UDC 615.771.7:582.542.1

10. **Akio Tanimura**[*1] : Studies on Anti-tumor Component in the Seeds of *Coix Lachryma-Jobi* L. VAR. *Ma-yuen* (ROMAN.) STAPF. II.[*2] The Structure of Coixenolide.

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In the course of a research on anti-tumor activity of the component of pearl barley (seeds of *Coix Lachryma-Jobi* L. VAR. *Ma-yuen* (ROMAN.) STAPF) against Ehrlich ascites sarcoma in mice, an oily compound was isolated and purified which showed a considerable potency in this test and was named coixenolide.[*2] The present paper deals with the chemical structure of coixenolide.

Analysis of the pure coixenolide (I), which revealed a single peak in the repeated column chromatography according to the method of Fillerup[1)] using varied series of mixed solvents of petroleum ether and ether, showed it corresponded to the chemical formula of $C_{38}H_{70}O_4$.

This compound (I) was negative to the sodium nitroprusside reaction and did not react with *p*-nitrobenzyl chloride. (I) was easily hydrolyzed by aqueous alkaline solution to give fatty acids and an alcoholic compound, which showed a glyceride- or wax-like property.

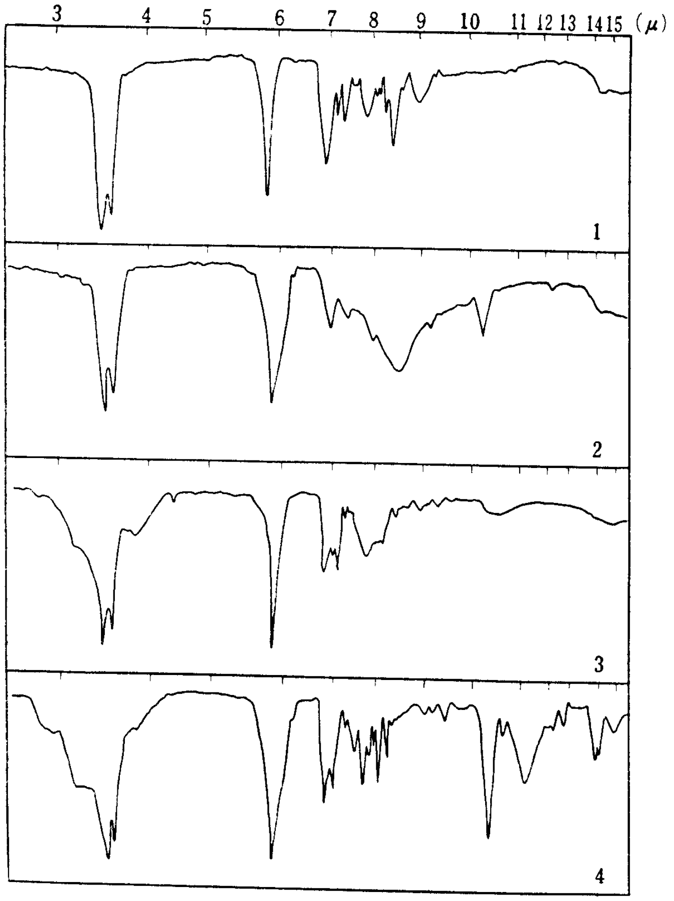

Fig. 1. Infrared Spectra

1. Tetrahydro-coixenolide (KBr)
2. Coixenolide (liq. cap.)
3. *cis*-9-Hexadecenoic acid (liq. cap.)
4. *trans*-11-Octadecenoic acid (KBr)

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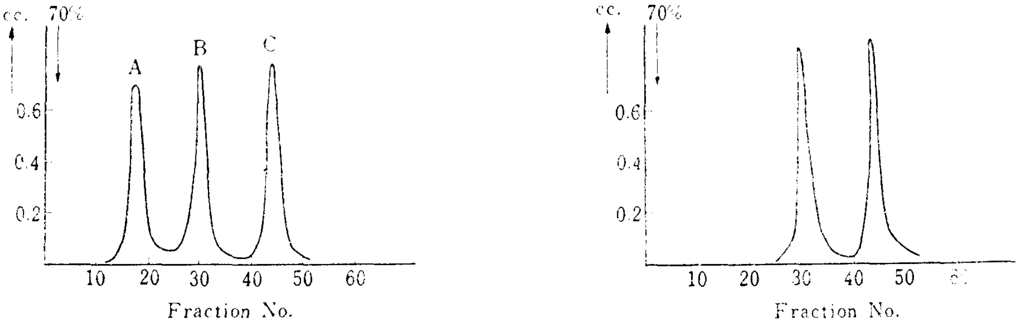
[*2] Part I : This Bulletin, **9**, 43 (1961).

