Nuclear Magnetic Resonance Studies of Cytochalasin E and Its Decomposition Product

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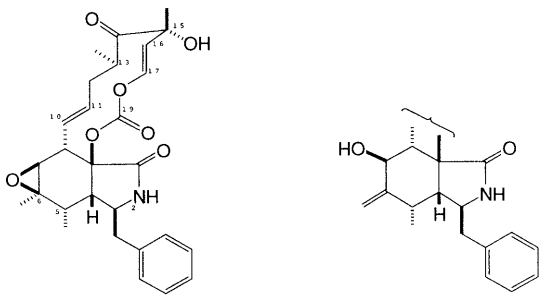
The proton and carbon-13 nuclear magnetic resonance signals of cytochalasin E (1) were assigned with the aid of ^{1}H–^{1}H, ^{1}H–^{13}C and ^{1}H–^{13}C long-range chemical shift correlation spectroscopy spectra, and the structure of the decomposition product (2) generated under neutral conditions was determined.

Keywords cytochalasin E; *Rosellinia necatrix*; ^{1}H–^{13}C long-range COSY spectrum; phytopathogenic fungus

Cytochalasins[1] have many interesting bioactivities against mammalian cells.[2] One member of this family, cytochalasin E(**1**), is a metabolite of a phytopathogenic fungus, *Rosellinia necatrix*,[1a] which causes white root rot of mulberry and fruit trees. Recently, one of the authors isolated an actinomycetes which suppresses the growth of this fungus.[3] In the course of a study on the molecular mechanism of this plant disease, we required the spectral data of cytochalasin E. Although the structure of **1** was elucidated by X-ray crystallography in 1973,[1b] the nuclear magnetic resonance (NMR) spectra of this compound were not fully discussed. Thus, we assigned the signals observed in the ^{1}H- and ^{13}C-NMR spectra of cytochalasin E, and obtained results that will make it easier to elucidate the structures of other cytochalasins or cytochalasin analogues.

The methanol extract of incubated hyphae of *Rosellinia necatrix*, isolated from the root of mulberry infected with white root rot was purified to afford cytochalasin E, the structure of which was confirmed by X-ray analysis.[4]

By analysis of the ^{1}H–^{1}H and ^{1}H–^{13}C chemical shift correlation spectroscopy (COSY) (Chart 1) and ^{1}H–^{13}C long-range COSY spectra, we arrived at the assignments shown in Table I.



1 **2**

Fig. 1. Structures of Cytochalasin E (**1**) and **2**

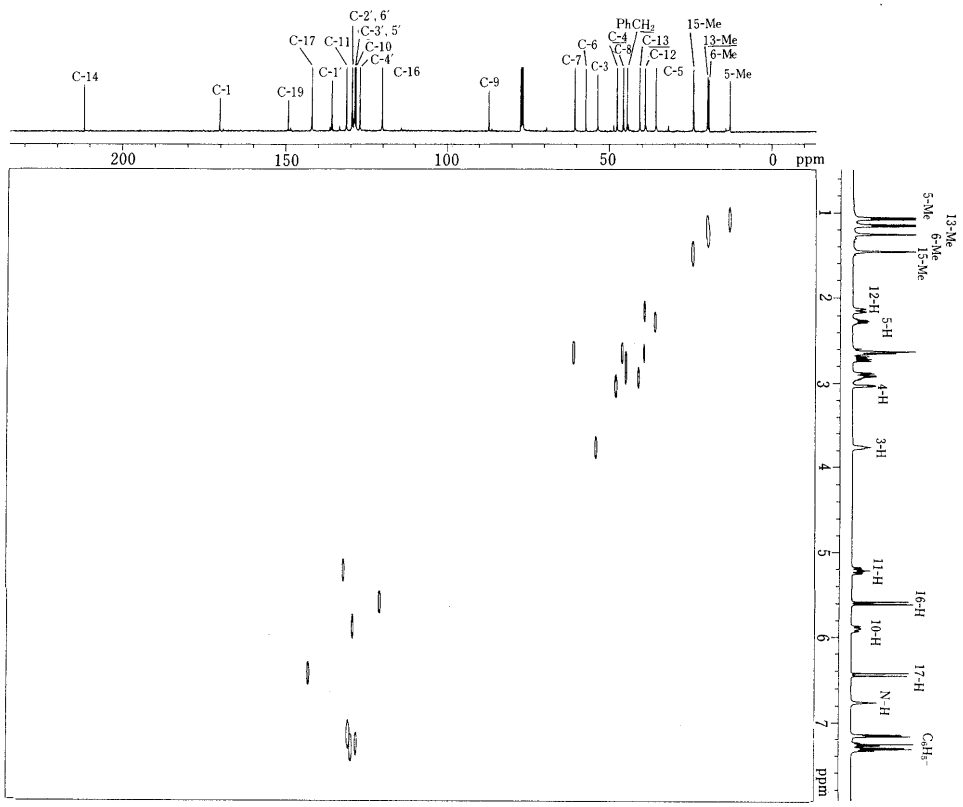


Chart 1. ^{1}H–^{13}C COSY Spectrum of Cytochalasin E (**1**)

