

Summary

The optical rotation of several rotenone derivatives has been determined. It has been found that one of the asymmetric centers in rotenone and its derivatives disappears when the lactone group is hydrogenated to a desoxy acid.

This observation together with a number of other known facts constitutes an argument against the presence in rotenone of the grouping characteristic of tubaic acid.

Rotenonic acid and dihydrorotenonic acid become optically inactive on treatment with alcoholic potash.

Rotenone and isorotenone are reduced with Clemmensen's reagent to isodesoxyrotenone.

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[CONTRIBUTION FROM THE INSECTICIDE DIVISION, BUREAU OF CHEMISTRY AND SOILS]

DEGUELIN. III. THE ORIENTATION OF THE METHOXYL GROUPS IN DEGUELIN, TEPHROSIN AND ROTENONE

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In a brief note recently published in *THIS JOURNAL*¹ it was reported that under certain conditions dehydrodeguelin was oxidized with potassium permanganate in such a manner that one of the main products of the reaction was an hydroxydimethoxybenzoic acid. The structure of this acid was established as that of one of three isomers, namely, 2-hydroxy-4,5-dimethoxy, 5-hydroxy-2,4-dimethoxy or 4-hydroxy-2,5-dimethoxybenzoic acid. The evidence upon which this was based was that upon methylation of the hydroxy acid, asaronic acid, 2,4,5-trimethoxybenzoic acid was obtained. The identity of asaronic acid was established by its melting point and also by the products obtained from it by bromination and nitration.

The hydroxy acid gave an intense blue color with ferric chloride, indicating that it was probably 2-hydroxy-4,5-dimethoxybenzoic acid. Attempts were made to synthesize this acid by diazotizing 2-amino-4,5-dimethoxybenzoic acid and replacing the diazonium group with hydroxyl. These experiments failed, however, so recourse was had to the procedure of decarboxylating the hydroxy acid by heating it with aniline and identifying the resulting dimethoxyphenol. This was accomplished without difficulty, and the properties of the phenol were established.

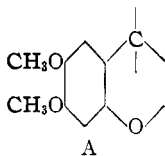
If the acid obtained from dehydrodeguelin is actually 2-hydroxy-4,5-dimethoxybenzoic acid, the phenol derived from it by decarboxylation would be 1-hydroxy-3,4-dimethoxybenzene. As this phenol has not been described, its synthesis was undertaken. The steps involved were the

¹ Clark, *THIS JOURNAL*, 53, 2007 (1931).

following: 4-nitroveratrole was reduced to 4-aminoveratrole. This in turn was diazotized, and the diazonium group was replaced by hydroxyl. The reaction gave a poor yield of a rather impure product, but it was possible to purify the substance and to obtain the pure phenol in ample quantity for analysis and comparisons.

The synthetic preparation had the same melting point (79–80°) as the natural phenol, and when the two substances were mixed no melting point depression occurred. It also gave the same color reaction with ferric chloride as did the natural compound, and the 2,5-dinitrobenzoyl derivatives of the two products were identical. This evidence shows that the hydroxydimethoxybenzoic acid obtained from dehydrodeguelin is 2-hydroxy-4,5-dimethoxybenzoic acid and that the methoxyl groups in deguelin are ortho to each other.

In view of the properties and reactions of deguelin it is justifiable to deduce that the formation of the free hydroxyl group in the hydroxydimethoxybenzoic acid arises from the rupture of an indifferent oxygen linkage such as exists in a furan or pyran ring. This and the considerations given above point to the conclusion that in deguelin there exists the atomic grouping indicated by formula (A).



In the second report upon deguelin² it was shown that the derric acid or the methoxyl-containing portions of the deguelin and rotenone molecule are identical. It has also been demonstrated³ that tephrosin, upon the loss of one mol of water, is converted into dehydrodeguelin. It follows, therefore, that the structure of the methoxyl-containing portion of tephrosin is the same as that in deguelin and rotenone, and that *the structural grouping given above which is present in deguelin also exists in rotenone and tephrosin.*

Without entering into an analysis of the structure of rotenone recently proposed by Takei, Miyajima and Ono,⁴ it may be definitely stated that the portion of their formula involving the methoxyl groups is erroneous, as they propose that the methoxyl groups are para to each other and that the linkage between the methoxylated benzene ring and the remainder of the molecule is through a single carbon atom ortho to a methoxyl group.

