Tetrahedron Letters No. 5, pp 489-492, 1977. Pergamon Press. Printed in Great Britain.

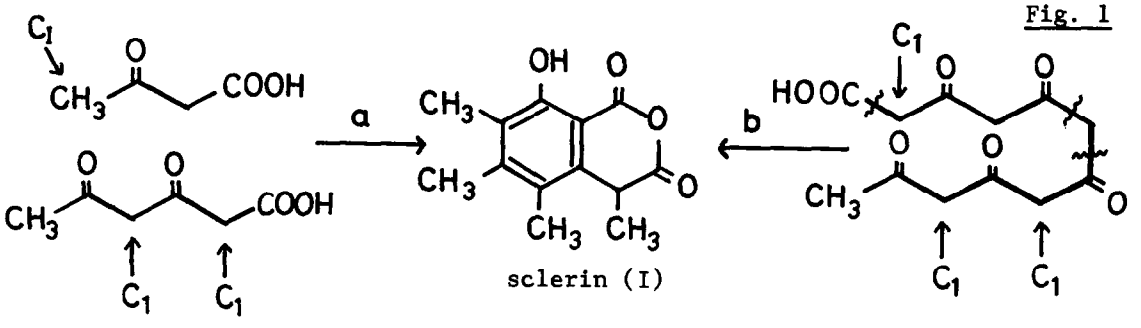
BIOSYNTHESIS OF SCLERIN

Mikio Yamazaki* and Yukio Maebayashi
Research Institute for Chemobiodynamics, Chiba University,
Izumicho, Narashino, Chiba, Japan
Takashi Tokoroyama
Faculty of Science, Osaka City University,
Sugimotocho, Sumiyoshiku, Osaka, Japan

(Received in Japan 16 December 1976; received in UK for publication 6 January 1977)

The previous result of feeding experiments with ^{14}C labeled compounds by Tokoroyama et al[1] suggested that sclerin might be biosynthesized by condensation of two separate polyacetyl chains of comparatively small size without prior cyclisation (route a in Fig. 1). Some recent findings in the biosynthesis of citromycetin[2] and mollisin[3] which have been proved to be formed by condensation of two polyacetyl chains may support above suggestion. In the sclerin biosynthesis suggested as above, one C_1 unit among three must be incorporated onto the head carbon of one of the polyacetyl chains.

Fig. 1



However, no other examples of such C-methylation to the head methyl carbon has so far been demonstrated in the biosynthesis of natural polyketides except one in the barnol biosynthesis.[4] Moreover, sclerotinin A and B[5] and sclerone[6] which are co-metabolites of sclerin in the Sclerotinia fungus may be smoothly formed by simple cyclisation of single chain through acetate-malonate pathway similarly to the citrinin-type compounds. In fact, sclerotinin A has been isolated from Penicillium citrinum along with citrinin.[7]

