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Cinnatriacetins A and B, New Antibacterial Triacetylene Derivatives from the Fruiting Bodies of *Fistulina hepatica*

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In the course of our screening program for antimicrobial agents, we isolated two novel triacetylene derivatives named cinnatriacetin A (**1**) and cinnatriacetin B (**2**), from the fruiting bodies of the Japanese edible mushroom 'kanzotake', *Fistulina hepatica*. In this paper, we report isolation, characterization, structure elucidation, and biological activities of **1** and **2**.

F. hepatica was grown on a sawdust based substrate consisting of *Fagaceae* sawdust (81% dry wt.) and malted rice (19%) with water added to give final moisture content of 58%. Polyethylene bags (20 cm × 12 cm × 43 cm) were filled with the sawdust based substrate to 2.5 kg (wet wt.). The bags were then autoclaved at 121°C for one hour, cooled and inoculated with 17 g sawdust spawn. The bags were incubated in darkness at 25°C for fruiting initiation. After 25 days, the culture condition was changed to produce fruit bodies; temperature and relative humidity were maintained at 20°C and 90% and light treatment was made at over 200 lux. After cultivation for a further 19 days, the fruit bodies (2.3 kg) were collected and extracted with acetone and the extract was concentrated to a small volume. The aqueous residue was then extracted with ethyl acetate. The solvent layer was dried over Na_2SO_4 and concentrated to give an oily residue. This material was applied to a silica gel column developed with a mixture of chloroform-methanol (25:1). The crude active material thus obtained was subjected to Toyopeal HW-40 column chromatography developed with a mixture of chloroform-methanol (1:1). Further purification was performed by preparative MPLC (C18) using 80% aqueous methanol (pH 3.3). The active fraction was finally subjected to preparative HPLC using a PEGASIL ODS column with 73% aqueous methanol (pH 3.3) to give two active components which were separately concentrated to dryness to yield pale brown powders of **1** (7.3 mg) and **2** (0.6 mg).

The physico-chemical properties of **1** and **2** are shown in Table 1. They are soluble in methanol, ethyl acetate, chloroform and insoluble in water. Their molecular formulae were determined both to be $C_{23}H_{20}O_5$ by HRFAB-MS suggesting close structural similarity between them.

The ^1H and ^{13}C NMR data of **1** and **2** are summarized in Table 2. All one-bond ^1H-^{13}C connectivities were established by a heteronuclear multiple-quantum coherence (HMQC) experiment.

The ^1H-^1H COSY and HMBC experiments on **1** revealed a partial structure, HOOC1–$C2H_2$–$C3H_2$–$C4H_2$–C5H=C6H–$C7H_2$–, in addition to a *p*-substituted cinnamic acid moiety (Fig. 2). The isolated methylene protons H14 (δ_H 4.83) were assigned to an oxymethylene function adjacent to a triple bond due to the characteristic high field chemical shift of C14 (δ_C 52.9). The oxymethylene protons were long-range coupled to an ester carbonyl carbon C1′ (δ_C 167.9), the presence of which was supported by an IR absorption at 1706 cm^{-1}. The remaining six quaternary carbons C8, C9, C10, C11, C12 and C13 were ascribed to triacetylene groups from their characteristic chemical shifts (δ_C 59.7 to 80.4). Connectivities between the partial structures thus obtained (C1∼C7 and C14∼C9′) were elucidated by ^{13}C-^1H long-range correlations from two terminal methylene protons H7 (δ_H 3.12) and H14 (δ_H 4.83) to triacetylene carbons (C8∼C13). In order to explain the chemical shifts of C1 (δ_C 177.4) and C7′ (δ_C 161.5) and the molecular formula of **1**, C1 and C7′ are assigned to a carboxylic acid and a free phenolic carbon, respectively. The geometrical configurations of **1** were elucidated to be 5Z and 2′E by coupling constants of the olefinic protons ($J_{5\sim6}$=11.0 Hz and $J_{2'\sim3'}$=16.0 Hz). Thus, the structure of **1** was elucidated as shown in Fig. 1.

The molecular formula of **2** was determined to be the same as that of **1** by the HRFAB-MS data. The ^1H and ^{13}C NMR spectra of **2** were quite similar to those of **1** except for two olefinic proton chemical shifts (H2′ and H3′). These data indicated that **2** is a geometrical isomer of **1** at the C2′–C3′ double bond. The geometries of **2** were elucidated to be 5Z and 2′Z by the coupling constants of the relevant olefinic protons ($J_{5\sim6}$=11.0 Hz

Table 1. Physico-chemical properties of cinnatriacetin A and cinnatriacetin B.

	cinnatriacetin A	cinnatriacetin B
Appearance	pale brown powder	pale brown powder
Molecular formula	$C_{23}H_{20}O_5$	$C_{23}H_{20}O_5$
HRFAB-MS		
Found	399.1237 $(M+Na)^+$	399.1250 $(M+Na)^+$
Calcd	399.1208	399.1208
UV λ_{max}^{MeOH} nm(ε)	314 (14400)	314 (14100)
IR υ (KBr)cm^{-1}	3230, 2217, 1706, 1629 1602, 1440	3230, 2217, 1706, 1629 1602, 1440

Table 2. ^{13}C and ^{1}H chemical shifts of cinnatriacetin A and cinnatriacetin B in CD_3OD.

