[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

THE SYNTHESIS OF GLUCOSIDOFERULIC ACID

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The natural occurrence of hydroxycinnamic acids and their internal lactones, the coumarins, in the free state and in glucosidic combinations has been demonstrated repeatedly. While ferulic acid (3-methoxy-4-hydroxycinnamic acid) has been observed in the free state by Ponti[1] in the extract from the macerated plants of *Ajuga iva*, the isolation of its glucoside does not seem to have been reported. Power and Tutin,[2] however, have shown it to be an integral portion of the chalcone, homoeriodictyol (2,4,6-trihydroxyphenyl-4-hydroxy-3-methoxystyryl ketone) in *Eriodictyon californicum*, and the occurrence of its related alcohol, coniferyl alcohol (3-methoxy-4-hydroxycinnamyl alcohol) in the glucoside coniferin is well known. The present communication describes the synthesis of ferulic acid *d*-glucoside. The procedure adopted in this study was that of the interaction of the sodium salt of the methyl ester of ferulic acid with acetobromoglucose in cold alcoholic solution. This plan materially shortened the usual method of agitation of an ether solution of the acetylated sugar halide with an alkaline aqueous solution of the phenolic acid ester. The resulting tetra-acetylglucosidoferulic acid methyl ester was simultaneously de-esterified and de-acetylated by treatment with cold barium hydroxide, yielding directly the desired glucosidoferulic acid, which was obtained in crystalline form. Its rotation shows it to be the β-glucoside, as would be expected from its synthesis from acetobromoglucose.

Experimental

Methyl Ferulate.—Ferulic acid was prepared by the Knoevenagel condensation of vanillin with malonic acid in the presence of pyridine and piperidine according to the directions of Robinson and Shinoda.[3] The acid (4 g.) was esterified by boiling for four hours under a reflux condenser with 25 cc. of absolute methyl alcohol and 1 cc. of concd. sulfuric acid. The oily ester was precipitated by addition of 75 cc. of water, then extracted with ether and the ether solution was washed with dilute sodium carbonate, dried over calcium chloride, and evaporated until free of ether. The oily ester (3.6 g. or 84% of the theoretical) was used directly in the condensation with acetobromoglucose.

Methyl Tetra-acetyl-β-*d*-glucosidoferulate (Methyl 3-Methoxy-4-tetra-acetyl-glucosido-cinnamate).—A solution of 3.4 g. of methyl ferulate in 25 cc. of absolute alcohol was treated with an amount of alcoholic potassium hydroxide (17.6 cc. containing 0.0517 g. per cc.) sufficient exactly to neutralize the phenolic hydroxyl. The solution was cooled in ice and an ether solution of 6.7 g. of acetobromoglucose rapidly added.

[1] Ponti, *Gazz. chim. ital.*, **39**, II, 349 (1920).

[2] Power and Tutin, *J. Chem. Soc.*, **91**, 887 (1907).

[3] Robinson and Shinoda, *ibid.*, **127**, 1973 (1925).

Following an immediate cloudiness a rapid precipitation of potassium bromide took place. Following a period of standing the salt was removed and the ether–alcohol solution evaporated to a sirup by an air current. Upon standing crystallization occurred, which was aided by cautious addition of 50% ethyl alcohol. The separated crystals were filtered off, washed and recrystallized from 50% ethyl alcohol; yield 5.6 g. (64% of the theoretical).

Methyl tetra-acetylglucosidoferulate separated from its solutions in 50% ethyl alcohol in colorless brilliant platelets, almost needle-like in character. It melts to a clear oil at 142–143° (corr.). A determination of its rotation in chloroform gave a value of $[\alpha]_D^{20}$ −32.3° (0.2408 g. in 25 cc. of $CHCl_3$ solution gave a reading of 0.623° to the left in a 2-dm. tube). An acetyl determination by the method of Kunz[4] indicated that simultaneous de-acetylation and de-esterification took place (0.2304 g. substance consumed 10.8 cc. of $N/5$ NaOH; calcd., 10.7 cc.). A micro combustion was performed by Dr. R. T. K. Cornwell, to whom thanks are herewith expressed.

Anal. Subs., 3.943 mg.: CO_2, 8.095 mg.; H_2O, 1.943 mg. Calcd. for $C_{25}H_{30}O_{13}$: H, 5.62, C, 55.74. Found: C, 55.99; H, 5.51.