Experimental

2-Hydroxy-4,5-dimethoxybenzoic Acid.—A mechanically stirred suspension of 5 g. of dehydrodeguelin in 300 cc. of acetone, cooled to 0°, was oxidized with 18 g. of potassium permanganate, added in 2-g. portions. Upon completion of the reaction the manganese dioxide was removed by filtration and digested for thirty minutes with 400 cc. of boiling water. The aqueous extract and the water used to wash the residual manganese dioxide were combined, heated to boiling and acidified to Congo red with

² Clark, *THIS JOURNAL*, 53, 2669 (1931).

³ Clark, *ibid.*, 53, 729 (1931).

⁴ Takei, Miyajima and Ono, *Ber.*, 64, 1000 (1931).

dilute sulfuric acid. The liquid was immediately filtered and concentrated under reduced pressure to approximately 30 cc. As the distillation proceeded, the acid gradually separated as gray to brownish aggregates of crystals. The average yield in a number of experiments was 1 g. from 5 g. of starting material. The melting point was usually 210° , with decomposition.

To recrystallize the acid, 1 g. of the material was suspended in 100 cc. of hot water and sufficient alkali to cause complete solution was added. The liquid was then made acid to litmus with acetic acid and filtered through norit. The clear colorless filtrate was acidified to Congo red with dilute sulfuric acid and set aside to crystallize. The acid separated as irregularly shaped plates and rods, most of which melted with decomposition at 210° . Some samples, however, melted as high as 213° . An alcoholic solution of the acid gave an intense blue color with ferric chloride.

Anal. Calcd. for $C_9H_{10}O_5$: mol. wt. 198.1; OCH_3 (2), 31.4; C, 54.54; H, 5.09. Found: acid equivalent, 202; OCH_3 , 31.1; C, 54.46; H, 5.20.

Asaronic Acid from the Above.—One gram of the hydroxydimethoxybenzoic acid was dissolved in 25 cc. of water containing a little potassium hydroxide.

During half an hour, while the solution was kept below 30° and frequently shaken, 5 g. of dimethyl sulfate and 2.2 g. of potassium hydroxide in 50% aqueous solution were added simultaneously in small portions. Two and two-tenths grams more of potassium hydroxide was then added at one time, and the solution was heated to boiling and refluxed for thirty minutes.

After cooling, the reaction mixture was made acid to Congo red with dilute sulfuric acid, whereupon 0.2 g. of unchanged material separated immediately. This material was removed by filtration, and soon the trimethoxybenzoic acid began to crystallize from the filtrate. The yield was 0.6 g. The acid was purified by dissolving it in hot water, filtering the solution through norit and allowing the liquid to cool. It separated as colorless crystals which melted at 144° , the melting point recorded for asaronic acid, and it gave no color test with ferric chloride.

Anal. Calcd. for $C_{10}H_{12}O_5$: methoxyl, 43.9. Found: methoxyl, 43.7.

The asaronic acid was further identified by nitration and bromination as follows.

1-Nitro-2,4,5-trimethoxybenzene.—One-tenth gram of trimethoxybenzoic acid, dissolved in 1 cc. of acetic acid, was treated with 0.04 cc. of fuming nitric acid. After a short interval, scratching the test-tube containing the red solution caused the nitro compound to separate. It was filtered, washed with acetic acid and dried. Its melting point was 128 – 129° . Upon recrystallization from alcohol it separated as long yellow rods and needles with a melting point of 130° , which is the same as the melting point recorded by Fabinyi and Székí⁶ who prepared the material from an authentic sample of asaronic acid.

Bromination of the Asaronic Acid.—A cooled solution of 0.12 g. of the trimethoxybenzoic acid in 0.7 cc. of acetic acid was treated with 0.25 cc. of a 12% acetic acid solution of bromine. The trimethoxybromobenzene was separated by adding approximately 5 g. of ice to the reaction mixture and neutralizing the solution with sodium hydroxide. The product was removed by filtration and recrystallized from alcohol, from which it separated as rods and prisms whose melting point was 54 – 55° . Fabinyi and Székí⁶ record the melting point of the bromo derivative from asaronic acid as 54.5° .

Hydroxydimethoxybenzene from the 2-Hydroxy-4,5-dimethoxybenzoic Acid.—One gram of the acid was heated with 2 cc. of aniline until the evolution of gas ceased, and a homogeneous solution resulted. The liquid was then cooled, and 25 cc. of water

⁵ Fabinyi and Székí, *Ber.*, 39, 3681 (1906).

