CINATRINS, A NOVEL FAMILY OF PHOSPHOLIPASE A_2 INHIBITORS

I. TAXONOMY AND FERMENTATION OF THE PRODUCING CULTURE; ISOLATION AND STRUCTURES OF CINATRINS

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Cinatrins A, B, C_1, C_2 and C_3, a family of phospholipase A_2 inhibitors were isolated from the fermentation broth of *Circinotrichum falcatisporum* RF-641. They were found to be novel spiro-γ-dilactones and γ-lactones derived from 1,2,3,5-tetra or 1,2,3(or 1,2,4)-trihydroxypentadecane-1,2,3-tricarboxylic acids. Structures were elucidated by MS and NMR studies and chemical transformations. The structure of cinatrin C_3 was confirmed by X-ray crystallographic analysis, and its absolute configuration was determined by comparison of the CD spectra with related compounds.

In a screening program for unique microbial products with pharmacological activity, the phospholipase A_2 (PLA_2) inhibitors cinatrins A (**1a**), B (**2a**), C_1 (**3a**), C_2 (**4a**) and C_3 (**5a**) were isolated from the fermentation broth of *Circinotrichum falcatisporum* strain RF-641. The taxonomy, fermentation, isolation, physico-chemical properties and structures of cinatrin congeners are described in this paper. The biological properties are reported in a companion paper.[1]

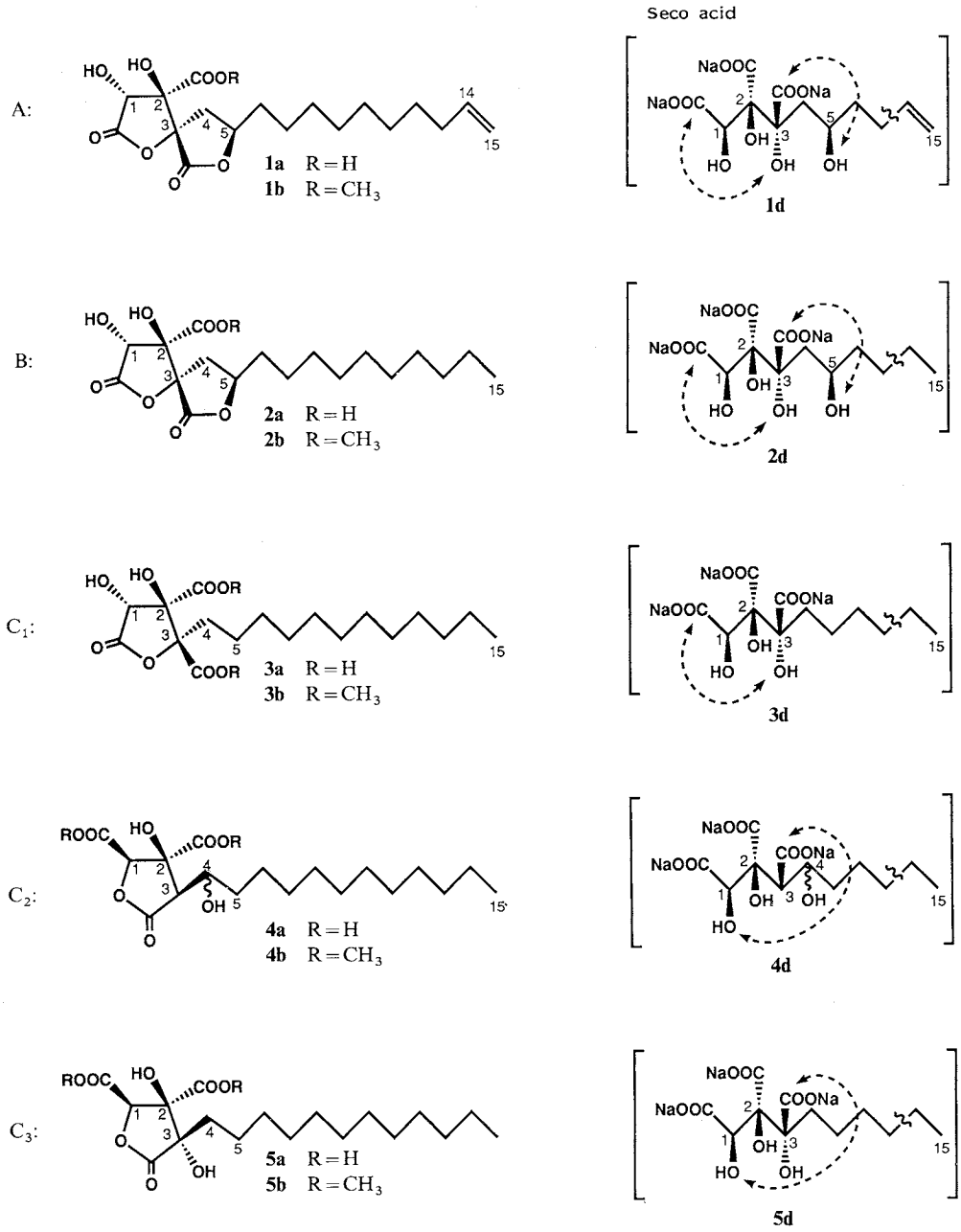
Taxonomy

Strain RF-641 does not show typical morphological properties on agar media, and the properties shown on a leaf of Indian-rubber-tree (*Ficus elastica*) are described. Colonies are punctiform to effused, dark brown to black, hairy, and composed of dark, branched and anastomosing hyphae bearing setae and sporogenous cells. Setae arising from dark brown, thick-walled and swollen cells of the superficial mycelium, are numerous, simple, erect, thick-walled, sparsely, and indistinctly septate, roughened, dark brown, opaque, darker near the base, paler towards the apex which is circinate or spirally twisted. Sporogenous cells are numerous, arising laterally on the superficial hyphae, obclavate to lageniform, thin-walled, subhyaline. Conidia are adherent, persisting at the bases of the setae in the form of a whitish pellicle, falcate with acute ends, $18.5 \sim 20.0 \times 1.7 \mu m$. Based on the taxonomic properties described above, strain RF-641 was identified as *Circinotrichum falcatisporum* Pirozynsky (1962).[2] Strain RF-641 has been deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, under the accession No. FERM P-10681.

