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THE BIOSYNTHESIS OF COLLETOTRICHINS ISOLATED FROM *Colletotrichum nicotianae*

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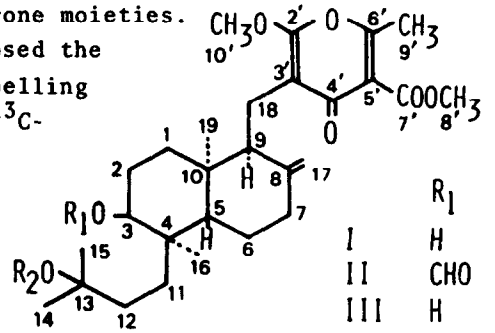
Colletotrichin (I) is a phytotoxic substance isolated from fungi[1,2] and the structure has been rigorously established by X-ray chrystallography[2,3]. In the course of our continuous searching for the related substances, we isolated two new compounds, colletotrichin B (II) and C (III) from the culture filtrate of *Colletotrichum nicotianae*[4]. These compounds consist of unique norditerpene and polysubstituted γ-pyrone moieties.



	R_1	R_2
I	H	H
II	CHO	H
III	H	CHO

In the previous paper[5], we proposed the biosynthetic pathway of I from the labelling patterns with ^{13}C-formate, 1-^{13}C-, 2-^{13}C- and 1,2-^{13}C-acetates.

Here we wish to report the biosynthetic pathway of I, II and III from the results of labelling studies of II and III with ^{13}C-formate, 1-^{13}C-, 2-^{13}C- and 1,2-^{13}C-acetates and 5-^{13}C-mevalonolactone and that of I with 5-^{13}C-mevalonolactone.

Colletotrichum nicotianae was inoculated to 500 ml Erlenmeyer flasks containing 120 ml of the medium (sucrose 2.0 % and Yeast extract 0.001 %) and incubated aerobically at 26.5 °C. After 2 days, ^{13}C-labelled precursors were separately added and after a further 4 days, I, II and III were isolated in a pure state from ethyl acetate soluble neutral fraction of the culture filtrate.

The signals in ^{13}C-nmr spectra of I, II and III were assigned as shown in Table. The signals at 160.6 of II and 161.0 ppm of III appeared as doublet were assigned to formyl carbon of each compound.

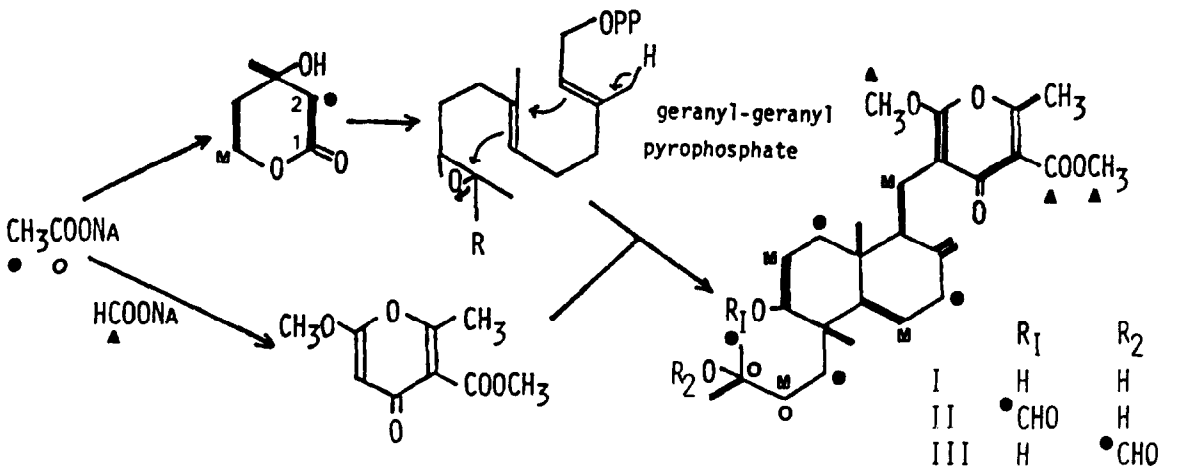
In the ^{13}C-nmr spectra of 5-^{13}C-mevalonolactone labelled I and III, the signal intensities of carbons C-2, -6, -12 and -18, were increased by approximately two fold.

