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9,10,13-TRIHYDROXY-11,15-OCTADECADIENOIC ACID AND RELATED FATTY ACIDS IN THE ROOTS OF KIDNEY BEAN (PHASEOLUS VULGARIS L., "BENI-KINTOKI")

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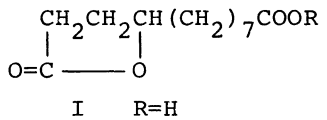
In connection with studies on characterization of natural hatching-stimulants of soybean cyst nematode eggs, several fatty acids were isolated newly from the roots of kidney bean and identified as 4-hydroxy-1,12-dodecanedioic acid γ-lactone, 9,10,13-trihydroxy-11- and 15-octadecenoic acids, and 9,10,13-trihydroxy-11,15-octadecadienoic acid.

The hatching of eggs of the soybean cyst nematode (Heterodera glycines) has recently been found to be stimulated by the root diffusates of several host plants.[1] In a continuing study[2] aimed at isolation of such stimulants from the roots of kidney bean (Phaseolus vulgaris L., Beni-kintoki), we newly isolated several fatty acids with a few aromatic acids. The present paper describes the isolation and structure determination of the acids.

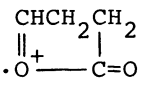
Aqueous, acidic extracts (66.5 g) with the relevant biological activity at $10^{-5\sim6}$ g/ml in water, obtained from the dried roots (135 kg) collected in July at Memuro, Hokkaido, were fractionated by chromatography over active charcoal with aqueous acetone to give oily mixtures (5.2 g), which were active at 10^{-7} g/ml. Further chromatography of the mixtures over silica gel with ethyl acetate-chloroform-acetic acid separated oil (fraction B, 360 mg) with activity at $10^{-8\sim9}$ g/ml. As one of clues for identification of the active principle(s), we examined components of fractions (A and C) which were eluted just before and after the highly active fraction B. The fraction A (145 mg, inactive) was then submitted to preparative TLC over silica gel to give lactonic acid (I, 13 mg) along with veratric acid (2 mg), decane-1,10-dioic, undecane-1,11-dioic and dodecane-1,12-dioic acids (total 11 mg) and syringic acid (3 mg). On the other hand, the fraction C (480 mg, active at 10^{-6} g/ml), when treated with methanol-ethyl acetate, separated crystalline acid (24 mg), which on fractional recrystallization gave 9,10,13-trihydroxyoctadecanoic acid (II, 16 mg), mp 139-139.5°C, [the methyl ester (IIA), mp 120-120.5°C], which was identical with the known sample[3] in the mixed mp, Mass, IR and NMR spectra. The filtrate was then converted into the p-bromophenacyl esters, which were further purified by argentation chromatography over silica gel followed by recrystallizations to give three unsaturated acid esters (IIIA, 20 mg; IVA, 21 mg; VA, 3 mg) in, at least chemically, pure state.

Compound I (inactive), mp 48.5-50°C (isopropyl ether-n-hexane); IR ($CHCl_3$),

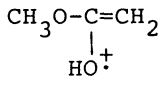
ν_{max} 1760, 1705 and 1170 cm^{-1}, was converted into the methyl ester (IA), $C_{13}H_{22}O_4$, mp 20-22°C; Mass, m/e 242 (M^+), 224 ($M^+ - H_2O$), 211 ($M^+ - CH_3O$), 192 (224 - CH_3OH), 151 (224 - 31 - CH_2CO), 85 (base peak, Ia), and 74 (Ib); NMR ($CDCl_3$), δ 1.1 ~ 2.6 (18H, broad $9CH_2$), 2.26 (2H, t J = 7 Hz, CH_2COOCH_3), 3.60 (3H, s, $COOCH_3$), 4.46 [1H, m, W_H = 18 Hz, CH(O)]. These spectra indicated the compound to be represented by formula I.[4)]