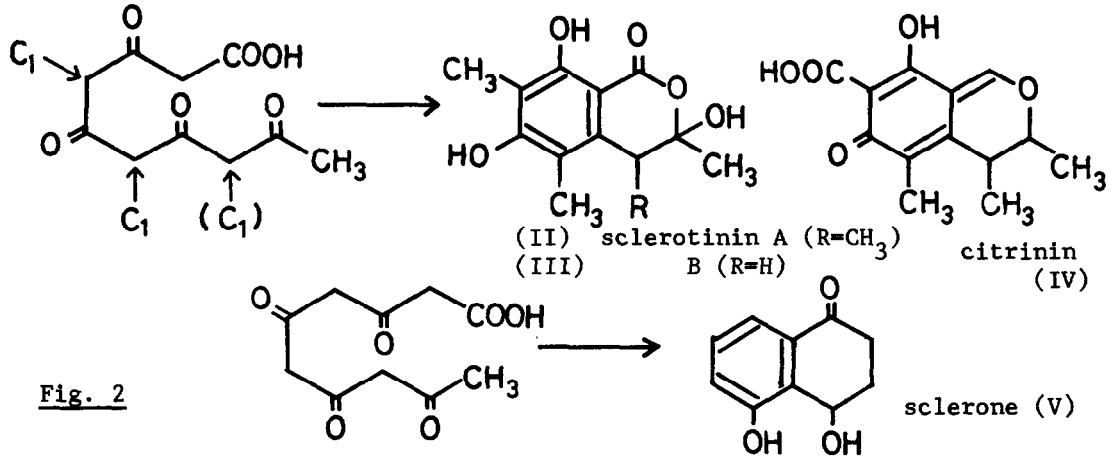


Fig. 2

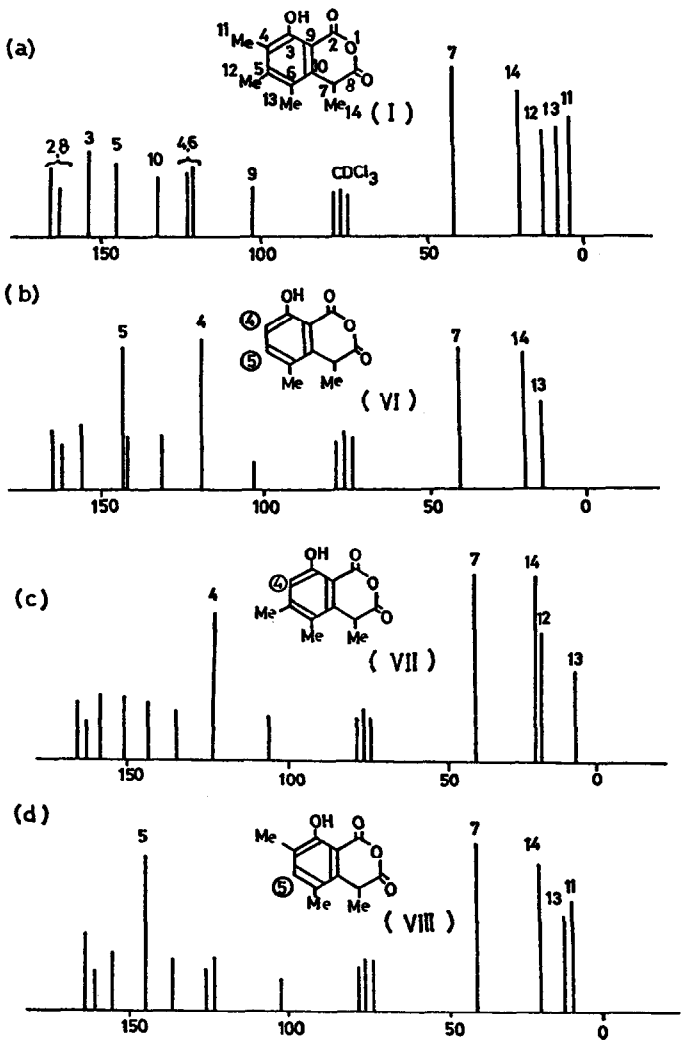
The situation as above lead us to examine again which way, route a and b in Fig. 1, sclerin is formed through, using ^{13}C-nmr spectrometry. Sclerin contains three adjacent methyl groups on its aromatic ring and it is quite difficult to distinguish these methyl groups in the spectrum. The signals including that of aromatic methyls appeared in the spectrum were identified ultimately by comparing to that of three synthesized sclerin-related compounds (VI, VII and VIII) as shown in Fig. 3. On the aromatic carbons, parameters for a substitution effect were also used for calculation of chemical shifts. Assignment of the carbonyl carbons was made by observation of broadening of the signal at the lowest field (C=O at 8) which had long range coupling with methine proton at 7, in the off-resonance decoupling experiment.

Sclerotinia sclerotiorum was cultured in a 2% yeast extract solution with 2% malt extract added. At the 8th day of cultivation, 250mg of H^{13}COONa (65.4% enriched) was added to the media and the culture was incubated for more 6 days at 25°C. The addition of ^{13}C labeled compounds was employed in the same way through the investigation.

The result of the first experiment using ^{13}C-formate proved the incorporation of C_1 unit as the three methyl groups which were C-11, 13 and 14, and it was quite agreeable with that of previous experiment using ^{14}C tracers.

