Biosynthesis of a Carbocyclic Pentose Analogue Linked to Bacteriohopanetetrol from the Bacterium *Methylobacterium organophilum*

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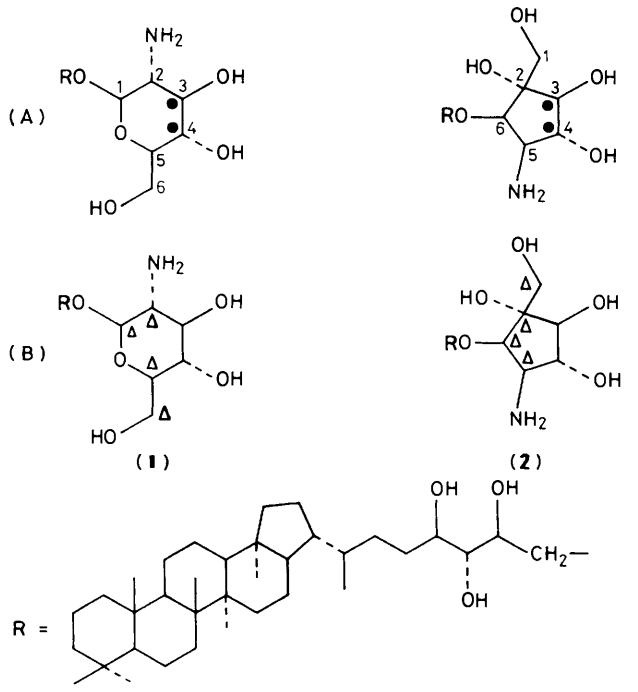
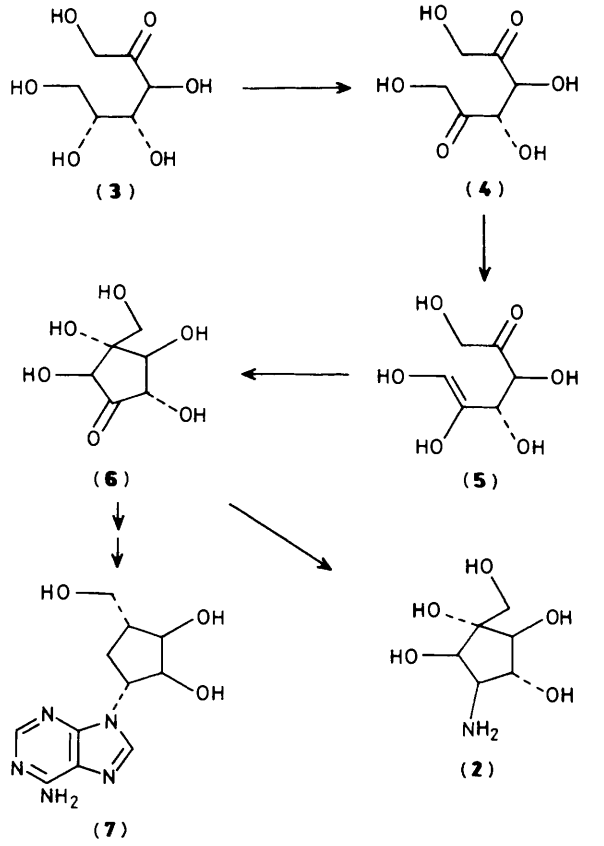
The labelling patterns resulting from incorporations of [1-^{13}C]- and [2-^{13}C]-acetate into a novel carbocyclic pentofuranose analogue linked *via* an ether bond to bacteriohopanetetrol in several bacteria were consistent with a reaction sequence starting from a 2-ketohexose and identical with those involved in the conversion of D-glucose into *myo*-inositol.

Systematic study of the complex hopanoids, a triterpenoid series widespread in prokaryotes[1] and acting as membrane reinforcers in these microorganisms,[2] has permitted us to identify a novel carbocyclic pentofuranose analogue linked *via* an ether bond to the primary hydroxy group of bacteriohopanetetrol (**2**) in three different bacteria: *Methylobacterium organophilum*,[3] *Rhodopseudomonas acdophila*,[4] and *Zymomonas mobilis*.[3,5] Biosynthetic studies on the formation of the bacteriohopane skeleton were performed by feeding *Methylobacterium organophilum* grown for 4 days at 30 °C on the ammonium/mineral salts medium of Whittenbury *et al.*[6] with ^{13}C-labelled acetate as sole carbon and energy source. The results showed that exogenous acetate is not directly incorporated into the isoprenoid biosynthetic pathway and that the C_5



