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STRUCTURES OF ISODOMOIC ACIDS A, B AND C, NOVEL INSECTICIDAL AMINO ACIDS FROM THE RED ALGA CHONDRIA ARMATA

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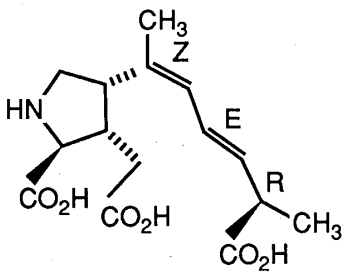
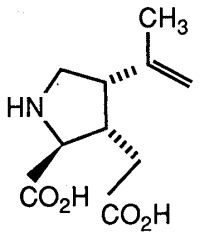
Three novel amino acids with insecticidal activity, isodomoic acids A, B and C, were isolated from water extracts of the red alga Chondria armata. Their structures were elucidated by spectroscopic analysis.

KEYWORDS —— red alga; Chondria armata; Rhodomelaceae; amino acid; insecticidal activity; isodomoic acid; ^{1}H-^{1}H shift correlated spectroscopy (COSY); NOE difference experiment; ^{13}C-NMR spectrum

Chondria armata and Digenea simplex, red algae belonging to the Rhodomelaceae, were used in Japan as a vermifuge. Domoic acid (I) and α-kainic acid (II) were isolated as the active components from C. armata and D. simplex respectively.[1,2] C. armata's fly-killing properties were known to the inhabitants of Yakushima island, Kagoshima.



domoic acid (I)



α -kainic acid (II)

Recently, we reported that domoic acid (I) is strongly insecticidal against the American cockroach (Periplaneta americana) when injected subcutaneously. It also induces significant contraction of the hindgut excised from the American cockroach.[3] Its extremely strong insecticidal activity prompted us to re-examine its constituents. We have fractionated the water extract by means of an assay based on insecticidal activity against the American cockroach. From the insecticidal fraction, we isolated three novel amino acids closely related structurally to domoic acid (I). This is a report on the structures of these amino acids, named isodomoic acid A (III), B (IV) and C (V). The dried red alga (1 kg) was extracted with 1 l of water. The water

extract was first chromatographed on Lichroprep Rp-18, followed by separation on DEAE-cellulose (OH^- form). Gel-filtration (Sephadex LH-20 and G-10) of the active fractions gave domoic acid (I) (140 mg), isodomoic acid A (III) (4 mg), B (IV) (10 mg), and crude isodomoic acid C (V) (3 mg). The crude isodomoic acid C was purified by column chromatography on Lichroprep Rp-8 to give pure isodomoic acid C (IV) (1 mg).

Isodomoic acids A (III), B (IV) and C (V) had the following physical characteristics: (III), $C_{15}H_{21}O_6N$ [SIMS, m/z 312$(M+1)^+$], mp(dec.) 185-187°C, $[\alpha]_D^{25}$ -70.0(c=0.1, H_2O), $\Delta\varepsilon_{210}$ -7.31, λ_{max}nm: 214(ε 9850), $\nu_{max}cm^{-1}$: 3400, 3200-2940, 1690, 1640, 1580, 1390; (IV), $C_{15}H_{21}O_6N$ [SIMS, m/z 312$(M+1)^+$], mp(dec.) 182-183°C, $[\alpha]_D^{25}$ -8.1(c=0.14, H_2O), $\Delta\varepsilon_{204}$ +1.62, λ_{max}nm: 211(ε 8670), $\nu_{max}cm^{-1}$: 3420-2950, 1690, 1640, 1580, 1390; (V), $C_{15}H_{21}O_6N$ [SIMS, m/z 312$(M+1)^+$], mp(dec.) 257-260°C, $[\alpha]_D^{25}$ -30.0(c=0.015, H_2O), $\Delta\varepsilon_{220}$ -0.19, λ_{max}nm: 213(ε 5030), $\nu_{max}cm^{-1}$: 3360-2950, 1652, 1640, 1575, 1399. These amino acids were positive (yellow color) with ninhydrin and displayed acidic behavior on paper electrophoresis.