Fermentation

A slant culture of strain RF-641 was inoculated into a seed medium (100 ml) containing Polypepton 1.0%, glucose 2.0%, beef extract 0.3%, yeast extract 0.2%, NaCl 0.1%, and tap water (pH 7.0) in a 500-ml Sakaguchi flask, and cultured at 28°C for 72 hours on a rotary shaker at 120 rpm. The seed culture was used at the rate of 4% to inoculate 100 ml of the production medium in each of one hundred 500-ml Erlenmeyer flasks and cultivation was carried out at 28°C for 96 hours under agitation at 180 rpm. The

Fig. 1. Structures of cinatrins.



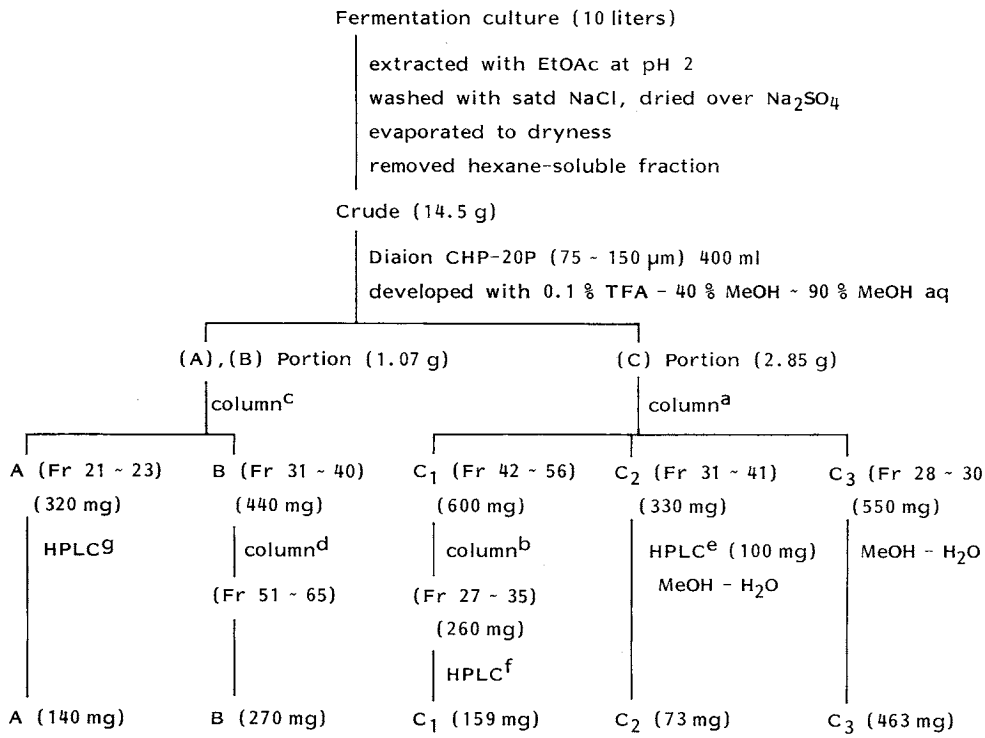
production medium contained 20% potato decoction 1 liter and sucrose 20 g (pH 7.0).

Isolation

The purification procedures of the cinatrins are outlined in Fig. 2. After the fermentation culture (10 liters) was adjusted with 2 N HCl to pH 2, cinatrins were extracted with EtOAc (3 liters). The organic layer was worked up in the usual manner to give a crude extract.

This extract was combined with hexane (1 liter) to remove a hexane soluble fraction and obtain a

Fig. 2. Isolation of cinatrins.



Column chromatography: Column, LiChroprep RP-18, 25~40 μm (20 i.d. × 500 mm); flow rate, 5 ml/minute; detection, 210 nm; one fraction 15 g.

[a] Mobile phase, 0.1% TFA-50% MeOH, [b] 0.1% TFA-50% MeCN, [c] 0.1% TFA-55% MeCN, [d] 0.1% TFA-70% MeOH, preparative HPLC: column, Cosmosil 5C18 (20 × 150 mm); detection, UV 210 nm; flow rate, 8 ml/minute, [e] 0.1% TFA-50% MeCN; Rt 33 minutes (C_2); [f] 0.1% TFA-50% MeCN aq; Rt 28 minutes (C_1); [g] 0.1% TFA-55% MeCH aq; Rt 20 minutes (A).

crude material (14.5 g). This material was dissolved in 40% MeOH (100 ml) containing 0.1% trifluoroacetic acid (TFA), and adsorbed on a Diaion CHP-20P column (400 ml), which was developed with a gradient from 40% to 90% MeOH containing 0.1% TFA. Each fraction (Fr) weighed 15 g. The eluates from Fr 116 to 130 gave a mixture of C_1, C_2 and C_3 (2.85 g). The eluate from Fr 137 to 152 afforded a mixture of A and B (1.07 g). The former mixture was further purified by column chromatography into three portions, the C_3 portion (Fr 28~30, 550 mg), C_2 portion (Fr 31~41, 330 mg) and C_1 portion (Fr 42~56, 600 mg). Recrystallization of the C_3 portion from MeOH-H_2O gave pure cinatrin C_3 (463 mg, mp 205~207°C, colorless fine needles). The C_2 portion was purified by HPLC to afford cinatrin C_2 (100 mg), which was recrystallized from MeOH-H_2O to give pure cinatrin C_2 (73 mg, mp 152~154°C, colorless fine needles). The C_1 portion was subjected to column chromatography and gave an eluate of 260 mg, which was further purified by HPLC to yield pure cinatrin C_1 (159 mg, amorphous powder). The latter mixture (1.07 g) was subjected to column chromatography and gave A (Fr 21~23, 320 mg) and B portions (Fr 31~40, 440 mg). Pure cinatrin A (140 mg, colorless amorphous powder) was obtained by HPLC of the A portion, and pure cinatrin B (270 mg, colorless amorphous powder) was obtained by column chromatography of the B portion.