1) N. L. Fillerup, J. F. Mead : Proc. Soc. Exptl. Biol. Med., **83**, 574 (1953).

On catalytic hydrogenation of (I) with palladium-charcoal,[2] colorless crystals (II), m.p. 60°, were obtained. Analysis and molecular weight determination of (II) showed that it is represented by the molecular formula of $C_{38}H_{74}O_4$. The amount of hydrogen consumed to furnish (II) was two moles calculated for one mole of the original compound. Infrared spectrum of the tetrahydro derivative (II) of (I) thus obtained (Fig. 1-1) showed the presence of ester and $-(CH_2)_n-$ group, and lacked the absorption for free hydroxyl group.

(II) was hydrolyzed with alkali and the acidic hydrolysate was isolated as crystals melting at 59~61°, which showed no definite melting point even after repeated recrystallization, and its *p*-bromophenacyl derivative also did not show any definite melting point, melting at about 82~84°. The saturated acid thus obtained was further treated on a reversed-phase column chromatography reported by Silk and Hahn[3,4] using Hyflo Super Cel treated with dimethyldichlorosilane and liquid paraffin as stationary phase and hydrous acetone as mobile phase (Fig. 2). The result of this chromatography revealed that this fatty acid fraction is an equimolar mixture of two fatty acids which were identified with palmitic and stearic acids.



(1) Separation of authentic specimens of myristic, palmitic, and stearic acids eluted with 70% acetone solution
A : Myristic acid C : Stearic acid
B : Palmitic acid

(2) Separation of acidic hydrolysate of tetrahydro-coixenolide eluted with 70% acetone solution

Fig. 2. Reversed-phase Column Chromatography

The alcoholic part obtained by alkaline hydrolysate of (II) gave negative acrolein test for glycerol and was oxidized with periodate to produce an aldehyde which was negative to formaldehyde detection by chromotropic acid.[5]

From the above-mentioned results, (II) was assumed to be an ester consisting of one mole each of palmitic and stearic acids, and a glycol having the molecular formula of $C_4H_{10}O_2$.

The infrared spectrum of (I) (Fig. 1-2) showed, besides the ester and $-(CH_2)_n-$ group, the presence of a *trans*-double bond (absorption at 10.36 μ), which was absent in that of (II), and again lacked the absorption of a hydroxyl. The mixture of fatty acids obtained by alkaline hydrolysis of (I) immediately decolorized bromine water and permanganate solution, indicating the presence of a double bond, while the alcoholic part did not decolorize either of these reagents.

The unsaturated fatty acid thus obtained was also proved by reversed phase chromatography to be a mixture of two fatty acids and these acids were purified by vacuum distillation to an acid (V) of $b.p_{0.1}$ 102°, and an acid (VI) of $b.p_{0.1}$ 125°.

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2) L. Crombie, J. L. Taylor : J. Chem. Soc., **1954**, 2816.
3) M. H. Silk, H. H. Hahn : Biochem. J., **56**, 574 (1954).
4) J. B. Wittenberg : *Ibid.*, **65**, 42 (1957).
5) E. Eegriwe : Z. anal. Chem., **110**, 22 (1937).

(V) came as a colorless viscous liquid, which, on chilling with dry-ice and addition of acetone-ethanol (1:2), solidified into a white amorphous mass melting at 0° to 1°, and analysed to have a molecular formula of $C_{16}H_{30}O_2$. From its infrared spectrum, which showed no absorption at 10.36 μ (Fig. 1-3), and melting point of its *p*-bromophenacyl derivative, m.p. 40°, this acid was assumed to be a *cis*-hexadecenoic acid.[6]

(VI) solidified to a faint yellow mass on addition of cold acetone and was recrystallized from the same solvent to white leaflets, melting at 42.5°, which analyzed to give the formula of $C_{18}H_{34}O_2$. The infrared spectrum of this acid, which indicated the presence of a *trans*-double bond[7,8] (Fig. 1-4), and melting point of its *p*-bromophenacyl ester,[9] m.p. 34°, were in good agreement with those of *trans*-octadecenoic acid.