From the next experiment in which (1-^{13}C)-sodium acetate(250mg, 88.2% enriched) was fed, significant intensification of signals corresponding to C-2, 3, 5, 10 and 8 was shown. On the other hand, from (2-^{13}C)-sodium acetate (250mg, 86.9%), carbons at 7, 9, 4, 6 and 12 were obviously enriched with ^{13}C. This result indicated that acetate was well incorporated into sclerin as previously expected.

Fig. 3
^{13}C-NMR spectra of sclerin and its derivatives



Chemical shifts of sclerin

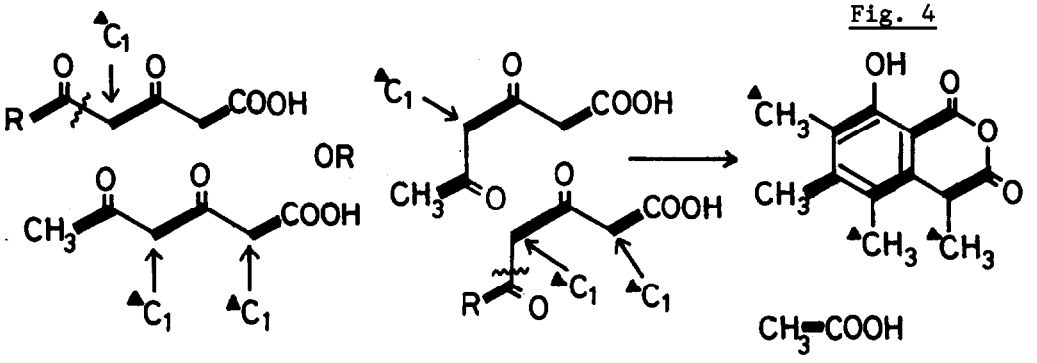
C No.	ppm from TMS	C No.	ppm from TMS
2	166.94 (s)*	9	101.97 (s)
3	159.42 (s)	10	135.33 (s)
4	124.84 (s)	11	12.47 (q)
5	148.62 (s)	12	18.12 (q)
6	125.20 (s)	13	15.14 (q)
7	39.29 (d)	14	22.91 (q)
8	169.37(b.s)		

* pattern of split in off-resonance decoupling.

By feeding (2-^{13}C)-malonate (250mg, 90.0%) with 99mg of non-labeled sodium acetate, the signal corresponding to the carbon at position 4 together with 6, 9 and 7 of sclerin was significantly intensified, however C-12 was not. This result revealed that C-12 was derived from the head carbon of the polyacetyl chain neverthless C-4 was not. Accordingly, the presence of route a for the sclerin biosynthesis seemed unlikely at the time. β

Lastly, (1,2-^{13}C)-sodium acetate (400mg, 90.0%) was fed to the fungus. If the path of the sclerin biosynthesis involved rupture of the ring once formed as shown in Fig. 1 (route b), one of two carbonyl carbons should be observed as singlet in the spectrum. However, in the spectrum of sclerin obtained here, no singlet were observed except three of the methyl carbons which were derived from C_1 units, but all of signals were observed as splitted by ^{13}C-^{13}C coupling. This result indicated that the presence of route b in the sclerin biosynthesis is also improbable. The pattern of incorporation of acetate into

Fig. 4



sclerin was confirmed by calculation of the coupling constant of each signals : 2-9 (70 Hz),3-4 (67 Hz), 5-12 (44 Hz), 6-10 (63 Hz) and 7-8 (55 Hz).as shown in Fig. 4.

Conclusively, we would like to propose a new hypothesis as that two separate polyacetyl chains condense to form a ring once and the head part of one of two chains may be lost perhaps by oxidation during the biosynthetic pathway.

References

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Addendum

After finished description of this manuscript, we have received a communication on the biosynthesis of sclerin (M.J.Garson and J.Staunton,Chem.Commun.,1976, 928). Although their results reported are not scarcely different from ours, a different hypothesis on the sclerin biosynthesis has been proposed there, suggesting further investigation must be done in near future to make it sure.