This result revealed that a terpene moiety of colletotrichins was biosynthesized from 4 moles of mevalonate, indicating geranyl-geranyl pyrophosphate pathway.

The labelling patterns of II and III biosynthesized from ^{13}C-formate, 1-^{13}C-, 2-^{13}C- and 1,2-^{13}C-acetates were consistent with those of I except for formyl growps.

Contrary to our expectation that formate as C_1 unit might be incorporated in each formyl growp of II and III, only three signals assignable to C-7', -8' and -10' carbons were enriched by approximately eight fold, whereas in the ^{13}C-nmr spectra of II and III labelled with 1,2-^{13}C-acetate, the signal intensities of all carbons including formyl carbons except for C-7', -8' and -10' carbons were increased.

It is noteworthy that the signal intensities of formyl carbons derived from 1,2-^{13}C-acetate were almost the same extent as those of other enriched carbons, 1-^{13}C-acetate was incorporated into adjacent C-12 and C-13, and 2-^{13}C-

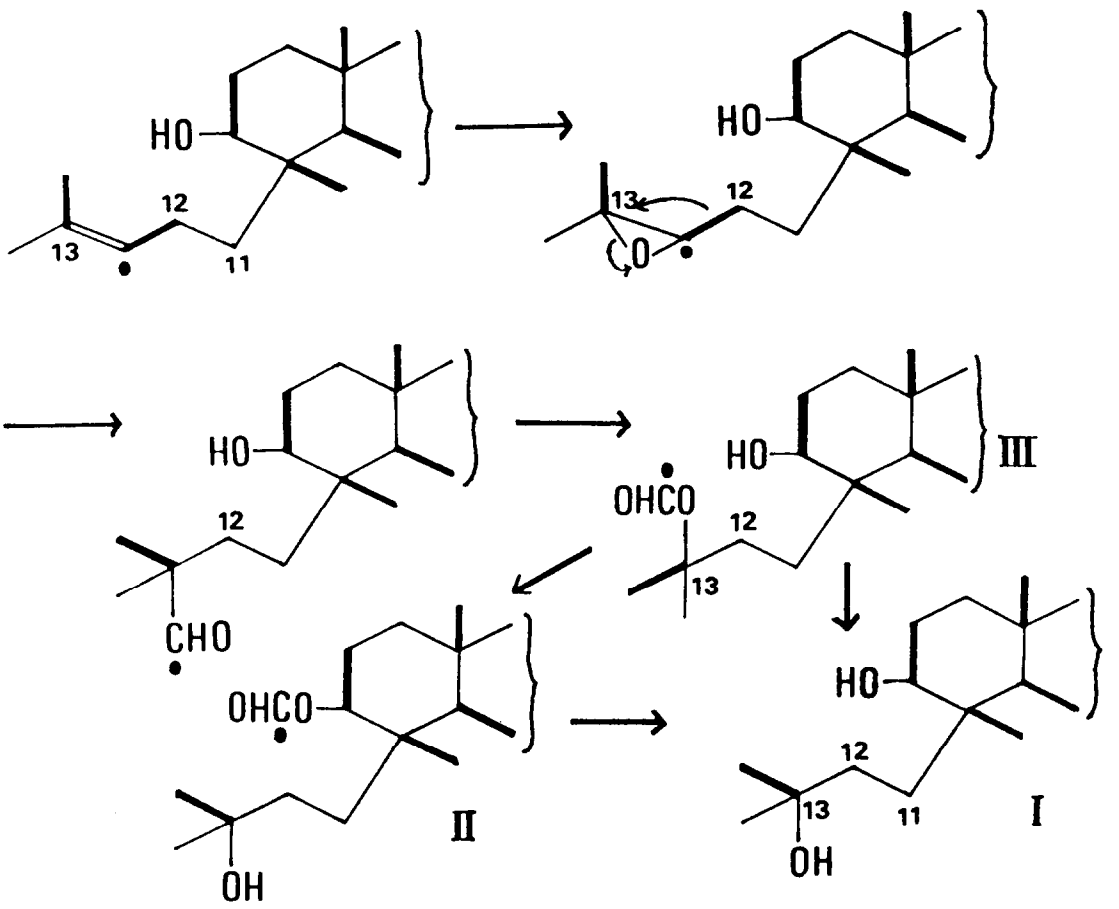


Scheme 1

acetate into formyl growps of II and III.

These results indicated that colletotrichins were biosynthesized through acetate to mevalonate, to geranyl-geranyl pyrophosphate. Moreover the pattern of acetate and mevalonate incorporation revealed that cleavage of the C-1 and C-2 bond in mevalonolactone occured during its conversion to isopentenyl pyrophosphate, and C-2, -6, -12 and -18 carbons of I, II and III were derived from C-5 of mevalonolactone.