In order to confirm the position of the double bond in these two unsaturated fatty acids, they were further oxidized with permanganate solution and peracetic acid.

On oxidation of (V) with hot permanganate solution, heptanoic acid was obtained as a monocarboxylic acid and nonanedioic acid as the dicarboxylic acid. By cold permanganate oxidation, (V) gave a dihydroxypalmitic acid (X), m.p. 124°, which was further oxidized with periodate to give heptanal and the aldehydo-acid converted to nonanedioic acid, m.p. 106°, by subsequent permanganate oxidation. In the case of peracetic acid oxidation of (V), the products were the same as by cold permanganate oxidation. These final degradation products were identified with the corresponding authentic specimens.

(VI) was also oxidized with hot permanganate to give heptanoic acid as a monocarboxylic acid and a dicarboxylic acid as white leaflets (XII), m.p. 110°.

The melting points of both (VIII) and that of its *p*-bromophenacyl derivative, m.p. 134°, were coincident with those of undecanedioic acid, and the melting points were not depressed when mixed with an authentic sample of undecanedioic acid synthesized from diethyl azelate.[10]

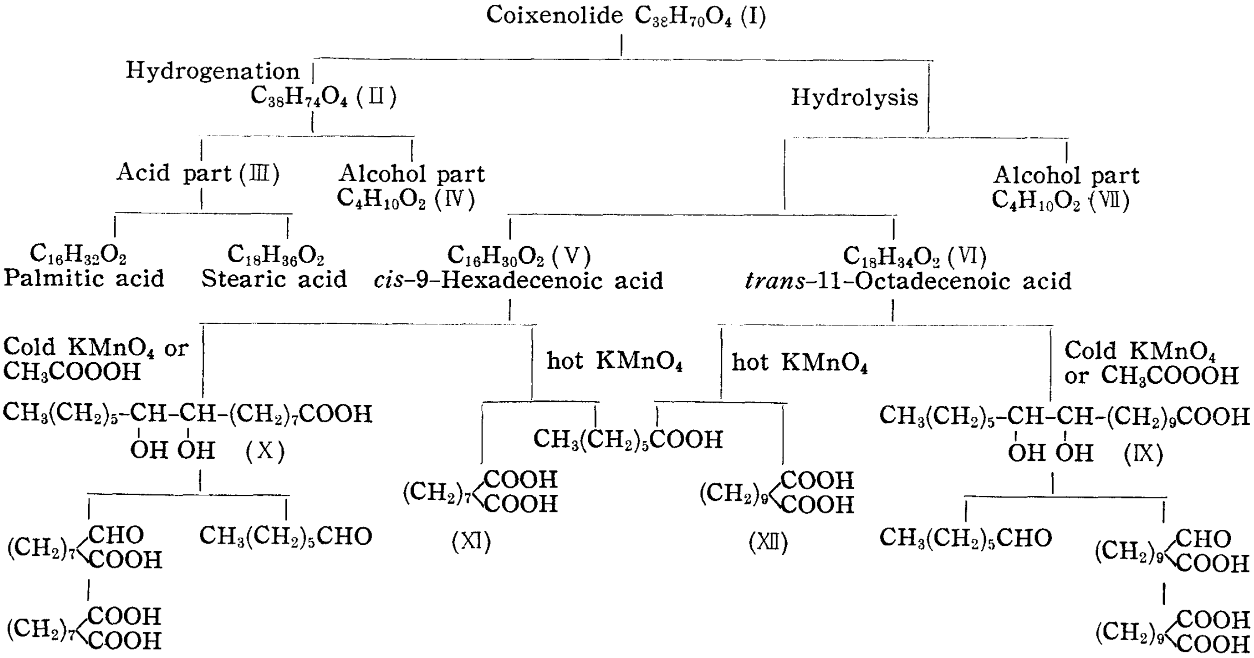

Chart 1. Oxidation Processes of Coixenolide

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6) B.W. Broughton, R.E. Bowman: J. Chem. Soc., **1952**, 671.
7) L. Crombie, B.P. Griffin: *Ibid.*, **1958**, 4436.
8) P.C. Rao, B.F. Daubert: J. Am. Chem. Soc., **70**, 1102 (1948).
9) F.M. Bumpus, W.R. Tayler: *Ibid.*, **72**, 2116 (1950).
10) N. Kananiwa: Ann. Report of Kanazawa Univ., **2**, 30 (1952).

