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# Altenin. II. The Structure of Altenin

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Altenin, a metabolite of *Alternaria Kikuchiana* Tanaka, produces the black spot disease on the leaves and fruits of the pear. The optimum conditions for a 200-l. tank culture of this fungus will be described. Altenin has been tentatively deduced as being an ethyl ester of a $C_7$-carboxylic acid. Of the four expected tautomeric structures, the probability of a furanose ring structure will be demonstrated. There is only one asymmetric center in altenin, at the position $\alpha$ to the ethoxycarbonyl group; this belongs to the L-lactic acid configuration.

It has been demonstrated that one of the metabolites of the fungus *Alternaria Kikuchiana* Tanaka produces the black spot disease on pear leaves and fruit.[1] This substance has been isolated from the culture filtrate and designated "Altenin."[2,3] Altenin is a faint yellow liquid. The spectral and chemical data of altenin allows one to tentatively deduce it as an ethyl $\alpha$-hydroxy carboxylate with a methyl reductone residue.

## Results and Discussion

Altenin gives only one spot on a silica-gel thin-layer chromatogram with a benzene-acetone (1 : 1 v/v) mixture; its $R_f$ value is 0.76, while that of methyl red, a pilot dye, is 0.37.

The molecular peak in the mass spectrum of altenin appears at *m/e* 218; altenin does not contain sulfur and halogen, as can be seen from the fact that the isotope peak, *m/e* 220 (M+2), is very weak compared with the molecular peak. From the molecular weight, 218, and the results of elementary analysis, the molecular formula of altenin may be determined to $C_9H_{14}O_6$. It is apparent that altenin does not contain an aromatic ring.

In an infrared absorption spectrum, an OH absorption at 3400 $cm^{-1}$ and a C=O absorption at

1) I. Hiroe, S. Nishimura and W. Sato, *Transaction of the Tottori Society of Agriculture Science (Tottori Nogeikagaku Kaishi)*, **11**, 291 (1958).

2) N. Sugiyama, C. Kashima, M. Yamamoto and R. Mohri, This Bulletin, **38**, 2028 (1965).

3) N. Sugiyama, C. Kashima, M. Yamamoto, T. Sugaya and R. Mohri, ibid., **39**, 1573 (1966).

1730 $cm^{-1}$ are observed. However there is no absorption band in the region between 1700 and 1600 $cm^{-1}$. This suggests that altenin has neither carboxyl group nor an olefinic double bond.

The nuclear magnetic resonance spectrum of altenin is given in Table I. The quartet at 4.83 $\tau$ is assigned to a methine proton, which is deshielded by an electron withdrawing group and which is coupled to a methyl group at 8.48 $\tau$. The quartet at 5.80 $\tau$ is assigned to an ester methylene proton, which is coupled with a methyl group at 8.71 $\tau$. Therefore, the presence of an ethoxycarbonyl group in altenin may be concluded. Similar to the methine proton of ethyl $\alpha$-hydroxy levulinate[4] at 5.90 $\tau$, the methine proton of altenin at 5.83 $\tau$ is deshielded by the oxygen atom. The doublet of 8.54 $\tau$ is coupled with the 5.83 $\tau$ methine proton. The broad band between 6.9 and 8.0 $\tau$ disappears upon deuterium exchange; it shows the presence of two hydroxyl protons. The nuclear magnetic resonance spectrum lacks signals due to a methyl group attached to a quaternary carbon or acetyl group.

The ultraviolet absorption spectrum shows an absorption maximum at 275 m$\mu$ ($\varepsilon$ 228) in ethanol; it also shows a small shift to a shorter wavelength in acidic ethanol. The optical rotatory dispersion spectrum of altenin is shown in Fig. 3, the specific rotation at 589 m$\mu$ being $-5.1°$.

