

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

DEGUELIN. I. THE PREPARATION, PURIFICATION AND PROPERTIES OF DEGUELIN, A CONSTITUENT OF CERTAIN TROPICAL FISH-POISONING PLANTS

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RECEIVED NOVEMBER 7, 1930

PUBLISHED JANUARY 12, 1931

In two recently published reports concerning the constituents of some tropical fish-poisoning plants^{1,2} attention was directed to a pale green substance which crystallizes in rod-like plates and melts at 171°. This material, which is highly toxic to fish, is an active constituent of derris and cubé roots, the leaves of *Cracca* (*Tephrosia*) *vogelii* and the roots of *Cracca* (*Tephrosia*) *toxicaria*.

For purposes of convenience the compound has been termed *deguelin*, derived from *Deguelia*, the generic name, which, under the American code of botanical nomenclature, supersedes that of derris.

Although deguelin occurs abundantly in cubé and derris roots, its preparation in a chemically pure condition is accomplished with difficulty, which fact has seriously limited the supply of material for study. However, with the information now available it is possible to state that deguelin is a dimethoxylactone, C₂₃H₂₂O₆. Some derivatives of the substance have been prepared and, in a preliminary way, its effectiveness as an insecticide has been investigated.³ This communication describes the procedure used in the preparation and purification of the compound, records some of its chemical and physical properties and presents the data upon which the statements regarding its composition and nature have been based.

Deguelin occurs in the non-crystallizable extractives of the four previously mentioned plant materials. Apparently, however, it exists in some form of combination, since it and the constituents with which it is associated are obtained only when the non-crystalline material, dissolved preferably in methyl or ethyl alcohol, is treated with an alkali. In many of the common solvents, the solubilities of all the constituents, including deguelin, are essentially the same, rendering the purification of any one of the substances difficult.

Deguelin showed no optical activity, but it may have existed in the plant in an optically active state and the drastic alkali treatment might have caused racemization. This point has not yet been studied.

When deguelin was first prepared it was purified by repeated recrystallization until it had a constant melting point of 165°. This material was optically uniform, and analytical data showed the composition indicated

¹ Clark, *Science*, 71, 396 (1930).

² Clark, *THIS JOURNAL*, 52, 2461 (1930).

³ Davidson, *J. Econ. Entomol.*, 23, 877 (1930).

by its formula to be constant. Later, in purifying another lot, the melting point constantly dropped until 165° was reached. Upon further recrystallization the melting point rose until at 171° it was constant. Both preparations, that is, the compound melting at 171° and that melting at 165° , had identical optical properties and gave identical analytical data. After obtaining the substance melting at 171° it has been impossible, so far, to obtain a pure sample melting at 165° . The melting point of 171° has, therefore, been recorded for the material.

A reaction of deguelin useful in permitting an attack upon the molecule is its oxidation with potassium ferricyanide. Under properly controlled conditions the reaction proceeds smoothly, yielding a yellow compound, dehydrodeguelin, $C_{23}H_{20}O_6$. On further oxidation with chromic acid, this substance forms dehydrodeguelone, $C_{23}H_{18}O_7$. This is interpreted as indicating an oxidation of a methylene to a carbonyl group.

Dehydrodeguelin reacts with alcoholic potassium hydroxide, forming a phenolic monocarboxylic acid, $C_{23}H_{24}O_8$. The reaction shows the presence in the molecule of a lactone group, but since it involves the addition of two moles of water instead of one, as would be expected, the reaction is abnormal and is analogous to the results obtained by Butenandt with dehydrorotenone.⁴

Evidence of a phenolic hydroxyl group in this acid, which has been designated as deguelic acid, was given by the claret color developed when an alcoholic solution of the acid was treated with ferric chloride. Attempts to form a crystalline acetyl derivative of the acid by using acetic anhydride and sodium acetate were unsuccessful. Instead, ring closure and dehydration occurred, resulting in the regeneration of the mother substance, dehydrodeguelin.