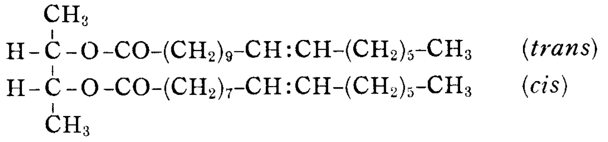
Cold permanganate oxidation of (VI) gave a dihydroxystearic acid (IX), m.p. 126°, and its further oxidation with periodate produced heptanal and ω-aldehydodecanoic acid which was converted to undecanedioic acid on treatment with permanganate. Products of peracetic acid oxidation of (VI) were the same as those from cold permanganate oxidation. The final oxidation products thus obtained were identified with authenic specimens and the route of this oxidation is summarized in Chart 1.

From these results, the structure of the unsaturated fatty acids, obtained from (I) should be *cis*-9-hexadecenoic acid for (V) and *trans*-11-octadecenoic acid for (VI).

Alcoholic substance (VII) obtained by hydrolysis of (I) showed no unsaturated property and was purified by vacuum distillation to an oil of $b.p_{0.5}$ 73°, which was analyzed to have a molecular formula of $C_4H_{10}O_2$. This compound was oxidized with sodium periodate and gave acetaldehyde as the only product, which was identified as its phenylhydrazone of m.p. 63°. (VII) was converted to its *p*-nitrobenzoate and 3,5-dinitrobenzoate which respectively melted at 193° and 247°, and were identified with the corresponding derivatives of authentic 2,3-butanediol.

The alcoholic fraction (IV) obtained from the hydrolysate of (II) was purified by distillation as above and the product was converted to similar derivatives which also showed no depression of the melting point on mixed fusion with those of authentic 2,3-butanediol.

In conclusion of the work described above, the neutral compound, coixenolide (I), isolated from the seeds of *Coix Lachryma-Jobi* L. VAR. *Ma-yuen* (ROMAN.) STAPF is a glycolide represented by the structure of 1-methyl-2-(*cis*-9-hexadecenoyloxy)propyl *trans*-11-octadecenoate.[*3]



$$\begin{array}{ll} \quad CH_3 & \\ H\text{-}\overset{|}{C}\text{-}O\text{-}CO\text{-}(CH_2)_9\text{-}CH{:}CH\text{-}(CH_2)_5\text{-}CH_3 & (\textit{trans}) \\ H\text{-}\overset{|}{\underset{|}{C}}\text{-}O\text{-}CO\text{-}(CH_2)_7\text{-}CH{:}CH\text{-}(CH_2)_5\text{-}CH_3 & (\textit{cis}) \\ \quad CH_3 & \end{array}$$

Experimental

Detection of Functional Groups in Coixenolide—a) Double Bond : $CHCl_3$ solution of (I) decolorized Br_2 while its Me_2CO solution decolorized 0.5% $KMnO_4$ solution immediately, indicating the presence of a double bond in the molecule.

b) Ferric Chloride Reaction : When a freshly prepared 5% $FeCl_3$ solution was added to several drops of the EtOH solution of (I), no special coloration was observed.

c) Detection of Carbonyl Groups : On addition of a drop of a freshly prepared 5% Na-nitroprusside and a few drops of 2*N* NaOH to a drop of EtOH solution of (I), no red or yellow coloration was observed.

