the same in the two cases.

6,7-DMQx (IX). To the final solution of the reduction product from 11.4 g. of 4,5-dinitroveratrole (I), without attempting to separate the pure free base, and at a temperature of 28–30°, there was added all at once 15.6 g. (10% excess) of finely powdered glyoxal sodium bisulfite, and the mixture stirred to effect solution. In a closed flask, shaken occasionally (2 hrs.), this solution separated a pale fluffy crystalline precipitate of the quinoxaline sought (IX). After 20 hrs.' standing at 28–30° the precipitate was filtered out, washed thoroughly with water, and dried at 50° to constant weight (about 20 hrs.); yield, 7.2 g.; m.p. 149–150°. The combined aqueous filtrates were extracted with benzene, the extract concentrated to crystals, and the latter dried for four hrs. at 50°. There was thus obtained 1.1 g. of pale yellow crystals, m.p. 147–148°, making a total yield of 8.3 g. By recrystallization from water, the m.p. was raised to 149.5–150.5°.

For analysis, a sample recrystallized from water was crystallized from alcohol, decolorizing with active carbon, and yielded long white needles, m.p. 150–151°.

Anal. Calc'd for $C_{10}H_{10}N_2O_2$: C, 63.2; H, 5.3; N, 14.7.

Found: C, 63.5; H, 5.4; N, 14.6.

In water, 100 cc. dissolved about 0.17 g. at 20°, and 2.1 g. at 95°; in alcohol, about 3.5 g. at 24°, and 12.5 g. at 60°; 100 cc. of glacial acetic acid dissolved about 5.0 g. at 25°, and 10 g. at 50°. It was moderately soluble in ether or benzene cold, quite soluble in hot benzene; very weakly basic, dissolving only in large excess of *N* hydrochloric acid; not volatile at 50° but, in thin layers at 100° lost about 0.6% per hour. Refluxed with 40% hydrobromic acid, a brown amorphous infusible material was obtained, insoluble in all solvents tried. With 30% hydrobromic acid, the original substance was recovered unaltered.

Nitration of 6,7-DMQx (IX). 6,7-DMQx was quite stable to concentrated nitric acid (sp. gr. 1.42) even at its b.p. Attacked by mixed acid, it was found impossible to obtain the mononitro derivative without some simultaneous hydrolysis of a methoxyl group; but no dinitro derivative was detected in any of the nitration experiments.

Eleven and four-tenths grams of (IX) was gradually fed to a mixture of 120 cc. of concentrated (95.5%) sulfuric acid + 30 cc. of concentrated nitric acid (sp. gr. 1.42), at 23–25°, during an hour, let stand for 40 hours longer at the same temperature and then poured into 240 g. of ice and 240 cc. of water. The mixture was diluted with ice-water to a volume

of 900 cc., the orange precipitate removed, washed repeatedly with water, and as much as possible of the adhering water removed by suction. This crude product was a mixture of soda-soluble and soda-insoluble nitration products, the separation of which proved very difficult.

The still moist cake, containing about 10.3 g. of dry material, was added to 340 cc. of water at 23–25°, and 2 *N* aqueous sodium carbonate solution added in 2-cc. portions, until a five minute test with alkacid paper (pale green color) was obtained. Total soda used was about 22 cc. The solution became blood-red, with some yellow solid in suspension.

It was believed that the soda-soluble substance was probably the *6-methoxy-7-hydroxy-8-nitroquinoxaline* (XI) because of the red color of its alkaline solution, its acidity to Congo, the fact that an alkoxyl group *ortho* to a nitro group is more readily hydrolyzed than when in the *meta* position, and by a preliminary determination of sodium in the sodium salt. The soda-insoluble constituent should then be the simple *8-nitro-6,7-DMQx* (X).

The soda-insoluble material was collected by extraction with benzene at 40–42°, the filtered benzene extract concentrated to crystals, and the latter dried at 50° to constant weight. The product was a pale brown crystalline cake; yield, 5.6 g. (39.7% of that calculated for a m.w. of 235). This was crystallized twice from alcohol in the presence of a decolorizing carbon, and then formed colorless hair-like needles, m.p. 122–124°.