⁶ Fabinyi and Székí, *ibid.*, 43, 2681 (1910).

and sufficient concentrated hydrochloric acid completely to dissolve the aniline were added. The liquid was extracted a number of times with ether. The extract was dried with sodium sulfate and concentrated to a small volume. Ten volumes of warm petroleum ether (boiling point 55–70°) was added to the concentrate, and the turbid liquid was allowed to crystallize. A good yield of the phenol was obtained. The melting point was 79–80°. It was recrystallized from *n*-butyl ether by the addition of petroleum ether.

The purified phenol separated as irregularly shaped rods and plates which melted at 79–80°. It gave a light pink color with ferric chloride.

Anal. Calcd. for $C_6H_{10}O_3$: C, 62.32; H, 6.54; OCH_3 (2), 40.3. Found: C, 62.54; H, 6.68; OCH_3 , 39.9.

3,5-Dinitrobenzoyl Derivative of the Above-Described Phenol.—A mixture of 120 mg. of the phenol, 190 mg. of 3,5-dinitrobenzoyl chloride and 2.5 cc. of pyridine was gently refluxed for thirty minutes. The reaction mixture was cooled and poured into 150 cc. of 2% sulfuric acid. The resulting milky suspension was then extracted with 150 cc. of ether and the extract was washed in the separatory funnel, first with 1% sodium hydroxide and then with water. The ethereal solution was dried with sodium sulfate and evaporated to dryness. The residue was dissolved in the necessary quantity of boiling alcohol, then an equal volume of hot water was added, and the solution was allowed to crystallize. The dinitrobenzoyl derivative which separated melted at 158°.

Anal. Calcd. for $C_{16}H_{12}O_8N_2$: N, 8.04. Found: N, 8.06.

Synthesis of 1-Hydroxy-3,4-dimethoxybenzene

4-Nitroveratrole.—This preparation was made by the method of Moureu,⁷ somewhat modified. Twenty grams of veratrole, dissolved in 20 cc. of acetic acid, was added dropwise to a continuously stirred ice-cooled solution of 25 cc. of concentrated nitric acid in 50 cc. of water. After all the veratrole had been added, the stirring was continued for two hours. The temperature of the reaction mixture was maintained at 0° for the first hour, then it was allowed to warm to room temperature. At the end of the specified time the nitroveratrole had completely crystallized. The product was removed by filtration, washed with water and dried.

It was recrystallized from 150 cc. of hot methanol upon the addition of sufficient water to cause a slight turbidity. The yield of crystalline nitro compound was 21.6 g. or 81.5%. Its melting point was 95–96°.

4-Aminoveratrole.—The preparation of this compound was also made by a modification of Moureu's method.⁷ A mixture of 30 g. of mossy tin, 24 g. of 1-nitro-3,4-dimethoxybenzene and 200 cc. of concentrated hydrochloric acid was allowed to react with occasional stirring. After the vigorous reaction which at first took place subsided, 30 g. more of tin was added. As the nitro compound was reduced the amine separated as the stannic chloride double salt. Upon completion of the reduction the mixture was cooled, the precipitate was removed by filtration and then dissolved in 300 cc. of water. Sodium hydroxide was added to the solution in sufficient quantity to dissolve the tin precipitate first formed and to render the liquid strongly alkaline. Much of the amine separated as pearlescent plates. These were not removed, however, but the amine was extracted directly with ether. The ethereal solution was dried with sodium sulfate and evaporated to dryness. The yield of crude amine was 15 g. It melted at 76°, and when it was recrystallized from ether or *n*-butyl ether, or when it was distilled under reduced pressure, it consisted of snow-white crystals, which melted at 80–81°, the melting point reported by Heinisch.⁸

⁷ Moureu, *Bull. soc. chim.*, [3] 15, 647 (1895).

⁸ Heinisch, *Monatsh.*, 15, 231 (1894).

1-Hydroxy-3,4-dimethoxybenzene.—Several procedures for preparing this substance were tried, but decomposing the diazonium compound with hot cupric sulfate solution gave the best results.⁹

A solution of 5 g. of 1-amino-3,4-dimethoxybenzene dissolved in 35 cc. of 10% sulfuric acid was cooled to 0° and diazotized with the theoretical quantity of sodium nitrite. Extreme caution was taken to prevent the presence of free nitrous acid in the final solution. After the diazonium solution had stood in an ice-bath for fifteen minutes, it was added dropwise to a solution of 100 g. of crystalline cupric sulfate dissolved in 100 cc. of water. This liquid was contained in a distillation apparatus, the flask of which was immersed in an oil-bath heated to 150°. At the same time live steam was passed through the apparatus and the distillate was collected.