Altenin behaves as a reducing agent in reaction with Tillman's reagent,[5] manganese dioxide[6]

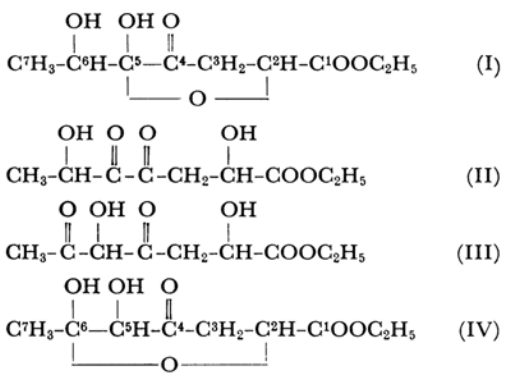


Fig. 1. Structure of altenin.

and Tollens' reagent. The red violet color of Tillman's reagent in acetic acid is decolorized; manganese dioxide is reduced, and silver nitrate is reduced to silver metal in an alkaline solution. These color reactions are characteristic of reductones, acyloins, and ascorbic acid. These properties suggest structures I to IV (Fig. 1) as conceivable for altenin.

4) A. Rossi and A. Lauchenauer, *Helv. Chim. Acta*, **30**, 1501 (1947).
5) F. Cramer and W. Krum, *Chem. Ber.*, **86**, 1586 (1953).
6) F. Feigl and H. T. Cardoso, *Rev. Brasil. Biol.*, **2**, 117 (1942).

The mass specturm of altenin is shown in Fig. 2. The base peak, *m/e* 45, might represents $CH_3$–CH–OH and $C_2H_5$–O groups. The peaks at *m/e* 45, 73 and 145 (M-73) suggest the presence of an ethoxycarbonyl group. The peaks at *m/e* 102 and 115 are obtained from the cleavage of the molecule into two parts; that at 102 might also be due to an O–CH–$COOC_2H_5$ fragment. The peak at *m/e* 85 can be accounted for by CH–$COOC_2H_5$ minus one proton, and that at 89 can be accounted for by $CH_3$–CH(–OH)–C(–O)$_2$ or $CH_3$–C(–O)$_2$–CH–OH; these high intensive peaks can not be expected from structures II and III. The structures I and IV, in which the lactol ring is present, thus seem to be more reasonable than structures II and III.

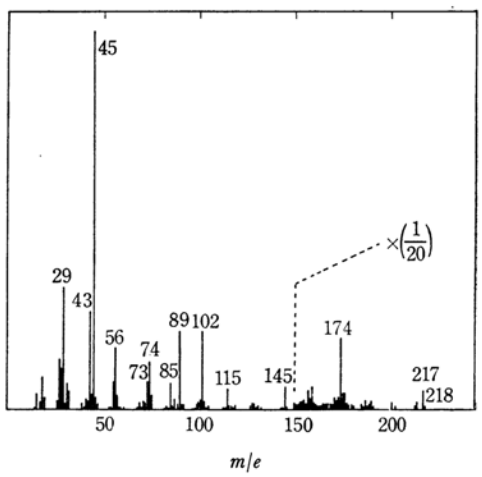


Fig. 2. Mass spectrum of altenin.

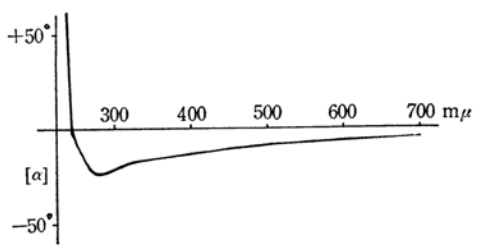


Fig. 3. ORD spectrum of altenin.

The hydroxyl group at C-6 is indicated by the splitting of the methine signal at 4.83 $\tau$ to a quartet. However a hydroxyl group of reductones can be rearranged through the endiol tautomer. The presence of an $\alpha$-hydroxy carbonyl (–CO–CH–OH) group in strucutres II and III, its absence in the structure I, and the absence of the methine proton at C-6 in IV suggest the preference of I to II, III and IV.