Further work upon this insecticide is in progress.

Experimental

The rotenone in a concentrated ethereal extract of derris root was removed by crystallization, and the mother liquors were concentrated to a thick sirup, practically free from ether; 100 g. of this material was dissolved in 300 cc. of hot methanol and treated with 20 cc. of aqueous 10% solution of sodium hydroxide. In a short time crystallization began and after twenty-four hours 27.5 g. of crude product was removed. This material was a mixture of several substances, three of which predominated, namely, toxicarol, deguelin and tephrosin.

A rough separation of toxicarol was obtained as follows. The crude mixture was dissolved in the minimum quantity of boiling chloroform. The solution was filtered through norit, and again heated to its boiling point. Twelve volumes of boiling alcohol were then added. The solution was vigorously boiled until it was practically free from chloroform, and by that time most of the toxicarol had separated. The crystals filtered from the boiling solution consisted of 10 g. of impure toxicarol. As the mother liquors cooled, 5 g. of light yellow crystals separated, whose melting point was around 168° . This fraction, which is richest in deguelin, was subjected to fractional crystalliza-

⁴ Butenandt, *Ann.*, **464**, 372 (1928).

tion from boiling 95% alcohol. As purification proceeded the melting point generally dropped somewhat and then rose eventually to 171°.

The substance crystallizes in plate-like rods many of which are six-sided. Its indices of refraction are: η_{α} , 1.570; η_{β} , 1.590 (common) and η_{γ} , > 1.739 both ± 0.003 . The extinction is inclined, and the elongation is negative. Most of the crystals do not extinguish sharply when examined in parallel polarized light with crossed nicols.⁵

Anal. Calcd. for $C_{23}H_{22}O_6$: C, 70.03; H, 5.63; OCH_3 (2), 15.7. Found: C, 69.99; H, 5.63; OCH_3 , 15.8.

There is much deguelin in the impure toxicarol and in the mother liquors from the crystalline material used for fractionation. However, the difficulty involved in separating it as such is too great for it to be of practical value. Some of the deguelin fractions obtained after roughly removing toxicarol may have melting points higher or even lower than 168, depending largely upon the quality of the derris root employed.

Deguelin from Cubé Root.—The non-crystallizable extractives from cubé root when treated with sodium hydroxide in the manner just outlined yield principally a mixture of deguelin and tephrosin. There is also a small quantity of a yellow substance present, but deguelin is much more readily obtained pure from cubé than from the crystalline mixture derived from derris root. Also, sodium carbonate may be substituted for sodium hydroxide to induce the crystallization of the mixture of compounds under discussion. With sodium carbonate crystallization is generally slower, and the two main constituents separate in large crystals, enabling one to make a rough mechanical separation, and thereby simplifying the purification of deguelin. The following experiment is representative of what may be obtained with a good specimen of cubé roots.

Rotenone mother liquors from 5.2 kg. of cubé roots were concentrated to a thick sirup. This material, which weighed 560 g., was dissolved in 11 liters of hot methanol, and 55 g. of dry sodium carbonate was added. The mixture was stirred occasionally for an hour and then allowed to crystallize for two days. The product was removed by filtration and washed first with methanol, and then with water to remove the excess of sodium carbonate. Upon suspending the mixture in methanol, tephrosin, which consisted of heavy prisms, quickly settled to the bottom of the container, and deguelin, in the form of thin plate-like rods, remained suspended sufficiently long to be decanted. The yield was 92 g. of crude tephrosin and 83 g. of deguelin. Upon concentrating the mother liquors under reduced pressure, 98 g. of a mixture of deguelin and tephrosin was obtained. Since this material was more valuable as a source of tephrosin, it was used for this purpose. The purity of the deguelin fraction was such that one recrystallization from *n*-butanol followed by two recrystallizations from ethanol gave a product whose melting point was 171° and which possessed the same optical properties recorded for deguelin from derris.