Table 1. Physico-chemical properties of cinatrins.

	A (**1a**)	B (**2a**)	C_1 (**3a**)	C_2 (**4a**)	C_3 (**5a**)
Appearance	Colorless powder	Colorless powder	Colorless powder	Colorless needles	Colorless needles
MP (°C)	170~172	190~192	162~164	152~154	205~207
$[\alpha]_D^{24}$ MeOH	−20.1° (*c* 0.303)	−24.4° (*c* 0.308)	−11.2° (*c* 0.314)	−54.5° (*c* 0.312)	−86.1° (*c* 0.519)
Molecular formula	$C_{18}H_{26}O_8$	$C_{18}H_{28}O_8$	$C_{18}H_{30}O_8$	$C_{18}H_{30}O_8$	$C_{18}H_{30}O_8$
Elemental analysis		$C_{18}H_{28}O_8 \cdot \frac{1}{2}H_2O$		$C_{18}H_{30}O_8 \cdot \frac{1}{4}H_2O$	
Calcd:		C 56.63, H 7.66		C 57.05, H 8.11	C 57.74, H 8.08
Found:		C 56.58, H 7.53		C 57.23, H 8.07	C 57.51, H 8.00
SI-MS (*m/z*, $(M+H)^+$)	371	373	375	375	467 $(M+Gly)^+$, 749 $(2M+H)^+$
UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$)	220 (sh, 11)	220 (sh, 10)	220 (sh, 11)	End absorption	220 (sh, 10)
IR (KBr) cm^{-1}	1776, 1747	1795, 1767, 1750 (sh)	1785, 1739	1777, 1725, 1689	1824, 1723, 1695
TLC[a] Rf	0.47	0.47	0.29	0.29	0.29
HPLC[b] Rt minutes	12.7	20.2	12.4	13.1	14.8

[a] Merck silica gel F-254; $CHCl_3$ - MeOH - H_2O, 2:2:1 (lower layer) - AcOH (9:1).

[b] Column, Cosmosil 5C18 (4.6 × 250 mm); mobile phase, 0.1% TFA - 55% CH_3CN aq; flow rate, 1 ml/minute; detect, UV 210 nm.

Results and Discussion

Structures of Cinatrins A, B, C_1, C_2 and C_3

Cinatrin congeners A (**1a**), B (**2a**), C_1 (**3a**), C_2 (**4a**) and C_3 (**5a**) were acidic in nature, soluble in methanol and dimethyl sulfoxide, slightly soluble in chloroform and substantially insoluble in water. They gave a positive color reaction with iodide, bromocresol green and phosphomolybdic acid. Their physico-chemical properties are listed in Table 1. The UV spectra showed end absorptions. The IR spectra of cinatrin congeners displayed absorption bands at $1824 \sim 1725\,cm^{-1}$, indicating the presence of both lactone and carboxylic acid moieties.

The chemical shifts of carbons and protons of cinatrins and their methyl esters are shown in Tables 2~4. Detailed NMR analyses such as ^1H-^1H homonuclear chemical shift correlation spectroscopy (HOMCOR), ^{13}C-^1H HETCOR and rotating frame Overhauser enhancement spectroscopy (ROESY) of cinatrin congeners revealed the structures and relative configurations shown in Fig. 1.

The structure of **5a** was established by X-ray analysis and the absolute configuration by CD. The absolute configurations of the other cinatrins (**1a**, **2a** and **4a**) were ambiguous. Since they were produced by fermentation of the same strain, it was assumed that the configurational situations about carbons 1, 2 and 3 of the precursors (tricarboxylic acids) are the same. The measured relative configurations of cinatrins offered support for this assumption.

Cinatrin C_3 (**5a**)

The molecular formula of **5a** was established as $C_{18}H_{30}O_8$ from the elemental analysis, SI-MS and ^{13}C NMR spectra. Methylation of **5a** with diazomethane afforded a dimethyl ester (**5b**). The structure of

Table 2. ^{13}C NMR data of cinatrins (a series) and their methyl esters (b series) (in DMSO-d_6 at 26°C).

Position	A		B		C_1		C_2		C_3	
	1a	**1b**	**2a**	**2b**	**3a**	**3b**	**4a**	**4b**	**5a**	**5b**
1	72.8 d	73.0 d	72.6 d	73.2 d	73.1 d	73.0 d	81.0 d	81.1 d	79.4 d	79.6 d
1-*C*O	172.8 s	172.1 s	172.4 s	172.6 s	173.6 s	172.6 s	166.4 s	165.8 s	167.5 s	166.9 s
1-COO*C*H$_3$	—	—	—	—	—	—	—	52.4 q	—	52.3 q
2	83.8 s	84.2 s	83.6 s	84.4 s	84.0 s	84.3 s	80.4 s	81.0 s	81.3 s	82.4 s
2-*C*O	169.9 s	168.7 s	169.5 s	169.1 s	170.4 s	168.8 s	171.7 s	170.8 s	170.4 s	169.4 s
2-COO*C*H$_3$	—	52.8 q	—	53.0 q	—	52.8 q	—	53.1 q	—	52.7 q
3	84.3 s	84.2 s	84.1 s	84.4 s	86.5 s	86.8 s	53.6 d	53.7 d	78.7 s	78.8 s
3-*C*O	172.7 s	172.0 s	172.3 s	172.4 s	170.6 s	168.9 s	173.2 s	172.8 s	174.6 s	174.4 s
3-COO*C*H$_3$	—	—	—	—	—	52.3 q	—	—	—	—
4	36.2 t	35.9 t	36.2 t	36.0 t	30.8 t	30.7 t	66.8 d	66.8 d	30.5 t	30.4 t
5	77.3 d	77.2 d	77.1 d	77.4 d	23.5 t	23.4 t	33.3 t	33.3 t	21.0 t	21.0 t
6	34.4 t	34.3 t	34.3 t	34.4 t	28.7 t	28.5 t	25.0 t	25.0 t	29.6 t	29.6 t
7	24.4 t	24.3 t	24.4 t	24.4 t	28.8 t	28.7 t	28.8 t	28.9 t	28.9 t	29.0 t
8	28.6 t	28.5 t	28.5 t	28.6 t	29.2 t	28.9 t	29.0 t	29.0 t	29.0 t	29.0 t
9	28.8 t	28.7 t	28.7 t	28.8 t	29.0 t	28.9 t	29.0 t	29.0 t	29.0 t	29.0 t
10	28.7 t	28.6 t	28.8 t	28.9 t	29.0 t	28.9 t	29.0 t	29.0 t	29.0 t	29.0 t
11	28.5 t	28.4 t	28.9 t	29.0 t	29.0 t	28.9 t	29.0 t	29.0 t	29.0 t	29.0 t
12	28.2 t	28.2 t	28.6 t	28.7 t	28.7 t	28.6 t	28.6 t	28.7 t	28.7 t	28.7 t
13	33.2 t	33.1 t	31.2 t	31.3 t	31.3 t	31.2 t	31.2 t	31.3 t	31.2 t	31.3 t
14	139.1 d	138.7 d	22.0 t	22.1 t	22.1 t	22.0 t	22.0 t	22.1 t	22.0 t	22.1 t
15	114.9 t	114.5 t	13.9 q	13.9 q	13.9 q	13.9 q	13.9 q	13.9 q	13.9 q	13.9 q

