Bacterial Sterol Surrogates. Biosynthesis of the Side-chain of Bacteriohopanetetrol and of a Carbocyclic Pseudopentose from ^{13}C-Labelled Glucose in *Zymomonas mobilis*

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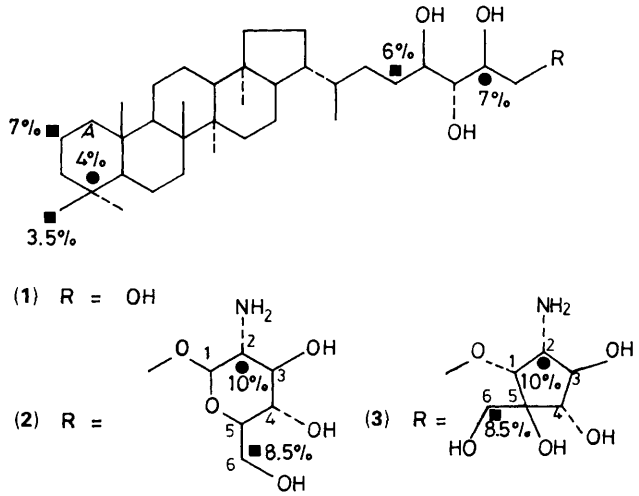
Incorporation of ^{13}C-labelled glucose into the triterpenoids of the hopane series of *Zymomonas mobilis* confirmed the following: (i) a D-ribose derivative is the precursor of the side-chain of bacteriohopanetetrol; (ii) the skeleton of a novel carbocyclic pentofuranose analogue derives from a hexose by formation of a carbon–carbon bond between C(1) and C(5); (iii) isoprenic units are not synthesized *via* direct incorporation of simple glucose catabolites into the isoprenoid biosynthesis.

The triterpenoids of the bacteriohopane family are essential metabolites of many prokaryotes; their presence is restricted solely to these micro-organisms.[1] These triterpenoids are equivalent to the eukaryotic sterols and were shown to modulate the properties of the cellular membranes of bacteria.[2] In the ethanol producing *Zymomonas mobilis*, the resistance to this membrane destabilizing solvent is related to the presence of hopanoids [(**1**), (**2**), and (**3**), Scheme 1]: the higher the ethanol concentration in the culture medium, the higher the hopanoid content of the cells.[3] This bacterium contains almost trace amounts of bacteriohopanetetrol (**1**), tetrol glycoside (**2**), and tetrol ether (**3**).[4] The structures were determined by us by comparison with the hopanoids of *Methylobacterium organophilum*.[4] The correct position of the ether linkage in hopanoid (**3**) was determined later on by Australian workers.[5]

The following interpretations were made from labelling patterns obtained previously from the incorporation of ^{13}C labelled acetate into the hopanoids of *Rhodospirillaceae* and *Methylobacterium organophilum*. Firstly, exogenous acetate from the culture medium is not incorporated directly into the isoprenoid biosynthesis.[6] Thus isoprenoids are synthesized in these prokaryotes either from different pools of C_2 unit precursors (obtained *e.g. via* the glyoxylate cycle and the Entner–Doudoroff degradation of glucose) or *via* a pathway which differs, in at least one of the first steps, from the previously accepted isoprenoid biogenetic scheme. Secondly, the precursor of the C_5 side-chain of the bacteriohopane skeleton is probably a D-ribose derivative arising from the non-oxidative pentose phosphate pathway.[6] Thirdly, the carbocyclic pseudopentose of the ether (**3**) might derive from a 2-ketohexose by a reaction sequence similar to that which leads from D-glucose into *myo*-inositol.[7]

Feeding experiments performed on *Zymomonas mobilis* using ^{13}C-labelled glucose gave direct proof of the intervention of hexose metabolism into the formation of the bacteriohopane side-chain and of the pseudopentose skeleton.

Indeed, in the bacteriohopane skeleton carbon atoms C(31) and C(34) of the side-chain derive from C(6) and C(2) of D-glucose, respectively. This shows that the precursor of the polyhydroxylated C_5 unit is a D-ribose derivative solely arising from the non-oxidative pentose phosphate pathway; the observed isotopic enrichments (Scheme 1) are in full agreement with this interpretation. Furthermore, the labelling pattern of the side-chains of glycoside (**2**) and ether (**3**) showed that exogenous D-glucose was directly converted into



Scheme 1. Labelling patterns of bacteriohopane derivatives (**2**) and (**3**) from *Zymomonas mobilis*: (A) after incorporation of [2-^{13}C]glucose (●, 10% isotopic enrichment); (B) after incorporation of [6-^{13}C]-glucose (■, 8.5% isotopic enrichment). *Zymomonas mobilis* has been grown on a defined medium (pH 5) containing ^{13}C-labelled glucose (20g/l) as the sole carbon source, citric acid (210 mg/l), biotin (1 mg/l), calcium panthotenate (1 mg/l), KH_2PO_4 (3.5 g/l), $MgSO_4 \cdot 7H_2O$ (2 g/l), NH_4Cl (1.6 g/l), and $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ (14 mg/l). Hopanoids have been isolated after acetylation and analysed as reported earlier.[4,7,10] Isotopic enrichments are directly indicated on the formulae of (**2**) and (**3**). All six isoprenic units of the triterpenic moiety of hopanoids (**2**) and (**3**) were identically labelled. Thus, for the sake of the clarity of the figure, the labelling pattern has only been indicated for the first isoprenic unit of ring *A*.

glucosamine and also showed correlation between the carbon atoms of the C_6 skeleton of the pseudopentose and those of D-glucose which are utilized as the sole carbon source by the bacterium.

Biosynthesis of this carbocyclic pentose analogue involves the formation of a carbon–carbon bond between C(1) and C(5) of a hexose precursor (this feature could not be determined by the experiments using ^{13}C-labelled acetate[6]) and a reaction sequence possibly starting from a D-fructose derivative and including the same reactions as those which lead from D-glucose to *myo*-inositol.[7,8] Similar or only slightly differing carbocyclic pseudopentoses have been found in the bacteriohopanetetrol derivatives in other bacteria,[4,9] antibiotics, and enzyme inhibitors from *Streptomyces spp.*[10] However, in the case of the adenosine analogue, aristeromycine, it could be shown that the pseudopentose moiety was synthesized *via* the formation of a carbon–carbon bond between C(2) and C(6) of the hexose frame-work.[11]

Finally, the labelling pattern of the pentacyclic triterpenic nucleus (Scheme 1) could not be interpreted by the direct incorporation of acetyl coenzyme A, synthesized from ^{13}C-labelled glucose, into the previously accepted isoprenoid biosynthetic pathway. This emphasizes a more general and long undisclosed problem concerning the first steps of the isoprenoid biosynthesis in prokaryotes. This problem was also revealed by the incorporation of ^{13}C-labelled acetate into the phytanyl chains of *Halobacterium cutirubrum*[12] or the hopanoids of *Rhodopseudomoas spp.* and *Methylobacterium organophilum*.[6] Thus, biosynthesis of mevalonic acid, which is the accepted precursor of isoprenic units and which has been reported to be incorporated into the polyterpenoids of several prokaryotes,[13] might either involve the intervention of distinct definite pools of C_2 unit precursors, or imply a different pathway from the classical one which starts from acetyl coenzyme A. This might be the case for *Zymomonas mobilis* since no thiolase activity related to the synthesis of acetoacetyl coenzyme A, the precursor of hydroxymethylglutaryl coenzyme A and consequently of mevalonic acid, could be detected.[14]

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