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Enduracidin, a New Antibiotic. VIII.[1] Structures of Enduracidins A and B

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From the structural investigation of acidic products obtained by hydrolysis of enduracidins and tetrahydroenduracidins, 10-methylundeca-2(*cis*)-4(*trans*)-dienoic acid (V) and (+)-10-methyldodeca-2(*cis*)-4(*trans*)-dienoic acid (VI) were found to be constituent of enduracidins A and B, respectively.

Consequently, the structural difference between enduracidins A and B was proved to be present in their unsaturated fatty acid moieties, and their full structures were assumed to be as shown in Fig. 10.

Enduracidin was obtained from the mycelium of *Streptomyces fungicidicus* B-5477[3] and found to be a novel basic peptide antibiotic containing chlorine in the molecule.[4] Enduracidin showed strong bactericidal activity both *in vitro* and *in vivo* against gram-positive bacteria including strains resistant to known antibiotics.[3,5]

Enduracidin was separated into enduracidins A (I) and B (II) by Amberlite XAD-2 column chromatography with the gradient elution of 0.05 N NaCl to 0.006 N HCl in 50% aqueous methanol,[6] but both components have closely similar physicochemical properties (Table I).

Table I. Properties of Enduracidins A anb B

Property	Enduracidin A·HCl (I)	Enduracidin B·HCl (II)
Melting point (°C)	240—245	238—241
Molecular wt. (x-ray)	*ca.* 2500	
Molecular formula	$C_{107}H_{138}O_{31}N_{26}Cl_2 \cdot HCl$	$C_{108}H_{140}O_{31}N_{26}Cl_2 \cdot HCl$
$[\alpha]_D^{23}$ (0.5%, DMF[a])	+92°	+92°
UV absorption ($\lambda_{max}^{0.1\,N\ HCl}$ nm)	231, 272	231, 272

a) DMF: dimethylfolmamide

Hori, *et al.*[1b] investigated structures of the peptide moieties of I and II, and found that they are composed of seventeen amino acids, and that sixteen of seventeen amino acids form a macrocyclic peptide lactone, a cyclodepsipeptide.

In this paper the chemical structures of novel fatty acids obtained from I and II are discussed and the difference in the chemical structure between I and II is proved to lie in their fatty acid moieties. Finally the full structures of I and II are presented.

The infrared spectra of I and II possess the characteristic absorption at 1750 cm^{-1} corresponding to an ester or lactone function. On treatment with 1 N NaOH at room temperature, the absorption at 1750 cm^{-1} disappeared, but no change was observed in the characteristic

1) *a*) Abstract of this work was reported by K. Mizuno, M. Asai, S. Horii, M. Hori, H. Iwasaki, and J. Ueyanagi, on *Antimicr. Agents & Chemoth.*, **1970**, 6 (1971); *b*) Part VII: M. Hori, H. Iwasaki, S. Horii, I. Yoshida, and T. Hongo, *Chem. Pharm. Bull.* (Tokyo), **21**, 1175 (1973).
2) Location: *Juso, Higashiyodogawa-ku, Osaka.*
3) E. Higashide, K. Hatano, and M. Shibata, *J. Antibiotics*, **21**, 126 (1968).
4) M. Asai, M. Muroi, N. Sugita, H. Kawashima, K. Mizuno, and A. Miyake, *J. Antibiotics*, **21**, 138 (1968).
5) K. Tsuchiya, M. Kondo, T. Oishi, and T. Yamazaki, *J. Antibiotics*, **21**, 147 (1968).
6) M. Hori, N. Sugita, and M. Miyazaki, *Chem. Pharm. Bull.* (Tokyo), **21**, 1171 (1973).

ultraviolet spectrum and the amino acid composition. These saponification products were assumed to be linear peptides formed by cleavage of the lactone bond in the depsipeptide structure[1b] of I and II, and were designated as enduracidic acids A and B.

C_{12}- and C_{13}-Saturated Acids obtained from Tetrahydroenduracidins

Hydrogenation of enduracidins over platinum black or palladium charcoal afforded tetrahydroenduracidins having the same amino acid composition. The ultraviolet absorption ranging from 260 to 280 nm of enduracidins decreased remarkably in tetrahydroenduracidins, indicating the presence of an unsaturated component besides amino acids.

