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Altenin. IV. Compounds related to Altenin

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Three compounds, ethyl 5-hydroxy-4, 6-dioxoheptanoate, ethyl 2-methyl-5-hydroxy-5-(1-hydroxyethyl)-4-oxotetrahydrofuroate and 5-phenyl-2-ethoxy-2-(1-hydroxyethyl)-3-oxotetrahydrofuran, which have structures closely related to altenin, were synthesized from 3-acetoxy-2, 4-dioxopentane. These compounds produced the black spot disease in pears. As these compounds all possess the reductone grouping, it appears that this grouping is responsible for the phytopathogenic activity.

The black spot disease of pears is caused by a metabolite of the fungus *Alternaria Kikuchiana* Tanaka, "altenin,"[1,2] the structure of which was determined as being ethyl 5-hydroxy-5-(1-hydroxyethyl)-4-oxotetrahydrofuroate (I).[3] This structure indicates the possibility that it could exist in one of the tautomeric forms, II, III, IV or V. The α-hydroxyester grouping, which is part of the tautomeric structure II or III, is frequently found in biological substances, *e. g.*, ethyl lactate and ethyl citrate. The α-hydroxyl group of II or III is capable of closing the hemirketal ring as in I. In addition the α-hydrogen in I may be expected to play some role in the altenin activity. In order to acquire some information on the role of groups responsible for the phytopathogenic activity of altenin, three analogous compounds, ethyl 5-hydroxy-4, 6-dioxoheptanoate (VIII), ethyl 2-methyl-5-hydroxy-5-(1-hydroxyethyl)-4-oxotetrahydrofuroate (X), and 5-phenyl-2-ethoxy-2-(1-hydroxyethyl)-3-oxotetrahydrofuran (XII), were synthesized and tested for their pathogenic activities.

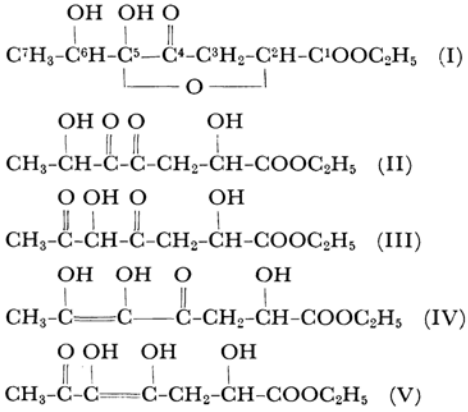

Fig. 1.

Results and Discussion

As VIII lacks the hydroxyl group at C-2, it cannot cyclize to the furanose ring structure as

1) N. Sugiyama, C. Kashima, M. Yamamoto and R. Mohri, This Bulletin, **38**, 2028 (1965).
2) N. Sugiyama, C. Kashima, M. Yamamoto, T. Sugaya and R. Mohri, *ibid.*, **39**, 1573 (1966).
3) N. Sugiyama, C. Kashima, Y. Hosoi, T. Ikeda and R. Mohri, *ibid.*, **39**, 2470 (1966).

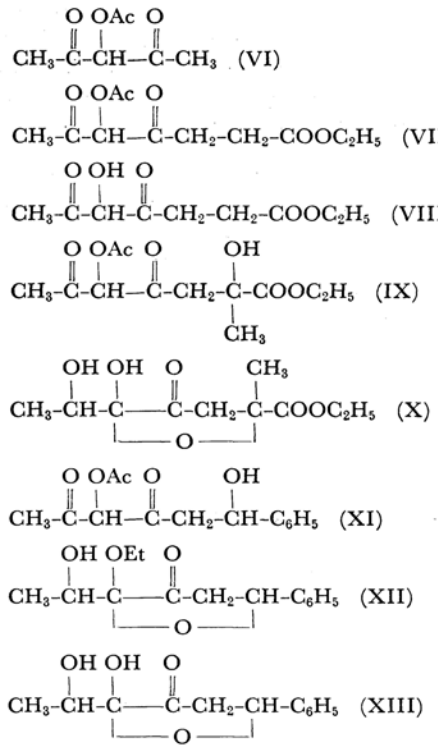

Fig. 2.

in I. VIII was synthesized as follows. 3-Acetoxy-2, 4-dioxopentane (VI)[4] was condensed with ethyl bromoacetate by sodium amide in liquid ammonia.[5] The condensation product showed in its infrared absorption spectrum the absorption bands at 1750 ($\nu_{C=O}$), 1720 ($\nu_{C=O}$) and 1240 cm^{-1} (δ_{C-O}). It showed the characteristic red color of β-dicarbonyl compounds with ferric chloride, and a negative Beilstein test. The R_f value on silica gel thin-layer chromatography was 0.60 while that of VI was 0.71. From these data and the elemental analysis, the structure of this product was confirmed as ethyl 5-acetoxy-4, 6-dioxoheptanoate (VII). After removal of the protecting acetyl group from VII by hydrochloric acid in ethanol,[6] the product showed infrared absorption bands at 3400 (ν_{O-H}), 1060 (δ_{C-O}), 1725 ($\nu_{C=O}$) and 1230 cm^{-1} (δ_{C-O}), but did not show the characteristic absorption bands of carboxyl group. It reduced Tillman's reagent. Together with the results of elemental analysis, structure VIII is assigned to this hydrolysis product.