Catalytic Reduction of (I)—A solution of 0.50 g. of (I) in 10 cc. of AcOEt was added dropwise into a suspension of 20 mg. of Pd-C and the mixture was shaken in H_2 atmosphere. After a lag of about a few min., the consumption of H_2 started and 39.5 cc. of H_2 (theoretical amount, 39.0 cc.) was absorbed in 4 hr., shaking at 760 mm. Hg. The catalyst was filtered off and washed with hot AcOEt. The filtrate and washings were combined and the solvent was evaporated under a reduced pressure. The white solid that separated was recrystallized from EtOH-AcOEt (5:1) to white leaflets, m.p. 60°; yield, 0.32 g. *Anal.* Calcd. for $C_{38}H_{74}O_4$: C, 76.70; H, 12.54; mol. wt., 594.97. Found : C, 76.65; H, 12.39; mol. wt. (Akiya-Barger's method), 590.

Hydrolysis of Reduced Product (II)—A solution of 500 mg. of white crystals, obtained by the catalytic reduction of (I), dissolved in a mixture of 5 cc. of MeOH and 1 cc. of 0.1*N* NaOH in MeOH, was refluxed for 3 hr. on a boiling water bath. After cooling and acidification with HCl, the reaction mixture was extracted three times with Et_2O. Et_2O extract was dried over Na_2SO_4, and the solvent was evaporated. The separated white crystals were recrystallized from 70% EtOH to colorless crystals (III), m.p. 59~61°. In spite of repeated recrystallization, the crystals showed no definite m.p. Its *p*-bromo-phenacyl derivative also did not show a definite m.p., melting at about 82~84°.

[*3] The possibility that coixenolide is an equimolecular mixture of two glycolides, 2,3-butanediol di-*cis*-9-hexadecenoate and 2,3-butanediol di-*trans*-11-octadecenoate, could not be excluded since these two compounds are not separable by the method used in this work.

The aqueous layer left after Et_2O extraction was concentrated under a reduced pressure and NaCl that separated was filtered off to give a pale yellow oil. After complete drying, the oil was extracted with anhyd. pyridine to separate the remaining NaCl. From the extract, pyridine was removed *in vacuo* and a pale yellow oil (IV) was obtained. Yield, 55 mg.

Reversed-phase Column Chromatography of Acidic Substance (III)—Further separation of the fatty acids (III), obtained on hydrolysis of (II), was performed by reversed-phase column chromatography.[11]

i) Preparation of Non-wetting Kieselguhr : Hyflo Super Cel was dried at 110° after separation from fine particles by decantation in water and was exposed to the vapor of dimethyldichlorosilane for 5 hr. in a slightly evacuated desiccator. The material was washed with anhyd. MeOH to remove free acid and dried at 110°. The mull obtained was completely nonwetting when shaken with H_2O.

ii) Preparation of Pure Paraffin : Liquid paraffin was dissolved in petr. ether and passed through a column of alumina. After removal of the solvent, pure paraffin thus obtained was used as the stationary phase in this chromatography.

iii) Preparation of Solvent for Elution : Me_2CO of 40, 65, 70, 75, and 83% aqueous solutions were used, and all the solvents were equilibrated with liquid paraffin before elution, and passed through a column (10×50 mm.) of above kieselguhr powder impregnated with liquid paraffin to remove the emulsion of paraffin in the solvent.

iv) Preparation of the Column : A mixture of 20 g. of the non-wetting kieselguhr impregnated with liquid paraffin and 200 cc. of boiled 83% Me_2CO placed in a 500-cc. flask was transferred cautiously into the column (0.8×850 mm.) by Komagome pipette so as not to introduce air bubbles. The column was eluted with 40 cc. of 40% Me_2CO before loading the acids.

v) Loading of Fatty Acids : The acid mixture was dissolved in a small amount of anhyd. Et_2O, added with appropriate amount of non-wetting kieselguhr impregnated with liquid paraffin, and Et_2O was completely evaporated. Diatomaceous earth, which adsorbed fatty acids, was mixed with 5 cc. of 40% Me_2CO, previously equilibrated with paraffin. The mixture was boiled to expel air and poured into the column through Komagome pipette, a small amount of kieselguhr being placed at the top of the column as a protecting band. The column was maintained at 35° by circulation of H_2O of that temperature through the jacket.

vi) Elution and Titration : For a control chromatography, a mixture of authentic myristic, palmitic, and stearic acids was used. In one run, 50 cc. of 65% Me_2CO, 25 cc. of 70% Me_2CO, and 200 cc. of 75% Me_2CO previously equilibrated with paraffin were successively used. In the second run only 70% Me_2CO similarly triturated was used. Eluate was collected in 60 drops each by a drop-counting fraction collector, at a flow rate of 50~60 cc./hr. For the complete separation of these acids, the second run was found to be the most suitable. Each tube containing the eluate was boiled before titration and titrated with 0.01*N* NaOH in MeOH, using a solution of Bromothymol Blue in 70% Me_2CO as an indicator.

