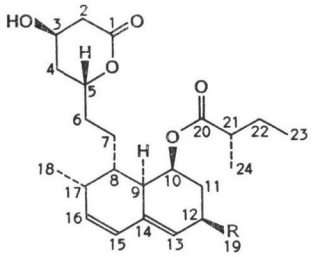
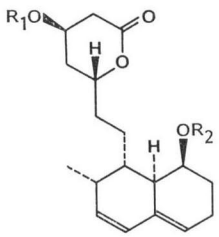
BIOSYNTHESIS OF ML-236B (COMPACTIN) AND MONACOLIN K

Sir:

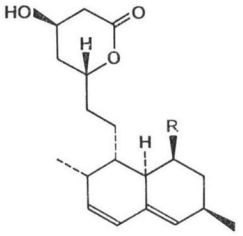
The fungal metabolites, ML-236B[1] (compactin)[2] **1** and monacolin K[3] (mevinolin)[4] **2**, are potent competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis. They reduce effectively plasma cholesterol levels in various mammalian species including man, and are thereby effective in the therapy of hypercholesterolemia[5,6]. Furthermore, **1** has been used as an important biological tool for research of the regulation of the synthesis of polyisoprenoids such as cholesterol, ubiquinone, dolichol and juvenile hormone[5,7]. Their



1 R = H
2 R = Me



3 R_1 = Ac, R_2 = H
4 R_1 = R_2 = Ac
5 R_1 = Ac, R_2 = COCH(Me)C_2H_5
6 R_1 = R_2 = H (ML-236A)



7 R = H (Monacolin L)
8 R = OH (Monacolin J)

Fig. 1. Part of the two-dimensional ^1H-^{13}C chemical shift correlation map of ML-236B (**1**) in $CDCl_3$.

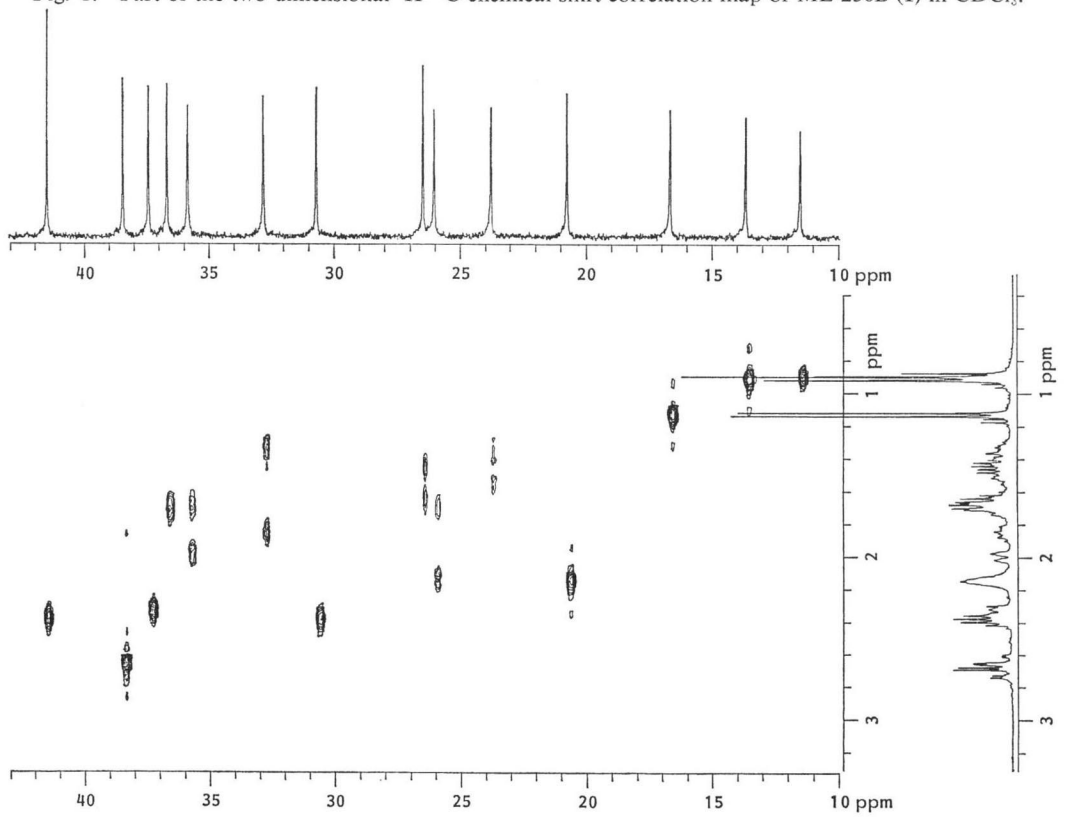


Table 1. Incorporation of sodium [1-^{13}C]acetate and sodium [2-^{13}C]acetate in ML-236B (**1**) and monacolin K (**2**).

