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STUDIES ON THE BIOSYNTHESIS OF RADICLONIC ACID

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Hitherto many biosynthetic studies on microbial metabolites of polyketide origin have been well-documented.[1] When these products possess C-methyl groups or oxygenated equivalents on their main frameworks, one of the two following pathways is involved in their biosyntheses, i.e. (a) condensation of acetic acid molecules with the incorporation of C_1 units from methionine or (b) condensation of propionic acid molecules. In most cases, however, fungal metabolites are produced only by pathway (a), and the exception of this trend is, to the best of our knowldge, the case of aurovertin[2], in which one propionic acid molecule was incorporated into the starter unit of a polyketide chain.

In this regard, the biosynthesis of a metabolite of *Penicillium.* sp., radiclonic acid[3], seemed to be an interesting target for us, since it possesses as a fungal metabolite unusually many C-methyl or its oxygenated substituents on alternate carbons of the main skeleton suggesting the possible involvement of pathway (b)[3] in its biosynthesis (Fig. 1).

We wish to report the biosynthetic result obtained by ^{13}C-nmr spectroscopy.

In the ^{13}C-nmr spectrum[4] of dimethyl radiclonate I, 24 carbon signals were well resolved except for overlapping of two methoxy signals. On the basis of chemical shift trend[5], selective (long range[6]) proton decoupling experiments, calculated chemical shift values[5] and comparison to ozonolysis products II and III[3], the assignments of I were made as shown in Table.

For ^{13}C-labeling studies, *Pen.* sp. was inoculated to 500ml Erlenmeyer flasks containing 100 ml of the medium (glucose 4% and corn steep liquor 2.2%) and incubated stationary at 28°C. After 3 days, ^{13}C-labeled precursors ($^{13}CH_3CO_2Na$, $CH_3{}^{13}CO_2Na$ and $H^{13}CO_2Na$, ~90% enriched) were separately added and after a further 4 days radiclonic acid was isolated as dimethyl ester I from the acidic fraction of the mycelium extract.[3]

In the ^{13}C-nmr spectrum of the $CH_3{}^{13}CO_2Na$ labeled I, the signal intensities of carbons 1, 3, 5, 7, 9, 11, 13 and 15 are increased by approximately three fold, whereas the resonances due to carbons 2, 4, 6, 8, 10, 12, 14 and 16 are enriched by the same degree (3-4 fold) in the ^{13}C-nmr spectrum of I labeled with $^{13}CH_3CO_2Na$. The label of $H^{13}CO_2Na$ (in place of methionine) was also efficiently incorporated into carbons 17, 18, 19, 20, 21, 22 and 23 (6-7 times). However, several attempts to label I with $CD_3CD_2CO_2Na$ (checked by mass spectrometry) were unsuccessful.

Thus, it has been proved that radiclonic acid is biosynthesized *via* pathway (a) in the same manner as most fungal metabolites, and the expected pathway (b) has been excluded (Fig. 1).

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4) ^{13}C-nmr spectra were obtained on a JEOL FX-100 spectrometer at 25.05MHz, in $CDCl_3$. spectral width 5KHz, data points 16K, pulse width 7μsec, repetition time 2sec. TMS as internal standard

5) G. C. Levy and G. L. Nelson; "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Wiley-Interscience, New York, 1972. J. B. Stothers; "Carbon-13 NMR Spectroscopy", Academic Press, New York, 1972.

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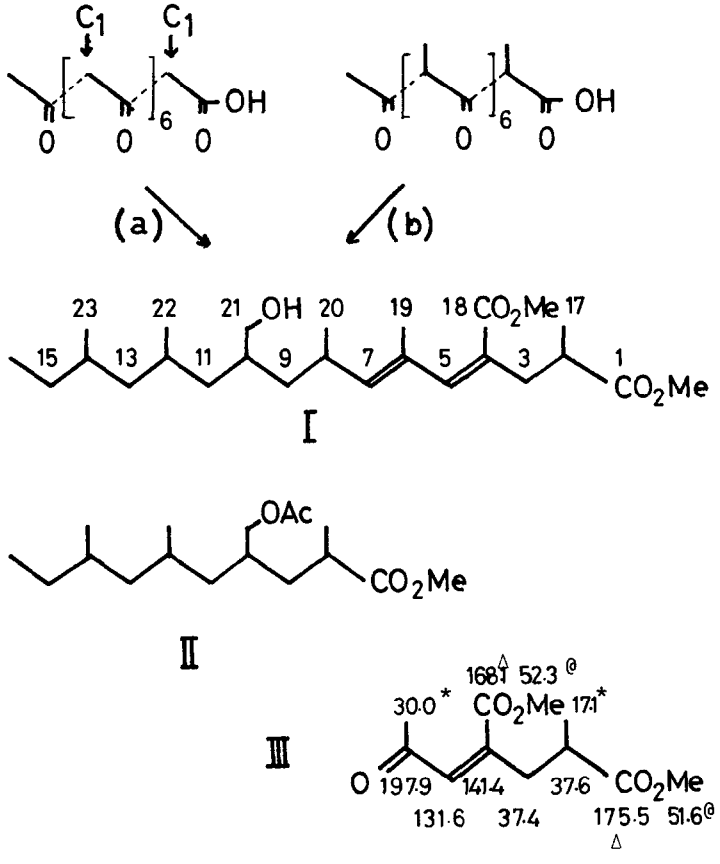
TABLE

carbon	δ_C^{TMS} I	II	calcd.
1	176.0#		
2	38.7∇		
3	39.4*		
4	127.0#		
5	140.0*		
6	130.0*		
7	140.4*	176.7Δ	
8	30.5#†	37.0*	
9	39.2#@	35.8#	
10	36.0#†	32.8#	
11	39.7∇@	39.6Δ	
12	27.5	27.4	28.3
13	45.1	44.8	43.8
14	31.5	31.4	32.5
15	29.0	28.9	29.8
16	11.1	11.1	10.9
17	16.7∇		
18	170.0*		
19	15.0*		
20	20.8#	17.4#	
21	66.3†	67.5†	
22	20.7	20.4	20.6
23	19.9	19.8	19.6
OCH_3	51.5†	51.4†	

Fig-1



Basis of the assignments. ___ calculation, * selective (long range) decoupling, # elimination, † chemical shift and multiplicity in the off-resonance spectrum, ∇ comparison to II or III, Δ comparison to I. @ assignments may be exchanged.