Tetrahydro-I and -II were boiled with 6N HCl for 6 hours, and the hydrolyzate was extracted with ether. The ether extract was treated with diazomethane, and the resulting methyl ester was subjected to gas chromatography. Fatty acid methyl esters, III-Me and IV-Me were isolated by preparative gas chromatography from tetrahydro-I and -II, respectively and subjected to mass spectrum analysis (Fig. 1). Parent peaks (M^+) in 214 and 228 *m/e*

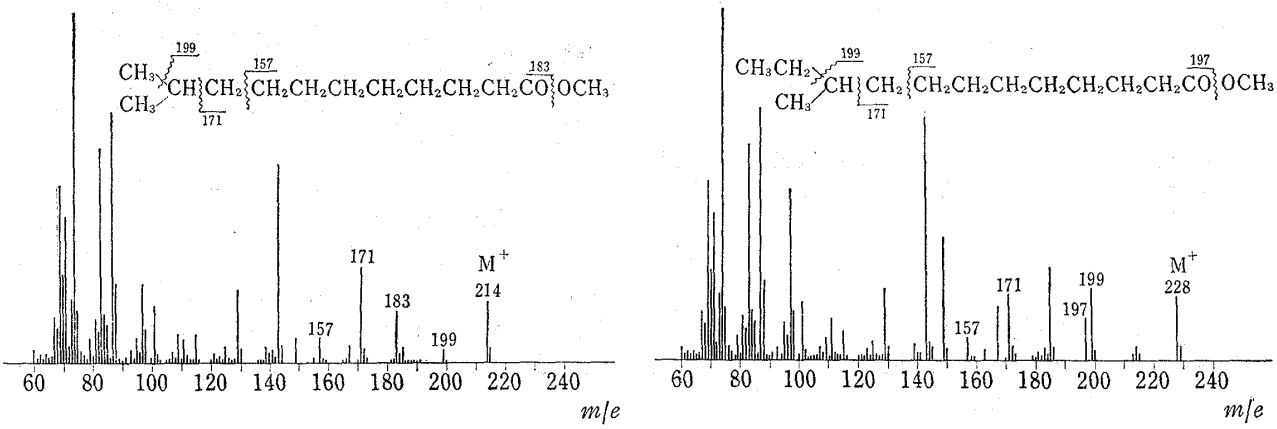
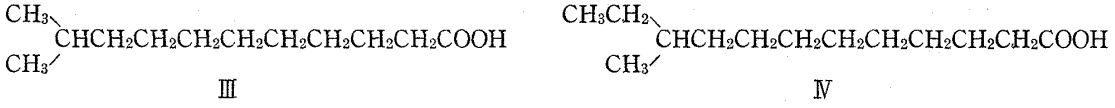


Fig. 1. Mass Spectra of C_{12}-Saturated Acid Methyl Ester (III-Me) (left) and C_{13}-Saturated Acid Methyl Ester (IV-Me) (right)

showed that III-Me and IV-Me are C_{12}-saturated acid methyl ester ($C_{13}H_{26}O_2$) and C_{13}-saturated acid methyl ester ($C_{14}H_{28}O_2$), respectively. Elemental analysis supports this assignment. Infrared spectrum of III-Me suggests the presence of gem-methyl group (1370, 1385 cm^{-1}). Nuclear magnetic resonance (NMR) and mass spectra of III-Me agree well with those of methyl 10-methylundecanoate obtained from aspartocin.[7] Comparison of NMR and mass spectra of IV-Me with those of methyl 9-methylund ecanoate obtained from aspartocin[7] led to the estimation of the chemical structure of IV-Me as methyl 10-methyldodecanoate. Of the two saturated fatty acid methyl esters, only IV-Me contains an asymmetric carbon atom; thus it would be expected to account for the observed optical activity. Milburn and Truter[8] reported that synthetic (+)-IV and (+)-10-methyldodecanol had $[\alpha]_D^{20}$ +5.9° and +7.3°, respectively. Because our IV-Me had $[\alpha]_D^{23}$ +5.4°, it is reasonable to assume that IV is the dextrorotatory isomer.

Consequently, the chemical structures of C_{12}-saturated acid obtained from tetrahydro-I and C_{13}-saturated acid obtained from tetrahydro-II were assumed to be 10-methylundecanoic acid (III) and (+)-10-methyldodecanoic acid (IV), respectively.



7) W.K. Hausmann, A.H. Struck, J.H. Martin, R.H. Barritt, and N. Bohonas, *Antimicr. Agents & Chemoth.*, **1963**, 352 (1964).
8) A.H. Milburn and E.V. Truter, *J. Chem. Soc.*, **1954**, 3341.

Novel Diene Carboxylic Acids obtained from I and II

Acid hydrolysis of enduracidins with 6N HCl resulted in a degradation of carboxylic acids moiety, showing complicated multiple peaks in gas chromatography. Good yield of the unsaturated carboxylic acids, V and VI, was accomplished by boiling enduracidins or enduracidic acids in 0.05N HCl for 30 hours. V and VI were extracted with ether from the hydrolyzates of I and II, respectively, and purified by vacuum distillation. V and VI absorbed 2 moles of hydrogen by catalytic hydrogenation and were converted into III and IV. Consequently, V and VI were proved to be C_{12}-dienoic acid and C_{13}-dienoic acid.