Dehydrodeguelin.—Ten grams of deguelin dissolved in 500 cc. of boiling ethanol was treated with 150 cc. of a hot aqueous solution of 18 g. of potassium ferricyanide and 2.8 g. of potassium hydroxide. The mixture was allowed to stand until the next day and then quickly diluted with 1.5 liters of water. Dehydrodeguelin separated as large yellow crystals. The residual organic material dispersed in the milky mother liquor was carefully decanted. The crystals were then washed with water by decantation, transferred to a filter and finally washed with methanol. The product was 5.4 g. of dehydrodeguelin, consisting of bright yellow pointed rods and needles whose melting point was 233°.

For preparative purposes dehydrodeguelin may be obtained by oxidizing crude

⁵ The optical data recorded in this communication were determined by George L. Keenan of the Food, Drug and Insecticide Administration of the U. S. Department of Agriculture. Appreciation is gratefully acknowledged for this valuable cooperation.

deguelin from derris. For example, 50 g. of crude deguelin in 10-g. portions gave by the above described procedure 19.6 g. of pure dehydrodeguelin, whose melting point was 233°. The material was recrystallized from a chloroform solution by adding 5 volumes of methanol. The melting point and appearance did not change. The crystals have straight extinction and negative elongation. The refractive indices are: η_{α} , 1.635 (shown lengthwise); η_{β} , 1.687 (shown crosswise) and η_{γ} , higher than 1.74.

Anal. Calcd. for $C_{23}H_{20}O_6$: C, 70.39; H, 5.14; OCH_3 (2), 15.8. Found: C, 70.20; H, 5.17; OCH_3 , 15.8.

Several preparations of this compound had a melting point of 228°, which was not altered by repeated recrystallization. The analytical and optical data, nevertheless, were identical with those of the specimens melting at 233°.

Dehydrodeguelone.—Two grams of dehydrodeguelin dissolved in 30 cc. of boiling acetic acid was oxidized with 15 cc. of a 10% acetic acid solution of crystalline sodium dichromate (theory requires 10 cc.). After five minutes three volumes of methanol were added, immediately causing 0.26 g. of the oxidation product to separate. It consisted of bright yellow rods which melted at 287° (corr.).

This compound was recrystallized from its solution in chloroform by adding eight volumes of hot ethanol. The material separated as groups of bright yellow slender rods which began to contract and darken at about 280° and melt at 292° (corr.). It showed inclined extinction and negative elongation. Its indices of refraction were η_{α} , 1.505; the β value was not determinable and the maximum (γ) value was greater than the refractive index of methylene iodide (η_D , 1.74). However, many of the rods matched this liquid.

Anal. Calcd. for $C_{23}H_{18}O_7$: C, 67.96; H, 4.47; OCH_3 (2), 15.3. Found: C, 67.80; H, 4.58; OCH_3 , 15.5.

Deguelic Acid.—To a 10% alcoholic solution of potassium hydroxide, made by adding 30 cc. of an aqueous solution of 15 g. of the alkali to 120 cc. of 95% ethanol, 5 g. of dehydrodeguelin and 10 g. of zinc dust were added, and the mixture was gently boiled for thirty minutes. After the zinc had settled, the clear liquid was decanted and cooled by adding crushed ice. The flask containing the zinc dust was washed with water, and the liquid was filtered from the zinc and added to the main reaction mixture, which was then diluted to 750 cc. The solution was made acid to congo red with sulfuric acid and extracted with ether. The ethereal solution was washed with water, dried with sodium sulfate, concentrated to 75 cc. and allowed to crystallize. The yield was 2.2 g. Upon allowing the mother liquor to evaporate a second crop of crystals was obtained, giving in all a yield of 53–55% of the starting material. The crude acid had a melting point of 183–185°. It was recrystallized by dissolving 1 g. in 20 cc. of boiling ethanol and adding an equal volume of water. The yield was 95%, and the substance had a melting point of 188°. A second recrystallization gave a colorless preparation whose melting point was 189° (corr.). It consisted of rods, square plates and needles. The extinction was inclined, and the sign of elongation was positive: η_{α} , 1.520; η_{β} , occurring infrequently, was 1.665; η_{γ} , >1.74. The most significant optical feature possessed by the substance, observed with convergent polarized light (crossed nicols), was the frequent occurrence of interference figures with the optic axis inclined. Many such crystals or fragments do not extinguish readily with crossed nicols (parallel polarized light) but remain bright when the microscope stage is rotated.