In the case of chromatography for the acidic hydrolyzate (III), palmitic and stearic acids were detected, when both of above two solvent systems were applied. On quantitative estimation of these by alkali titration, (III) was found to be a mixture of equimolar amount of palmitic and stearic acids. These acids, fractionated by the use of 70% Me_2CO, were isolated and recrystallized from 70% EtOH. Crystals, obtained from fraction Nos. 27~32, showed m.p. 63°. *Anal.* Calcd. for $C_{16}H_{32}O_2$: C, 74.94; H, 12.58. Found : C, 74.88; H, 12.50.

p-Bromophenacyl Ester : m.p. 86°. *Anal.* Calcd. for $C_{24}H_{37}O_3Br$: C, 52.08; H, 6.69. Found : C, 51.87; H, 6.42.

No depression of m.p. was observed when this acid and its *p*-bromophenacyl ester were admixed with authentic palmitic acid and its *p*-bromophenacyl ester. Crystals (obtained from fraction Nos. 41~46), m.p. 70°. *Anal.* Calcd. for $C_{18}H_{36}O_2$: C, 75.99; H, 12.76. Found : C, 75.86; H, 12.54. *p*-Bromophenacyl ester, m.p. 90°. *Anal.* Calcd. for $C_{26}H_{41}O_3Br$: C, 64.85; H, 8.58. Found : C, 65.09; H, 8.33. No depression was observed when this acid and its *p*-bromophenacyl derivative were admixed with authentic stearic acid and its *p*-bromophenacyl ester.

Hydrolysis of (I) and Detection of Resulting Unsaturated Fatty Acids and Alcohol—i) Acid Fraction : A mixture of 100 mg. of (I) with 0.1*N* KOH in EtOH was refluxed for 3 hr. on a water bath. The reaction mixture was acidified with HCl and extracted several times with Et_2O. The Et_2O extracts were combined, dried over Na_2SO_4, and the solvent was evaporated to give an oil. Yield, 72 mg. As it was assumed that this oil is a mixture of two unsaturated fatty acids, this was further fractionated by reversed-phase column chromatography. Isolation of these componental fatty acids was effected by fractional distillation under a diminished pressure and furnished oily acids of $b.p_{0.1}$ 102° (V) and $b.p_{0.1}$ 125° (VI). *Anal.* Calcd. for $C_{16}H_{30}O_2$: C, 75.53; H, 11.89; COOH, 17.69. Found (V): C, 75.50; H, 11.74; COOH, 17.94.

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[11] T. Yamakawa : J. Biochem. (Tokyo), **43**, 63 (1956).

p-Bromophenacyl Ester : m.p. 39°. *Anal.* Calcd. for $C_{24}H_{35}O_3Br$: C, 63.85; H, 7.82. Found : C, 63.99; H, 7.57.

The other oily acid of b.p$_{0.1}$ 125°(VI) crystallized on addition of cold Me_2CO and showed m.p. 37°. This was repeatedly recrystallized from cold Me_2CO to show a final m.p. of 42.5°. *Anal.* Calcd. for $C_{18}H_{34}O_2$: C, 76.54; H, 12.13; COOH, 15.94. Found : C, 76.55; H, 11.97; COOH, 15.99.

p-Bromophenacyl Ester : m.p. 34°. *Anal.* Calcd. for $C_{26}H_{39}O_3Br$: C, 65.12; H, 8.20. Found : C, 65.01; H, 8.06. (VI) and its *p*-bromophenacyl ester showed no depressions on admixtures with *trans*-11-octadecenoic acid and its *p*-bromophenacyl ester.

ii) Alcohol Fraction : The aqueous layer, left after extraction of the acidic substances, was concentrated under a reduced pressure and separated NaCl was filtered off. The filtrate was completely dried and the residue was extracted with anhyd. pyridine. On removal of pyridine from the extract, a pale yellow oil was obtained. Yield, 14 mg. Purification was effected by distillation under a reduced pressure to obtain a colorless viscous liquid. *Anal.* Calcd. for $C_4H_{10}O_2$: C, 53.31; H, 11.19. Found : C, 53.21; H, 11.32.

