Phytochemistry, Vol. 20, No. 10, pp. 2339–2342, 1981.
Printed in Great Britain.

0031–9422/81/102339–04 $02.00/0

BIOSYNTHESIS OF PR TOXIN FROM [1,2-^{13}C]ACETATE: OCCURRENCE OF INDUCED ^{13}C–^{13}C COUPLING

SERGE MOREAU*, ALAIN LABLACHE-COMBIER† and JEAN BIGUET*

* I.N.S.E.R.M. U-42, 369 rue Jules Guesde, 59650 Villeneuve d'Ascq, France; † Laboratoire de Chimie Organique Physique, Associé à l'E.N.S.C.L., E.R.A. C.N.R.S. no. 827, Université des Sciences et Techniques de Lille I, 59655 Villeneuve d'Ascq Cedex, France

(*Revised received* 11 *March* 1981)

Key Word Index—PR toxin; biosynthesis; ^{13}C NMR; induced ^{13}C–^{13}C coupling.

Abstract—The biosynthesis of PR toxin was studied by incorporation of [1,2-^{13}C]acetate. The biosynthesis of the eremophilane skeleton of PR toxin follows the scheme proposed by Robinson involving a 1,2-methyl shift from a eudesmane skeleton. Induced coupling, arising from farnesyl pyrophosphate being formed from more than one labelled acetate unit, was observed and explained. The intervention of an abnormal isopentenyl pyrophosphate unit to account for satellite bands is discounted.

INTRODUCTION

Secondary metabolites from *Penicillium roqueforti*, a fungal species used in the ripening of Roquefort cheese include alkaloids [1,2] and sesquiterpenoid metabolites such as PR toxin [3] and related compounds [4, 5]. As shown by X-ray diffraction studies, the absolute stereochemistry of PR toxin is characteristic of the eremophilane series [6]. We report here our investigation of the biosynthesis of the eremophilane skeleton using the technique of [1,2-^{13}C]acetate incorporation into PR toxin and ^{13}C NMR spectroscopy of the resulting labelled compounds.

RESULTS AND DISCUSSION

In the case of the eremophilane type sesquiterpenoids, the currently accepted biosynthetic scheme involves a 1,2-shift of an angular methyl group in a eudesmane precursor. The incorporation of the intact acetate units, and the carbon arising from C-2 of the mevalonate as originally postulated by Robinson [7] are shown in Fig. 1. The assigned proton noise decoupled natural abundance ^{13}C NMR spectrum of PR toxin is shown in Fig. 2. The 90-MHz spectrum obtained after ^{13}C-labelling experiments is shown in the same figure. The ^{13}C NMR spectrum of [1,2-^{13}C]acetate enriched PR toxin is characterized by sharp singlets (C-15, C-3, C-9, C-12 and C-10) and as anticipated, multiplets arising from coupled carbon nuclei from intact acetate units. The determination of the coupled carbons based on coupling constants measurements is given in Table 1. It is immediately evident that our results are in complete accordance with the scheme proposed by Robinson. The carbon atoms C-3, C-9, C-12 arise from the C-2 of mevalonate while C-15 and C-10 (which also exhibits singlets) arise from the cleavage implicated in the 1,2 shift

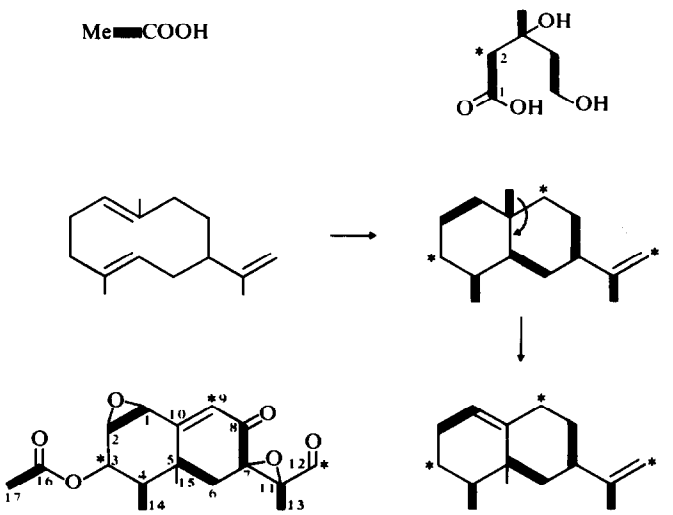

Fig. 1. Incorporation of acetate units into the eremophilane skeleton according to Robinson.