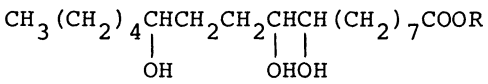
I R=H
IA R=CH_3



Ia

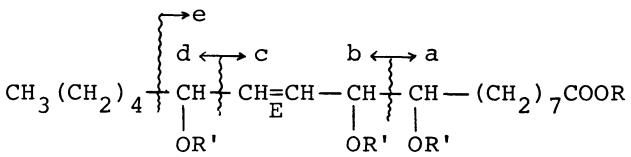


Ib

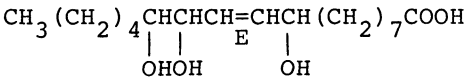


II R=H
IIA R=CH_3

Compound III (inactive), regenerated from the p-bromophenacyl ester (IIIA) of mp 127-128°C (ethyl acetate-methanol), gave the methyl ester (IIIB), $C_{19}H_{36}O_5$, mp 88.5-90.5°C (ethyl acetate); IR (KBr), ν_{max} 3270, 1735 and 968 cm^{-1}; NMR ($CDCl_3$), δ 0.90 (3H, broad t, CH_3), 1.2 ~ 1.8 (23H, broad, $10CH_2$ and 3OH; 20H on addition of D_2O), 2.30 (2H, t J = 7 Hz, CH_2COOCH_3), 3.60 [1H, broad, CH(OH)], 3.66(3H, s, $COOCH_3$), 4.12 [2H, broad, CH(OH)CH=CHCH(OH)], and 5.77 (2H, m, W_H = 9 Hz, CH(OH)CH=CHCH(OH)]. These spectra and the Mass spectrum of the trimethylsilyl derivative (IIIC); m/e 545 ($M^+ - 15$), 460 [$M^+ - C_6H_{12}O$ (hexanal)], 455 (545 - 90), 439 ($M^+ - 31 - 90$), 399 (IIIe - 90), 387 (IIIc), 301 (IIIb), 297 (IIIc - 90), 259 (IIIa), 227 (IIIa - 32), 173 (base peak, IIId), 155 [$\overset{+}{O}{\equiv}C(CH_2)_7CHO$], 147 [$(CH_3)_2Si{=}\overset{+}{O}Si(CH_3)_3$], 129 [$\overset{+}{C}_3H_4OSi(CH_3)_3$], 103 [$\overset{+}{C}H_2OSi(CH_3)_3$], 83 (IIId - 90), 75 [$H\overset{+}{O}{=}Si(CH_3)_2$], and 73 [$(CH_3)_3\overset{+}{S}i$],[5)] indicated that the compound could be formulated either as III[6)] or III'. Oxidation of IIIB (0.23 mg) with potassium periodate gave methyl 9-oxononanoate (IIID), which was transformed into the 2,4-DNP. This hydrazone proved to be identical with the corresponding authentic sample,[7)] mp 56-56.5°C (ethanol), by TLC and HPLC, which was prepared by oxidation of oleic acid with potassium permanganate[8)] and then with potassium periodate[9)] followed by treatment with 2,4-dinitrophenylhydrazine and with diazomethane. No spot corresponding to hexanal 2,4-DNP could be detected on the TLC of oxidation products of IIIB.



III R=R'=H
IIIA R=$CH_2COC_6H_4Br$, R'=H
IIIB R=CH_3, R'=H
IIIC R=CH_3, R'=$Si(CH_3)_3$

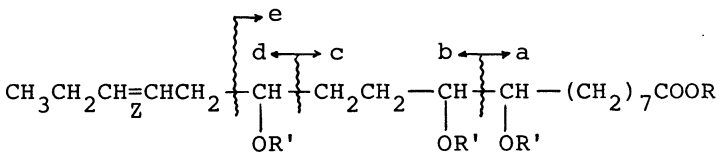


III'

$OHC(CH_2)_7COOCH_3$

IIID

Compound IV (inactive), obtained by hydrolysis of the ester IVA of mp 112-113°C (ethyl acetate), afforded the methyl ester (IVB), $C_{19}H_{36}O_5$, oil; IR (direct), ν_{max} 3300 and 1735 cm^{-1}, no strong maximum near 970 cm^{-1}, and its trimethylsilyl derivative (IVC); Mass, m/e 529 (M^+ - 31), 491 (IVe), 455 (M^+ - 15 - 90), 439 (529 - 90), 401 (IVe - 90), 389 (IVc), 311 (401 - 90), 301 (IVb), 299 (IVc - 90), 259 (IVa), 227 (IVa - 32), 171 (IVd), 155, 147, 129, 103, 81 (IVd - 90), 75, and 73 (base peak). These spectra and the NMR spectrum ($CDCl_3$) of IVA; δ 0.97 (3H, clear t, J = 7 Hz, $C\underline{H}_3$), 1.35 ~ 1.64 (16H, broad, $8C\underline{H}_2$), 2.07 and 2.28 (each 2H, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 2.48 (2H, t J = 7 Hz, $C\underline{H}_2COOCH_2$), 2.61(3H, broad s, $3O\underline{H}$; disappeared on addition of D_2O), 3.62 [3H, broad, $3C\underline{H}(OH)$], 5.27 (2H, s, $COC\underline{H}_2OOC$), 5.48 (2H, m, $C\underline{H}{=}C\underline{H}$), 7.60 and 7.76 (each 2H, d J = 10 Hz, arom $\underline{H}$), indicated that the compound was (Z)-9,10,13-trihydroxy-15-octadecenoic acid (IV).



