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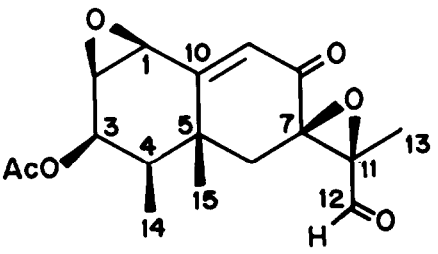
BIOSYNTHESIS OF PR TOXIN BY PENICILLIUM ROQUEFORTI, PART 2
EVIDENCE FOR AN HYDRIDE SHIFT FROM ^{2}H N.M.R. SPECTROSCOPY

by Charles P. Gorst-Allman*, and Pieter S. Steyn,
National Chemical Research Laboratory,
Council for Scientific and Industrial Research,
P.O. Box 395, Pretoria 0001, South Africa

The biosynthesis of the eremophilane sesquiterpenoid PR toxin is shown to involve an 1,2-hydride migration from C(5) to C(4) of the molecule.

The biosynthesis of PR toxin (1), the major toxin produced by strains of Penicillium roqueforti, has been shown to occur via a standard isoprene pathway.[1] In view of the importance of P. roqueforti in the dairy industry, and the production of PR toxin by certain strains used in the ripening of Roquefort cheese,[2] additional investigations were undertaken to examine this biosynthesis in more detail. In particular it has been shown that an 1,2-hydride shift from C(5) to C(4) occurs during the bioformation of the structurally related sesquiterpenes capsidiol[3], petasin[4] and rishitin.[5] It was of interest to examine if such a shift occurs during the biogenesis of PR toxin, especially as PR toxin possesses the opposite stereochemistry to capsidiol at C(3) and C(4) and to petasin at C(3).



(1)

Accordingly, cultures of P. roqueforti were supplemented every 24 hours with [4-$^2H_2$]-mevalonic acid lactone and the isolated PR toxin subjected to ^{2}H n.m.r. spectroscopy. The resultant spectrum showed only two signals, at δ 3.63 and δ 1.75 corresponding to H(1) and H(4) respectively.[6] The identity of these signals was confirmed by comparison with a ^{2}H n.m.r. spectrum of PR toxin isolated from a medium containing 20% D_2O. In this case, an

uniformly labelled product was obtained with ^{2}H chemical shifts corresponding to those reported for the ^{1}H n.m.r. spectrum of PR toxin[6]. This confirms an 1,2-hydride migration from C(5) → C(4) during the biosynthesis of PR toxin, as label from C(4)of mevalonic acid lactone should appear at C(5) of PR toxin (Scheme). The folding of the intermediate must be such that both methyl groups [at C(4) and C(10)] occupy the β-axial orientation. Equatorial attack at C(1), followed by subsequent transannular migrations will give rise to an intermediate having the required PR toxin stereochemistry with respect to the C(4) and C(5) methyl groups (Scheme). Subsequently oxidation and acetylation complete the biogenesis.

During our initial study on PR toxin biosynthesis, anomalous couplings were observed at C(3), C(9) and C(12) in the ^{13}C n.m.r. spectrum of [1,2-$^{13}C_2$]acetate-derived PR toxin. Multiple labelling giving rise to inter-acetate couplings,[7] and an abnormal isopentenyl pyrophosphate unit[8] have been used to explain such irregular labelling. We have further investigated this phenomenon by incorporation experiments with [2,3-$^{13}C_2$]mevalonic acid lactone. The ^{13}C n.m.r. spectrum of [2,3-$^{13}C_2$]mevalonate-derived PR toxin showed three intact units, C(3)-C(4), C(9)-C(10), and C(11)-C(12), with satellite signals due to carbon-carbon couplings (Table). This confirms that the only intact units are those to be expected from a normal isoprene pathway, and thus that the anomalous labelling observed in the acetate feeding experiment must arise from multiple labelling.