Analysis of various samples of this product showed that it was not the pure 8-nitro-6,7-DMQx (X) expected although, as noted beyond, the 8-amino compound (XII) was obtained from it by direct reduction.

It was only slightly soluble in ether or boiling water; fairly soluble in hot cyclohexanol or glacial acetic acid; quite soluble in cold benzene, dioxane, or nitroethane. Its solubility in 100 cc. of ethanol at 23° was about 0.9 g.; at 75°, 4 g.; in 100 cc. of benzene at 23°, about 8.3 g. It was not volatile at 50°, or with steam. In a thin layer at 100°, it lost about 0.1% hourly. Suspended in boiling 3 *N* sodium hydroxide it was slowly hydrolyzed to the following compound.

6-Methoxy-7-hydroxy-8-nitroquinoxaline (XI). The original aqueous solution of the soda-soluble product from which the soda-insoluble substances had been removed by filtration and benzene extraction, was made strongly acid to Congo with 3 *N* hydrochloric acid, the pale yellow precipitate removed, washed thoroughly with water, and dried at 50–52°; yield, 3 g. (22.2% of that calculated for m.w. of 221). This crude material was purified by reconversion into its sodium salt with sodium carbonate and decolorization of the hot clear red aqueous solution of the latter. As it cooled, the solution separated the sodium salt in bright orange needles, which were removed, washed with acetone, and air-dried to constant weight. Further drying at 100° showed a loss of only 1.2%. From an aqueous solution of this purified sodium salt, the free hydroxy compound was precipitated with 3 *N* hydrochloric acid, washed with water, and dried to constant weight at 50°.

Anal. Calc'd for $C_9H_7N_3O_4$: C, 48.47; H, 3.17; N, 19.0.
Found: C, 48.48; H, 3.26; N, 18.92.

The compound appeared in pale yellow crystals, which began to darken at 235° and then charred without melting. Heated slowly on a porcelain dish, it suddenly flared without previous melting. In hot solvents, it was difficultly soluble in water, ether, ethanol, benzene, or nitroethane; and only slightly soluble in glacial acetic acid, dioxane, or cyclohexanol. Although the aqueous solution contains only a small amount of the compound, it was fairly acid to Congo. It gave a negative test for sulfur, and was not volatile with steam, or at 50°, but in a thin layer at 100° lost about 0.1% hourly. At 23°, a solution of 1 g. in 2.5 cc. of 2 *N* sodium carbonate and 65 cc. of water, is about saturated; at 95°, the same result was secured with 10 cc. of water. Reduction of this compound was not attempted, because of lack of material.

Analysis of the purified sodium salt showed 9.72% sodium; calc'd for $C_9H_6N_3NaO_4$, 9.47% Na.

This sodium salt was very resistant to alkylation, and experiments conducted with methyl iodide, methyl sulfate, or methyl *p*-toluenesulfonate, failed to yield any 8-nitro-

6,7-DMQx. The same was the case with the free hydroxy compound in pyridine solution. Diazomethane was not tried.

8-Amino-6,7-DMQx (XII). To a mixture of 13.8 g. of finely divided iron in 83 cc. of water was added 2.8 cc. of 2 *N* acetic acid. The mixture was warmed to 93–95°, and 5.5 g. of the crude nitro compound (X) was fed in gradually (30–35 mins.). After 10 mins. further stirring at 93–95°, 83 cc. of hot water was added, then (5 mins. later) 5.5 cc. of 2 *N* sodium carbonate solution, and finally 0.6 g. of active carbon, bringing the temperature finally to 98°. The mixture was filtered, the clear pink filtrate cooled to about 25°, seeded with a few crystals of (XII) prepared from 3,4,5-triaminoveratrole (XV) (see beyond), and left for 20 hours in the refrigerator. The separated garnet crystals were collected, washed with cold water, and dried for 6 hours at 50°; yield, 1.7 g.; m.p. 101–103°.

This crude product was dissolved to a clear red solution in 100 cc. of benzene. A single extraction of this solution with 13 cc. of *N* hydrochloric acid, and treating this extract with about 1 g. of powdered sodium carbonate yielded 0.9 g. of a brown crystalline material, m.p. 101–103°. The residual clear yellow benzene solution was washed with dilute sodium carbonate solution and concentrated to pale orange crystals, m.p. 106–107.5°; yield, 0.7 g. Further purification was effected by crystallization from water, decolorization with active carbon, and a final crystallization from isopropanol, giving coarse bright yellow needles, m.p. 107.5–108.5°. Mixed with an equal weight of (XII) prepared from (XV), the m.p. was 107–108°.