Position	ML-236B (**1**)			Monacolin K (**2**)		
	Chemical shift[a]	Enrichment factor[b]		Chemical shift[a]	Enrichment factor[b]	
		[1-^{13}C]	[2-^{13}C]		[1-^{13}C]	[2-^{13}C]
1	170.67	2.5		170.73	2.5	
2	38.44		3.1	38.47		2.5
3	62.12	3.2		62.16	2.7	
4	35.84		3.3	35.88		2.1
5	76.26	2.6		76.38	1.8	
6	32.82		2.7	32.82		2.0
7	23.78	2.8		24.06	1.9	
8	36.67		2.4	36.45		1.9
9	37.40	2.7		37.19	2.0	
10	67.51		2.6	67.84		1.8
11	26.03	2.3		32.54	2.1	
12	20.74		2.7	27.28		1.9
13	123.33	2.9		129.35	1.8	
14	133.38		2.1	131.41		1.7
15	127.96	3.3		128.13	2.0	
16	132.37		2.7	132.83		2.0
17	30.70	2.9		30.53	2.0	
18	13.64		2.1	13.70		2.1
19	—	—	—	22.65	(11)[c]	
20	176.55	2.0		176.67	1.7	
21	41.50		2.3	41.32		2.1
22	26.48	2.0		26.60	1.8	
23	11.49		2.0	11.51		2.0
24	16.64	(73)[c]		16.02	(10)[c]	

[a] Relative to TMS in $CDCl_3$.

[b] Ratios between peak heights of the observed resonances of ^{13}C enriched and natural abundance sample recorded under identical conditions.

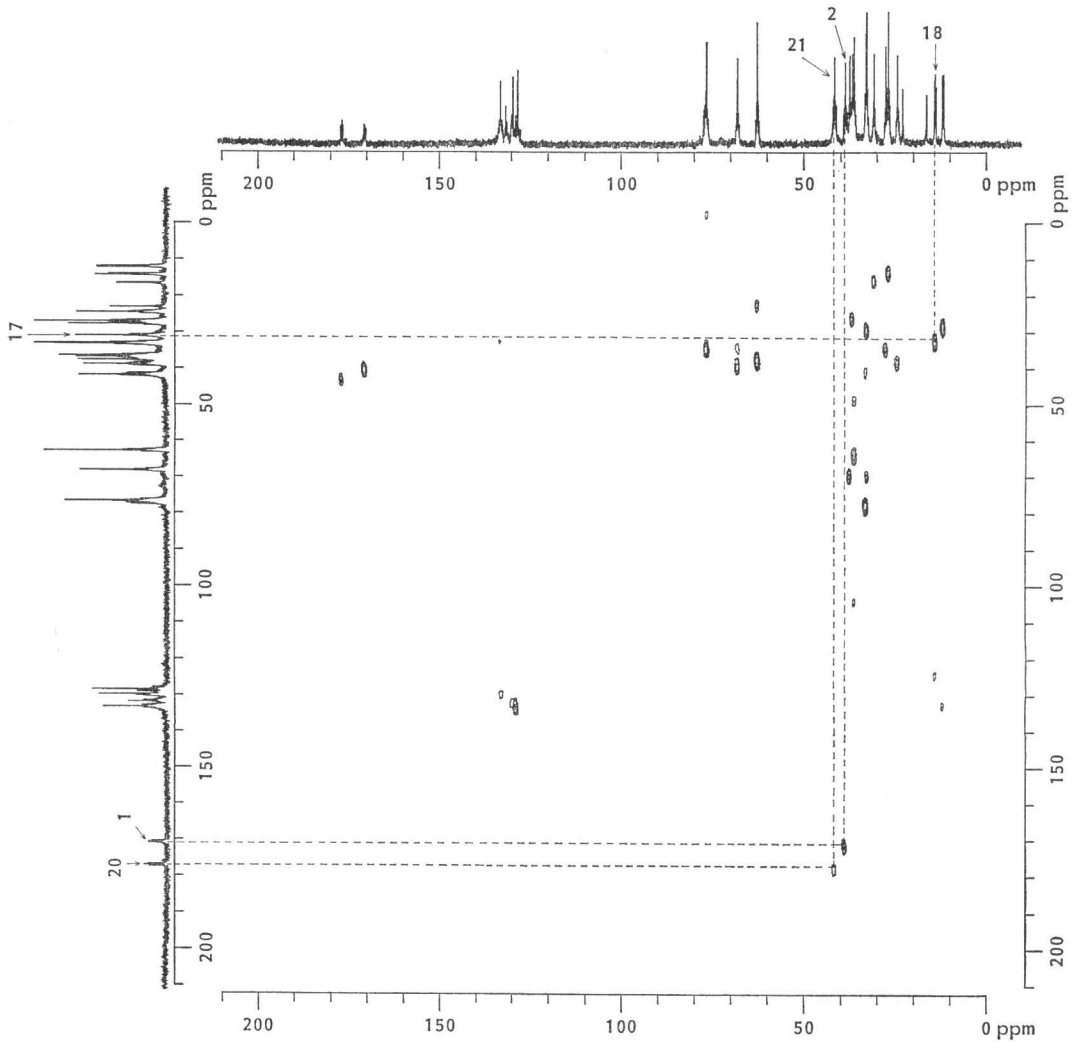
[c] Enrichment factors in incorporation study of [*methy*-^{13}C]methionine.

biological and pharmacological significance as well as structural novelty have inspired extensive efforts for total syntheses[8~10] and chemical[11,12] and microbial modifications[13~16] of **1** and **2**. Recent report for the biosynthesis[17] of **2** prompted us to disclose herein our work on a total assignment of NMR spectra and a biosynthetic investigation of **1** and **2** using ^{13}C labeled precursors. Part of this work has been published[18,19].