β-*d*-Glucosidoferulic Acid (3-Methoxy-4-β-*d*-glucosido-cinnamic Acid).—For saponification and de-acetylation of the tetra-acetylated methyl ester glucoside an aqueous 6% solution of barium hydroxide was used. Two grams of the finely powdered acetylated glucoside was shaken with 100 cc. of the alkali solution for eighteen hours, when solution was complete. The barium was precipitated successively as the carbonate and sulfate and the precipitate thoroughly extracted with boiling water. Upon concentrating the filtrate and washings under reduced pressure at 40° to a small volume, the glucoside of ferulic acid separated in colorless needles. It is almost insoluble in cold water. It was repeatedly recrystallized from boiling water for purification; yield, 1.1 g.

Glucosidoferulic acid crystallizes from boiling water, in which it is readily soluble, in colorless, brilliant, long, acicular needles containing one molecule of water of crystallization. This is retained at room temperature and ordinary humidity, but is rapidly lost over calcium chloride even at room temperature, the brilliancy of the crystal being replaced by a dull cottony appearance. Upon heating the glucosido acid *in vacuo* at 110° some loss of carbon dioxide occurs and at 180° an amount comparable to one molecule, although partial sublimation of the residue prevented an accurate determination. It is conceivable that the corresponding styrene derivative is formed as has been demonstrated in the case of caffeic acid (3,4 dihydroxycinnamic acid).[5]

The acid melts at 198–199° (corr.) without appreciable decomposition to a colorless oil. Its solutions rotate the plane of polarized light to the left, its $[\alpha]_D^{21}$ value being −36.6° (0.2433 g. in 10 cc. of absolute alcohol solution gave a value of −0.890° in a 1 dm. tube). A micro analysis was carried out.

Anal. Subs., 6.549 mg.: CO_2, 12.302 mg.; H_2O, 3.489 mg. Calcd. for $C_{16}H_{20}O_9 \cdot H_2O$: C, 51.31; H, 5.93. Found: C, 51.22; H, 5.96.

The writer takes this opportunity to express to Dr. C. S. Hudson his appreciation of the many kindnesses extended during the course of this research.

Summary

By the interaction of acetobromoglucose and the sodium salt of methyl ferulate, crystalline methyl ferulate β-*d*-glucoside-tetra-acetate has been

[4] Kunz and Hudson, THIS JOURNAL, **48**, 1982 (1926).

[5] Kunz-Krause, *Ber.*, **30**, 1617 (1897).

synthesized. Through alkaline saponification it has been converted to crystalline *β-d*-glucosidoferulic acid, a glucoside which is closely related to the naturally occurring coniferin.

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

THE TOXICITY OF ROTENONE, ISOROTENONE AND DIHYDROROTENONE TO GOLDFISH[1]

BY W. A. GERSDORFF

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The method used by the author for the study of toxicity in which the goldfish serves as the test animal has been described in a previous paper.[2] This method was used in studying the relative toxicities of rotenone, and two of its derivatives, isorotenone and dihydrorotenone. These substances were prepared by F. B. LaForge and L. E. Smith of this Division[3] in their researches to determine the chemical structure of rotenone.

The chemical structure of these compounds is not yet known. The empirical formula of rotenone is $C_{23}H_{22}O_6$. It possesses a ketone group, two methoxyl groups, a lactone group and an oxygen atom which is probably in the form of an ether linkage. LaForge and Smith reported that dihydrorotenone was one of the products of the catalytic reduction of rotenone, the reaction involving the simple reduction of an unsaturated bond in the rotenone molecule. It is now known that the double bond reduced is that in an isopropylene group. Isorotenone differs from rotenone in the position of its double bond. The double bond of the isopropylene group in rotenone has migrated and an isopropyl group has formed. The chemical relationship of these compounds will be discussed in a forthcoming paper by H. L. Haller of this Division.

The data are given in Tables I to III. The survival time curves and velocity of fatality curves which were plotted from these data are given in Figs. 1 to 3. In the former the ordinates are survival times in minutes; in the latter, the reciprocals of the survival times multiplied by 100. In both kinds of curves the abscissas are concentrations in milligrams per liter.

These curves resemble those given by Powers[4,5] to show the general type of toxic action to goldfish and that given by Carpenter[6] to show the

[1] Presented as a part of the Insecticide Symposium before the Division of Agricultural and Food Chemistry at the 79th Meeting of the American Chemical Society, Atlanta, Ga., April 7 to 11, 1930.

[2] W. A. Gersdorff, THIS JOURNAL, **52**, 3440–3445 (1930).

[3] F. B. LaForge and L. E. Smith, *ibid.*, **51**, 2574–2581 (1929).

[4] Edwin B. Powers, *Ill. Biol. Mono.*, **4**, No. 2 (1917).

[5] Edwin B. Powers, *Ecology*, **1**, 95–112 (1920).

[6] Kathleen E. Carpenter, *Brit. J. Exptl. Biol.*, **4**, 378–390 (1927).