Anal. Calc'd for $C_{10}H_{11}N_3O_2$: C, 58.53; H, 5.37; N, 20.49.
Found: C, 58.80, 58.69; H, 5.50, 5.22; N, 19.86, 20.98.

3,4,5-Trinitroveratrole (XIV), prepared by adding veratrole to a mixture of concentrated nitric acid and concentrated sulfuric acid, essentially as described by Frisch and Bogert (1), was purified by crystallization from benzene, in the presence of a decolorizing carbon and a suitable filter-aid, instead of using alcohol as the solvent. The purified product melted at 144.5–145.5°. in agreement with the literature (20, 21). It was difficultly soluble in water, heptane, or methanol, cold or hot; only slightly soluble in toluene or *n*-butanol cold, easily soluble hot. In 100 cc. of alcohol, its solubility was approximately 0.4 g. at 19°, 4 g. at 75°; in 100 cc. of benzene, 3.3 g. at 18°, 33.3 g. at 80°.

3,4,5-Triaminoveratrole (XV). The reduction of the trinitro derivative (XIV) was conducted in a 2-liter 3-necked round-bottom Corning flask, carrying a thermometer (with bulb in the mixture) in one side opening, and a rubber stopper in the other, with the central opening available for a reflux condenser.

There was first placed in the flask 33.5 g. (0.6 atom) of steel card teeth (No. 33 W & M gage wire) This excess (200%) of iron was found necessary for complete reduction There was then added 190 cc. of U.S.P. alcohol and 105 cc. of concentrated hydrochloric acid (sufficient to convert the base into its trihydrochloride and all iron into ferrous chloride). The temperature of the mixture rose to about 41° and there was some evolution of gas. To the mixture, 1.4 cc. of 10 *N* stannous chloride solution was run in, and a minute later 9.1 g. (0.033 mole) of trinitroveratrole. After warming carefully on the water-bath so that the reflux temperature (85–86° in the mixture) was reached in about 17 mins., and continuing this refluxing for an hour longer, the charge was cooled to 70°, the flask removed and 500 cc. of water added A Hopkins bulb was fitted in the central opening, connected with a descending condenser and exactly 570 cc. distilled off (90 mins.). The temperature of the mixture rose gradually from the initial b.p. of 87°, to about 106°. The distillate contained all of the original alcohol and some hydrochloric acid. Some metallic iron was still present in the flask. The mixture was cooled to 65°, the flask removed, stoppered loosely, cooled to 25°, and decanted through a small bare Büchner funnel into a 750-cc. Erlenmeyer flask. The residual iron was rinsed with two 25-cc. portions of water and the washings added to the main filtrate, giving a total volume of about 245 cc. To prevent air oxidation of the base during subsequent partial neutralization, 8 g. of anhydrous sodium sulfite powder was dusted in, and dissolved quite rapidly with little heat effect. The solution, still decidedly acid to Congo, and at a temperature of 25–30°, was treated with small portions of

2 *N* aqueous sodium carbonate. Foam was collapsed by addition of a few drops of ether. The last 5 cc., at the end-point gave no foam, but a small precipitate of ferrous carbonate persisted, and the solution was acid to litmus, but neutral to Congo; total sodium carbonate solution required was about 132 cc. *Most of the ferrous chloride remained in solution at this point.*

One g. of filter-aid and 1 g. of active carbon were added, the whole mixed for 5 mins. at 25–30°, and filtered with suction. The filtrate was pale apple-green and quite sensitive to air oxidation. The best yields of the glyoxal condensation product were secured from this liquor, by carrying out the reaction in the suction flask.