Anal. Calcd. for $C_{23}H_{24}O_8$: mol. wt. 428.3; C, 64.47; H, 5.65; OCH_3 (2), 14.5. Found: acid equivalent, 424; C, 64.32; H, 5.78; OCH_3 , 14.7.

Dehydrodeguelin from Deguelic Acid.—A mixture of 0.25 g. of dry sodium acetate, 1 g. of deguelic acid and 4 cc. of acetic anhydride was refluxed for fifteen minutes. The condenser was then disconnected, and about 3 cc. of the liquid was removed by distillation and the residue carefully treated with methanol to decompose the remaining an-

hydride. Finally 15 cc. of methanol was added, causing the crystallization of 0.24 g. of brown rods, whose melting point was 228°. The material was purified by filtering its chloroform solution through norit, evaporating the filtrate to a small volume and adding 10 volumes of methanol. The substance separated as irregular-shaped salmon-colored plates and rods, which melted at 232°. Recrystallization of the compound in the same manner gave a product whose melting point was 233° (corr.). When this preparation was mixed with an authentic sample of dehydrodeguelin, the melting point was not depressed. Also, oxidation with chromic acid gave dehydrodeguelone. In spite of these facts and the fact that the analytical data given below were the same as those for dehydrodeguelin, the optical properties of the material were not the same as those recorded for this compound. However, when a specimen of the substance was dissolved in chloroform and the solution treated with several volumes of methanol in which a few crystals of pure dehydrodeguelin were suspended, the material separated possessed the optical properties recorded for dehydrodeguelin.

Anal. Found: C, 70.40; H, 5.27; OCH₃, 15.9.

Summary

1. The preparation, purification, and some properties of deguelin, a constituent of several tropical fish-poisoning plants, are described.

2. Evidence is presented to show that deguelin is a dimethoxylactone, C₂₃H₂₂O₆.

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[CONTRIBUTION FROM THE NICHOLS LABORATORY, NEW YORK UNIVERSITY]

PREPARATION OF CERTAIN BROMINATED CINCHOPHENS

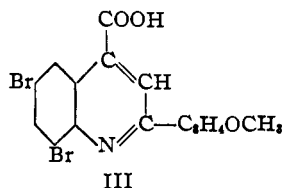
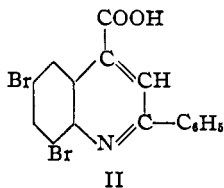
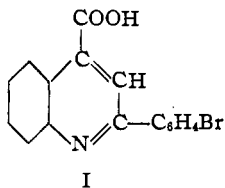
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RECEIVED NOVEMBER 10, 1930

PUBLISHED JANUARY 12, 1931

It has been the purpose of this investigation to synthesize some brominated derivatives of cinchophen (2-phenylcinchoninic acid) in order to study later the physiological effects of the introduction of halogen. Condensations of 5,7-di-bromoisatin, as well as of isatin, with certain methyl ketones have been effected by means of the Pfitzinger¹ method.

By the interaction of isatin and *p*-bromoacetophenone in the presence of potassium hydroxide in water and alcohol, 2-phenyl-4'-bromocinchoninic acid (I) was obtained. In the same manner, 5,7-dibromoisatin and acetophenone reacted to give 6,8-dibromo-2-phenylcinchoninic acid (II), while *p*-methoxyacetophenone and 5,7-dibromoisatin yielded 6,8-dibromo-2-phenyl-4'-methoxycinchoninic acid (III)



¹ Pfitzinger, *J. prakt. Chem.*, 56, 292 (1897).