Ethyl 2-methyl-5-hydroxy-5-(1-hydroxyethyl)-4-oxotetrahydrofuroate (X) was synthesized by condensation of VI with ethyl pyruvate followed by hydrolysis with hydrochloric acid. The condensation was carried out in liquid ammonia with potassium amide.[7] In the infrared absorption spectrum, the condensation product showed bands at 1720 ($\nu_{C=O}$) and 1220 cm^{-1} (δ_{C-O}), and the hydrolyzed product showed bands at 3400 (ν_{OH}), 1720 ($\nu_{C=O}$), 1230 (δ_{C-O}) and 1090 cm^{-1} (δ_{C-O}). The latter product was capable of reducing Tillman's reagent.

5-Phenyl-2-ethoxy-2-(1-hydroxyethyl)-3-oxotetrahydrofuran (XII) was synthesized by condensation of VI with benzaldehyde to IX and subsequent ethanolysis. The condensation product showed infrared absorption bands due to the carbonyl group at 1715 and 1260 cm^{-1} and those due to the aromatic ring at 1500, 760 and 700 cm^{-1}. Its color reaction with ferric chloride was red. These data supports XI as the structure of the condensation product. Treatment of XI with hydrochloric acid in ethanol resulted in ethanolysis and furanose ring formation to afford XII. The infrared absorption spectrum of XII showed hydroxyl group absorption bands at 3400 and 1040 cm^{-1}, carbonyl group absorption bands at 1730 and 1260 cm^{-1}, and aromatic ring absorption bands at 1490, 760 and 700 cm^{-1}. In the ultraviolet absorption spectrum, absorption maxima found at 253, 258, 264 and 293 mμ indicated the aromatic ring. From the fact that XII shows a positive Tillman's test, it appears that XII is readily hydrolyzed to a reductone (XIII).

TABLE 1. THE PHYTOPATHOGENIC ACTIVITIES OF COMPOUNDS, I, VIII AND X

Compound	Concentration mol/l	Activity mm^2
I	10^{-2}	10.1
VIII	10^{-2}	5.7
X	10^{-2}	12.6

The phytopathogenic activities of compounds, VIII, X and XII were examined and the results are listed in Table 1. From the fact that XII exhibits pathogenic activity, obviously the ethoxycarbonyl group of altenin has no influence. The phytopathogenic activity of X is nearly equal to that of altenin, but VIII is weaker. These facts suggest that the hydroxyl group at C-2 in altenin (I) has some effect, but the α-hydrogen at C-2 shows little or no effect on the phytopathogenic activity. From these facts, it is presumed that the most effective grouping in the altenin molecule is the reductone or acyloin grouping.

The details of the phytopathogenic activities of reductones and acyloins on pear leaves will be described in the following paper.

4) A. Combes, *Compt. rend.*, **111**, 421 (1890).
5) K. G. Hampton, T. M. Harris and C. R. Hauser, *J. Org. Chem.*, **30**, 61 (1965).
6) N. Sugiyama, C. Kashima, M. Yamamoto and R. Mohri, This Bulletin, **40**, 345 (1967).
7) R. L. Light and C. R. Hauser, *J. Org. Chem.*, **26**, 1716 (1961).

Experimental

Ethyl 5-Acetoxy-4, 6-dioxoheptanoate (VII). To 350 m*l* of liquid ammonia in 500 m*l* three necked flask, 1.1 g of sodium and catalytic amount of ferric nitrate were added and the content of the flask was stirred for one hour at −78°C. Blue color of solution was changed to dark gray, and sodium amide was produced inliquid ammonia. To this solution of sodium amide was added 4.0 g of VI dissolved in 6 m*l* of anhydroul ether. After one hour stirring at −33°C, the color of the mixture became brown. To the solution was added 3.5 g of ethyl bromoacetate of 7 m*l* of ether solution with stirring, and the stirring was continued for 3 hr at −33°C. After removal of the ammonia by standing at the room temperature, the residue was cooled and 20 m*l* of hydrochloric acid and 50 m*l* of ether were added carefully. The aqueous layer was extracted with three 30 m*l* portions of ether, and combined ether solution was dried over anhydrous sodium sulfate. The ether was removed, and the residue was passed through silica gel (merck 7729) column with benzene-acetone (9 : 1 v/v) mixture. On evaporation of the solvent from the eluted solution, which showed the positive ferric chloride test, there obtained a yellow liquid (1 g). Its R_f value on silica gel (Wakogel B-5) thin-layer chromatography with benzene-acetone (1 : 1 v/v) was 0.60 while that of VI was 0.71.