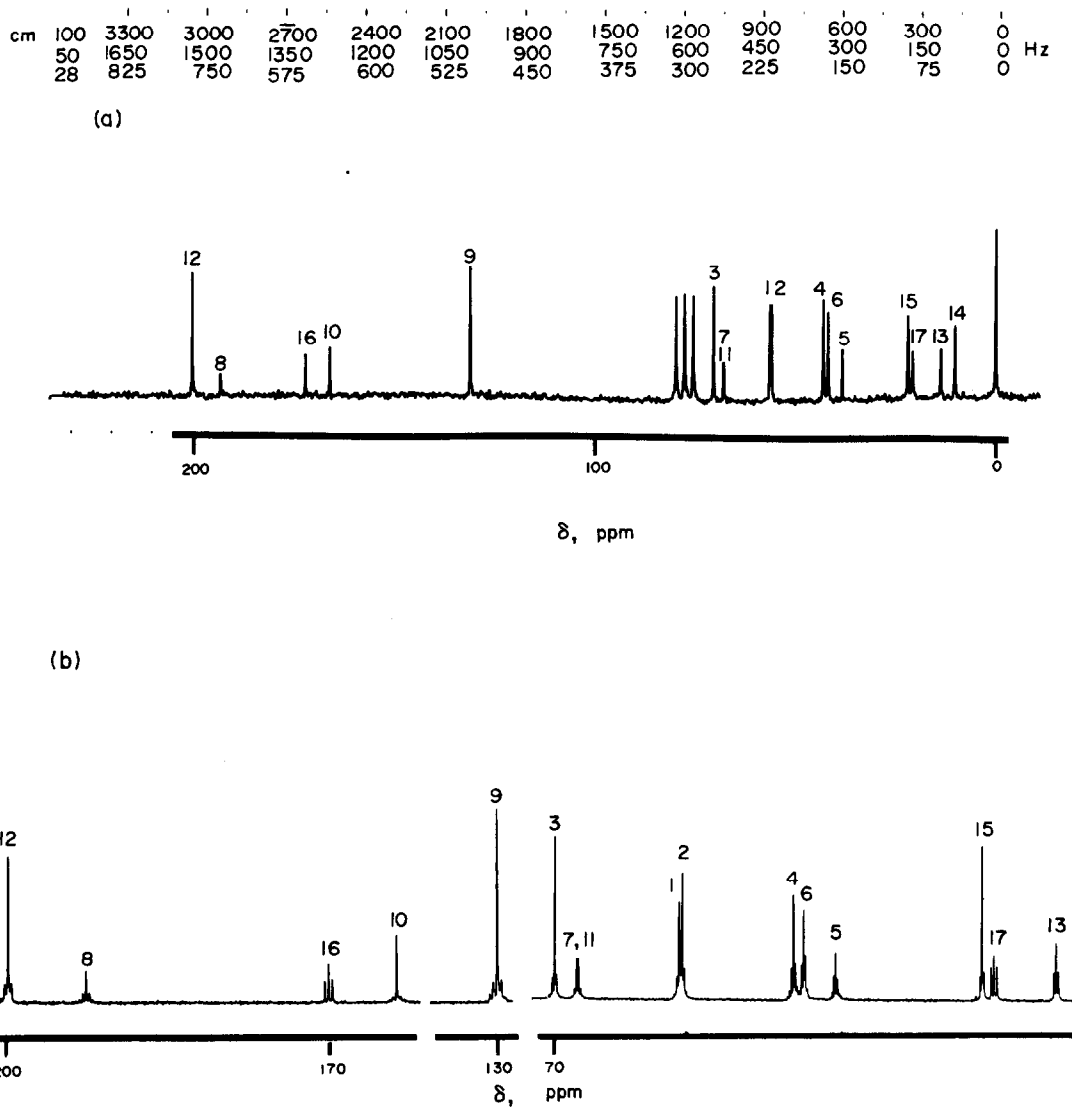

Fig. 2. The PFT ^{13}C NMR spectra of PR toxin in $CDCl_3$. (a) Natural abundance (25.2 MHz); (b) labelled with 90% enriched [1,2-^{13}C]acetate (90 MHz).

previously mentioned. Finally, the distribution of the intact units is also consistent with the terpenoid origin of the molecule.

