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ASSIGNMENT OF ^{13}C-NMR SPECTRUM AND BIOSYNTHESIS OF COLLETOTRICHIN

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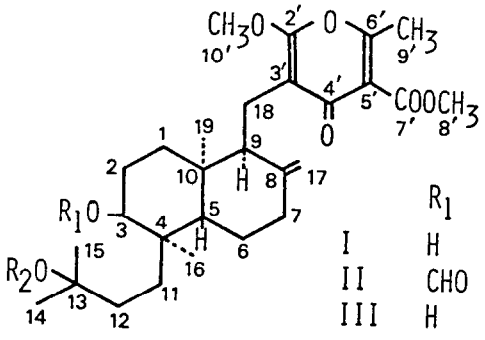
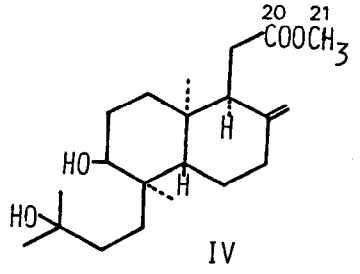
Colletotrichin (I) is a phytotoxic substance isolated from *Colletotrichum nicotianae*[1] and *C. capsi*[2], and the structure has been independently determined by X-ray analysis of itself[1] and its acetate[3]. In addition to I, we isolated two closely related compounds, colletotrichins B (II) and C (III)[4] from *C. nicotianae* and established the structures. The structural features of colletotrichins consisting of anomalous norditerpene and polysubstituted γ-pyrone moieties have stimulated us to study the biosynthesis of colletotrichins. Here we wish to report the assignment of ^{13}C-nmr spectra of colletotrichins and biosynthesis of I in *C. nicotianae* from ^{13}C-formate, 1-^{13}C- , 2-^{13}C- and 1,2-^{13}C-acetate.

The chemical shifts and multiplicities of off-resonance decoupled spectra of I, II and III along with IV[5] are shown in Table 1. ^{13}C-Nmr chemical shifts of I were assigned from the multiplicities, litereture values[6] and the correlation to those of II, III and IV. Three signals at 28.7, 31.7 and 32.6 ppm due to methylene carbons, C-1, C-6 and C-11, could not be assigned.

^{13}C-Signals attributed to polysubstituted γ-pyrone moiety were firmly established by the measurement of proton coupled spectra and also by selective proton decoupling experiments. A quartet due to C-6' (160.8 ppm) in the proton coupled spectrum was selectively decoupled to a singlet by irradiation at C-9' methyl protons.



	R_1	R_2
I	H	H
II	CHO	H
III	H	CHO

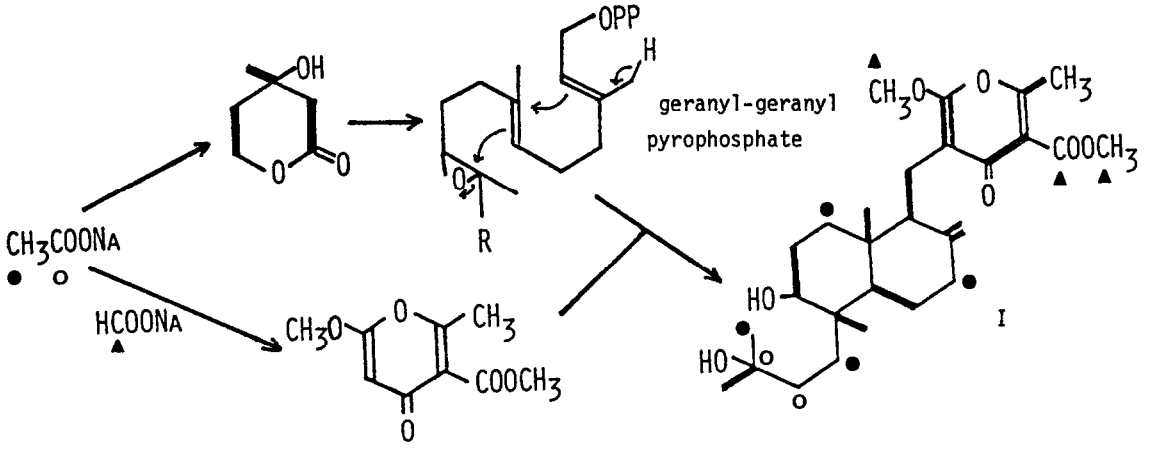


IV

Since irradiation at methoxyl protons of C-8' and C-10' enhanced the signal intensity at 163.5 ppm and the signal at 166.2 ppm appeared as a singlet accompanying the enhancement of intensity, the former signal was assigned to C-2' and the latter to C-7'. Discrimination between the signals at 106.0 and 120.2 ppm was also accomplished by the selective proton decoupling experiment (irradiation at OCH_3 signal). In this case, the signal at 120.2 ppm was observed as a sharp quartet coupled with C-9' methyl protons and on the other hand that at 106.0 ppm was rather broad due to coupling with protons attached to C-17 and C-18.

From these evidences, the former was assigned to C-5' and the latter to C-3'. Further, the signal at 177.1 ppm was attributed to C-4'.