	cinnatriacetin A		cinnatriacetin B	
No	δ_C	δ_H (J/Hz)	δ_C	δ_H (J/Hz)
1	177.4		177.3	
2	34.2	2.28 (t, J=7.5)	34.1	2.29 (t, J=7.5)
3	25.6	1.65 (m)	25.5	1.67 (m)
4	27.4	2.15 (m)	27.4	2.11 (m)
5	133.2	5.55 (dtt, J=11, 7.5, 1.5)	133.2	5.55 (dtt, J=11, 7.5, 1.5)
6	123.6	5.45 (dtt, J=11, 7.5, 1.5)	123.6	5.43 (dtt, J=11, 7.5, 1.5)
7	18.1	3.12 (d, J=7)	18.1	3.11 (d, J=7)
14	52.9	4.83 (s)	52.6	4.80 (s)
1'	167.9		166.9	
2'	113.9	6.36 (d, J=16)	115.0	5.77 (d, J=13)
3'	147.7	7.65 (d, J=16)	146.4	6.92 (d, J=13)
4'	126.9		127.3	
5',9'	131.4	7.45 (d, J=8.5)	133.8	7.65 (d, J=8.5)
6',8'	116.8	6.79 (d, J=8.5)	115.8	6.76 (d, J=8.5)
7'	161.5		160.3	

Triacetylene signals from C8 to C13 are as follows; cinnatriacetin A, δ_C 80.4, 72.7, 71.4, 65.3, 64.7, 59.7; cinnatriacetin B, δ_C 80.4, 72.7, 71.4, 65.3, 64.7, 59.7.

and $J_{2'\sim3'}=12.9$ Hz). Thus, the structure of **2** was determined to be a stereoisomer of **1** at the C2'–C3' double bond as shown in Fig. 1. Treatment of a methanol solution of **2** with light (about 1000 lux) at room temperature for 12 hours gave **1**. Thus, **1** might have been formed from **2** through light treatment for the formation of fruit bodies.

Many kinds of polyacetylene derivatives are known to

Fig. 1. Structures of cinnatriacetin A (**1**) and B (**2**).

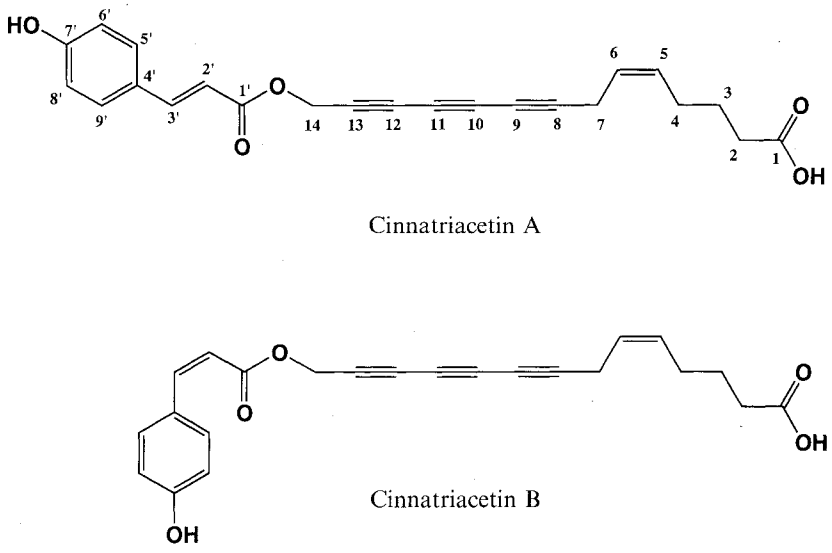

Fig. 2. Structure elucidation of cinnatriacetin A by ^{1}H-^{1}H COSY and HMBC experiments.

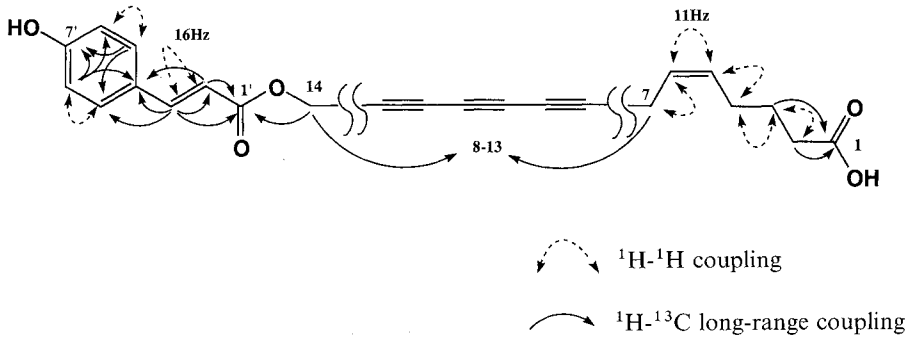

Table 3. Antimicrobial activities of cinnatriacetin A and cinnatriacetin B.

Test organism	cinnatriacetin A	cinnatriacetin B
Staphylococcus aureus IFO 12732	12.4	13.4
Bacillus subtilis ATCC 6633	13.2	14.0
Bacillus cereus IAM 1110	11.8	11.6
Bacillus coagulans IFO 12583	13.6	14.2
Escherichia coli IAM 12119	0	0
Saccharomyces cerevisiae IFO 0205	0	0
Saccharomyces bailii IFO 1137	0	0

Samples (20 μg) were applied on 8 mm paper disks.
Values are diameters (mm) of inhibitory zones.

be widespread in Basidiomycetes[1~3]. About 70% of those polyacetylenic compounds belong to the C_9 and C_{10} acyclic series; thus the structures of cinnatriacetins containing a cinnamate moiety with a C_{14} polyacetylene chain are uncommon[1~6].

Some polyacetylene derivatives possess antimicrobial activities, such as agrocybin[1], biformin[5] and marasin[6]. They were reported to be active against some kinds of bacteria and fungi. However, **1** and **2** were active only against Gram-positive bacteria as shown in Table 3. Tested so for, they were inactive against Gram-negative bacteria, yeast and other fungi.

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