Table 3. ^{1}H NMR data of cinatrins (in DMSO-d_6 at 26°C).

Position	1a	2a	3a	4a	5a
1	4.74 (s)	4.73 (s)	4.56 (s)	5.12 (s)	5.32 (s)
1-OH	6.70 (br)	6.65 (br)	6.39 (br)	—	—
2-OH				6.30 (br)	6.25 (br)
3	—	—	—	3.15 (d, J=5.8)	—
3-OH	—	—	—	—	Not determined
4a	2.33 (dd, J=14.1, 10.0)	2.32 (dd, J=14.1, 10.0)	1.55 (m)	3.82 (m)	a, b 1.69 (m)
4b	2.39 (dd, J=14.1, 5.8)	2.39 (dd, J=14.1, 5.8)	2.04 (m)		
4-OH	—	—	—	4.82 (m)	—
5	4.54 (m)	4.53 (m)		1.52 (m)	1.41 (m)
6	1.64 (m)	1.65 (m)		1.38 (m)	
7	1.34 (m)	1.34 (m)			
8					
9	(C-8∼C-11)	(C-8∼C-14)	(C-5∼C-14)	(C-7∼C-14)	(C-6∼C-14)
10	∼1.26 (m)	∼1.26 (m)	∼1.24 (m)	∼1.24 (m)	∼1.24 (m)
11					
12	1.34 (m)				
13	2.02 (m)				
14	5.79 (m)				
15	4.94 (m), 4.98 (m)	0.86 (t like)	0.86 (t like)	0.86 (t like)	0.86 (t like)

J=Hz.

Table 4. ^{1}H NMR data of cinatrin methyl esters (in DMSO-d_6 at 26°C).

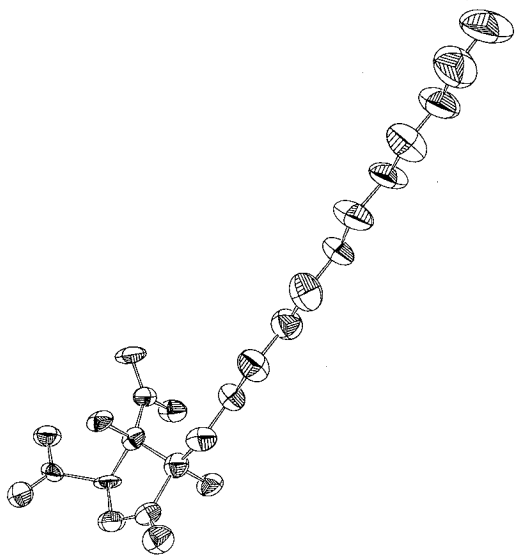
Position	1b	2b	3b	4b	5b
1	4.76 (d, J=6.7)	4.76 (d, J=6.6)	4.52 (d, J=6.8)	5.26 (s)	5.51 (d, J=0.8)
1-OH	6.81 (d, J=6.7)	6.85 (d, J=6.6)	6.59 (d, J=6.8)	—	—
1-COOCH$_3$	—	—	—	3.67 (s)	3.67 (s)
2-OH	7.34 (s)	7.39 (s)	7.06 (s)	6.65 (s)	6.74 (d, J=0.8)
2-COOCH$_3$	3.75 (s)	3.76 (s)	3.70 (s)	3.78 (s)	3.73 (s)
3	—	—	—	3.22 (d, J=5.8)	—
3-OH	—	—	—	—	6.41 (s)
3-COOCH$_3$	—	—	3.73 (s)	—	—
4a	2.31 (dd, J=14.3, 9.9)	2.31 (dd, J=14.3, 9.9)	1.43 (m)	3.83 (m)	a, b, 1.40 (m)
4b	2.39 (dd, J=14.3, 5.7)	2.39 (dd, J=14.3, 5.9)	2.06 (m)		
4-OH	—	—	—	4.94 (d, J=4.8)	—
5	4.53 (m)	4.54 (m)		1.48 (m)	
6	1.64 (m)	1.64 (m)			
7	1.34 (m)	1.33 (m)			
8	(C-8∼C-11)	(C-8∼C-14)	(C-5∼C-14)	(C-6∼C-14)	(C-5∼C-14)
9	∼1.26 (m)	∼1.25 (m)	∼1.24 (m)	∼1.24 (m)	∼1.24 (m)
10					
11					
12	1.34 (m)				
13	2.02 (m)				
14	5.79 (m)				
15	4.93 (m), 4.99 (m)	0.86 (t like)	0.86 (t like)	0.86 (t like)	0.86 (t like)

J=Hz.