Ethyl $\alpha$-hydroxy levulinate has a structure similar to II and III, and it cannot cyclize to lactol linkage. However, the doublet of methylene

protons of altenin at 8.54 $\tau$ is more shielded than the $\beta$-methylene protons of ethyl $\alpha$-hydroxy levulinate at 6.97 $\tau$. In general, the $\alpha$-methylene protons of aliphatic ketones and cyclohexanones are deshielded but the deshielding effect of the carbonyl group in cyclopentanone is weak[7] and the $\beta$-methylene protons of tetrahydrofurane is not influenced by the oxygen atom.[7] Therefore, the methylene protons of C-3 of I may be expected to be at a high field.

The peak intensity of altenin at 7.98 $\tau$ is about one half that of a proton; it is probably to be assigned to the methyl group at C-7 of III and IV. The signal of acetyl protons of diacetyl carbinol appears as a singlet at 7.80 $\tau$. Since the peak ratio at 8.48 and 7.98 $\tau$ (2.5 : 0.5) is nearly proportional to the ratio of tautomers (I and II): (II and IV), the tautomers I and II may be thought to comprise 85 per cent, while III and IV comprise 15 per cent.

These four tautomeric structures are also found in osones. Petuely[8] reported that D-glucosone preferred the lactol ring form to the open-chain form. Bayne[9] obtained the triisopropylidene glucosone, and demonstrated the presence of a furanose ring. Therefore, it seems most probable for altenin to have the structure I, which has a furanose ring.

Since the carbon at C-6 is enolizable to its endiol tautomer, the only asymmetric center in altenin is the carbon C-2. The specific rotation of altenin is similar to that of $\alpha$-hydroxy heptanoic acid $[\alpha]_D$ $-4.4°$, which has L-lactic acid configuration.

The substance of structure I or its tautomers was synthesized by the condensation of 3-acetoxy acetylacetone and ethyl glyoxylate with potassium amide; it was thus demonstrated that it showed the same phytopathogenic activity and $R_f$ value as altenin. The details of this will be reported in the near future.

## Experimental

**The Cultivation of the Fungus Alternaria Kikuchiana Tanaka.**—The culture broth (3 l.) of *Alternaria Kikuchiana* Tanaka (No. 5), which was cultured on an ager (0.1%) slant, was transferred into a 200 l. fermenter tank charged with 120 l. of a medium containing sucrose (4.0%), monobasic potassium phosphate (1.1%), dibasic ammonium phosphate (0.2%), potassium chloride (0.05%) and magnesium sulfate (0.05%); it was then cultivated at 30°C for 8 days under aeration (20 l./min.) with stirring. On the second or third day, the fermenter was filled with foam. An antifoam agent (silicon oil) was added on the third day. The activity test with pear leaves showed the highest value on the ninth day; this cultivation was 1.22 times as active as that of the stationary cultivation using the Czapek-Dox medium.

**Material.**—From the culture filtrate of this fungus, altenin was isolated by a previously-reported method.[3] Altenin is a yellow liquid, which is unstable above 60°C. The $R_f$ value of altenin on silica-gel thin-layer chromatography with a benzene-acetone (1 : 1 v/v) mixture was 0.76, while that of methyl red, a pilot dye, was 0.37. The $R_f$ value on paper (Toyo paper No. 53) chromatography with water saturated with benzene was 0.87.

Found*1: C, 50.95; H, 7.56. Calcd. for $C_9H_{14}O_6$: C, 49.54; H, 6.47.

**Mass Spectrum.***2—The mass spectrum of altenin was measured by a Hitachi RMU-6D mass spectrometer; it vaporized at 80°C, and ionized at 70 V., and its ions accelarated at 1800 V. The molecular peak was observed at *m/e* 218, and the base peak, at *m/e* 45.

**Infrared and Ultraviolet Absorption Spectra.**—In the infrared absorption spectrum (liquid film), absorptions were observed at 3440, 2980, 1740, 1450, 1380, 1270, 1200, 1130, 1100, 1050, 1020, 940, 860 and 760 $cm^{-1}$. The ultraviolet absorption spectrum showed an absorption maximum at 276 m$\mu$ ($\varepsilon$ 228) in ethanol and at 273 and 370 m$\mu$ in acidic ethanol.