From the data of labelled II and III together with those of I, the mechanism involving cyclisation (via. sacculatal type cyclisation[6]) of geranyl-geranyl pyrophosphate, followed by epoxidation of terminal isopentenyl double bond, cleavage and Baeyer-Villiger type reaction would account for the biosynthetic pathway of colletotrichins as shown in Scheme 2.



Scheme 2

Acknowledgement

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Chemical Shifts and Observed Labelling Patterns of Colletotrichins

Carbon		I	II	III					$J_{^{13}C-^{13}C}$ (Hz)
C-1[b]	t[f]	32.6	33.0	32.6		●	Δ		
C-2	t	25.6	22.9	25.8	o		Δ	M	37
C-3	d	71.6	75.5	71.8		●	Δ		37
C-4[e]	s	38.0	37.7	38.0	o		Δ		37
C-5	d	40.2	41.0	40.2		●	Δ		35
C-6	t	23.1	22.9	22.9	o		Δ	M	35
C-7[b]	t	31.7	31.2	31.4		●	Δ		
C-8	s	149.6	148.4	148.8	o		Δ		72
C-9	d	56.0	55.9	55.8		●	Δ		34
C-10[e]	s	39.4	38.4	39.2	o		Δ		36
C-11[b]	t	28.7	29.0	26.5		●	Δ		
C-12	t	35.7	36.2	34.0	o		Δ	M	
C-13	s	71.2	70.9	84.3	o		Δ		39
C-14	q	28.2	29.2	26.7		●	Δ		39
C-15	q	31.0	29.4	28.4		●	Δ		h
C-16[a]	q	22.8	22.9	22.8		●	Δ		37

Carbon		I	II	III					$J_{^{13}C-^{13}C}$ (Hz)
C-17	t	109.6	109.6	109.4		●	Δ		72
C-18	t	20.1	19.9	20.0	o		Δ	M	34
C-19[a]	q	18.9	18.5	18.9		●	Δ		36
C-2'	s	163.5	163.0	163.0	o		Δ		89
C-3'	s	106.0	105.4	105.5		●	Δ		89
C-4'	s	177.1	176.6	176.4	o		Δ		54
C-5'	s	120.2	119.9	119.8		●	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		●	Δ		

Measured in $CDCl_3$ solution, in ppm downfield from internal TMS. [a,c,e]Assignment may be reversed. [b]Assignment may be changed. [f]Multiplicity. ▲ ^{13}C-Formate. o 1-^{13}C-Acetate. ● 2-^{13}C-Acetate. Δ 1,2-^{13}C-Acetate. M 5-^{13}C-Mevalonolactone.

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