A similar study, reported on capsidiol [8,9], has led to the same conclusion. An alternative proposal involving a sequence of spiro-rearrangements has been suggested for the eudesmane–eremophilane conversion [10]. Our results rule out this suggestion for the biosynthesis of the metabolites of *P. roqueforti*. Careful examination of the NMR spectrum of labelled PRT revealed small but easily distinguishable satellite resonances around the sharp singlets exhibited by C-15, C-9, C-3, C-12 (Fig. 2), and even associated with the multiplets attributed to C-5, C-6 and C-4 (Fig. 3).

Table 1. Values of ^{13}C–^{13}C spin–spin coupling

J C-1, C-2 = 25 Hz
J C-4, C-14 = 36 Hz
J C-5, C-6 = 34 Hz
J C-7, C-8 = 52 Hz
J C-11, C-13 = 45 Hz
J C-16, C-17 = 59 Hz

The existence of such satellites could only be due to spin–spin coupling. These satellite signals originated from coupling within acetate units. Here we propose that our satellite observations arise from couplings between carbon nuclei from adjacent acetate units. The occurrence of multiply labelled compounds from [^{13}C]acetate has been already mentioned [11,12,8] and seems to be a general phenomenon. The proposal of an 'abnormal' isopentenyl pyrophosphate unit (to account for satellite band of C-2 from mevalonate) in which part of the terminal olefinic carbon is derived from C-(3') of mevalonate can be discarded in our experiment since satellite signals clearly occur for C-15, a carbon not implicated in isopentenyl phosphate isomerization.