Table

^{13}C N.m.r. data for [2,3-$^{13}C_2$]mevalonic acid-derived PR toxin

Carbon atom	$^{1}J(C,C)$/Hz
3	38.4
4	37.4
9	65.3
10	65.2
11	52.3
12	52.5

Acknowledgement

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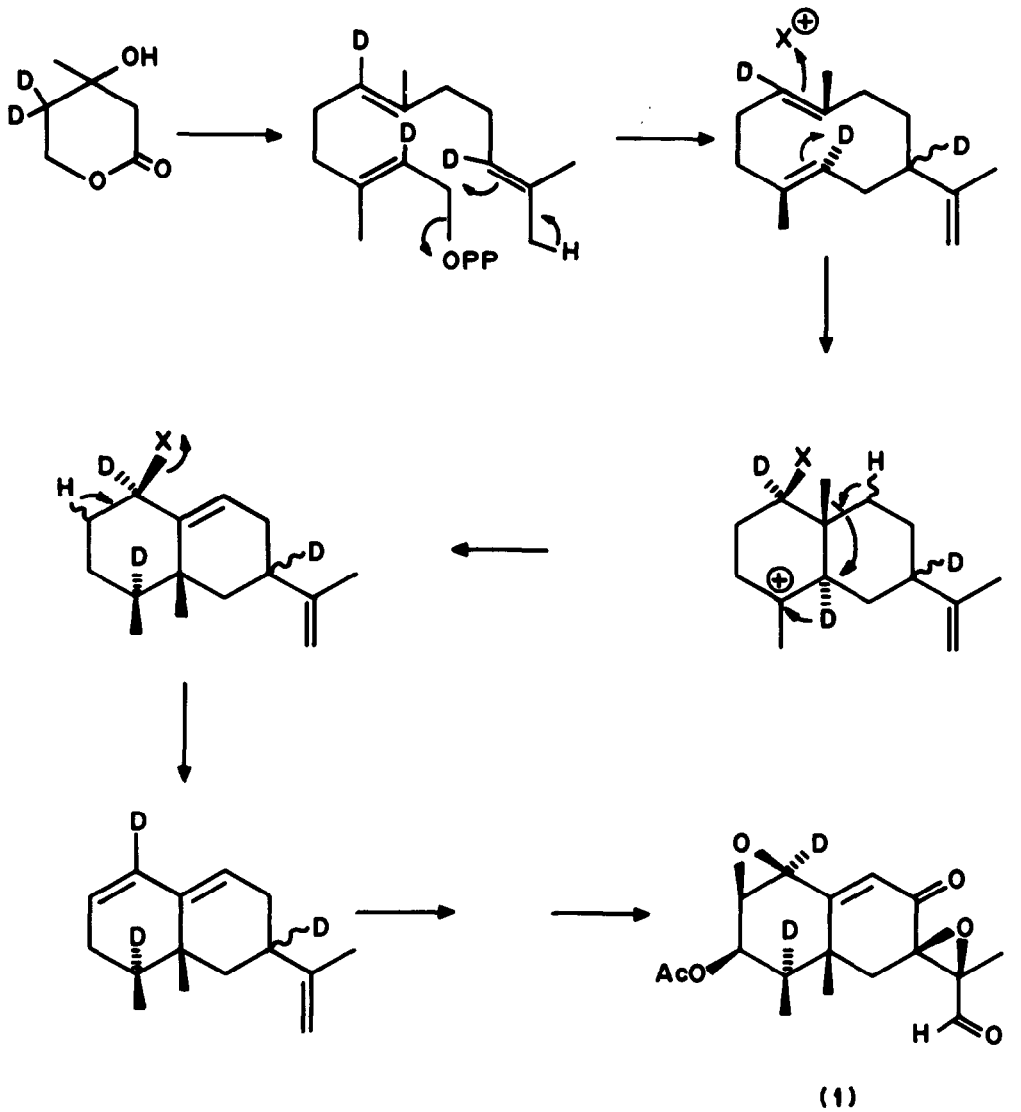

(1)

SCHEME. PROPOSED BIOSYNTHETIC PATHWAY IN THE FORMATION OF PR TOXIN

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