

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

TEPHROSIN. I. THE COMPOSITION OF TEPHROSIN AND ITS RELATION TO DEGUELIN

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Hanriot isolated from the leaves of *Cracca (Tephrosia) vogelii* a substance which he named tephrosin.¹ To this material he assigned the formula $C_{31}H_{26}O_{10}$ and reported its melting point as 187° . His results were later confirmed by work published in the Bulletin of the Imperial Institute for 1915² except that the melting point of tephrosin was given as 192° .

In the studies in progress in this Laboratory upon the constituents of certain tropical fish-poisoning plants, an examination of the leaves of *Cracca vogelii* from Africa and Sumatra resulted in the isolation of a physiologically active crystalline material corresponding to that obtained by Hanriot. The preparation was a mixture of at least two compounds which were carefully separated and characterized. One was deguelin, m. p. 171° ,³ and the other was a colorless crystalline substance with a melting point of $197-198^{\circ}$.

At the time these experiments were being carried on, the same compounds, together with several others, were also found in the rotenone mother liquors from derris and cubé roots.⁴ When working with these materials difficulty in separating the individual constituents by fractional crystallization was experienced, due to the similarity of the solubilities of the several compounds in many solvents. However, as the separation proceeded, a fraction with a melting point of $180-185^{\circ}$ was invariably obtained. It was unusually hard to resolve, but optical methods showed it to be essentially deguelin and the compound melting at 198° . This preparation was similar to the one obtained by the writer from *Cracca vogelii* and it appears likely that it was such a mixture that Hanriot described as tephrosin. Since a mixture of these two substances melting at 187° would contain more of the compound melting at 198° than deguelin, the name tephrosin should be assigned to the former substance. In this and subsequent communications upon the subject this name will be adopted.

To obtain tephrosin, and the substances with which it is associated, it is necessary to treat the plant extractives in which it is found with an alkali.⁴ A mixture is thus obtained from which tephrosin may be separated by fractional crystallization, but since purification in this way is un-

¹ Hanriot, *Compt. rend.*, **144**, 150 (1907); *Compt. rend. soc. biol.*, **62**, 384 (1907).

² Anon., *Bull. Imp. Inst.*, **13**, 61 (1915).

³ Clark, *Science*, **71**, 396 (1930).

⁴ Clark, *THIS JOURNAL*, **53**, 313 (1931).

usually difficult, it is expedient, after several fractionations, to destroy the contaminating materials (mostly deguelin) by selective oxidation. In the experimental part directions are given for conducting such a procedure.

Tephrosin, prepared by either method, crystallizes in colorless prisms whose melting point is 198° (corr.), but this value varies somewhat depending upon the rate of heating. The substance has the molecular formula $C_{23}H_{22}O_7$ and contains two methoxyl groups.

When tephrosin is dissolved in a mixture of one volume of concentrated sulfuric acid and two volumes of acetic acid and the solution maintained at about 60° for fifteen minutes, tephrosin loses a molecule of water, forming dehydrodeguelin.⁴ The same result is also obtained when tephrosin is boiled with a mixture of acetic anhydride and sodium acetate. The reactions show that tephrosin is intimately related to deguelin and possibly is a hydroxydeguelin. As yet direct evidence for an hydroxyl group in tephrosin has not been obtained, but in certain derivatives of this group of compounds an hydroxyl group may be present that gives extremely poor yields or fails entirely to form acyl or alkyl derivatives. Since dehydrodeguelin is a lactone it follows that tephrosin is a lactone also.

Another reaction presented as confirmatory evidence of the C_{23} formula for tephrosin is its behavior toward potassium permanganate. When oxidized with this reagent in an acetone solution, tephrosin yields a dicarboxylic acid, $C_{23}H_{22}O_{11}$, which melts at 221° . This acid has been designated as tephrosindicarboxylic acid.

Experimental

Tephrosin from *Cracca Vogelii*.—Two and four-tenths kg. of the ground leaves of *Cracca vogelii* were percolated with ether and the extract was concentrated to a thick sirup. When this sirup was dissolved in 400 cc. of hot alcohol and allowed to cool, 1 g. of fine colorless waxy crystals separated. (Since this material was not of immediate interest it was not investigated.) One hundred cc. of the alcoholic filtrate from the waxy crystals, representing 600 g. of leaves, was dissolved in 750 cc. of boiling alcohol; 750 cc. of hot water was added, and the resulting turbid liquid was filtered through norit. The filtrate was concentrated under reduced pressure until the extractives separated as a gummy material which adhered to the flask. The residual liquid was decanted, and the flask was dried with a current of air. The gummy material was dissolved in 100 cc. of boiling methanol and treated with 0.5 g. of dry sodium carbonate. Crystallization began at once and was completed after several hours. The yield was 2 g. or 0.33%.

The crude crystalline material was dissolved in boiling alcohol, filtered through norit and allowed to crystallize. The compound which separated was identified as deguelin by its melting point (171°), mixed melting point and optical properties. The deguelin mother liquors were concentrated to one-fourth their volume and allowed to crystallize. A small quantity of colorless prisms, m. p. $188-191^{\circ}$, was obtained. This substance was dissolved in a small quantity of hot chloroform and about 20 volumes of methanol added. The resulting solution was evaporated to one-half its volume and set aside to crystallize. This procedure was repeated until the product had a constant melting point of $197-198^{\circ}$.