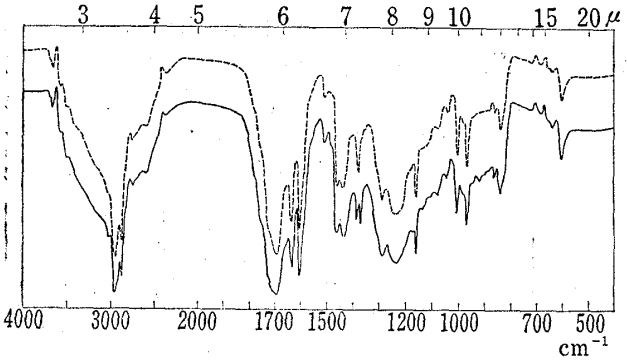


Fig. 2. IR Spectra of C_{12}-Dienoic Acid (V) (solid line) and C_{13}-Dienoic Acid (VI) (broken line) in $CHCl_3$

Both dienoic acids have the identical ultraviolet absorption and show the maximum ε 18100 at 260 nm, which resembles that of 2(*cis*)-4(*trans*)-sorbic acid (λ_{max} 257 nm, 17000,[9a] 21000[9b]). The ultraviolet absorption of enduracidin is well accounted for as the sum of the absorptions of these dienoic acids, and 4-hydroxyphenylglycine (K_1)[4] and 3,5-dichloro-4-hydroxyphenylglycine (K_2).[4]

V has characteristic gem-methyl absorptions at 1370 and 1385 cm^{-1}, while VI has one absorption at 1380 cm^{-1} (Fig. 2), but, in the remaining region, V and VI have the closely similar infrared absorption spectra. Absorptions of olefins ranging from 800 to 1000 cm^{-1} were compared with those of four stereoisomers of sorbic acid[9c] (Table II), and the absorptions of V and VI were found to resemble those of 2(*cis*)-4(*trans*)-sorbic acid.

Table II. IR Absorption of Dienecarboxylic Acids (cm^{-1})

	trans olefin		*cis* olefin	
2(*trans*)–4(*trans*)–Sorbic acid[a]	998	945	—	
2(*trans*)–4(*cis*)–Sorbic acid[a]	990	949	—	683
2(*cis*)–4(*trans*)–Sorbic acid[a]	996	958	837	—
2(*cis*)–4(*cis*)–Sorbic acid[a]		—	829	662
C_{12},C_{13}–Dienoic acid	1000	960	840	—

a) Data taken from ref. 9c

The configuration of 2(*cis*)-4(*trans*)-diene carboxylic acid was also confirmed by the comparison of NMR spectra (Fig. 3, 4) of the dienoic acids with those of methyl sorbates.[10] Chemical shifts (τ: 2-H 4.52, 3-H 3.48, 4-H 2.72, 5-H 4.07) and coupling constant (cps: $J_{2,3}$ 11.2, $J_{4,5}$ 15.5) of four protons adjacent to two double bonds of V and VI have the values near to those of methyl 2(*cis*)-4(*trans*)-sorbate.

V and VI were oxidized with potassium permanganate. In water-soluble fractions of the oxidation products of both dienoic acids, oxalic acid was detected by paper chromatography. The ether-soluble fractions were purified by vacuum distillation. Distilled oils showed parent peaks (M^+) 144 *m/e* and 158 *m/e* in the mass spectra corresponding to C_7H_{15}-COOH (VII) and $C_8H_{17}COOH$ (VIII), respectively.

9) a) U. Eisner, J.A. Elvidge, and R.P. Linstead, *J. Chem. Soc.*, **1953**, 1372; b) J.L.H. Allan, E.R.H. Jones, and M.C. Whiting, *ibid.*, **1955**, 1862; c) J.L.H. Allan, G.D. Meakins, and M.C. Whiting, *ibid.*, **1955**, 1874.

10) J.A. Elvidge and P.D. Ralph, *J. Chem. Soc.* (*B*), **1966**, 243.

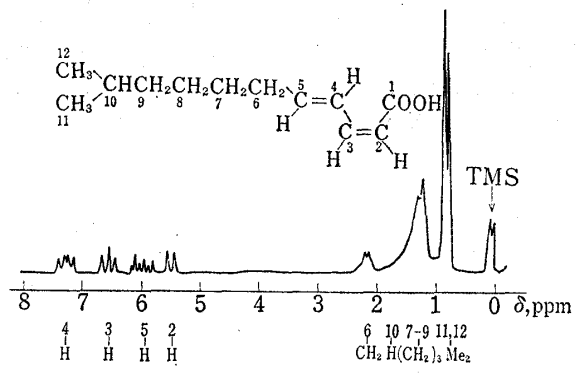


Fig. 3. NMR Spectrum of C_{12}-Dienoic Acid (V) in CCl_4 (100 MC)