Complete assignment of NMR spectra is essential for ^{13}C incorporation studies. Therefore, the ^{1}H NMR (360 MHz) spectral positions of **1** and **2** were first determined by use of extensive decoupling experiment and two-dimensional homonuclear chemical shift correlation spectroscopy (COSY)[20]. Based on the assignment of all hydrogens of **1**, ^{13}C NMR (90.5 MHz) spectral positions of **1** were determined by DEPT and heteronuclear chemical shift correlation spectroscopy (Fig. 1) except two carbonyl (170.67 and 176.55 ppm: C-1, C-20) and two methyne carbons (30.70 and 41.50 ppm: C-17, C-21) with complete overlapping hydrogens at 2.38 ppm. Discrimination of them was achieved conventionally by comparison with the spectra of derivatives of **1**. Both resonances corresponding to 176.55 and 41.50 ppm are missing in the methylbutyrate removed compounds such as **3** and **4**, while they are retained in methylbutyrate ester **5**. The ones corresponding to 30.70 and 170.67 ppm are found in all of them. Thus, these four signals are apparently assignable to C-20, C-21, C-17 and C-1, respectively. Subsequent assignment of ^{13}C NMR of **2** was carried out in a similar fashion, and both assignments are summarized in Table 1: they are quite similar, but the signals of C-11, -12, -13 and -14 of **2** shift considerably from those of **1** owing

Fig. 2. A two-dimensional Inadequate spectrum of ^{13}C NMR of monacolin K (**2**) derived from [1,2-$^{13}C_2$]-acetate.

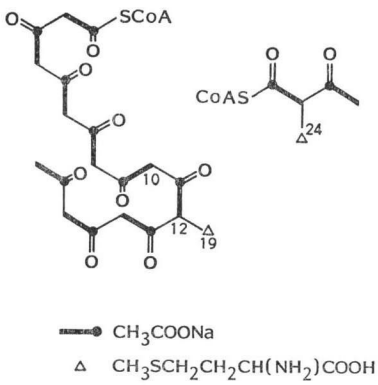
Dashed lines show the correlations of the signals in question.



to methyl substitution on C-12. However, since there remained a slight ambiguity in both assignments with regard to the signals which relied on the comparison of the spectra of related compounds, direct correlation of the carbon resonances was performed by two-dimensional Inadequate NMR experiment[21] (*vide infra*), and the above assignments were confirmed.

Feeding experiments were carried out using as precursors sodium [1-^{13}C]- and sodium [2-^{13}C]-acetate, sodium [1-^{13}C]- and [3-^{13}C]propionate, and [*methyl*-^{13}C]methionine both for **1** and for **2**. Cultures of *Penicillium citrinum* NRRL-8082 and *Monascus ruber* M-4681 were spplemented with one of the precursors after 4~6 days of inoculation. After further 2 and 5 days the cultures were harvested, from which **1** and **2** were isolated respectively by silica gel column chromatography[1,3]. ^{13}C NMR spectra of **1** and **2** derived from [1-^{13}C]acetate showed the same enrichment pattern of the eleven alternative carbons (Table 1). [2-^{13}C]Acetate enriched alternatively the other eleven carbons of **1** and **2** (Table 1). Whereas no incorporation of propionate was observed in either **1** or **2**, significant enrichment of methionine label was seen at C-24 both in **1** and in **2**

Fig. 3. A possible incorporation pattern of acetate and methionine into monacolin K molecule.



and, in addition, at C-19 in **2**. These results clearly indicate that the main skeleton of **1** and **2** is a polyketide of acetate origin. More interestingly, the butyrate substituent is also composed of acetate, and both additional methyl groups of C-19 and C-24 derive from methionine. The proton noise decoupled ^{13}C NMR and two-dimensional Inadequate NMR spectra[21] (Fig. 2) of **2** (~30 mg) derived from doubly labeled sodium [1,2-$^{13}C_2$]acetate clearly showed the direct correlation of twenty-two carbons and the mode of acetate incorporation. Thus, the tentative assignment of the four signals which relied on the conventional method proved to be correct, and they unambiguously demonstrate the polyketide origin of eleven intact acetates and its mode of folding, assuming a linear intermediate, as indicated in Fig. 3. Furthermore, preliminary investigations of the primary precursors of **1** and **2** using ^{14}C labeled acetate indicate the enzymatic hydroxylation and subsequent methylbutyration at C-10 occur after the construction of their hexahydronaphthalene moieties, and any compounds of monacolin series such as **2**, **7** and **8** were not produced by *P. citrinum*, the culture producing congeners of ML-236B. Therefore, the methyl group (C-19) of monacolin series may be incorporated biosynthetically into a linear intermediate before its cyclization.

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