The tephrosin thus obtained consists of stout six-sided colorless prisms. The extinction is inclined and the elongation is negative. In convergent polarized light with crossed nicols, partial biaxial figures are common. The optic axis of fragments is usually inclined. The refractive indices are: n_{α} , 1.590 (common); n_{β} , indeterminate; n_{γ} > 1.74. An intermediate index, n_i , 1.680 is also common.⁵

Anal. Calcd. for $C_{23}H_{22}O_7$: C, 67.30; H, 5.41; OCH_3 (2), 15.1. Found: C, 67.31; H, 5.47; OCH_3 , 15.2.

Tephrosin from Derris and Cubé Roots.—In the recent article upon deguelin⁴ details were given concerning the methods for obtaining a crystalline mixture from the rotenone mother liquors of derris and cubé roots. Since tephrosin is so intimately associated with deguelin in these mixtures, details for preparing a crude tephrosin was of necessity included in the preparation of deguelin. The fraction (m. p. 180–185°) which contained the most tephrosin was subjected to fractional crystallization until a product having a melting point of 198° was obtained. This was analytically and optically identical with tephrosin from *Cracca* leaves.

A more convenient method of preparing pure tephrosin is to destroy the deguelin in the mixture melting at 180–185° with chromic acid. This may be done as follows: 25 g. of the crude mixture (m. p. 180–185°) is dissolved in 500 cc. of boiling acetic acid. To this solution is added 40 cc. of a 10% acetic acid solution of crystalline sodium dichromate, and after the mixture has been allowed to react for five minutes, 500 cc. of boiling water is added. The turbid liquid is immediately filtered through norit and allowed to crystallize. Usually 12 g. of tephrosin with a melting point of 192–193° is obtained. When this product is recrystallized two or three times from 95% alcohol, it has a melting point of 197–198°. Tephrosin prepared in this manner has the same optical properties as the specimens obtained by fractional crystallization.

Anal. C, 67.32; H, 5.36; OCH_3 , 15.6.

The Action of Sulfuric and Acetic Acids upon Tephrosin.—One gram of tephrosin was warmed to about 60° for fifteen minutes with a solution of 3 cc. of concentrated sulfuric acid in 6 cc. of acetic acid. Fifty cc. of water was then added to the mixture and the precipitate formed was removed and washed with water until it was free from sulfuric acid. The product was dried and dissolved in a small volume of hot chloroform, filtered through norit and five volumes of methanol added to the filtrate. Crystallization began at once, yielding 0.8 g. of pure dehydrodeguelin. The product was identified by its melting point, 233°. When it was mixed with dehydrodeguelin from deguelin, no melting point depression occurred. It also possessed the optical properties characteristic of dehydrodeguelin.

Action of Acetic Anhydride upon Tephrosin.—A mixture of 0.5 g. of tephrosin, 0.25 g. of potassium acetate and 4 cc. of acetic anhydride was boiled for ten minutes. The condenser was then disconnected, and about two-thirds of the anhydride was removed by distillation. The remaining anhydride was carefully decomposed with methanol, after which ten volumes more of methanol were added. Crystallization began at once, yielding a product which melted at 228–230°. Upon recrystallization from chloroform and methanol it melted at 233°, and when it was mixed with dehydrodeguelin no depression of the melting point occurred. Its optical properties were the same as those of dehydrodeguelin.

Tephrosindicarboxylic Acid.—A constantly stirred solution of 5 g. of tephrosin in 300 cc. of acetone, maintained at a temperature between 15 and 20°, was treated with

⁵ The optical data recorded in this paper were determined by George L. Keenan of the Food and Drug Administration, U. S. Department of Agriculture. Appreciation is expressed for this coöperation.

10 g. of potassium permanganate until the color due to the permanganate ions disappeared. The resulting manganese dioxide was removed by filtration, washed with acetone and then digested for two hours with 500 cc. of boiling absolute alcohol. The hot alcoholic extract was filtered, concentrated to 40 cc. and allowed to crystallize.

The resulting potassium salt, which weighed 1.5 g., was then dissolved in 100 cc. of water and the solution filtered through norit. The filtrate was heated to boiling, and dilute sulfuric acid was added until an acid reaction to Congo red was obtained. Upon standing overnight the liquid deposited 1.4 g. of rod-like plates, which melted at 220° with decomposition.

The acid was recrystallized from 1.5% boiling aqueous solution, from which it separated as clear-cut tabular crystals and rods which melted at 221° with decomposition (corr.). The substance has inclined extinction, positive elongation and the following indices of refraction: n_{α} , 1.530; n_{β} , indeterminate, and n_{γ} , 1.640; both ± 0.003 .

Anal. Calcd. for $C_{23}H_{22}O_{11}$: mol. wt. 474.3; OCH_3 (2), 13.1; C, 58.21; H, 4.68. Found: acid equivalent (2 COOH), 472; OCH_3 , 13.1; C, 58.37; H, 4.73.

Summary

1. Deguelin, m. p. 171° , and a compound melting at 198° , were isolated from the leaves of *Cracca vogelii*, derris root and the roots of *Lonchocarpus nicou* (Cubé root).

2. These two substances form a mixture which is difficult to separate by fractional crystallization and which appears to be the material designated by Hanriot as *tephrosin*.

3. It is proposed that the name *tephrosin* should be retained for the compound with the melting point of 198° .

4. *Tephrosin* is a dimethoxy lactone, $C_{23}H_{22}O_7$, which, under the influence of certain dehydrating agents, loses one molecule of water, forming dehydrodeguelin.

5. It is suggested that *tephrosin* is a hydroxydeguelin.

6. The action of potassium permanganate upon *tephrosin* results in the formation of a dicarboxylic acid, $C_{23}H_{22}O_{11}$.

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