Ten minutes after the diazonium solution was added to the flask the cupric sulfate solution was diluted with two volumes of water, cooled and extracted several times with ether. The ether was removed from the extract, and the residue was dissolved by warming in 25 cc. of 2% sodium carbonate solution. The dark liquid was cooled and extracted with ether and, after the ether had been removed, the residue was dissolved in a large volume of boiling petroleum ether (boiling point 55–70°). After some time the phenol separated, partly as crystals and partly as a colored oil. The crystals were removed mechanically and the oil was dissolved in the boiling mother liquors. Upon standing, the solution yielded a further quantity of crystalline material. The process was repeated several times.

The distillate obtained during the addition of the diazonium solution to the cupric sulfate solution contained some of the phenol. This was recovered by evaporating the distillate under reduced pressure to approximately 75 cc. and extracting the liquid several times with ether. The residue obtained from the ether was dissolved in petroleum ether, from which it crystallized in a somewhat purer condition than did the fraction obtained from the flask. It was estimated that the quantity of the phenol formed by the reaction was approximately 1 g., but because of the difficulty of obtaining the substance with a reasonable degree of purity, the yield was small.

The slightly colored crystalline material described above was recrystallized from its solution in boiling petroleum ether (boiling point 55–70°). Often several days were required to obtain clean colorless crystals. The material melted at 79–80° and gave a light pink color with ferric chloride.

When the synthetic preparation was mixed with the phenol prepared by decarboxylating the hydroxydimethoxybenzoic acid, no depression of the melting point occurred.

Anal. Calcd. for $C_8H_{10}O_3$: C, 62.32; H, 6.54; OCH_3 (2), 40.3. Found: C, 62.20; H, 6.43; OCH_3 , 40.2.

3,5-Dinitrobenzoyl Derivative.—This preparation was made by the same procedure as that described before for the natural phenol. It melted at 158° and when mixed with the 3,5-dinitrobenzoyl derivative of the phenol obtained from the hydroxydimethoxybenzoic acid there was no depression of the melting point.

Anal. Calcd. for $C_{13}H_{12}O_5N_2$: N, 8.04. Found: N, 8.06.

Summary

1. One of the main products of the permanganate oxidation of dehydrodeguelin is 2-hydroxy-4,5-dimethoxybenzoic acid, demonstrating that in deguelin, tephrosin and rotenone the methoxyl groups are ortho to each other.

⁹ German Patent 167,211; *Friedländer*, **8**, 128 (1908).

2. The position of the free hydroxyl group in the hydroxydimethoxybenzoic acid indicates that the methoxylated benzene ring in the three substances mentioned above are united with the remainder of the molecule through a carbon atom and an indifferent oxygen atom which are ortho to each other.

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PHOSPHORUS IN GLYCOGEN¹

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Glycogen from various sources has phosphorus closely associated with it.² A part of this phosphorus dialyzes out readily through a collodion membrane and is obviously phosphate or a nearly related phosphorus-bearing ion, while the remainder is not so removed. This residual phosphorus may be insoluble and non-diffusible either because of its attachment to an organic residue or because it is in the form of an insoluble inorganic salt. The results of this investigation show that the major amount of non-dialyzable phosphorus is attached to a nitrogenous residue.

That this bound phosphate is firmly held to some system is evidenced by the fact that the severe treatment with hot aqueous alkali in the usual Pflüger method³ for the isolation of glycogen from animal tissue does not hydrolyze all the material to give simple phosphate ion.

For example, a specimen of rat liver glycogen prepared by the Pflüger method and subjected to long dialysis still contained 0.024% of "bound" phosphorus, while two different specimens of sea scallop (muscle glycogen) similarly treated still contained, respectively, 0.013 and 0.018% phosphorus. An undialyzed sample of Kahlbaum's glycogen contained 0.1% phosphorus.

Sources of Glycogen.—At first an attempt was made to obtain the material from the liver of calves and sheep by removing the organ immediately after slaughtering and dropping it into liquid air. The resulting friable mass was ground in a mortar and carried through the Pflüger method.³ The yields on these test runs were negligible, due either to the starved condition of the animals or to fear, both factors causing depletion of the storage carbohydrate.

Liver fairly rich in glycogen was obtained finally by decapitating adult

¹ The material for this paper is taken from the first part of a dissertation submitted by J. J. McBride to the Faculty of Pure Science of Columbia University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

² Samec and Isajević, *Compt. rend.*, **176**, 1419 (1923); Petree and Alsberg, *J. Biol. Chem.*, **82**, 385 (1929).

³ Pflüger, *Pflüger's Arch.*, **93**, 81 (1903).