[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, NORTH DAKOTA AGRICULTURAL EXPERIMENT STATION, AND DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

The Constitution of Linocinnamarin[1]

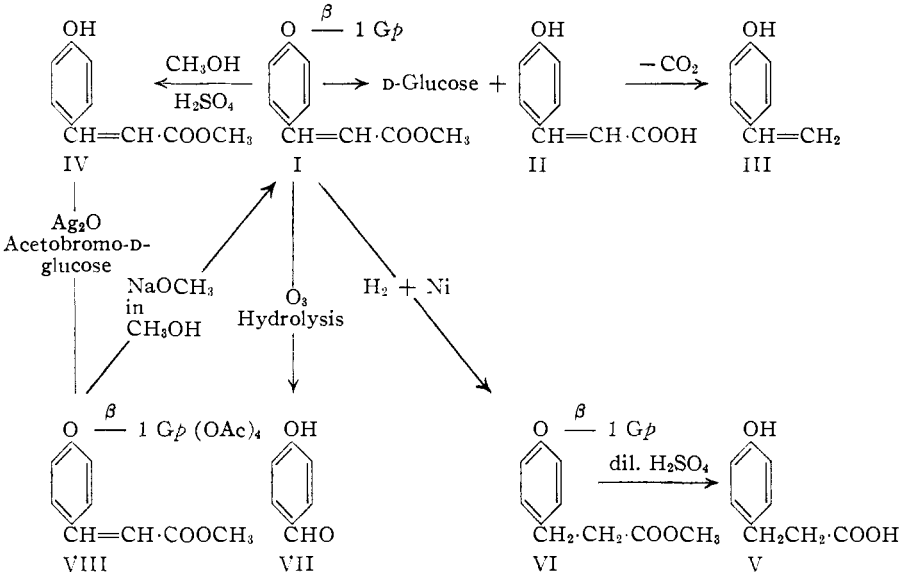
BY HAROLD J. KLOSTERMAN, F. SMITH AND C. O. CLAGETT

RECEIVED JULY 29, 1954

The crystalline glucoside formed when an extract from the ground seed of flax (*Linum usitatissimum*) is treated with methanolic alkali, is shown to be methyl 4-(β-D-glucopyranosido)-hydroxycinnamate. The structure I assigned to this new glycoside called *linocinnamarin*, is proved by degradation and by synthesis.

In a previous paper[2] it was shown that the action of alkali in methanol upon a substance extracted from flaxseed meal afforded β-hydroxy-β-methylglutaric acid and two glycosides, one a yellow amorphous compound and the other a colorless crystalline compound, which were not identified. The crystalline glycoside has now been shown to be the β-D-glucopyranoside of methyl 4-hydroxycinnamate and it is proposed to call this new glucoside "linocinnamarin."

The structure I assigned to linocinnamarin is based upon the following experimental evidence. Elementary analysis, methoxyl and saponification determinations suggested the formula $C_{16}H_{20}O_8$. Linocinnamarin showed strong selective absorption in the ultraviolet region of the spectrum indicating the presence of a conjugated system of double bonds.



Upon hydrolysis with dilute mineral acid I yielded D-glucose, carbon dioxide and an unidentified phenolic substance which appeared to be a polymer. This polymeric substance may well be derived from the 4-hydroxystyrene (III) formed when 4-hydroxycinnamic acid (II) undergoes decarboxylation.[3] When I was boiled with methanol containing sulfuric acid, decarboxylation did not occur and there was formed methyl 4-hydroxycinnamate (IV). These facts suggested that linocinnamarin (I) was a glucoside in which the aglycon moiety was a methyl 4-hydroxycinnamate residue (IV); such a glucoside corresponds to the formula $C_{16}H_{20}O_8$ which agreed with the elementary analysis of I. Further support for formulating linocinnamarin as I was afforded by the fact that reduction of I with hydrogen in the presence of a Raney nickel catalyst proceeded at room temperature with consumption of one mole of hydrogen per mole of linocinnamarin and afforded the dihydrolinocinnamarin (VI). When VI was hydrolyzed it afforded D-glucose and 4-hydroxydihydrocinnamic acid (V). Moreover, when I was ozonized and the resulting ozonide subjected to reductive cleavage followed by acid hydrolysis, there was formed crystalline 4-hydroxybenzaldehyde (VII), which was further characterized by its oxidation to 4-hydroxybenzoic acid.