To isolate the crude base, after the addition of 8 g. of sodium sulfite, the solution was diluted to 400 cc., and powdered sodium carbonate added at 25–30° until all of the iron was precipitated and Brilliant Yellow paper showed an alkaline reaction (red). The solution was heated to 70–75° and rechecked for alkalinity, adding more carbonate if necessary. A filter paper spot test with aqueous sodium sulfide showed no iron present. After 10 minutes' heating at 70–75°, a little filter-aid was dropped in, and the mixture filtered with suction. The material on the filter was washed with four 50-cc. portions of hot water, the combined filtrates bleached with a little sodium hydrosulfite, and extracted with chloroform in a continuous extraction apparatus and in an inert gas. The yield of base was 85%, m.p. 146-150°. [Literature (1), 150–152°.] It was easily soluble in water, aniline or hot alcohol; only slightly soluble in cold alcohol, hot benzene, or hot chloroform; difficultly soluble in ether.

Condensation of 3,4,5-triaminoveratrole with glyoxal. As noted in the description of the reduction of the trinitroveratrole (XIV), the best yields of the quinoxaline (XII) were obtained by using the final reduction liquor in the suction flask.

To that liquor, at 25–30°, 10.4 g. (10% excess) of finely powdered glyoxal sodium bisulfite was added, and the mixture stirred for 15 minutes. The flask was then stoppered, shaken occasionally during 2 hrs., and left for 20 hrs. at 25–30°. A crop of brilliant canary crystals separated. Without removing these crystals, the mixture was extracted with benzene, and the solvent removed from this extract. The residue, dried for 2 hrs. at 50°, formed a pale brown crystalline mass, m.p. 106–107°; yield, 4.25 g. Recrystallized from water, in the presence of active carbon, it formed yellow crystals, m.p. 106.5–107.5°; yield, 3.4 g. From the filtrates and mother liquors, an additional 0.2 g. was recovered of the same m.p. As noted in the foregoing, a 50:50 mixture of this compound with the 8-amino-6,7-DMQx (XII) prepared by direct reduction of 8-nitro-6.7-DMQx (X), showed the m.p. 107–108°.

One hundred cc. of water dissolved about 0.4 g. at 22°, and 3.6 g. at 90°. It was moderately soluble in ether or alcohol, and quite soluble in benzene; difficultly soluble in dilute acetic acid, sodium carbonate, hydroxide, or bisulfite. In dilute hydrochloric acid, it dissolved to a clear ruby solution, from which the bright yellow original substance was reprecipitated by alkali. About 0.3 g. per liter was carried over in the steam distillate.

Anal. Calc'd for $C_{10}H_{11}N_3O_2$: C, 58.5; H, 5.3; N, 20.5; M.W., 205.
Found: C, 59.2; H, 5.1; N, 20.5; M.W. (by titration with 0.1 *N* sodium nitrite), 205.

8-(N^4-Acetylsulfanilamido)-6,7-DMQx (XIII). To a well stirred solution of 12 g. of powdered 8-amino-6,7-DMQx (XII) in 75 cc. of dry pyridine, was added gradually (20 mins.) 18 g. or 1.32 times the calculated quantity of freshly prepared and finely powdered acetylsulfanilyl chloride, maintaining the temperature of the mixture at 25–28° during the addition and for 15 minutes after the addition was completed. After it had been heated for an hour at 100°, it was cooled to about 23°, the flask closed and left for 15 hrs. at laboratory temperature. It was then poured into an excess of cold water, chilled, stirred, allowed to settle, and the crystalline solid filtered out, washed thoroughly with water, and dried for 10 hrs. at 50–55°; yield, 18.2 g.; m.p. about 234–235° (with decomposition).

To the combined mother liquors was added 51 cc. of 3 *N* aqueous sodium acetate solution, and the mixture was distilled with steam until 1600 cc. of distillate was collected. The

liquid remaining in the distilling flask was concentrated to 120 cc., filtered hot, the insoluble crystalline cake washed with hot water, and dried for four hours at 50–55°. There was thus recovered an additional 4.4 g. of the product sought (XIII), making a total of 22.6 g., or 98% of that calculated.

For analysis, 1 g. of this crude product was digested with 25 cc. of 50% acetic acid, 0.1 g. of filter-aid, and 0.1 g. of active carbon, for 30 minutes at 100°, the mixture filtered hot, the filter paper washed with a little hot acetic acid, the pale salmon filtrate allowed to cool to 25°, then left in the refrigerator overnight. The crystalline precipitate was washed once with 4 cc. of 50% acetic acid, followed by three 4-cc. portions of water, and the pale yellow crystals dried for 5 hrs. at 50–55°. These crystals melted at 238.5–239°, with sudden decomposition. A repetition of this purification process failed to alter the m.p.