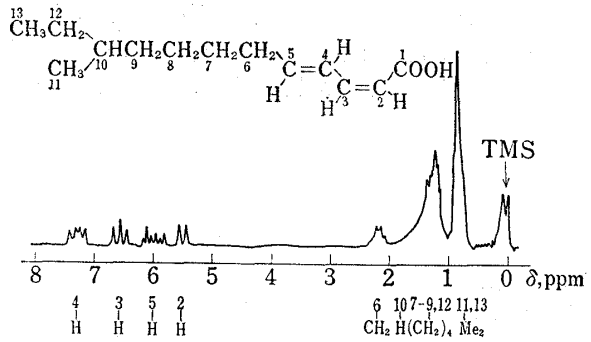
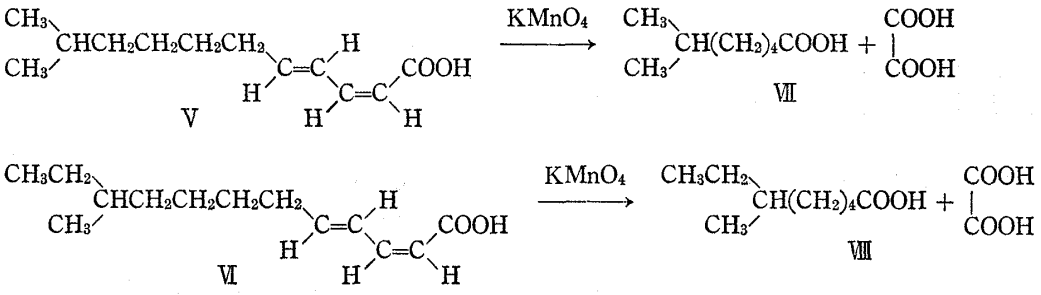


Fig. 4. NMR Spectrum of C_{13}-Dienoic Acid (VI) in CCl_4 (100 MC)

These physicochemical properties led to the conclusion that V in enduracidin A is 10-methylundeca-2(*cis*)-4(*trans*)-dienoic acid, and VI in enduracidin B is (+)-10-methyldodeca-2(*cis*)-4(*trans*)-dienoic acid.



Amino Acid Sequence Adjacent to the Diene Carboxylic Acids

For the conclusive evidence to the amino acid sequence adjacent to the diene carboxylic acid obtained by acid hydrolysis procedure,[1b] aqueous barium hydroxide solution of I was refluxed for 8 hours to give a mixture of several acidic substances, and all of them have an ultraviolet absorption maximum at 261 nm. The mixture of these acids was converted into methyl esters and the methyl esters were purified by preparative thin-layer chromatography to obtain a main compound (IX). The elemental analysis of IX agreed with the molecular formula $C_{18}H_{29}O_5N$. Acid hydrolysis of IX gave aspartic acid. Analysis of mass (Fig. 5)

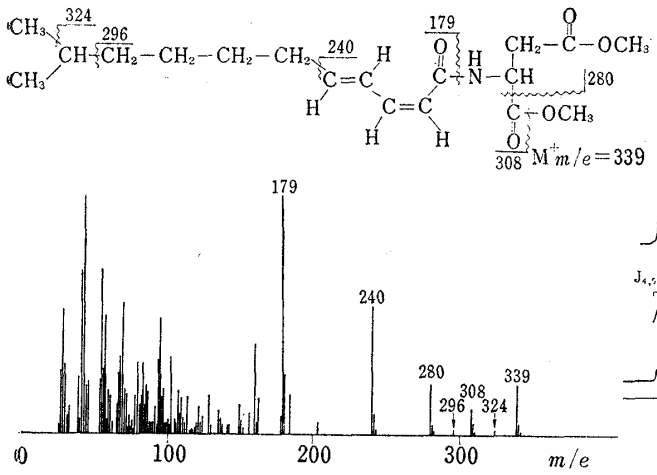


Fig. 5. Mass Spectrum of Compound (IX)

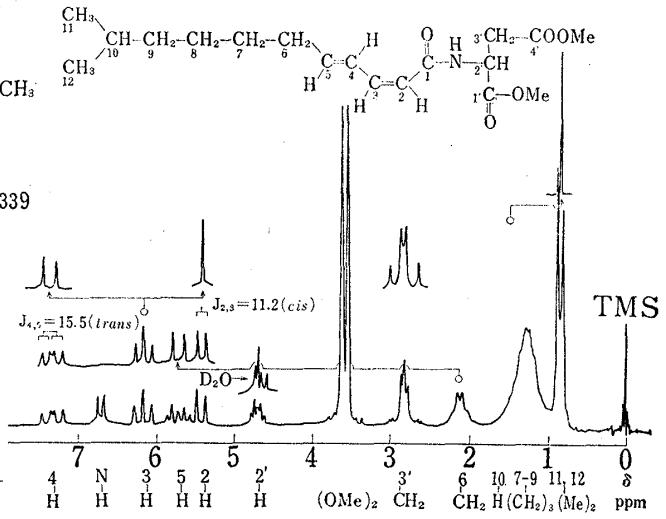


Fig. 6. NMR Spectrum of Compound (IX) in CCl_4 (100 MC)

and NMR (Fig. 6) spectra with extensive decoupling experiments led to the determination of the structure of IX as dimethyl N-[10-methylundeca-2(*cis*)-4(*trans*)-dienoyl]-aspartate. The same treatment of II afforded compound (X), $C_{19}H_{31}O_5N$. From the data of hydrolysis, mass (Fig. 7) and NMR (Fig. 8) spectra, the structure of X was determined as dimethyl N-