Final proof of the structure of linocinnamarin was provided by its synthesis. This was accomplished by the reaction of acetobromo-D-glucose with methyl 4-hydroxycinnamate (IV) in the presence of silver oxide to give methyl 4-(β-tetra-*O*-acetyl-D-glucopyranosido)-hydroxycinnamate (VIII). Catalytic deacetylation of VIII with sodium methoxide[4] furnished methyl 4-(β-D-glucopyranosido)-hydroxycinnamate which proved to be identical in every respect with the natural linocinnamarin (I) obtained from flaxseed. This new glucoside is believed to have the β-configuration for the reason that it exhibits a levo-specific rotation ($[\alpha]^{27}D$ − 73°, methanol) and because the Koenigs–Knorr method of glycoside synthesis[5] using acetobromo-D-glucose invariably yields β- rather than α-anomers.

Experimental

Isolation and Characterization of Linocinnamarin (I).— The isolation of linocinnamarin has been described.[2] A slight variation from the original procedure has simplified

(1) Paper No. 3201, Scientific Journal Series, Minnesota Agricultural Experiment Station. This work will form part of a thesis to be submitted to the graduate school of the University of Minnesota for the degree of Ph.D. Published by permission of the Director, North Dakota Agricultural Experiment Station.

(2) H. J. Klosterman and F. Smith, THIS JOURNAL, **76**, 1229 (1954).

(3) W. von Miller and F. Kinkelin, *Ber.*, **22**, 1715 (1889).

(4) G. Zemplén, *ibid.*, **59**, 1254 (1926); **60**, 1555 (1927).

(5) W. Koenigs and E. Knorr, *ibid.*, **34**, 957 (1901).

its isolation. A solution of the amorphous extract (15 g.) in a 1:1 mixture of 1,4-dioxane and methanol was treated with sodium methoxide as before. After about 12 hours the alkaline solution was evaporated to near dryness. The residue was dissolved in 50 ml. of dry methanol and poured into 200 ml. of dry ether. A flocculent precipitate formed which was readily removed from the clear solution by centrifugation. The nearly colorless ethereal solution was evaporated to a sirupy residue which consisted of linocinnamarin and methyl β-hydroxy-β-methylglutarate. Treatment of the residue with 10 ml. of ice-water caused the linocinnamarin to separate at once as fine needles. These were collected and recrystallized from aqueous methanol to give fine needles (0.75 g.) of linocinnamarin monohydrate, $[\alpha]^{27}D$ −70° in methanol (*c* 1). Upon heating the water of crystallization was lost and the resulting anhydrous product melted at 167°.

Anal. Calcd. for $C_{16}H_{20}O_8 \cdot H_2O$: C, 53.6; H, 6.2; MeO, 8.65; H_2O (loss on drying), 5.03. Found: C, 53.4; H, 6.3; MeO, 8.6; H_2O (loss on drying), 5.04.

Anhydrous linocinnamarin, $[\alpha]^{27}D$ −73° (*c* 1, methanol), was also obtained by recrystallization of the monohydrate from anhydrous methanol or by vacuum drying.

Anal. Calcd. for $C_{16}H_{20}O_8$: C, 56.5; H, 5.9; MeO, 9.1. Found: C, 56.5; H, 6.0; MeO, 9.1.

The ultraviolet absorption spectrum of linocinnamarin is characteristic of 4-hydroxycinnamyl derivatives. Two absorption maxima were found (max. 225 mμ, ε 11.3 × 10^3; max. 297 mμ, ε 22.7 × 10^3; 95% ethanol).

Hydrolysis of Linocinnamarin and Characterization of D-Glucose.—A solution of linocinnamarin (0.1 g.) in 10 ml. of *N* sulfuric acid containing 20% methanol by volume was placed in a sealed tube and heated in a boiling water-bath for 6 hr. When the cooled tube was opened, the presence of a slight internal pressure was apparent. The oily material which had separated from solution could not be characterized. It was weakly acidic and was probably formed by polymerization of *p*-hydroxystyrene (III) resulting from the decarboxylation of *p*-hydroxycinnamic acid (II). The acidic solution was treated with a little decolorizing carbon, filtered and neutralized by passage over an anion-exchange resin (Duolite A-4). Evaporation of the neutral eluate *in vacuo* to dryness yielded D-glucose, recognized by paper partition chromatography and by conversion to glucose-*p*-nitroanilide,[6] m.p. and mixed m.p. 184°.

Hydrogenation of Linocinnamarin (I).—Linocinnamarin (I) (1.0 g.) in methanol (50 ml.) was subjected to hydrogenation at 12 atm. pressure in the presence of Raney nickel catalyst. Hydrogen was taken up at room temperature, the pressure drop corresponding to approximately one mole of hydrogen uptake per mole of linocinnamarin. After removal of the catalyst, the methanol was evaporated to give a sirup, probably dihydrolinocinnamarin (VI), which failed to crystallize. However, upon acid hydrolysis 4-hydroxydihydrocinnamic acid (V) was formed, m.p. and mixed m.p. 126°. Calcd. equiv. wt., 166. Found: equiv. wt., 167.