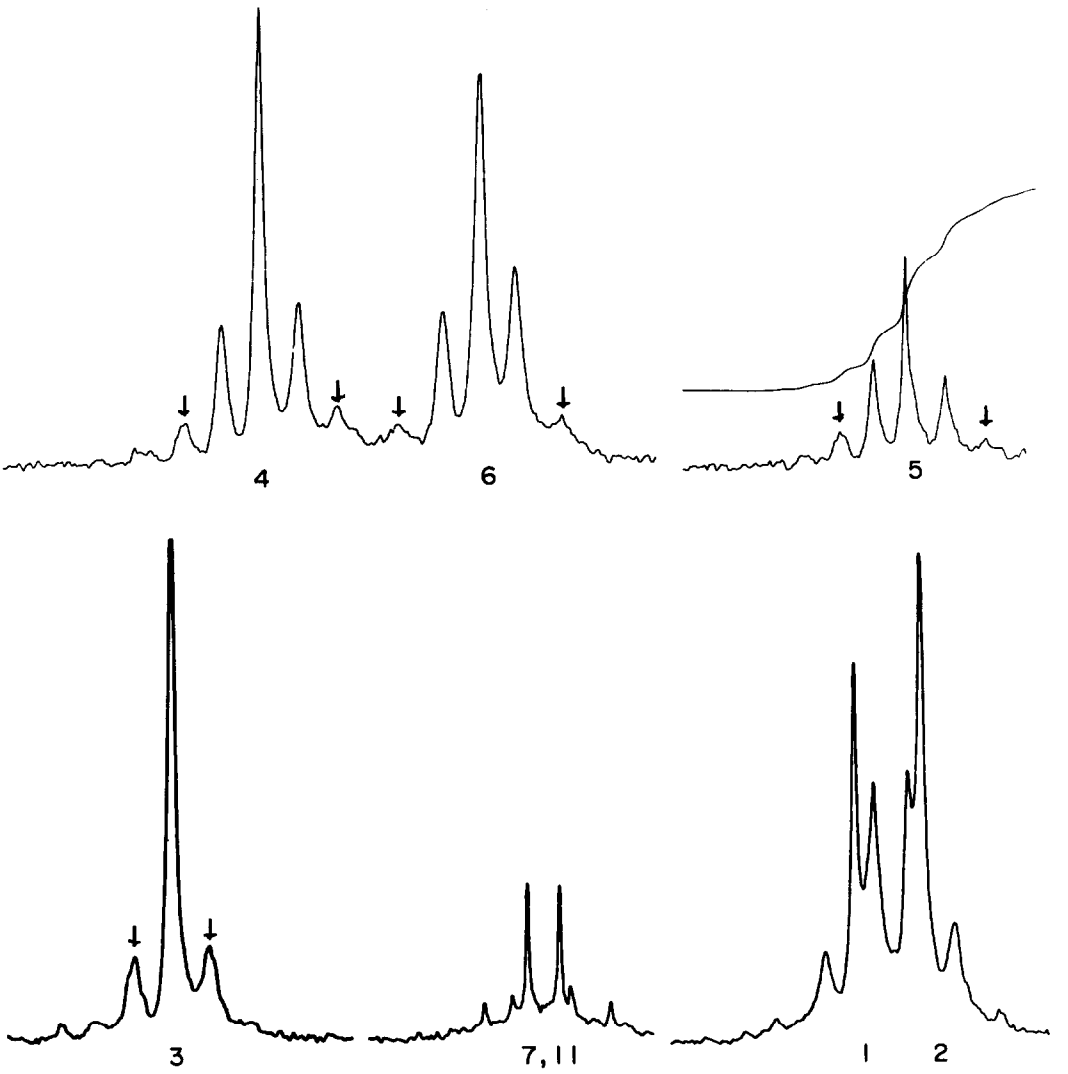

Fig. 3. Satellites' resonances from various peaks (arrows).

EXPERIMENTAL

Penicillium roqueforti cultures. 10^6–10^8 spore of *P. roqueforti* washed from potato dextrose agar slants were inoculated in 800-ml Roux bottles containing 150 ml of medium (2% yeast extract and 15% sucrose in demineralized water) sterilized by filtration on membrane filters (0.2 μm). Ten Roux bottles were incubated as stationary cultures in the dark at 25° for 14 days.

Incorporation of [1,2-^{13}C]*acetate.* Labelled NaOAc (1 g) was dissolved in demineralized water (50 ml) and sterilized by filtration on membrane filters (0.2 μm). One ml of this solution is added to each Roux bottle at days 7, 8, 9, 11 and 13.

Purification of PR toxin. On day 14 the cultures were filtered and the medium extrd by $CHCl_3$. PR toxin is purified and cryst. as previously described [4]. We obtained 65 mg of pure labelled PR toxin.

NMR experiments. ^{13}C NMR spectra were recorded on a Brücker WP 60 (15.06 MHz) and WP 360 (90 MHz). Samples were dissolved in $CDCl_3$. The measurement of the percentage enrichment at each labelled position is obtained by the following equation, % enrichment: $1.1\ I_L/I_C = \frac{18}{81} I_L$ for a 90% enriched precursor, where I_L is the sum of the intensities of the two lateral resonances; I_C the intensity of the central peak. Table 2 lists the results obtained for most of the carbon atoms. The average value of percentage enrichment is 1.7%.

Table 2. Per cent ^{13}C incorporation of various carbon atoms

	% Enrichment	Total abundance
C-1	3	4.1
C-2	1.1	2.2
C-4	1	2.1
C-5	2	3.1
C-6	1	2.1
C-8	0.3	1.4
C-13	1.7	2.8
C-14	2.2	3.3
C-16	2	3.1
C-17	2	3.1

Acknowledgements—We are grateful to the NMR service of Rhône Poulenc St. Fons (France) for 90 MHz ^{13}C NMR measurements, and Mme A. Masset for her excellent technical assistance.

REFERENCES

1. Ohomomo, S., Sato, T., Utagawa, T. and Abe, M. (1975) *Agric. Biol. Chem.* **39**, 1333.
2. Scott, P. M., Merrien, M. A. and Polonsky, J. (1976) *Experientia* **32**, 140.
3. Wei, R. D., Schnoes, H. K., Hart, P. A. and Strong, F. M. (1975) *Tetrahedron* **31**, 109.
4. Moreau, S., Gaudemer, A., Lablache-Combier, A. and Biguet, J. (1976) *Tetrahedron Letters* 833.
5. Moreau, S., Cacan, M. and Lablache-Combier, A. (1977) *J. Org. Chem.* **42**, 2632.
6. Baert, F., Foulon, M., Odou, G. and Moreau, S. (1980) *Acta Crystallogr. Sect. B* **36**, 402.
7. Robinson, R. cited by Penfold, A. E. and Simonsen, J. L. (1939) *J. Chem. Soc.* 87.
8. Baker, F. W. and Brooks, C. J. W. (1976) *Phytochemistry* **15**, 689.
9. Stothers, J. B. and Ward, E. B. (1978) *Can. J. Chem.* **56**, 645.
10. Dunham, D. J. and Lawton, R. G. (1971) *J. Am. Chem. Soc.* **93**, 2075.
11. Bradshaw, P. W., Hanson, J. R. and Siverns, M. (1977) *J. Chem. Soc. Chem. Commun.* 869 and (1978) *J. Chem. Soc. Chem. Commun.* 303.
12. Baker, F. C., Brooks, C. J. W. and Hutchinson, S. A. (1975) *J. Chem. Soc. Chem. Commun.* 293.