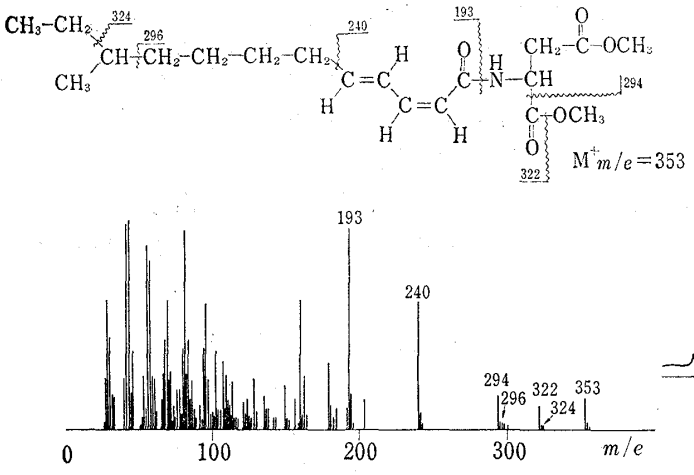


Fig. 7. Mass Spectrum of Compound (X)

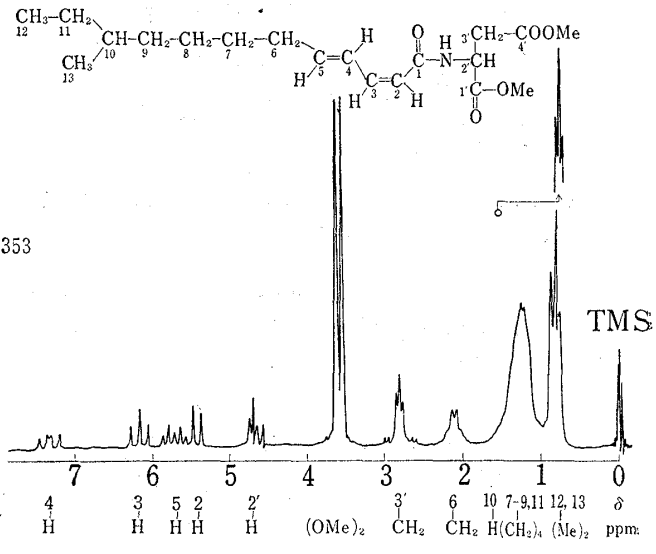
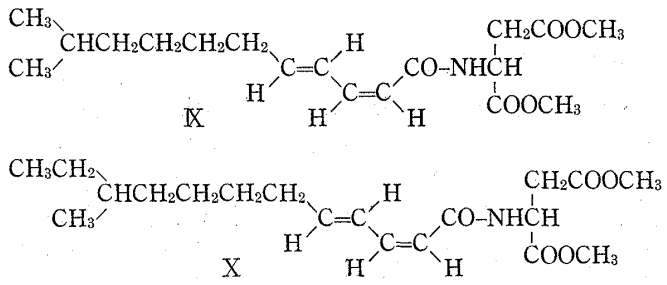


Fig. 8. NMR Spectrum of Compound (X) in CCl_4 (100 MC)



IX

X

[10-methyldodeca-2(*cis*)-4(*trans*)-dienoyl]-aspartate. Besides these two compounds, minor products were also isolated by preparative thin-layer chromatography and their structures were estimated to be methyl esters of C_{12}- and C_{13}-dienoyl→Asp→Thr, and C_{12}- and C_{13}-dienoyl→Asp→Gly, of which the latters are supposed to be products by secondary reaction, *i.e.*, removal of acetaldehyde from the threonine moiety of the formers, because acetaldehyde was detected as a volatile component during the hydrolysis.

N-Terminal amino acid in enduracidins was examined by dinitrophenylation (DNP) method. When DNP-derivatives of I and II were hydrolized, δ-DNP-ornithine, O-DNP-K_1 and O-DNP-K_2 were obtained, but no N-terminal amino acid was detected. From these experiments, N-terminal moieties were assumed to be C_{12}-dienoyl→Asp→Thr for I and C_{13}-dienoyl→Asp→Thr for II.

Structures of Enduracidins A (I) and B (II)

From the findings hitherto obtained and the peptide sequences of enduracidins elucidated by Hori, *et al.*,[1b] the full structures of enduracidins A (I) and B (II) were proposed as shown in Fig. 9.