TABLE I. Assignments of Signals in the ^{1}H- and ^{13}C-NMR Spectra of **1**

Position	^{13}C-NMR	^{1}H-NMR
1	170.2 (s)	
2		6.76 (br s, –NH)
3	53.6 (d)	3.76 (br s)
4	47.7 (d)	3.03 (dd, J=3, 5 Hz)
5	35.8 (d)	2.26 (m)
6	57.3 (s)	
7	60.6 (d)	2.63 (m)
8	45.7 (d)	2.63 (m)
9	87.1 (s)	
10	128.4 (d)	5.89 (dd, J=8, 15 Hz)
11	131.4 (d)	5.22 (m)[5]
12	39.1 (t)	2.63 (m)
		2.14 (br d)
13	40.8 (d)	2.95 (m)
14	211.8 (s)	
15	77.7 (s)	
16	120.3 (d)	5.59 (d, J=11 Hz)
17	142.1 (d)	6.43 (d, J=11 Hz)
18		
19	149.3 (s)	
5-Me	13.1 (q)	1.06 (d, J=7 Hz)
6-Me	19.7 (q)	1.25 (s)
13-Me	20.0 (q)	1.14 (d, J=7 Hz)
15-Me	24.3 (q)	1.46 (s)
Ph-CH_2	44.6 (t)	2.90 (dd, J=5, 13 Hz)
		2.70 (dd, J=7, 13 Hz)
1′	135.9 (s)	
2′,6′	129.7 (d)	7.15 (d, J=7 Hz)
3′,5′	128.9 (d)	7.32 (t, J=7 Hz)
4′	127.2 (d)	7.26 (t, J=7 Hz)

While measuring the NMR spectra, it was found that cytochalsin E was labile in $CHCl_3$ even at room temperature and decomposed to a single product (**2**). The ^{1}H-NMR spectrum of this compound had one less methyl signal and two more olefinic proton signals in comparison with that of cytochalasin E. More detailed examination showed that the methine signal at δ 2.63 in **1** was shifted to δ 3.81 in **2** and the coupling pattern also changed. As a result, the structure of the decomposition product was characterized as shown in Fig. 1.

Experimental

Cytochalasin E *Rosellinia necatrix* isolated by one of the authors was incubated in the PD-medium at 25 °C for 2 months. The MeOH extract of the grown hyphae was subjected to silica gel column chromatographies ($CHCl_3$: MeOH = 50 : 1, hexane : AcOEt = 1 : 1) to yield cytochalasin E as colorless plates of $[\alpha]_D$ −80.0° (c=0.3, $CHCl_3$), mp 213.2—214.0 °C.

Transformation of 1 to 2 Compound **1** (10 mg) was allowed to stand at room temperature in $CHCl_3$ (0.5 ml). After 2 d, the solvent was evaporated off and the residue was subjected to preparative thin layer chromatography (hexane : AcOEt = 1 : 3) to afford **2** (2 mg) and recovered starting material (7 mg).

Compound 2 ^{1}H-NMR ($CDCl_3$) δ: 7.32 (2H, t, J=7 Hz, 3′,5′-H), 7.25 (1H, t, J=7 Hz, 4′-H), 7.13 (2H, d, J=7 Hz, 2′,6′-H), 6.60 (1H, d, J=12 Hz, 17-H), 5.73 (2H, m, NH and 10-H), 5.63 (1H, d, J=12 Hz, 16-H), 5.41 (1H, br s, 6=CH_2), 5.36 (1H, dd, J=4, 11 Hz, 11-H), 5.18 (1H, br s, 6=CH_2), 3.81 (1H, br d, J=11 Hz, 7-H), 3.35 (1H, br s, 3-H), 3.30 (1H, m, 5-H), 1.51 (3H, s, 15-Me), 1.17, 1.15 (each 3H, d, J=7 Hz, 2 × Me).

References and Notes

1) *a*) D. C. Aldridge, B. F. Burrows and W. B. Turner, *J. Chem. Soc., Chem. Commun.*, **1972**, 148; *b*) G. Büchi, Y. Kitaura, S. Yuan, H. E. Wright, J. Clardy, A. L. Demain, T. Glinsukon, N. Hunt and G. N. Wogan, *J. Am. Chem. Soc.*, **95**, 5423 (1973); *c*) D. C. Aldridge, J. J. Armstrong, R. N. Speake and W. B. Turner, *J. Chem. Soc.* (*C*), **1967**, 1667; *d*) D. C. Aldridge and W. B. Turner, *ibid.*, **1969**, 923; *e*) H. Minato and H. Matsumoto, *ibid.*, **1970**, 38; *f*) H. Minato and T. Katayama, *ibid.*, **1970**, 45.

2) For example, N. K. Wessells, B. S. Spooner, J. F. Ash, M. O. Bradley, M. A. Luduena, E. L. Taylor, J. T. Wrenn and K. N. Yamada, *Science*, **171**, 135 (1971).

3) The identification and biological features of this actinomycetes will be reported by K. Takahashi *et al.*

4) We thank Dr. Setoguchi (Yoshitomi Seiyaku Co.) for his help in this.

5) The value of the chemical shift of this signal was not compatible with that reported by Büchi *et al.*[1b]