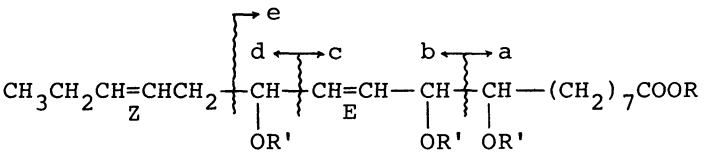
IV R=R'=H

IVA $R=CH_2COC_6H_4Br$, R'=H

IVB $R=CH_3$, R'=H

IVC $R=CH_3$, $R'=Si(CH_3)_3$

Compound V (active) was isolated by hydrolysis of the p-bromophenacyl ester (VA) of mp 114-115°C (ethyl acetate); NMR ($CDCl_3$), δ 0.96 (3H, clear t J = 7 Hz, $C\underline{H}_3$), 1.35 ~ 1.62 (12H, broad, $6C\underline{H}_2$), 2.07 and 2.28 (each 2H, m, $2C\underline{H}_2CH{=}CH$), 2.47 (2H, t, $C\underline{H}_2COOCH_2$), 2.60 (3H, broad s, $3O\underline{H}$; disappeared on addition of D_2O), 3.60 and 4.12 [1H and 2H, broad, $3C\underline{H}(OH)$], 5.27 (2H, s, $COC\underline{H}_2OOC$), 5.47 (2H, broad, $C\underline{H}{=}C\underline{H}$), 5.78 [2H, m W_H = 9 Hz, $CH(OH)C\underline{H}{=}C\underline{H}CH(OH)$], 7.60 and 7.76 (each 2H, d J = 10 Hz, arom $\underline{H}$). The derived methyl ester (VB), $C_{19}H_{34}O_5$, oil; IR (direct), ν_{max} 3360, 1735 and 973 cm^{-1}, was converted into the trimethylsilyl ether (VC), which showed the following Mass spectrum; m/e 543 (M^+ - 15), 460 [M^+ - $C_6H_{10}O$ (hexenal)], 453 (543 - 90), 437 (M^+ - 31 - 90), 399 (Ve - 90), 387 (Vc), 299 (Vb), 297 (Vc - 90), 259 (Va), 171 (Vd), 155, 147, 129, 103, 81 (Vd - 90), 75, and 73 (base peak). All these spectra were consistent with the assigned structure; namely, compound V was (11E,15Z)-9,10,13-trihydroxy-11,15-octadecadienoic acid.



V R=R'=H

VA $R=CH_2COC_6H_4Br$, R'=H

VB $R=CH_3$, R'=H

VC $R=CH_3$, $R'=Si(CH_3)_3$

Of these unsaturated fatty acids, only one compound V stimulated the hatching of the nematode eggs, though the activity was very weak ($10^{-5\sim6}$ g/ml in water at room temperature) and might be caused by contamination of highly active principle(s). However, in view of the chromatographic procedure, the isolation of these compounds suggests that the principle(s) would possess structure analogous to the acids.

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References and Footnotes

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4) This lactonic acid was isolated newly from natural sources, but the acid with the same (planar) formula had been obtained by oxidation of 9-hydroxy-12-octadecenoic acid with potassium permanganate. F. D. Gunstone, J. Chem. Soc., 1274 (1952).
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6) Graveland, Tsuchida, Heimann and their coworkers obtained compound III, as a mixture of compounds III and III', by enzymatic oxidation of linoleic acid in a dough, by irradiation and aerial oxidation of sesame oil, and by enzymatic oxidation of linoleic acid hydroperoxide in oats, respectively. A. Graveland, J. Amer. Oil Chem. Soc., 47, 352 (1970); M, Tsuchida, T. Miura, and K. Miyaki, Yukagaku, 21, 269 (1972); W. Heimann and P. Dresen, Helv. Chim. Acta, 56, 463 (1973). We expressed our sincere thanks to Dr. Masako Tsuchida, National Institute of Health, Tokyo, for providing us samples and spectra of a mixture of III and III'.
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