5a, which had been postulated by detailed NMR studies of **5a** and **5b**, was determined by X-ray crystallographic analysis to be 1,2,3-trihydroxypentadecane-1,2,3-tricarboxylic acid, (3→1)-γ-lactone as shown in Fig. 3. The carbon and proton signals of **5a** and **5b** in the ^{1}H and ^{13}C NMR spectra were reasonably assigned (see Tables 2, 3 and 4). Since the X-ray diffraction of **5a** only revealed the relative

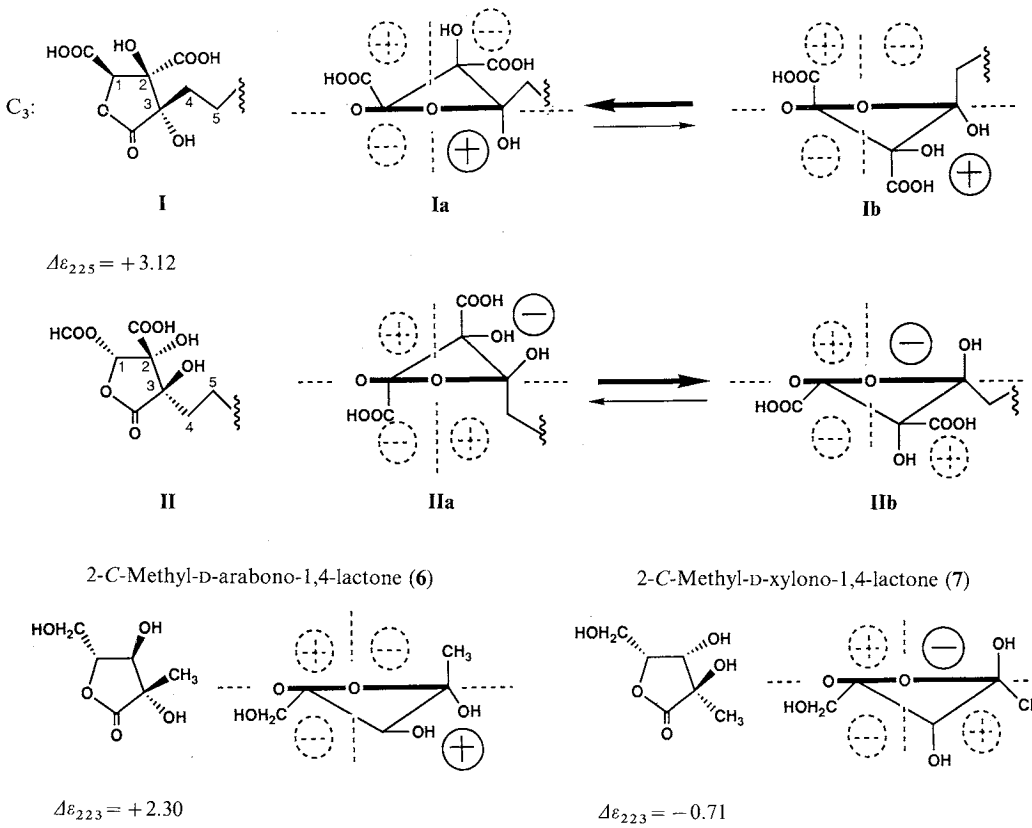
configuration, we used CD spectroscopy to determine its absolute configuration. As shown in Fig. 4, if the configuration of **5a** were **I**, its more stable conformation would be **Ia**. On the other hand, if the configuration of **5a** were **II**, its more stable conformation would be **IIb**. It was assumed from the octant rule that the Cotton effect of **Ia** would show a plus signal due to the effect of the 3-α-hydroxyl moiety, while that of **IIb** would indicate a minus signal due to the effect of the 3-β-hydroxyl moiety. Since a plus Cotton effect ($\Delta\varepsilon = +3.12$) was observed in the CD spectrum of **5a**, its absolute configuration was considered to be **I**. The same findings were obtained from the CD spectra of **6** and **7**, which were related to **5a** and its enantiomer. Therefore, the absolute configuration of **5a** was determined to be **I** by comparison of its CD with that of 2-*C*-methyl-D-arabono-1,4-lactone (**6**).[3]

Fig. 3. A perspective view of cinatrin C_3 (**5a**) by ORTEP drawing.



Thermal ellipsoids are represented with 50% probability.

Fig. 4. CD analysis of cinatrin C_3 (**5a**).



Cinatrin C_1 (**3a**): 1,2,3-Trihydroxypentadecane-1,2,3-tricarboxylic Acid, (1→3)-γ-Lactone

The molecular formula of **3a** was established to be $C_{18}H_{30}O_8$, which was identical to that of **5a**, from the SI-MS and ^{13}C NMR spectra. Methylation of **3a** with diazomethane afforded a dimethyl ester (**3b**), and acetylation of **3b** gave a mono-*O*-acetate. On the base of detailed NMR analysis, the structure of **3a** was assumed to be a lactone isomer of **5a** (see Tables 2 and 3). Compared with **3a** or **3b**, the acetate exhibited an expected downfield shift (*i.e.* acetylation shift) of the proton on carbon 1 ($\Delta\delta$ 1.15 ppm). The structure of **3a** was confirmed by chemical transformation. Cinatrin C_1 (**3a**) was hydrolized by sodium hydroxide solution at room temperature for 2 hours to give a seco acid (**3d**), which was relactonized by treatment with an acid. The resulting product was determined to be a mixture of **3a** and **5a** (1 : 1) by HPLC. According to the same procedure, cinatrin C_3 (**5a**) was converted into a mixture of **5a** and **3a** in equal amounts. This indicated that these lactones (**3a** and **5a**) were produced by translactonization *via* the seco acid (**3d**=**5d**). Since cinatrin C_3 (**5a**) had been previously determined to be the (3→1)-γ-lactone, **3a** must be the (1→3)-γ-lactone as shown in Fig. 1.