Bis-*p*-nitrobenzoate : Needles (EtOH), m.p. 193°. *Anal.* Calcd. for $C_{18}H_{16}O_8N_2$: C, 55.67; H, 4.15; N, 7.21. Found : C, 55.91; H, 3.96; N, 7.52.

Bis-3,5-dinitrobenzoate : Recrystallized from EtOH, m.p. 247~248°. *Anal.* Calcd. for $C_{18}H_{14}O_{12}N_4$: C, 45.20; H, 2.95; N, 11.71. Found : C, 45.42; H, 2.77; N, 12.04.

On admixture of these two derivatives with the corresponding derivatives of 2,3-butanediol, no depression of the m.p. was observed.

Oxidation of (V) and (VI) with Hot Potassium Permanganate—i) Oxidation : A solution of 100 mg. of (V) or (VI) dissolved in 1 cc. of 3.75% KOH solution was added dropwise with stirring into 15 cc. of aqueous solution containing 300 mg. of $KMnO_4$. The reaction mixture was heated to 70° for 20 min. on a water bath, cooled, neutralized with 5 cc. of dil. H_2SO_4(1:4), and heated to 95° on a boiling water bath. The reaction mixture was filtered to separate the precipitate which was washed with 50 cc. of hot H_2O and 100 cc. of hot Me_2CO. The filtrates and washings were combined and concentrated to about 50 cc.

ii) Monocarboxylic Acid : The mixture of the product was separated by steam distillation into monocarboxylic and dicarboxylic acids. Monocarboxylic acid in the distillate was extracted with Et_2O, and 42 mg. and 33 mg. of pale yellow oil were obtained from (V) and (VI), respectively. On distillation under a reduced pressure both the monocarboxylic acids gave a common fraction boiling at 105~113°/10 mm. Hg. These distillates were analyzed by paper chromatography and reversed-phase column chromatography, and both were found to be constituted from heptanoic acid. *Anal.* Calcd. for $C_7H_{14}O_2$: C, 64.58; H, 10.84; COOH, 34.58. Found : C, 64.35; H, 10.66; COOH, 35.03.

p-Bromophenacyl Ester : m.p. 72°. *Anal.* Calcd. for $C_{15}H_{19}O_3Br$: C, 55.04; H, 5.81. Found : C, 55.36; H, 5.63. The m.p. showed no depression on admixture with *p*-bromophenacyl ester prepared from an authentic specimen.

iii) Dicarboxylic Acid : From the residue of steam distillation of the oxidation mixture of (V), white fine crystals (XI), m.p. 106°, were separated, and from that of (VI), fine crystals (XII), m.p. 111°, were obtained. As will be described later, (XI) and (XII) were proved to be nonanedioic acid and undecanedioic acid, respectively, from their m.p.s, analyses, and m.p.s of their derivatives.

Oxidation of (V) and (VI) with Peracetic Acid—To 20 mg. of (V) or (VI) dissolved in 2 cc. of anhyd. glacial AcOH, 0.5 cc. of 30% H_2O_2 was added and the mixture was allowed to stand for several days. After the reaction was completed, AcOH was evaporated *in vacuo*, 0.5 cc. of *N* NaOH was added to the residue, and the mixture was heated for 5 hr. on a boiling water bath. The reaction mixture was neutralized and extracted with Et_2O. The product from the Et_2O extract was recrystallized from Et_2O or MeOH. A dihydroxy-C_{16} acid, m.p. 124°, was obtained from (V); yield, 17 mg. *Anal.* Calcd. for $C_{16}H_{32}O_4$: C, 66.63; H, 11.18; COOH, 15.61. Found : C, 66.74; H, 11.20; COOH, 15.87. A dihydroxy-C_{18} acid, m.p. 126°, was obtained from (VI); yield, 15.5 mg. *Anal.* Calcd. for $C_{18}H_{36}O_4$: C, 68.31; H, 11.46; COOH, 14.22. Found : C, 68.20; H, 11.47; COOH, 14.00.