Many kinds of fatty acid have so far been found as components of various antibiotics, but the conjugated diene carboxylic acids are the first discovered in this field. The differecne in the chemical structure between enduracidins A and B is only one methylene group in the fatty acid moieties. There are many cyclodepsipeptide antibiotics, but such a large lactone ring has not yet been found. In these respects, enduracidins A and B can be said to be very unique cyclodepsipeptide antibiotics.

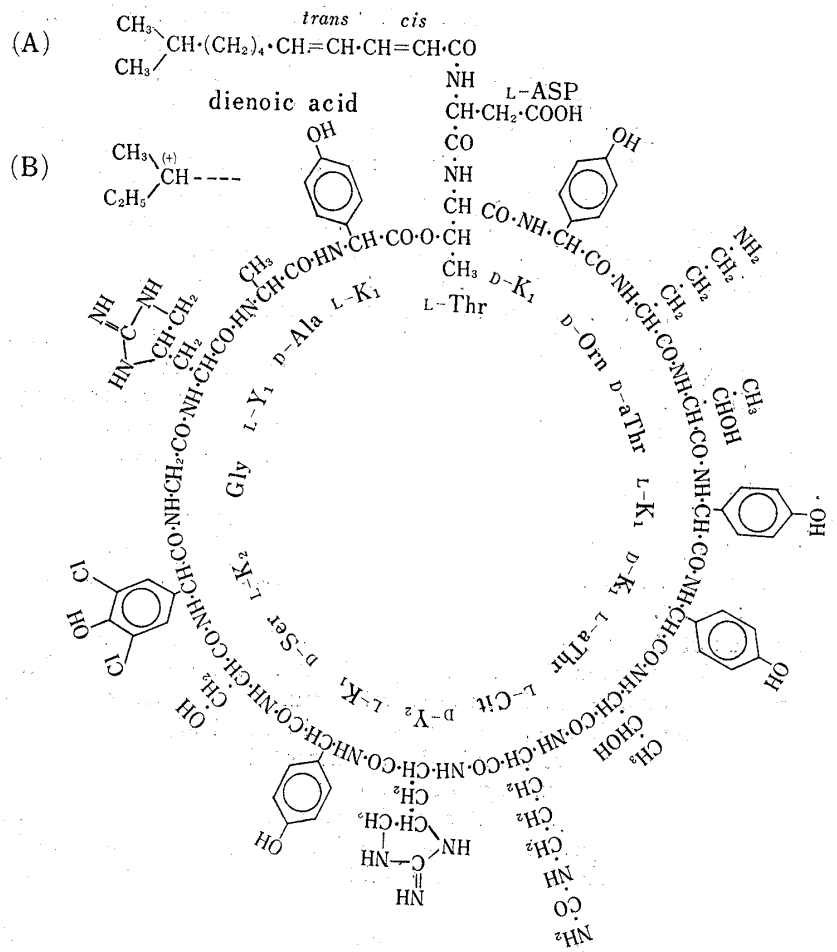


Fig. 9. Structures of Enduracidins A and B

Experimental

Enduracidic Acids——I or II (5 g) was dissolved in 1N NaOH (50 ml) and allowed to stand at room temperature for 3 to 10 hr. The reaction mixture was neutralized to pH 6 with dil. HCl under stirring to obtain precipitate. The precipitate was collected by filtration and washed several times with H_2O, and dried to afford enduracidic acid A or B (4.5 g). Colorless powder. The following properties were the same between enduracidic acids A and B. Paper electrophoresis (pH 1.8 HCOOH·AcOH, 3 kV, 1 hr): enduracidic acid A or B, 12 cm toward the cathode; I or II, 15 cm toward the cathode. Paper chromatography (Whatman No. 1) *n*-BuOH: AcOH: H_2O (4: 1: 5), enduracidic acid A or B *Rf* 0.42, I or II *Rf* 0.45. The 1750 cm^{-1} band which is present in the IR spectra of I and II are missing in enduracidic acids A and B. UV $\lambda_{max}^{0.1N\ HCl}$ nm ($E_{1cm}^{1\%}$): 231 (205), 272 (122). Amino acid analysis: Asp 1.0, Thr 2.91, Ser 0.95, Cit 0.51, Gly 1.01, Ala 1.00, K_1 4.35, K_2 1.11, Orn 1.47, Y_1[11] 1.12, Y_2[11] 1.01.