Scheme 1. Labelling patterns of the bacteriohopane derivatives (**1**) and (**2**) from *M. organophilum*: (A) after incorporation of [1-^{13}C]-acetate (●) (10% isotopic enrichment); (B) after incorporation of [2-^{13}C] acetate (△) (10% isotopic enrichment). The numbering of (**2**) has been chosen on the assumption that the cyclopentane moiety arises from a D-hexose by formation of a carbon–carbon bond between C-2 and C-6 as reported for the biosynthesis of aristeromycin.[9] Hopanoids were isolated as reported earlier.[3] N.m.r. spectra were recorded for solutions in $CDCl_3$ at 100 MHz with a Bruker W400 spectrometer. Signal assignments were supported by ^{1}H homonuclear *J*-correlated spectra (COSY) and by ^{1}H/^{13}C heteronuclear ^1J-correlated spectra and were modified in comparison with our previous tentative assignments. ^{13}C N.m.r. data for acetylated (**1**): δ 99.1 (C-1), 55.0 (C-2), 72.1 (C-3), 68.5 (C-4), 71.7 (C-5) and 61.9 (C-6); for acetylated (**2**): δ 64.5 (C-1), 76.5 (C-3), 75.6 (C-3), 79.1 (C-4), 57.3 (C-5), and 81.6 (C-6).

non-isoprenoid polyhydroxylated straight chain arises from a D-ribose derivative derived from the non-oxidative pentose phosphate pathway and linked *via* C-5 to the hopane skeleton.[4,5] These labelling experiments also threw light on the biosynthesis of the carbocyclic pentose analogue of the ether (**2**). On the one hand the labelling pattern of the glucosamine moiety of the bacteriohopanetetrol glycoside (**1**) from this bacterium corresponded exactly to that expected from direct incorporation of exogenous acetate into the gluconeogenesis (Scheme 1). The ^{13}C n.m.r. spectrum of the acetylated glycoside (**1**) derived from [1-^{13}C]acetate showed enrichment at C-3 and C-4 (3 and 4 times natural abundance, respectively) whereas the same glycoside derived from [2-^{13}C]acetate showed significant enhancement at C-1, C-2, C-5, and C-6 with similar isotopic abundances. On the other hand the locations of the labelled positions and the isotopic abundances observed in the methylcyclopentane moiety of the bacteriohopane ether (**2**) were identical with those of the glucosamine moiety of the glycoside (**1**), and strongly suggested that a hexose could be the precursor of this C_6 carbon skeleton. Indeed the same enzymic reaction sequence leading from D-glucose to the cyclitol *myo*-inositol[7] would give the carbocyclic pentofuranose from a 2-ketohexose (Scheme 2).



Scheme 2. Postulated biogenetic pathway for the biosynthesis of the carbocyclic pentofuranose.

The relative stereochemistry of the substituents on the cyclopentane ring has been determined by other authors using the nuclear Overhauser effect.[8] Thus if a β-configuration is assigned to the amino group, the relative stereochemistry of the other functional groups is as indicated in Scheme 1. In particular the relative configurations of C-3 and C-4 are consistent with the role of a D-fructose derivative (**3**) as a possible precursor (Scheme 2). However our labelling experiments did not permit us to determine completely the mode of formation of the five-membered ring. Since the diketo intermediate (**4**) possesses C_2 symmetry, its enzymic enolisation could give either the ene-1,2-diol or the ene-5,6-diol (**5**), both leading to the same cyclic compound (**6**) with the same labelling pattern by formation of a carbon–carbon bond either between C-1 and C-5 or between C-2 and C-6. This hypothesis appears consistent with the results obtained from the incorporation of [1-^{13}C]- and [2-^{13}C]-D-glucose into the nucleoside aristeromycin (**7**): the formation of the cyclopentane moiety of this antibiotic proceeds with the formation of a carbon–carbon bond between C-2 and C-6 of D-glucose.[9] As all intermediates in Scheme 2 possess D-*threo* stereochemistry at C-3 and C-4, this would imply in the case of a common biosynthetic pathway for both types of cyclopentanoid an epimerisation at C-4, α to the C-5 carbonyl group of the intermediate (**6**), in the biosynthesis of aristeromycin, as well as further dehydration and reduction steps at C-2 and C-6.

These data suggest a common origin for all carbocyclic pentofuranose analogues and the possibility of a wide distribution of this new family amongst prokaryotes. Similar structures have been found in other metabolites from *Streptomyces* species, such as the nucleoside analogues adecypenol[10] and

neplanocin[11] and the chitinase inhibitor allosamidin.[12] Furthermore the C_7 carbocyclic hexopyranose analogues have been known for many years. They are incorporated into bacterial oligosaccharides and are potent inhibitors of α-glycosidases.[13] It remains to determine the biological activity associated with this new cyclitol type, and to elucidate completely, by incorporation of ^{13}C-labelled hexoses, the biosynthetic pathway leading to these polyfunctionalised carbocyclic pentofuranose analogues.

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