Anal. Calc'd for $C_{18}H_{18}N_4O_5S$: C, 53.73; H, 4.48.
Found: C, 53.87; H, 4.78.

The crude product (m.p. 234–235°) was difficultly soluble in water, isopropanol, or toluene, when hot; and slightly soluble in hot *n*-butanol. In boiling ethanol, about 1.4 g. dissolved in 100 cc., and 0.3 g. at 21°. In 100 cc. of 50% acetic acid, 0.8 g. dissolved at 21°, and about 5 g. at 100°. It was difficultly soluble in cold 3 *N* hydrochloric acid, and dissolved in hot 3 *N* sodium hydroxide with hydrolysis.

8-Sulfanilamido-6,7-DMQx (XIII). The above acetyl derivative was hydrolyzed by digestion at 100° with 3 *N* hydrochloric acid. After cooling, the product was precipitated with aqueous sodium carbonate, filtered out, and washed with water. The crude product was decolorized and crystallized from alcohol and further purified by crystallization from 1-nitropropane. It then formed pale yellow prisms, m.p. 217.5–218.5°, with some decomposition; and was readily soluble in dilute sodium hydroxide.

Anal. Calc'd for $C_{16}H_{16}N_4O_4S$: C, 53.33; H, 4.44.
Found: C, 53.64; H, 4.82.

Pharmacological tests were carried out on this compound in the Merck & Co. laboratories, and it was found to have practically no bacteriostatic or bactericidal properties. It was also subjected to screening tests by the Survey of Antimalarial Drugs (SN 9162), and found to be without promise as an antimalarial.

8-(beta-Diethylaminoethylamino)-6,7-DMQx (XIII). Great difficulty was experienced in attempting to replace a hydrogen of the 8-amino group by a dialkylaminoalkyl group, irrespective of the method employed. Either no reaction at all occurred, or the products were resinous.

Attempts were made to react the amine (XII) with *beta*-diethylaminoethylchloride, in the presence of various condensing agents, including phenol (22), and pinene (23). *beta*-Diethylaminoethylpyridinium benzenesulfonate was also tried, and likewise the condensation with 1-diethylamino-4-pentanone, followed by reduction of the resultant azomethine. Some measure of success, however, was achieved in the following experiment.

A mixture of 7.5 g. of (XII) with 75 cc. of fused phenol formed a bright orange solution when warmed on a steam-bath. In this hot solution, there was dissolved 9.4 g. of *beta*-diethylaminoethyl chloride hydrochloride. After heating the liquid for 7 hrs. at 100°, it was cooled to 20–25° and 6.75 g. of Eastman "Sodium Phenoxide Anhydrous" fed in. Warmed to complete solution and heated at 100° for 17 hrs., it was then cooled to 20–25°, diluted with 75 cc. of water, acidified with 16 cc. of 3 *N* hydrochloric acid and most of the phenol blown out with steam. The mixture remaining in the flask was cooled to 20–25°, filtered, 3 *N* sodium hydroxide added to strong alkaline reaction, and the mixture extracted with benzene. The benzene extract was shaken first with anhydrous sodium sulfate, then with active carbon, and finally with Filter-cel and filtered. After elimination of the solvent, the pressure was reduced and, on redistillation at 1 mm. pressure, two fractions were collected: (a) thin, pale yellow oil, b.p. about 130°, 1.2 g.; (b) viscous orange oil, b.p. about 205°, 5.7 g. (51.3% yield). For analytical samples, these fractions were redistilled at 1 mm., taking the middle cuts to ampoule receivers.

Fraction (*a*). Slightly soluble in cold water, to alkaline reaction, still less soluble in

hot, halogen-free, volatile with steam; soluble in dilute hydrochloric acid, and with difficulty in dilute sodium hydroxide.

Anal. Calc'd for $C_{12}H_{19}NO$: C, 74.61; H, 9.84.
Found: C, 73.5; H, 10.4.