**Nuclear Maguetic Resonance Spectrum.***3—An Hitachi H-60 high resolution nuclear magnetic resonance spectrometer was used; the solvent was a mixture of carbon tetrachloride and deuterochloroform for altenin, and only deuterochloroform for ethyl $\alpha$-hydroxy levulinate. For the deuterium exchange of the hydroxyl proton, deuterium oxide was added to the altenin solution. TMS was used as the inner

TABLE I. NUCLEAR MEGNETIC RESONANCE SPECTRUM OF ALTENIN

| $\tau$, p. p. m. | Intensity | Coupling | $J$, c. p. s. |
|---|---|---|---|
| 4.83 | 1H | quartet | 7.0 |
| 5.80 | 2H | quartet | 7.0 |
| 5.83 | 1H | triplet | |
| 6.9—8.0 | 2H | multiplet | |
| 7.98 | 0.5H | singlet | |
| 8.46 | 2.5H | doublet | 7.0 |
| 8.54 | 2H | doublet | 7.0 |
| 8.71 | 3H | triplet | 7.0 |

TABLE II. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF ETHYL $\alpha$-HYDROXY LEVULINATE

| $\tau$, p. p. m. | Intensity | Coupling | $J$, c. p. s. |
|---|---|---|---|
| 5.78 | 2H | quartet | 7.0 |
| 5.90 | 1H | triplet | 8.0 |
| 6.97 | 2H | doublet | 8.0 |
| 7.80 | 3H | singlet | |
| 8.72 | 3H | triplet | 7.0 |

7) H. Primas, K. Frei and Hs. H. Gunthard, *Helv. Chim. Acta*, **41**, 35 (1958).
8) F. Petuely, *Montash. Chem.*, **83**, 765 (1952).
9) S. Bayne, G. A. Collie and J. A. Fewster, *J. Chem. Soc.*, **1952**, 2766.

*1 Elementary analyses were made by Mr. Kyusaburo Furuhashi of Department of Chemistry, Tokyo Kyoiku University.
*2 The mass spectra were measured by courtesy of Dr. Hiroshi Satoh of Hitachi Co. Ltd.
*3 The NMR spectra were measured by courtesy of Dr. Kisaburo Yamazaki and Dr. Takamichi Ichimura of Yamanouchi Pharmaceutical Co. Ltd.

standard. The $\tau$ value, intensity, coupling type and coupling constant are listed in Tables I and II.

**Optical Rotatory Dispersion Spectrum.**[*4]—The optical rotatory dispersion spectrum of altenin, which was recorded on a ORD/UV-5 spectrometer made by the Japan Spectroscopic Co., Ltd., in methanol at 28°C, is shown in Fig. 3. Altenin showed a levo specific rotation at 589 m$\mu$ and an inversion from levo to dextro specific rotation at 245 m$\mu$.

**The Color Reactions.**—*With Tillman's Reagent.*—The silica-gel thin layer chromatogram of altenin was obtained by spraying it with Tillman's reagent dissolved in acetic acid. After a few minutes' warming, the color of the thin layer became pink, while the altenin spot remained colorless.

*With Manganese Dioxide.*—Manganese dioxide was prepared on the filter paper using aqueous potassium permanganate. A drop of an altenin solution was put on a test paper, and after a few minutes the paper was immersed in an aqueous benzidine hydrochloride solution. The paper color became blue, while the altenin spot remained colorless. In this test, ascorbic acid exhibits a strongly positive, while acetoin exhibits only a weak positive, reaction.

*With Silver Nitrate.*—The paper strip on which altenin had been developed by water saturated with benzene was immersed in a silver nitrate acetone solution, then in an alcoholic sodium hydroxide solution and finally in an aqueous sodium thiosulfate solution. The spot of altenin was blackened by the reduction of silver ions to metal.

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[*4] ORD spectra were recorded by courtesy of Dr. Takuzo Nishimura of Sankyo Co., Ltd.