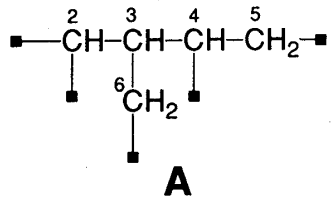
The COSY spectra of III, IV and V indicate the presence of a common partial structure A. The δ values for methine protons (at δ 3.96 in III, δ 4.04 in IV, and δ 4.08 in V) are similar to those of the signal due to the C-2 proton of amino acids such as L-proline. The δ values for methylene protons (at δ 3.45 and 3.67 in III, δ 3.45 and 3.60 in IV, and δ 3.43 and 3.59 in V) and other methylene protons (at δ 2.45 and 2.61 in III, δ 2.38 in IV, and δ 2.27 and 2.41 in V) reveal that the former are linked to a nitrogen atom while the latter are adjacent to a carboxylic acid function. These findings together with a positive ninhydrin color test demonstrate that these amino acids have a 4-substituted-2-carboxy-3-caboxymethyl pyrrolidine (the partial structure B, i.e., proline moiety). In fact, the δ and J values of III and domoic acid (I) are similar to each other except for the peak due to the C-4 proton signal, while those for IV and V also resemble those of α-kainic acid (II) except for the peak attributable to the C-4 proton (see Table I). Furthermore, as shown in Table II, the ^{13}C-NMR chemical shifts of III and IV are quite similar to those of the corresponding carbon signals on the pyrrolidine rings of domoic acid (I) and α-kainic acid (II) respectively. This indicates that the conformation of the pyrrolidine ring for III is the same as that of domoic acid (I), whereas the conformation for IV and V is identical with that of α-kainic acid (II).

The IR and UV spectra of III, IV and V have an α,β-unsaturated carboxylic acid in their side chains. The COSY spectra of III, IV and V indicate the presence of the partial structures C, D and E, respectively, in their side chains. The configurations of the two double bonds (C-1'-C-2' and C-4'-C-5') in the side chains for III and IV (Fig. 1) (Z and E in III, and E and E in IV) are indicated by the NOE difference experiments and ^{13}C-NMR spectral data (see Table II). On the other hand, the configuration of the C-4'-C-5' double bond for V is E. Thus, although there was an allylic coupling between the C-4' proton and the C-5' methyl protons in V, there was no NOE between the C-4' proton and C-5' methyl protons. The linkage of the pyrrolidine ring and the side chain for III, IV and V was proved the following facts. Irradiation of the C-1' methyl signal (at δ 1.75 in III and δ 1.72 in IV) in III and IV enhanced both the C-2α (at δ 3.96 in III and δ 4.04 in IV) and the C-5α protons (at δ 3.45 in III and δ 3.46 in IV). But in V, there was an NOE between the Ha proton (at δ 4.93) of the C-1' methylene group and the C-5α proton (at δ 3.59) and an allylic coupling between the Hb proton (at δ 5.15) of the C-1' methylene group and C-4β proton (at δ 2.99).

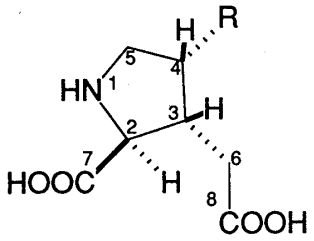
Table I. ^{1}H-NMR Spectral Data (in D_2O, 360 MHz)

	I	II	III	IV	V
C-2H	3.98 d 8.2	4.12 d 3.6	3.96 d 7.5	4.04 d 3.5	4.08 d 3.0
C-3H	3.05 dddd 8.2, 7.6, 5.1, 9.1	3.11 dddd 3.6, 7.2, 6.3, 8.3	2.98 dddd 7.5, 8.0, 6.0, 9.0	2.99 dddd 3.5, 7.0, 8.6, 7.5	3.09 dddd 3.0, 6.9, 8.6, 7.5
C-4H	3.82 ddd 7.6, 7.2, 7.6	3.03 ddd 7.2, 7.2, 11.7	3.62 ddd 8.0, 7.0, 10.0	2.91 *	2.99 ddd 6.9, 7.2, 12.1
C-5H$_2$	3.49 dd 7.2, 12.3 / 3.70 dd 7.6, 12.3	3.45 dd 11.7, 11.7 / 3.65 dd 7.2, 11.7	3.45 dd 10.0, 11.0 / 3.67 dd 7.0, 11.0	3.46 dd 12.0, 12.0 / 3.60 dd 7.0, 12.0	3.43 dd 12.1, 12.1 / 3.59 dd 7.2, 12.1
C-6H$_2$	2.50 dd 9.1, 16.8 / 2.75 dd 5.1, 16.8	2.40 dd 6.3, 16.6 / 2.49 dd 8.3, 16.6	2.45 dd 9.0, 16.0 / 2.61 dd 6.0, 16.0	2.38 d 7.0	2.27 dd 8.6, 16.8 / 2.41 dd 7.5, 16.8

*Patterns are unclear due to overlapping of the signals.