Ozonization of Linocinnamarin (I).—A solution of linocinnamarin (1.0 g.) in methyl acetate (200 ml.) was treated with ozone at 0° until ozone was detected in the effluent gas. Acetic acid (10 ml.) was added followed by zinc dust (1 g.) and water (10 ml.). After one hour the unreacted zinc was filtered off and the solvents evaporated under reduced pressure. The residue was hydrolyzed in boiling 1 *N* sulfuric acid (50 ml.) for four hours. An ether extract of the cooled solution was washed with dilute sodium bicarbonate, dried and evaporated to give 4-hydroxybenzaldehyde (VII) m.p. and mixed m.p. 117°.

Oxidation of VII (35 mg.) by potassium permanganate (35 mg.) in dilute sodium hydroxide (5 ml., *N*/10 molar) proceeded at room temperature. After 5 minutes a little sodium bisulfite was added to dissolve the manganese dioxide and the clear solution carefully acidified with dilute sulfuric acid. The solution was extracted repeatedly with ether and the combined extracts were evaporated to give 4-hydroxybenzoic acid which crystallized spontaneously, m.p. and mixed m.p. 212° (after recrystallization from water), yield 20 mg.

Methanolysis of Linocinnamarin (I).—A solution of linocinnamarin (1.0 g.) in methanol (50 ml.) containing sulfuric acid (2 g., sp. gr. 1.84) was heated under reflux for four hours. The solution was cooled, neutralized with solid sodium bicarbonate, evaporated to 15 ml. under reduced pressure and poured into hot water (30 ml.). Methyl 4-hydroxycinnamate (IV), m.p. and mixed m.p. 136°, separated in quantitative yield.

Synthesis of Linocinnamarin. Methyl 4-(β-Tetra-*O*-acetyl-D-glucopyranosido)-hydroxycinnamate (VIII). (Linocinnamarin Tetraacetate).—The procedure used was an adaptation of the Robertson and Waters[7] variation of the Koenigs and Knorr[5] reaction. From methyl 4-hydroxycinnamate[8,9] (3 g.) acetobromo-D-glucose (30 g.), silver oxide (16 g.) and quinoline (30 ml.) there was obtained 8.5 g. of crude linocinnamarin tetraacetate (VIII) which was recrystallized twice from methanol, $[\alpha]^{26}D$ −18.3° in chloroform (*c* 6). Linocinnamarin tetraacetate is bimorphic. With rapid heating the crystals melt at 138° but with slow heating, however, a transition occurs at about 140° and the resulting crystals melt at 166°.

Anal. Calcd. for $C_{24}H_{28}O_{12}$: C, 56.7; H, 5.55. Found: C, 56.8; H, 5.8.

Methyl 4-(β-D-Glucopyranosido)-hydroxycinnamate, Linocinnamarin (I).—The above acetyl derivative VIII (2.0 g.) was deacetylated by the Zemplén method.[4] The crude product was recrystallized from aqueous methanol to give 1.2 g. of linocinnamarin monohydrate, m.p. 167°. The anhydrous product, m.p. 167°, $[\alpha]^{27}D$ −73° in methanol (*c* 1) was obtained by drying the monohydrate for 2 hours at 100° and 2 mm. pressure. When mixed with the natural product there was no depression of the melting point.

Anal. Calcd. for $C_{16}H_{20}O_8$: C, 56.5; H, 5.9; MeO, 9.11. Found: C, 56.4; H, 6.1; MeO, 9.2.

Methyl 4-(β-Tetra-*O*-acetyl-D-glycopyranosido)-hydroxydihydrocinnamate. (Tetraacetyldihydrolinocinnamarin).—The acetate VIII (1.6 g.) was hydrogenated in methanol at room temperature using Raney nickel catalyst and a hydrogen pressure of 20 atm. After removal of the catalyst by filtration the solution was concentrated to give 1.5 g. of tetra-*O*-acetyldihydrolinocinnamarin, m.p. 112°, $[\alpha]^{26}D$ −23° in methanol (*c* 3.5); $[\alpha]^{26}D$ −15° in chloroform (*c* 5).

Anal. Calcd. for $C_{24}H_{30}O_{12}$: C, 56.5; H, 5.9. Found: C, 56.3; H, 5.9.

FARGO, NORTH DAKOTA
ST. PAUL, MINNESOTA

(6) F. Weygand, W. Perkow and P. Kuhner, *Ber.*, **84**, 594 (1951).

(7) A. Robertson and R. B. Waters, *J. Chem. Soc.*, 2729 (1930).

(8) F. von Konek and E. Pacsu, *Ber.*, **51**, 855 (1918).

(9) H. Schmid and P. Karrer, *Helv. Chim. Acta*, **28**, 725 (1945).