tive if present occurs in mere traces. The tetraethoxy and tetrabutoxy derivatives obtained as above are of especial interest as representing the first known derivatives of the hypothetical tautomeric barbital. They possess in addition to ether linkages in positions 4 and 6, an acetal structure in the 2-position. Hence they may be regarded as acetals of the ethers of enolic barbital. The isomeric 1,3,5,5-tetraethylbarbituric acid, but without the acetal grouping in position 2, has been prepared by Fischer and Dilthey[4] from ethyl diethylmalonate and *sym.*-diethylurea. This also was a high-boiling liquid.

Summary

2,2,4,6-Tetrachloro-5,5-diethyldihydropyrimidine, a product obtained by chlorination of barbital with phosphorus pentachloride, reacts with sodium alkoxides to form the corresponding 2,2,4,6-tetraalkoxy-5,5-diethyldihydropyrimidines. Hydrolysis by concentrated hydrochloric acid converts the tetraalkoxy derivative into diethylacetic acid and ammonium chloride, the latter in almost quantitative yield, showing that the alkyl is actually linked to oxygen. These tetraalkoxy derivatives represent acetals of the ethers of a hypothetical enolic barbital.

A series of 2,4,6-trialkoxy derivatives was also prepared from 2,4,6-trichloro-5-*sec.*-butylpyrimidine.

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[CONTRIBUTION FROM THE SWAN-MYERS DIVISION OF ABBOTT LABORATORIES]

STUDIES ON POLLEN AND POLLEN EXTRACTS. VII. A GLUCOSIDE FROM CERTAIN GRASS POLLENS

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RECEIVED MAY 4, 1931 PUBLISHED JULY 8, 1931

The existence of glucosides in pollen has been previously recognized. Heyl[1] found the coloring substances of ragweed pollen to be entirely glucosidic, and was able to isolate and identify glucosides of quercitin and isorhamnetin in the alcoholic extract. More recently, Fukuda[2] has isolated a glucoside of isorhamnetin from an alcoholic extract of the pollen of *Typha angustata Bory et Chaub.*

A new glucoside has been isolated in this Laboratory from two grass pollens. The first preparation of this compound was made from the pollen of orchard grass (*Dactylis glomerata L.*) and for this reason we are proposing the name "dactylin." An identical compound was found to separate from an extract of timothy pollen (*Phleum pratense L.*).

[4] Fischer and Dilthey, *Ann.*, **335**, 349 (1904).

[1] F. W. Heyl, THIS JOURNAL, **41**, 1285–1289 (1919).

[2] Masao Fukuda, *Chem. Abstracts*, **22**, 1993 (1928), from *Bull. Soc. Chem. Japan*, **3**, 53–56 (1928).

Experimental

Isolation and Purification.—A distilled water extract of either grass pollen (previously ether extracted) was found to deposit a pale yellow crystalline substance after standing for a few hours. After twenty-four hours at 2–5° a good yield of the crystals was obtained. A second smaller crop was recovered after longer standing.

The crystals were separated from the water extract by centrifugation. They were then purified by two recrystallizations from boiling water, washed with alcohol and ether and dried. The melting point, never sharp, is not appreciably changed by the second recrystallization. As the yield of crude crystals is less than 0.5% of the weight of pollen used, the quantity of purified material available for a study of the properties of dactylin was quite small.

Properties.—Dactylin separates as pale yellow tasteless needles, which may be dried at 100°. Decomposition begins slightly below the melting point and the material fuses to a darkened mass before it becomes truly liquid. This behavior is shared by many of its derivatives. The "melting points" as recorded represent the temperatures at which the crystals fuse to form a viscid mass. On this basis the melting point of dactylin from either source or mixed is 183–185° (corr.).

This glucoside is very sparingly soluble in alcohol, glycerol, or cold water, readily soluble in boiling water and practically insoluble in ether, chloroform, acetone, carbon disulfide, benzene, or glacial acetic acid. It is freely soluble in pyridine, with which it forms a compound, m. p. 261–263° dec. (corr.). In cold water which contains sufficient alkali to raise the P_H to about 8.4 or greater, dactylin dissolves, giving an intense yellow solution.