A solution of 12 mg. of the dihydroxy acid dissolved in 1 cc. of EtOH, added with 2 cc. of 1% $NaIO_4$ solution was allowed to stand for 20 min. and, after addition of 10 cc. of H_2O, the reaction mixture was extracted with Et_2O. The Et_2O solution was further extracted with 3% Na_2CO_3 solution to separate neutral and acidic products. Et_2O layer was concentrated and the aldehyde contained in it was converted to its semicarbazone by the usual method. From both of dihydroxy-C_{16} and -C_{18} acids, the same semicarbazone, m.p. 109°, was obtained. *Anal.* Calcd. for $C_8H_{17}ON_3$: N, 24.54. Found : N, 25.02.

The Na_2CO_3 solution was acidified with HCl and extracted with Et_2O. The solvent was evaporated from the extract and the residue was oxidized with $KMnO_4$ solution. Nonanedioic acid was obtained from the dihydroxy-C_{16} acid and was recrystallized from hot H_2O, m.p. 106°; yield, 7.3 mg. Undecanedioic acid from dihydroxy-C_{18} acid and recrystallized from hydr. Me_2CO, m.p. of 110°; yield, 8.1 mg. *Anal.* Calcd. for $C_9H_{16}O_4$: C, 57.42; H, 8.57; COOH, 47.94. Found : C, 57.22; H, 8.50; COOH, 48.32. *Anal.* Calcd. for $C_{11}H_{20}O_4$: C, 61.09; H, 9.32; COOH, 41.63. Found : C, 61.13; H, 9.30; COOH, 41.52. These products did not show m.p. depession on admixture with respective authentic specimens.

Oxidation of (V) and (VI) with Cold Potassium Permanganate—Each of 20 mg. of (V) and (VI) was converted to the potassium salt by treatment with 0.2*N* KOH, and added with 20 cc. of H_2O and 0.1 g. of KOH. To each mixture was added dropwise a solution of 0.1 g. of $KMnO_4$ dissolved in 7 cc. of H_2O at 5° and set aside for 48 hr. at room temperature. The reaction mixture was filtered, the filtrate was acidified with 0.5*N* HCl, and extracted three times with Et_2O. Et_2O was removed from the extract, after drying over Na_2SO_4, and remaining white solid was recrystallized from Et_2O or MeOH. A dihydroxy-C_{16}-acid, m.p. 124°, was obtained from (V); yield, 15.8 mg., and, a dihydroxy-C_{18}-acid, m.p. 126°, from (VI); yield, 16.0 mg. $NaIO_4$ oxidation of both hydroxy fatty acids gave the same heptanal, identified as its semicarbazone. Aldehyde-fatty acid was oxidized with $KMnO_4$ solution to furnish a nonanedioic acid from (V) and undecanedioic acid from (VI). These dicarboxylic acids did not show depression of m.p. on admixture with respective authentic specimens.

The author is deeply grateful to Professor T. Ukita for his kind and unfailing guidance throughout the course of the present work. Thanks are also due to the members of the Central Analysis Room of this Faculty, and to the members of the Photo-Instrument Room of National Institute of Hygienic Sciences. This study was partly supported by a Grant-in-Aid for Scientific Research provided by the Ministry of Education, which is gratefully acknowledged.

Summary

The structure of coixenolide (I), which was isolated from the seeds of *Coix Lachryma-Jobi* L. VAR. *Ma-yuen* (ROMAN.) STAPF and which has a growth-inhibiting activity against Ehrlich ascites sarcoma cells in mice, was investigated. Unsaturated fatty acids, obtained by hydrolysis of (I), was found to be an equimolar mixture of *cis*-9-hexadecenoic acid and *trans*-11-octadecenoic acid.

Alcoholic part of (I) was identified with 2,3-butanediol. The total structure of (I) was elucidated as 1-methyl-2-(*cis*-9-hexadecenoyloxy)propyl *trans*-11-octadecenoate.

(Received April 28, 1960)