BIOSYNTHESIS OF ROSAMICIN

Sir:

Rosamicin is a 16-membered macrolide antibiotic produced[1] by *Micromonospora rosaria*. Its structure (**1**) has been elucidated[2] using chemical degradations and spectroscopic determinations. Rosamicin is highly active (*in vitro* and *in vivo*) against gram-positive bacteria and also possesses significant activity against gram-negative bacteria.

We became interested in the biosynthesis of rosamicin because we needed specifically labelled material for drug metabolism studies. Using ^{13}C-labelled precursors and established ^{13}C-nmr signal assignments, we have determined the pattern of isotopic incorporation into rosamicin from acetic and propionic acids and methionine. With this information specifically ^{14}C-labelled rosamicin could then be prepared.

The ^{13}C- chemical shift values of rosamicin are summarized in Table 1. These figures are based on the literature data[3] on related macrolide antibiotics and also from our own independent studies on rosamicin and its analogs.

In a typical experiment *Micromonospora rosaria* (culture number NRRL 3718) was inoculated into a medium (50 ml) containing yeast extract (5 g), fish solubles (1 g), corn steep liquor solids (1 g), starch (30 g), calcium carbonate (1 g) in tap water (1 liter). The flasks were then shaken at 28°C and after a few hours the labelled precursors (^{13}C or ^{14}C) were added and shaking continued for some more time. The harvested broth was adjusted to pH 9.5 and the crude rosamicin extracted into chloroform. It was purified using preparative tlc. The incorporation results are summarized as follows.

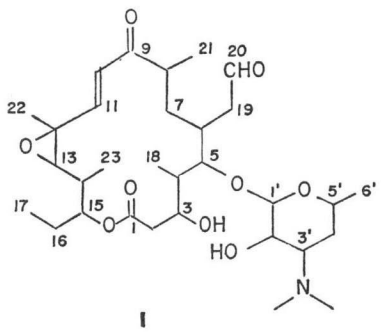
L-[Methyl-^{13}C] methionine was significantly incorporated into the dimethylamino function of the desosamine moiety of rosamicin. Incorporation of [1-^{13}C] acetate enriched carbons 1 and 9 although several other carbon atoms were somewhat enriched. This is probably due to indirect incorporation of acetate units. [2-^{13}C] Acetate was also incorporated poorly but enriched mainly carbon atoms 2 and 10.

[1-^{13}C] Propionate enriched specifically carbons 3, 7, 11, 13, 15 and [3-^{13}C] propionate enriched carbon atoms 17, 18, 21, 22 and 23. Propionate, unlike acetate, did not give much indirect incorporation. In a recent publication[4] it was reported that butyrate is incorporated well into the four carbon unit of 16-membered macrolide antibiotics corresponding to carbon atoms 5, 6, 19 and 20 in rosamicin. To test this hypothesis we investigated the incorporation of [1-^{13}C] butyrate in rosamicin and found that it was very well incorporated and caused significant enrichment of only carbon atom 5.

The percentage of incorporation of various precursors were as follows: ^{14}C-propionates (1.8%), L-[methyl-^{14}C] methionine (3.9%) and 1-^{14}C-butyrate (4.9%).

The macrolide ring of rosamicin is thus biosynthesized from one butyrate, two acetate and five propionate units. The methyl groups of the dimethyl amino group of desosamine are derived exclusively from L-methionine.

Table 1.*



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173.497 (C_1), 40.241 (C_2), 70.447 (C_3), 45.147 (C_4), 81.333 (C_5), 31.845 (C_6), 31.339 (C_7), 37.881 (C_8), 202.902 (C_9), 122.821 (C_{10}), 150.911 (C_{11}), 59.716 (C_{12}), 66.771 (C_{13}), 41.308 (C_{14}), 76.813 (C_{15}), 24.732 (C_{16}), 8.98 (C_{17}), 8.985 (C_{18}), 43.859 (C_{19}), 200.293 (C_{20}), 17.388 (C_{21}), 15.018 (C_{22}), 14.511 (C_{23}), 104.525 (C_1'), 70.447 (C_2'), 69.651 (C_3'), 28.502 (C_4'), 67.963 (C_5'), 21.107 (C_6')

* In the Table the chemical shift values are in δ and the assignments are indicated in parentheses.

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