Found: C, 53.33; H, 6.59%. Calcd for $C_{11}H_{16}O_6$: C, 54.09; H, 6.60%.

IR (liquid film): 1750, 1720, 1240 cm^{-1}.

Ethyl 5-Hydroxy - 4, 6 - dioxoheptanoate (VIII). Ten milliliters of crude VII was dissolve in 20 m*l* of ethanol. To this solution was added 1 m*l* of hydrochloric acid. The mixture was stirred for 8 hr at 50°C in nitrogen atmosphere. After removal of the ethanol under reduced pressure, the residue was chromatographed through silica gel (Merck 7729) column with benzene-acetone (4 : 1 v/v) mixture. On evaporation of the solvent from the eluted solution, which reduced the Tillman's reagent, the pale yellow liquid (500 mg) was obtained.

Found: C, 55.38; H, 7.03%. Calcd for $C_9H_{14}O_5$: C, 53.46; H, 6.98%.

IR (liquid film): 3400, 1060, 1725, 1230 cm^{-1}.

Ethyl 2-Methyl-5-hydroxy-5-(1-hydroxyethyl)-4-oxotetrahydrofuroate (X). By the similar procedure as described in the synthesis of VII, the potassium amide was obtained from 4.0 g of potassium and 150 m*l* of liquid ammonia. To the potassium amide solution was added 8 g of VI in 16 m*l* of ether. After stirring for one hour at −78°C, 6 g of ethyl pyruvate in 12 m*l* ether solution was added. The mixture was stirred for 4 hr at −78°C and then 5 g of ammonium chloride was added. After removal of ammonia, the residue was dissolved in water. The cooled aqueous solution was washed with ether and acidified with hydrochloric acid. The acidified solution was extracted with ether. The ether extract was dried over anhydrous sodium sulfate, and the solvent was removed. The residue, which contained IX, was dissolved in 20 m*l* of ethanol, and 0.5 m*l* of hydrochloric acid was added. The mixture was stirred for 11 hr at 45°C in nitrogen atmosphere. Removal of ethanol gave a residue, which was passed through silica gel (Merck 7729) column with benzene-acetone (4 : 1 v/v) mixture. On evaporation of the solvent from the eluted solution, which reduced the Tillman's reagent, the yellow liquid (200 mg) was obtained. Its R_f value on silica gel (Wakogel B-5) thin-layer chromatography with benzene acetone (1 : 1 v/v) mixture was 0.56, while that of methyl red, a pilot dye, was 0.15.

Found: C, 52.55; H, 6.74%. Calcd for $C_{10}H_{16}O_6$: C, 51.72; H, 6.94%.

IR (liquid film): 3400, 1720, 1230, 1090 cm^{-1}.

5-Phenyl-2-ethoxy - 2 - (1 - hydroxyethyl) - 3 - oxotetrahydrofuran (XII). To 200 m*l* of liquid ammonia solution of 5.4 g of potassium amide was added 7.5 g of VI in anhydrous ether. After stirring for one hour, 5 g of benzaldehyde was added and the stirring was continued for another 2 hr. The reaction mixture was treated with 5.4 g of ammonium chloride and let to stand at room temperature for removing the liquid ammonia. The residue was dissolved in 100 m*l* of water and was acidified with hydrochloric acid. The acidic solution was extracted with ether. The ether extract was dried over anhydrous sodium sulfate. The ether was removed and the residual oil was dissolved in 25 m*l* of ethanol containing 0.5 m*l* of hydrochloric acid. The mixture was stirred for 13 hr at 45°C. After removal of the volatile substance, the residue was passed through silica gel (Merck 7729) column with benzene-acetone (1 : 1 v/v) mixture. The eluted solution, which reduced the Tillman's reagent and manganese, dioxide was collected. On evaporation of the solvent from this solution, a yellow liquid (500 mg) was afforded. Its R_f value on silica gel (Wakogel B-5) thin-layer chromatography with benzene-acetone (2 : 1 v/v) mixture was 0.64.

Found: C, 76.87; H, 7.21%. Calcd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25%.

IR (liquid film): 3400, 1730, 1490, 1260, 1040, 760, 700 cm^{-1}.

NMR (in $CDCl_3$): 2.69 (5 H, singlet), 4.91 (1 H, triplet), 5.86 (2 H, quartet), 7.33 (3 H, doublet), 8.78 τ (3 H, triplet).

The Pathogenic Activities of VIII, X, XII and Altenin. The black spot test of the aqueous solution of VIII, X, XII and altenin were examined. The pH of the test solutions were adjusted 7.8 with ammonium acetate buffer. By the test on a same leaf, the pathogenic activities of VIII and X were compared with that of altenin in concentration of 10^{-2} mol/*l*. The results are listed in Table 1.

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