It is suggested that this product may be an impure ether of the following constitution: $Et_2N(CH_2)_2OPh$ (24, 25). Such compounds have been prepared by Levy and Ditz (25) and others, and were found to diminish, suppress, or reverse the pharmacodynamic action of adrenaline.

Fraction (b). Very slightly soluble in cold water to alkaline reaction, insoluble in hot, halogen-free, not volatile with steam; easily soluble in many of the usual neutral organic solvents, difficultly soluble in dilute sodium hydroxide. Soluble in dilute hydrochloric acid to a deep cherry solution; b.p. 175° at 0.5 mm. Attempts to form crystalline salts with inorganic or organic acids, by wet or dry methods, gave only hygroscopic gummy products.

Anal. Calc'd for $C_{16}H_{24}N_4O_2$: C, 63.16; H, 7.89; N, 18.42.
Found: C, 62.92; H, 7.70; N, 17.45, 17.63.

Through the courtesy of the Survey of Antimalarial Drugs, a sample of this product (SN 12,366) was examined pharmacologically and found to be practically devoid of any appreciable antimalarial effect.

Attempts to carry out this same phenol-phenate process, using $MeCHBr(CH_2)_3NEt_2$, or the corresponding chloride instead of $Cl(CH_2)_2NEt_2$, proved futile.

Production of 6-methoxy-7-hydroxyQx (VI) *from 8-amino-6,7-DMQx* (XII). A solution of 2.05 g. of (XII) in 30 cc. of water and 3 cc. of 13.5 *N* sulfuric acid, was diazotized with sodium nitrite at 0–5°. A slight excess of nitrous acid was destroyed by the addition of 0.1 g. of sulfamic acid, 27.5 cc. of 8.5 *N* sodium hydroxide added with cooling, and the mixture heated at 100°. Some suspended crystalline material, presumably a diazotate, began to dissolve at about 85° and evolution of gas occurred. After 90 minutes at 100°, the mixture was cooled to 20–25°, filtered, and the small amount (0.09 g.) of residue washed with water. To the filtrate and washings, amounting to a total of 77 cc., there was added 23 cc. of 9 *N* acetic acid, and the strongly acid mixture was digested at 100° for about 20 minutes, then cooled to 20°, the precipitate removed, washed with water and dried for 4 hrs. at 50–55°, giving a pale pink cake; yield, 0.91 g. Crystallized twice from 50% (by vol.) ethanol, there was obtained 0.74 g. of silvery nacreous flakes, m.p. 238–239°, apparently identical with the compound (VI) obtained from 4,5-diaminoguaiacol and glyoxal.

Subjected to a Kjeldahl analysis, with precipitated copper powder as digestion catalyst, it showed N 15.3% (calculated for a hydroxymethoxyquinoxaline, 15.9).

Diazotization reactions applied to 8-amino-6,7-DMQx. All attempts to obtain the corresponding 8-hydroxy, or 8-halogen-6,7-DMQx, from the 8-amino compound (XII), by the usual Sandmeyer or Gattermann reactions, were unsuccessful, but some of the observations made in the course of the experiments were interesting, especially when the decomposition of the diazonium compound was catalyzed by the use of moderately strong sulfuric acid, as in the following experiment.

(a) *Production of an iodo derivative.* To a clear red solution of 5 g. of the aminoDMQx (XII) in 147 cc. of 48.1% sulfuric acid, at 0–3°, finely powdered sodium nitrite (about 1.8 g.) was added gradually. Excess of free nitrous acid was destroyed by the addition of about 0.24 g. of sulfamic acid, and 5.3 g. of powdered potassium iodide was dropped in (as one lot), keeping the temperature at 0–3° and maintaining the stirring. With evolution of nitrogen, a brick-red precipitate separated and, after an hour, the temperature was raised to 23° and left at that point for 20 hours. After dilution with 366 cc. of water, a little sodium bisulfite solution was poured in to remove any free iodine, and the mixture was filtered. The precipitate was washed with small lots of water, until the washings gave a practically negative test with Congo, and was then dried at 55–57° to constant weight. This crude product formed a pinkish cake; yield, 6.55 g. It was decolorized and recrystallized from nitroethane, yielding nacreous pale yellow fine leaves, which decomposed around 210° without melting and, on analysis, gave figures which did not agree satisfactorily with those calculated for a monoiodo derivative of either 6-methoxy-7-hydroxy-, or 6,7-dimethoxy-Qx.