Cinatrin C_2 (**4a**): 1,2,4-Trihydroxypentadecane-1,2,3-tricarboxylic Acid, (3→1)-γ-Lactone

The molecular formula of **4a** was also determined to be $C_{18}H_{30}O_8$ by elemental analysis, SI-MS and ^{13}C NMR spectra. Methylation of **4a** with diazomethane afforded a dimethyl ester (**4b**), and acetylation of **4b** gave a monoacetate. Except for the carbonyl signals, the ^{13}C NMR spectrum of **4a** showed only one quaternary carbon signal at δ 80.4 (s, C-2) and three tertiary signals at δ 53.6 (d, C-3), 66.8 (d, C-4) and 81.0 (d, C-1). The dimethyl ester **4b** clearly exhibited signals of two hydroxy protons, which were not clear in **4a**. The proton signals revealing the partial structure near the C-3 and C-4 positions appeared at δ 3.22 (1H, d, J=5.8 Hz, 3-H), 3.83 (1H, m, 4-H) and 4.94 (1H, d, J=4.8 Hz, 4-OH). Based on the NMR data and comparison with that of **5a**, it was concluded that the structure of **4a** was a lactone similar to **5a** but that; the hydroxy group found at C-3 in **5a** was at the neighboring C-4 position in **4a**. As illustrated in Fig. 5, HETCOR analysis focusing on the long-range ^{13}C-^1H correlation confirmed the structure. The relative configuration was deduced from an observed NOE between 1-H and 3-H in spectrum of the ester **4b**. No NOE was observed between 2-OH and 1-H or 3-H in the same experiment.

Cinatrin B (**2a**): 1,2,3,5-Tetrahydroxypentadecane-1,2,3-tricarboxylic Acid, (1→3)-γ-Lactone, (3→5)-γ-Lactone

The molecular formula of **2a** was established as $C_{18}H_{28}O_8$ by elemental analysis, SI-MS and ^{13}C NMR spectra. Methylation of **2a** with diazomethane afforded a monomethyl ester (**2b**), and acetylation of **2b** gave a monoacetate. The ^{13}C NMR data of **2a** indicated the existence of three carbonyls assigned to the ester or carboxylic acid type. The ^1H NMR of **2b** exhibited two hydroxyl proton signals, a doublet and a singlet. These findings show that **2a** has a free carboxylic acid, two ester-type carbonyls, a secondary and a tertiary hydroxyl group. Reduction of **2b** with $LiAlH_4$, followed by acetylation with Ac_2O and pyridine, gave a pentaacetate (**2c**) which

Fig. 5. Long-range ^{13}C-^1H correlation of cinatrin C_2 (**4a**).

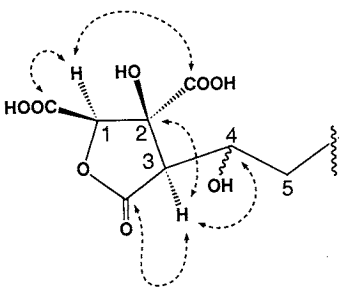


Fig. 6. Reduction product of cinatrin B methyl ester (**2b**).

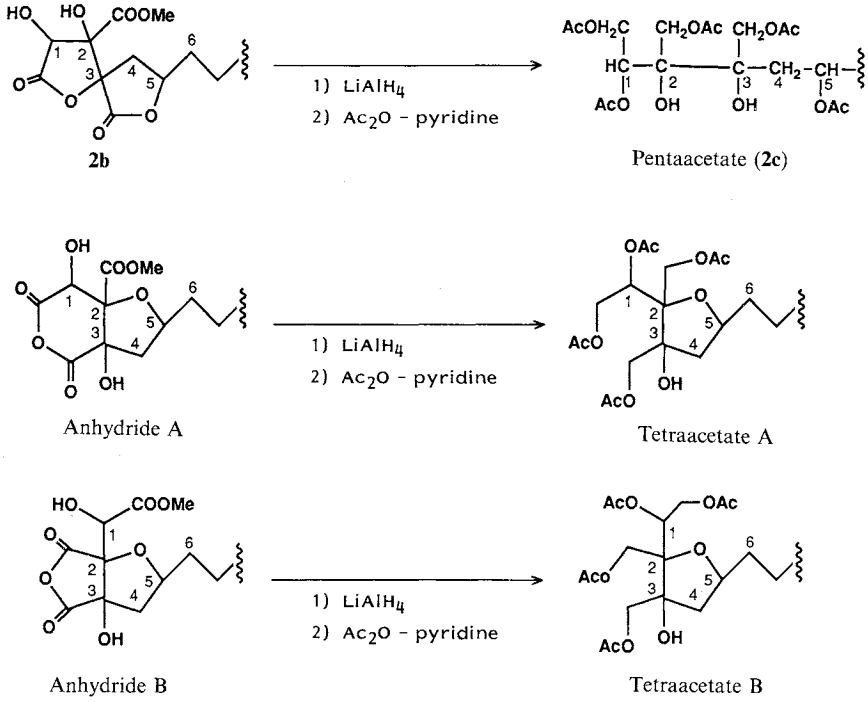
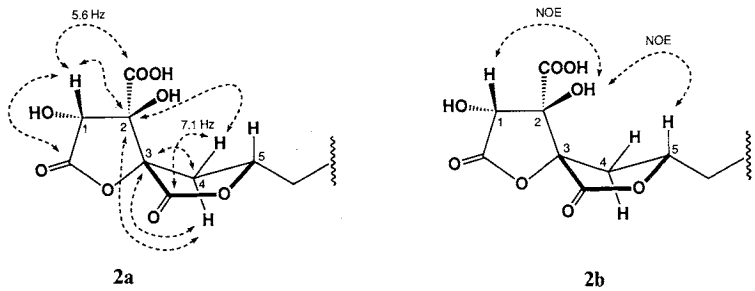


Fig. 7. Long-range ^{13}C-^{1}H correlation of cinatrin B (**2a**).



was found to be 1,2,3-tris(acetoxymethyl)pentadecane-1,2,3,5-tetraol 1,5-diacetate by ^{1}H and ^{13}C NMR spectra. If **2b** had other possible structures, such as the anhydrides A and B shown in Fig. 6, the tetraacetate compound would have been obtained by reduction of **2b**, followed by acetylation. From this finding, the structure of **2a** was concluded to be a spirodilactone.