Tetrahydroenduracidins——I or II (2 g) in 80% AcOH (30 ml) was hydrogenated over Pt (0.2 g) at atmospheric pressure (about 38 ml of H_2 was absorbed). The reaction mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was washed with acetone several times and dried to obtain colorless powder of tetrahydroenduracidin-HCl (1.8 g). Tetrahydroenduracidin A-HCl: $[\alpha]_D^{26}$ +82.8° (c=1, 70% MeOH). UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 264 (54.6), 310 (17.7); $\lambda_{max}^{0.1N\ HCl}$ nm ($E_{1cm}^{1\%}$) 230 (201), 276 (32.4), 282 (30.9); $\lambda_{max}^{0.1N\ NaOH}$ nm ($E_{1cm}^{1\%}$) 250 (289), 293 (68). *Anal.* Calcd. for $C_{107}H_{151}O_{35}N_{26}Cl_3$: C, 52.07; H, 6.17; N, 14.76; Cl, 4.31; H_2O, 2.92. Found: C, 52.05; H, 6.22; N, 14.27; Cl, 4.77; H_2O, 3.21. Tetrahydroenduracidin B-HCl: $[\alpha]_D^{26}$ +83.5° (c=1, 70% MeOH), UV λ_{max}^{MeOH} nm: ($E_{1cm}^{1\%}$) 264 (55.0), 310 (18.1); $\lambda_{max}^{0.1N\ HCl}$ nm ($E_{1cm}^{1\%}$) 230 (205), 276 (35.4), 282 (32.1); $\lambda_{max}^{0.1N\ NaOH}$ nm ($E_{1cm}^{1\%}$) 250 (291), 293 (70), *Anal.* Calcd. for $C_{108}H_{153}O_{35}N_{26}Cl_3$: C, 52.26; H, 6.21; N, 14.67; Cl, 4.29; H_2O, 2.90. Found: C, 52.07; H, 6.25; N, 14.21; Cl, 4.51; H_2O, 3.18.

11) S. Horii and Y. Kameda, *J. Antibiotics*, **21**, 665 (1958).

C_{12}- and C_{13}-Saturated Acids Methyl Esters (III-Me, IV-Me)——Tetrahydroenduracidins (5 g) in 6N HCl (250 ml) was boiled for 6 hr. After the reaction mixture was diluted twice with H_2O, the acidic solution was extracted three times with ether (100 ml). The ether solution was washed with H_2O and dried over Na_2SO_4. The evaporation of ether gave a yellow oil (396 mg). The oil was vacuum-distilled to obtain a colorless oil, bp 135—140° (1.2 mmHg). When the oil was allowed to stand in the refrigerator, a part crystallized to colorless needle, mp 33—35°. Methyl ester: Methylation was performed with CH_2N_2 in the usual way and purified by vacuum distillation, bp 150—155° (24 mmHg). Gas chromatography: Hitachi's type K-53, column: EGS 2 m, temperature 150°, carrier gas: He, flow rate: 0.7 ml/min. Methyl 10-methylundecanoate (III-Me): retention time: 3.6 min, colorless oil, $[\alpha]_D^{23}$ 0° (c=1, $CHCl_3$), mass spectrum (Fig. 1) m/e: 214 (M^+), 199 (M^+-CH_3), 183 (M^+-OCH_3), 171 (M^+-C_3H_7). *Anal.* Calcd. for $C_{13}H_{26}O_2$: C, 72.84; H, 12.23. Found: C, 73.06; H, 12.07. Methyl (+)-10-methyldodecanoate (IV-Me): retention time: 5.3 min, colorless oil, $[\alpha]_D^{23}$ +5.4° (c=1, $CHCl_3$), mass spectrum (Fig. 1) m/e: 228 (M^+), 199 (M^+-C_2H_5), 197 (M^+-OCH_3). *Anal.* Calcd. for $C_{14}H_{28}O_2$: C, 73.62; H, 12.36. Found: C, 73.67; H, 12.07.

C_{12}-Dienoic Acid (V)——I or enduracidic acid A (1 g, each) was boiled in 0.05N HCl (50 ml) for 30 hr. The reaction mixture was extracted three times with ether (20 ml) and the ether extract was dried over Na_2SO_4 after washing with H_2O. A yellow oil obtained by evaporation of ether was purified by vacuum distillation to obtain 10-methylundeca-2(*cis*)-4(*trans*)-dienoic aicd (V), 52 mg, bp 136—138° (0.8 mmHg), slightly yellow oil. *Anal.* Calcd. for $C_{12}H_{20}O_2$: C, 73.43; H, 10.27. Found: C, 73.56; H, 10.32. UV λ_{max}^{EtOH} nm (ε): 260 (18100). NMR (CCl_4): Fig. 3, IR ($CHCl_3$): Fig. 2.

C_{13}-Dienoic Acid (VI)——II or enduracidic acid B was hydrolyzed in the same manner to obtain (+)-10-methyldodeca-2(*cis*)-4(*trans*)-dienoic acid (VI), 45 mg, bp 138—140° (0.8 mmHg), slightly yellow oil. *Anal.* Calcd. for $C_{13}H_{22}O_2$: C, 74.24; H, 10.54. Found: C, 74.01; H, 10.73. UV λ_{max}^{EtOH} nm (ε): 260 (18150), NMR (CCl_4): Fig. 4, IR ($CHCl_3$): Fig. 2.