Found: C, 41.55; H, 3.0; I, 40.0; N, 10.43. Calc'd for $C_9H_7IN_2O_2$: C, 35.8; H, 2.3; I, 42.1; N, 9.3. Calc'd for $C_{10}H_9IN_2O_2$: C, 38.0; H, 2.9; I, 40.2; N, 8.9. The iodine was determined according to the Raney nickel-aluminum method of Schwenk, Papa, and Ginsberg (26).

Although quite insoluble in boiling water, it dissolved quite rapidly in cold dilute sodium carbonate solution, and then gave a strong test for iodide ion, thus suggesting the presence of a free hydroxyl group and an unusually mobile halogen. Acidification of this alkaline solution yielded an amorphous precipitate, which was insufficient for further investigation.

When some of this crystalline iodine compound was added to a solution of 1-diethylamino-4-aminopentane in *n*-butanol, and the crude product crystallized from 50% ethanol, silvery nacreous plates were obtained, melting at 238–239° to a dark red liquid, which gave a positive test for nitrogen, but a negative one for halogen. The yield from 3.2 g. of initial iodine compound was 1.2 g., and of purified product 1.0 g. It was difficulty soluble in hot water, ether, or *n*-hexane; fairly soluble in hot toluene or ethanol. In boiling 50% (by volume) ethanol, 100 cc. dissolved 2.9 g., and 0.2 g. at 4°. It was only slightly soluble in dilute acetic acid, but dissolved in 3 *N* hydrochloric acid or in cold 2 *N* sodium carbonate solution. Its solubilities, m.p., and other properties identified it as *6-methoxy-7-hydroxyQx* (VI), as produced from 4,5-diaminoguaiacol and glyoxal.

(b) *Decomposition of a diazoacetate.* 8-Amino-6,7-DMQx was diazotized in the usual way and normal sodium acetate solution was added. After the introduction of a small quantity of copper powder (in the absence of copper, this diazoacetate was unchanged by refluxing), the mixture was heated for an hour at 100°, nearly neutralized by anhydrous sodium carbonate, heated for 5 minutes, and filtered hot. By working up the precipitate and the solids separated from the filtrates and washings, an 11.9% yield of 6-methoxy-7-hydroxyQx (VI) was isolated, m.p. 238–239°.

From the mother liquors, there was recovered a compound, m.p. 161–162°, which was crystallized from a 1:1 mixture of benzene and *n*-heptane and decolorized. It then formed pale orange sandy crystals, m.p. 163–164°, difficultly soluble in hot water or boiling ether, fairly soluble in hot *n*-heptane; more easily soluble in ethanol, *n*-butanol, chloroform, ethyl acetate, or benzene, when hot. Its analysis showed C, 54.51; H, 3.20; N, 24.44.

By the action of hot 2 *N* sodium carbonate solution, or 3 *N* sodium hydroxide, it yielded 6-methoxy-7-hydroxyQx (VI), m.p. 238–239°.

The results of the experiments in which a methoxyhydroxyQx was formed from an initial dimethoxyQx indicate that one of the two methoxyl groups in the latter is very easily hydrolyzed.

SUMMARY

1. 4,5-Dinitroveratrol can be hydrolyzed by hydrobromic acid to a mixture of 4,5-dinitroguaiacol and 4,5-dinitropyrocatechol, both of which can be reconverted into the initial dinitroveratrole by methyl sulfate.

2. 4,5-Diamino derivatives of pyrocatechol, guaiacol or veratrole condense readily with glyoxal to the corresponding quinoxalines.

3. 3,4,5-Triaminoveratrole condenses with glyoxal to the 8-amino-6,7-dimethoxyquinoxaline and not to the 5-amino-7, 8-dimethoxy isomer.

4. From the 8-amino-6,7-dimethoxyquinoxaline, a sulfanilamido, and a *beta*-diethylaminoethyl derivative, were prepared and subjected to pharmacological tests by Merck & Co., and by the Survey of Antimalarial Drugs. The sulfanilamido derivative proved to be without appreciable antimalarial, bacteriostatic, or bactericidal properties, and the diethylaminoethyl derivative was practically devoid of antimalarial effect.

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