As illustrated in Fig. 7, the long-range ^{13}C-^{1}H correlation, $^{3}J_{C,H}$ values and NOEs among the protons 1-H, 2-OH and 5-H confirmed the structure and relative configuration; no NOE was observed between 2-OH and the protons on C-4. All the signals could be reasonably assigned for the structure of **2a** by detailed NMR experiments (Tables 2~4).

Cinatrin A (**1a**): 1,2,3,5-Tetrahydroxy-14-pentadecene-1,2,3-tricarboxylic Acid, (1→3)-γ-Lactone, (3→5)-γ-Lactone

The molecular formula of **1a** was established as $C_{18}H_{26}O_8$, which was two mass units less than that

of **2a**, by SI-MS and ^{13}C NMR spectra. Methylation of **1a** gave a monomethyl ester (**1b**), and acetylation of **1b** afforded a monoacetate. The ^{1}H and ^{13}C NMR spectral features of **1a** were quite similar to those of **2a**, although a significant difference was found in the side chain part. Cinatrin A had signals assigned to an olefinic terminal moiety in place of an ethyl terminal moiety for **2a**; ^{13}C NMR δ 114.9 (t, C-15) and 139.1 (d, C-14). Thus, **1a** was converted into **2a** by catalytic hydrogenation over 10% Pd-C in MeOH. Therefore, the structure of **1a** was determined to be 1,2,3,5-tetrahydroxy-14-pentadecene-1,2,3-tricarboxylic acid, (1→3)-γ-lactone, (3→5)-γ-lactone.

Experimental

NMR spectra were measured with a Varian XL-400 spectrometer in $CDCl_3$ or DMSO-d_6 solution with the internal standard TMS. Mass spectra were obtained with a Hitachi M-90 spectrometer, and IR spectra with a Jasco DS-403G spectrometer.

X-Ray Crystallographic Analysis of Cinatrin C_3 (**5a**)

The molecular structure of cinatrin C_3 (**5a**) was determined by X-ray analysis. Colorless plate crystals were obtained from methanol-water solution.

Crystal data: monoclinic; space group $P2_1$; $a=26.924(4)$, $b=6.803(1)$, $c=5.528(1)$ Å, $\beta=91.89(2)°$; $V=1011.9(3)$ Å^3; $Z=2$; $D_x=1.229$ g/cm^3. A crystal of dimensions $0.8\times0.6\times0.1$ mm was mounted on a Rigaku AFC-5R diffractometer. Intensities were measured using graphite monochromatized Cu Kα radiation by ω scans in the range $\theta\leqq60°$ with a scan width 4° and a constant scan rate of 3° minute^{-1}. A total of 1,649 unique reflections was measured and corrected for Lorentz and polarization factors, but not for absorption effects.

The structure was solved by MULTAN87.[4] A perspective view of the molecule drawing by ORTEP is shown in Fig. 3.[5] Hydrogen atoms were not found on the difference density map. Positional parameters and anisotropic thermal parameters of non-H atoms were refined by block diagonal least squares. From the least squares, hydrogen atoms of the hydrocarbons were calculated and fixed at their ideal positions. The temperature factor of each H atom was set equal to B_{eq} of the bonded atom. The weighting scheme used was $w=1/[\sigma^2(F_O)+0.00332|F_O|^2]$ for $w^{1/2}|\Delta F|\geqq3$, and $w=0$ for other cases. The final R value ($\Sigma|\Delta F|/\Sigma|\Delta F_O|$) was 0.076 for 1,073 observed reflections ($F_O>3\sigma$) and wR was 0.084, $S=1.1663$. Intermolecular short distances between oxygen atoms less than 3.2 Å are O(1)---O(9)(x, y, $z+1$)=3.17(1) Å, O(1)---O(12)(x, $y+1$, z)=2.98(1), O(7)---O(8)($-x$, $y-1/2$, $1-z$)=3.18(1), O(7)---O(8)($-x$, $y-1/2$, $-z$)=3.02(1), O(7)---O(11)($-x$, $y+1/2$, $-z$)=2.72(1), O(7)---O(12)($-x$, $y+1/2$, $1-z$)=3.04(1), O(8)---O(12)(x, $y+1$, z)=2.69(1), and O(9)---O(13) (x, y, $z-1$)=2.86(1).

Conversion of Cinatrin C_1 (**3a**) into C_3 (**5a**)

A mixture of cinatrin C_1 (4 mg) with 0.05 N NaOH (1 ml) was stirred for 2 hours at room temperature (disappearance of the starting material and production of the seco acid as monitored by HPLC). After the mixture was acidified at pH 1 by adding 0.05 N HCl (1.5 ml), it was heated at 45°C for 1 hour and left overnight at room temperature. The resulting mixture was extracted with EtOAc. The extract was washed with NaCl solution, dried over Na_2SO_4 and evaporated *in vacuo*. The product obtained was found to be a nearly 1 : 1 mixture of **3a** and **5a** by HPLC analysis (with cinatrin C_3, the same procedure gave a mixture of C_1 and C_3 in equal amounts).

Conversion of Cinatrin B Methyl Ester (**2b**) into 1,2,3-Tris(acetoxymethyl)pentadecane-1,2,3,5-tetraol 1,5-Diacetate (**2c**)

A mixture of **2b** (10 mg, 0.026 mmol) and $LiAlH_4$ (30 mg, 0.789 mmol) in anhydrous THF (3 ml) was refluxed for 3 hours. After excess $LiAlH_4$ was decomposed by adding 1 N HCl (1.5 ml) under cooling on an ice bath, the reaction mixture was concentrated to dryness *in vacuo*. The residue was extracted with a mixed solvent (2 ml) of $CHCl_3$-MeOH (8 : 2), and the extract was concentrated to dryness *in vacuo*. A solution of Ac_2O (1 ml) and pyridine (3 ml) was added to this residue, and the mixture was allowed to

Table 5. Methyl esters of cinatrins (yield and SI-MS).