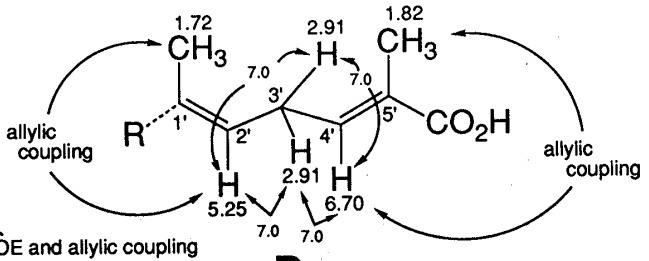
A



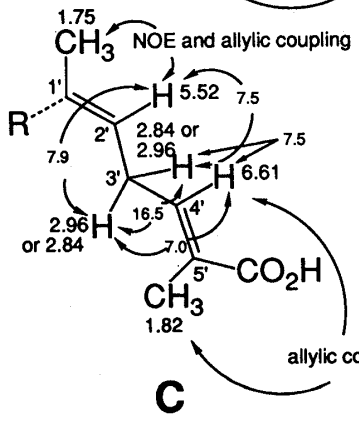
B

Table II. ^{13}C-NMR Spectral Data (in D_2O)

	I	II	III	IV
C-2	65.0	66.1	65.4	65.7
C-3	41.0	41.3	41.0	41.6
C-4	42.7	46.5	42.6	47.3
C-5	47.8	47.5	47.8	47.8
C-6	33.8	33.9	34.0	33.9
C-7	172.9	173.6	173.0	173.0
C-8	176.0	176.5	176.2	176.3
C-1'	132.4	140.6	131.9	132.2
C-1'-CH$_3$	22.2	22.9	22.1	16.8
C-2'	127.4	114.4	128.3	125.3
C-3'	131.6		28.0	28.1
C-4'	133.7		142.5	142.6
C-5'	43.4		128.8	125.5
C-5'-CH$_3$	17.2		12.5	12.5
C-5'-COOH	180.3		173.1	173.1



D



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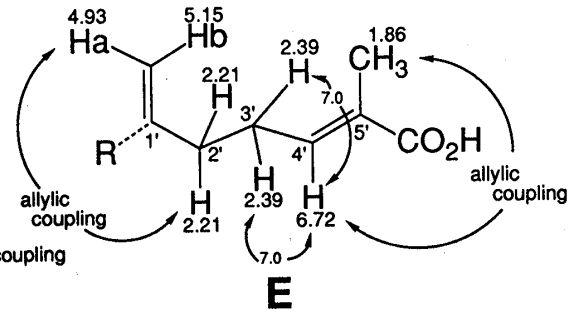
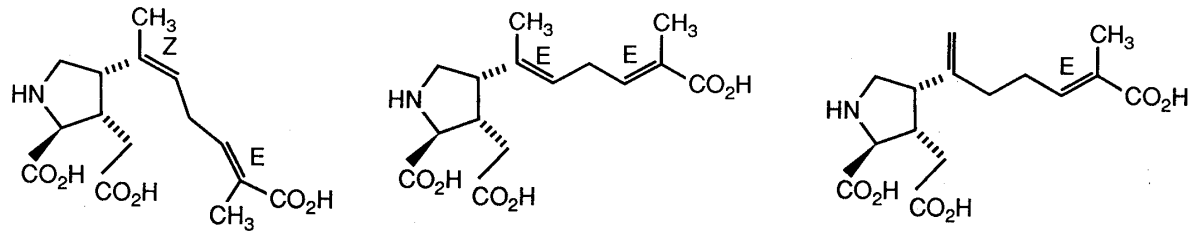


E

Thus, the stereostructures of isodomoic acids A, B and C have been established as shown in Fig. 1.



isodomoic acid A (III) isodomoic acid B (IV) isodomoic acid C (V)

Fig. 1

Isodomoic acids A, B and C were significantly insecticidal when they were injected subcutaneously into the abdomens of American cockroaches. Table III shows the minimum effective dose and the relative activity of the acids and their related compounds. As shown in Table III, the minimum doses of the acids are 3.2×10^{-8}, 3.2×10^{-8} and 6.4×10^{-8} mol, respectively.

Table III. Insecticidal Activity on Injection into American Cockroaches

	Minimum effective dose (mol)	Relative activity
Isodomoic acid A	3.2×10^{-8}	1.2
Isodomoic acid B	3.2×10^{-8}	1.2
Isodomoic acid C	6.4×10^{-8}	0.6
Domoic acid	2.6×10^{-9}	14.0
Natural pyrethrin	1.8×10^{-7}	0.2
γ-BHC	7.0×10^{-8}	0.5
DDT	3.7×10^{-8}	1.0

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