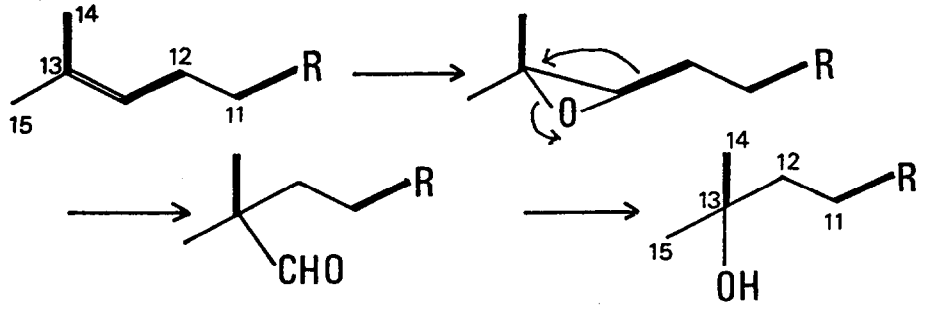
The fungus was cultured in the media fortified with 90% enriched ^{13}C-formate, 1-^{13}C-, 2-^{13}C- or 45% enriched 1,2-^{13}C-acetate. The resulting labeling pattern of I is illustrated in Scheme 1. In proton noise decoupling FT-^{13}C-nmr spectrum of I biosynthesized from 1,2-^{13}C-acetate, 20 signals with ^{13}C-^{13}C coupling were detected, indicating that 10 acetate units were incorporated. In addition to these carbons, the signals for C-1, C-7, C-11, C-12 and C-15 carbons have also enhanced intensities relative to the natural abundance of C-8' methoxyl signal without ^{13}C-^{13}C coupling. The pattern of acetate incorporation is consistent with the acetate to mevalonate, to geranyl-geranyl pyrophosphate route[7].



Scheme 1

Though we postulated in the previous paper[1] that pyrone might be derived from the cleavage of orsellinaldehyde derivative, the labeled results indicated that the pyrone originated from three moles of each acetate and formate followed by polyketide pathway. Thus it was suggested that I was biosynthesized through the combination of acetate-mevalonate-terpene pathway (via. sacculatal type cyclization[8]) with acetate-polyketide pathway as shown in Scheme 1.

It is noteworthy that signal intensities of enriched carbons in C-11~15 of labeled I was almost the same extent as those in remaining terpenyl moiety, and that 1-^{13}C-acetate was incorporated into both C-12 and C-13. Further, in the spectrum of I biosynthesized from



Scheme 2

Table 1. Chemical Shifts and Coupling Constants of Colletotrichins and C_{-20} Acid Methyl Ester

Carbon		I	II	III	IV				$J_{^{13}C-^{13}C}$
C-1[b]	t[i]	32.6	33.0	32.6	32.1		●	Δ	h
C-2	t	25.6	22.9	25.8	25.3	o		Δ	37
C-3	d	71.6	75.5	71.8	71.1		●	Δ	37
C-4[e]	s	38.0	37.7	38.0	37.6	o		Δ	37
C-5	d	40.2	41.0	40.2	40.2		●	Δ	35
C-6	t	23.1	22.9	22.9	22.9	o		Δ	35
C-7[b]	t	31.7	31.2	31.4	31.4		●	Δ	h
C-8	s	149.6	148.4	148.8	148.0	o		Δ	72
C-9	d	56.0	55.9	55.8	55.4		●	Δ	34
C-10[e]	s	39.4	38.4	39.2	39.3	o		Δ	36
C-11[b]	t	28.7	29.0	26.5	28.7		●	Δ	h
C-12	t	35.7	36.2	34.0	35.2	o[f]		Δ	h
C-13	s	71.2	70.9	84.3	71.3	o[f]		Δ	39
C-14	q	28.2	29.2	26.7	28.1		●	Δ	39
C-15	q	31.0	29.4	28.4	30.9		●	Δ	h
C-16[a]	q	22.8	22.9	22.8	22.2		●	Δ	37
C-17	t	109.6	109.6	109.4	110.1		●	Δ	72
C-18	t	20.1	19.9	20.0	34.4	o		Δ	34
C-19[a]	q	18.9	18.5	18.9	18.5		●	Δ	36
C-20	s	-	-	-	173.9				
C-21	q	-	-	-	51.4				
C-2'	s	163.5	163.0	163.0	-	o		Δ	89
C-3'	s	106.0	105.4	105.5	-		●[g]	Δ	89
C-4'	s	177.1	176.6	176.4	-	o		Δ	54
C-5'	s	120.2	119.9	119.8	-		●[g]	Δ	54
C-6'	s	160.8	160.4	160.5	-	o		Δ	52
C-7'	s	166.2	165.6	165.7	-			▲	
C-8'[c]	q	52.7	52.6	52.7	-			▲	
C-9'	q	18.0	18.0	18.1	-		●	Δ	52
C-10'[c]	q	56.2	55.9	56.0	-			▲	
R_1 or R_2	d	-	160.6	161.0	-				

Measured in $CDCl_3$ solution, in ppm downfield from internal TMS. [a,c,e] Assignment may be reversed. [b] Assignment may be changed. [f,g] Coupling constants ; J_f=38, J_g=12 Hz. [h]Uncoupled signal. [i] Multiplicity. ▲ ^{13}C-Formate. o 1-^{13}C-Acetate. ● 2-^{13}-C-Acetate. Δ 1,2-^{13}C-Acetate.

1,2-^{13}C-acetate, the signals due to adjacent C-11 and C-12 were equally enhanced but did not couple with each other. These facts indicated that one carbon corresponding to C-4 methylene of mevalonate was lost during the biosynthesis of I. The elimination of this carbon atom may proceed as shown in Scheme 2[9]. The confirmation of this proposed mechanism is now under investigation.

Acknowledgement

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