Cinatrins	Yield (%)	SI-MS (m/z $(M+H)^+$)
A (**1b**)	54	385 ($C_{19}H_{28}O_8+H$)
B (**2b**)	47	387 ($C_{19}H_{30}O_8+H$)
C_1 (**3b**)	63	403 ($C_{20}H_{34}O_8+H$)
C_2 (**4b**)	34	403 ($C_{20}H_{34}O_8+H$)
C_3 (**5b**)	64	403 ($C_{20}H_{34}O_8+H$)

Table 6. Sodium salts of cinatrins seco acids (yield and SI-MS).

Cinatrin	Yield (%)	HPLC[a] (Rt minutes)	SI-MS (m/z (molecular formula))
A (**1d**)	27	2.7	472 ($C_{18}H_{27}O_{10}Na_3$)
B (**2d**)	34	3.5	474 ($C_{18}H_{29}O_{10}Na_3$)
C_1 (**3d**)	38	6.3	458 ($C_{18}H_{29}O_9Na_3$)
C_2 (**4d**)	36	4.6	458 ($C_{18}H_{29}O_9Na_3$)
C_3 (**5d**)	39	6.3	458 ($C_{18}H_{29}O_9Na_3$)

[a] HPLC: column, Cosmosil-5C18 (4.6×150 mm); mobile phase, 0.1% TFA-CH_3CN (45:55); flow rate, 1 ml/minute; detection, UV 220 nm.

stand overnight at room temperature. After water (10 ml) had been added, the mixture was extracted twice with EtOAc (5 ml). The extracts were combined and washed with NaCl solution, dried over Na_2SO_4 and evaporated *in vacuo*, giving an oil (19 mg), which was further purified by TLC (Merck, KGF, 5% MeOH-CH_2Cl_2, Rf 0.4) to afford a pure compound (3.3 mg). **2c**: $C_{28}H_{48}O_{12}$; ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t like, CH_3), 1.26 (16H, s like, CH_2), 1.58 (2H, m, CH_2), 2.02~2.11 (8H, m, CH_2), 2.02, 2.07, 2.08, 2.10, 2.11 (each 3H, $OCOCH_3$), 4.11 (2H, d, J=12 Hz), 4.22 (1H, dd, J=3 and 12 Hz), 4.27 (1H, d, J=12 Hz), 4.29 (1H, d, J=12 Hz), 4.63 (1H, dd, J=3 and 12 Hz), 4.97 (1H, m), 5.54 (1H, dd, J=3 and 9 Hz). ^{13}C NMR ($CDCl_3$) δ 14.0, 20.7×2, 20.8, 21.0, 21.3, 22.7, 25.4, 29.3, 29.5, 29.6×3, 32.0, 36.3, 38.5, 63.5, 64.2, 65.6, 71.7, 73.0, 76.2, 76.7~77.4, 170.1×2, 170.6, 170.7, 171.9.

1,2,3-Tris(acetoxymethyl)pentadecane-1,2,3-triol 1-Acetate (**5c**)

5c ($C_{25}H_{44}O_{11}$) was obtained from cinatrin C_3 methyl ester (**5b**) according to the above procedure. ^{1}H NMR ($CDCl_3$) δ 0.85 (3H, t like, CH_3), 1.26 (18H, s like, CH_2), 1.58 (4H, br, CH_2), 2.03, 2.09, 2.10, 2.12 (each 3H, s, $OCOCH_3$), 4.12~4.30 (5H, m), 4.70 (1H, dd), 5.50 (1H, dd).

Conversion of **1a** into **2a**

Cinatrin A (**1a**) (4.9 mg) in MeOH (2 ml) was catalytically hydrogenated with 5% Pd-C (6.6 mg) under atmospheric pressure with absorption of hydrogen gas for 2 hours. After removal of the catalyst by filtration, evaporation of the solvent gave **2a** (4.9 mg), which was identified with an authentic sample by HPLC.

Preparation of Cinatrin Methyl Esters

An ether solution of diazomethane was added to a solution of cinatrin A (50 mg) in THF (5 ml) and Et_2O (10 ml) under ice-bath cooling, and then the mixture was allowed to stand at room temperature for 30 minutes. After excess diazomethane had been decomposed by adding three drops of acetic acid, the reaction mixture was evaporated to dryness *in vacuo*. The residue was subjected to preparative TLC (Merck, F-254, CH_2Cl_2-MeOH, 95:5, Rf 0.3) giving a crude product (48 mg). This was further purified by HPLC (column, Cosmosil 5C18 20×150 mm; mobile phase, 80% CH_3CN; flow rate, 6 ml/minute; detection, UV 220 nm; Rt 10.5 minutes) to afford cinatrin A methyl ester (**1b**) (28 mg, yield 54%). As shown in Table 5, cinatrins B, C_1, C_2 and C_3 methyl esters were obtained by the same procedure.

Preparation of Cinatrin Seco Acids

Cinatrin A (5 mg) was dissolved in 0.05 N NaOH (1 ml), and the mixture was allowed to stand at room temperature for 16 hours. After the reaction was completed, the reaction mixture was adsorbed on a column of Diaion HP-20 (2 ml), and then the column was washed with 20% NaCl solution (5 ml), followed by distilled water until the eluate was free of chloride ions. Cinatrin A seco acid was eluted with 50% MeOH. After the eluate was concentrated *in vacuo*, the product was lyophilized to give the sodium salt of cinatrin A seco acid (1.7 mg, yield 27%). As shown in Table 6, sodium salts of cinatrins B, C_1, C_2 and C_3 seco acid were obtained by the same procedure, and C_1 seco acid was found to be identical with C_3 seco acid.

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