A water solution of the glucoside gives a strongly positive Molisch test, and does not reduce Fehling's solution. With Millon's reagent it gives a red solution, soon changing to brown. If a trace of ferric chloride is added to the dactylin solution, a permanent dull purplish-green color is produced. No precipitate or characteristic color appears in the solution upon addition of barium chloride, silver nitrate, mercuric chloride, calcium chloride, or lead acetate. In cold concentrated sulfuric acid, the glucoside gives a yellow solution, which develops a greenish fluorescence upon standing.

A suspension of dactylin in water is not hydrolyzed by an active emulsin preparation within a week at 37°.

Hydrolysis readily occurs when the glucoside is heated with 5% sulfuric acid in a boiling water-bath. At first a clear solution is formed, but after a few minutes a yellow crystalline precipitate separates. There remains a colorless solution which reduces Fehling's reagent.

The yellow precipitate formed by acid hydrolysis, when washed with water until free from sulfates, then with alcohol and ether and dried at 100°, melts with decomposition at 298–300° (corr.). This melting point was checked with the preparation from each pollen and with a mixture. The crystals appear microscopically as small feathery collections of fine needles. The saturated solution of this material in alcohol, in which it is slightly soluble, gives no characteristic reaction with water solutions of calcium chloride, barium chloride, silver nitrate or mercuric chloride. Lead acetate increases the yellow color of the solution. With ferric chloride it gives a permanent brownish-purple color. When the compound is added to magnesium and glacial acetic acid, a brick-red color appears after the mixture is allowed to stand for a minute. Addition of hydrochloric acid changes the color to a cherry-red.

The soluble hydrolysis product gives no test for pentoses with phloroglucinol. It forms an osazone having the microscopic appearance of dextrosazone, and melting at 204–205° (corr.). This was checked by preparations from both sources.

In two hydrolyses, using crystals from the separate sources, the insoluble material was found to amount to 40.16 and 40.25% of the original glucoside.

Dactylin was found to contain only carbon, hydrogen and oxygen. Samples for analysis were dried at 100°.

Anal. Calcd. for $C_{23}H_{28}O_{15}$: C, 50.74; H, 5.15; for $C_{26}H_{32}O_{17}$: C, 50.65; H, 5.19; for $C_{29}H_{36}O_{19}$: C, 50.58; H, 5.23. Found: (source—orchard grass pollen) C, 50.59; H, 5.25. (Source—timothy pollen) C, 50.56, 50.64; H, 5.19, 5.06.

Which of the above formulas is preferable may be inferred from a consideration of the percentage of insoluble hydrolysis product formed. Assuming the reaction: $C_{23}H_{28}O_{15} + 2H_2O \longrightarrow 2C_6H_{12}O_6 + C_{11}H_8O_5$, the theoretical percentage of this product would be 40.4% (found, 40.2%). If this percentage is calculated, using either of the other above formulas, the values do not agree with the experimental within the limits of probable error. For this reason we believe the formula $C_{23}H_{28}O_{15}$ is justified.

Discussion

The method of isolation of dactylin raises the question as to whether it is originally present as such in the pollen or is formed in its water extract by the action of enzymes upon some parent substance. The latter theory is supported by the fact that the crystals will spontaneously precipitate from a water extract of pollen at the same temperature as that used for extraction. Moreover, in the presence of 50% glycerol (by volume), the precipitation of dactylin does not occur, although the compound after isolation is very difficultly soluble in glycerol.

The reaction with magnesium and acids makes it appear that this glucoside is closely related to those of the hydroxy flavones.[3]

The fact that it is not hydrolyzed by emulsin indicates that dactylin is not a β-glucoside. The sugar present is probably a hexose, two molecules of monosaccharide being present for each molecule of the insoluble hydrolysis product.

Further work is planned to determine the molecular formula and structure of this compound, when a sufficient supply of grass pollen becomes available.

Summary

A glucoside "dactylin" has been isolated from water extracts of orchard grass and timothy pollens, and its properties studied. Analytical results indicate $C_{23}H_{28}O_{15}$ as the empirical formula.

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[3] Keita Shibata, Yuji Shibata and Itizo Kaswagi, THIS JOURNAL, **41**, 208–220 (1919).