Permanganate Oxidation of V and VI——$KMnO_4$ (1.7 g) was dissolved in H_2O (23 ml) by stirring at 35°. V or VI (280 mg) and KOH (85 mg) was dissolved in H_2O (5 ml), and the resulting solution was added once to the $KMnO_4$ solution. After 30 min stirring, the reaction mixture was neutralized with dil. H_2SO_4 and warmed in the water bath. The precipitate, MnO_2, was filtered off and washed with H_2O (5 ml) and EtOH (5 ml). The filtrate and the washing were combined and extracted at pH 2 several times with ether and the ether solution was dried over anhydrous $MgSO_4$, then evaporated *in vacuo*. Vacuum distillation of the residue gave VII and VIII, respectively. Molecular weight (mass spectra): VII 144, VIII 158. The aqueous layer, after ether extraction, was readjusted to pH 3 with dil. H_2SO_4 and evaporated *in vacuo* to dryness. The residue was extracted with EtOH and the EtOH-soluble fraction was found to contain oxalic acid by paper chromatography.

Hydrolysis of Enduracidins with aq. Ba(OH)$_2$——The solution of I (5 g), in 4% $Ba(OH_2)$ (100 ml) was refluxed for 8 hr. A volatile product was collected in a 2,4-dinitrophenylhydrazine solution, and the resulting product was identified as acetaldehyde 2,4-dinitrophenylhydrazone by comparing melting point (mp 164°) and IR spectrum with the authentic sample. Precipitated Ba-salts formed was collected by filtration, washed with H_2O and dried to obtain colorless powder (750 mg). A suspension of the powder in MeOH (50 ml) was adjusted to pH 2 with dil. H_2SO_4 under stirring and filtered. Precipitate was treated twice in the same way. After dilution with H_2O (500 ml), the combined filtrate was extracted twice with EtOAc (250 ml). From the resulting EtOAc extract, acidic substances were transferred to 2% $NaHCO_3$ solution. Acidification of the solution to pH 2 with dil. H_2SO_4, extraction with EtOAc (150 ml), followed by evaporation *in vacuo* afforded a mixture of free acids (450 mg). Methylation was performed with 7% HCl–MeOH (20 ml) for 16 hr at room temperature. The reaction mixture was poured into ice–water and extracted with EtOAc. After washing with 2% $NaHCO_3$ and H_2O, the EtOAc solution was evaporated to obtain a mixture of methyl esters (480 mg). The mixture of methyl esters was separated by silica gel thin-layer chromatography (Merck, silica gel HF) with benzene: EtOAc (6.5: 3.5) into six compounds, when observed with ultraviolet light. Main compound (Rf 0.85) was dimethyl N-[10-methylundeca-2(*cis*)-4(*trans*)-dienoyl]aspartate (IX), syrup, 175 mg. *Anal.* Calcd. for $C_{18}H_{29}O_5N$: C, 63.69; H, 8.61; N, 4.13. Found: C, 63.36; H, 8.78; N, 4.05. UV λ_{max}^{MeOH} nm (ε): 261.5 (33400). Mass Spectrum m/e: 339 (M^+) (calcd. mol. wt. 339.4) Fig. 5. NMR (CCl_4) Fig. 6.

The same treatment of II gave dimethyl N-[10-methyldodeca-2(*cis*)-4(*trans*)-dienoyl]aspartate (X) (Rf 0.85) as a main product, syrup, 185 mg. *Anal.* Calcd. for $C_{19}H_{31}O_5N$: C, 64.56; H, 8.84; N, 3.96. Found: C, 64.36; H, 8.95; N, 3.75. UV λ_{max}^{MeOH} nm (ε): 261.5 (28400). Mass Spectrum m/e: 353 (M^+) (calcd. mol. wt. 353.4) Fig. 7. NMR (CCl_4) Fig. 8.

IX and X were hydrolyzed with 6N HCl at 110° for 24 hr. After dilution with H_2O the hydrolyzate was washed with ether and the aqueous phase was evaporated *in vacuo*. The residue was subjected to paper chromatography with BuOH: AcOH: H_2O (4:1:5) and 80% phenol, and ninhydrin-positive substance was identified as aspartic acid.

Minor compounds detected on the thin layer chromatogram were also isolated. From the hydrolyzate of I; dimethyl ester of C_{12}-dienoyl——> Asp——>Thr, Rf 0.28, syrup, mass spectrum m/e: 440 (M^+). Acid hydrolysis; Asp, Thr. Dimethyl ester of C_{12}-dienoyl——>Asp——>Gly, Rf 0.43, syrup. Mass Spectrum m/e:

396 (M^+). Acid hydrolysis: Asp, Gly. From the hydrolyzate of II; dimethyl ester of C_{13}-dienoyl⟶Asp⟶Thr, *Rf* 0.28, syrup, mass spectrum *m/e*: 454 (M^+). Acid hydrolysis; Asp, Thr. Dimethyl ester of C_{13}-dienoyl⟶Asp⟶Gly, *Rf* 0.43, syrup, mass spectrum *m/e*: 410 (M^+). Acid hydrolysis: Asp, Gly.

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