NOTES

BIOSYNTHESIS OF AURODOX (ANTIBIOTIC X-5108).

INCORPORATION OF ^{14}C-LABELLED PRECURSORS INTO AURODOX[1,2]

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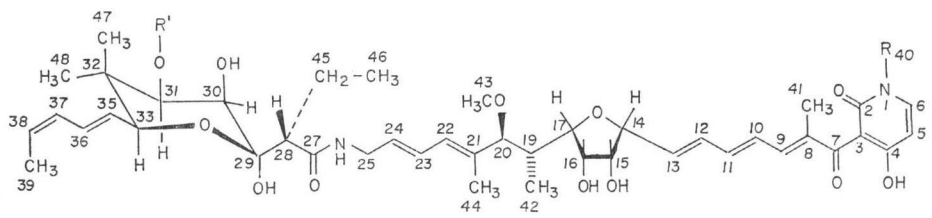
Streptomyces goldiniensis produces aurodox[3] (**1**) and small amounts of its N-demethyl homolog mocimycin (**2**)[4] which is probably identical[5] with kirromycin[6,7]. These antibiotics and a recently discovered disaccharide derivative of aurodox (**3**)[8] are primarily active against Gram-positive bacteria and enhance the growth of farm animals. The chemical structures, including stereochemistry, have been elucidated for aurodox and mocimycin[5,9]. Since aurodox is a member of a new class of antibiotics, biosynthetic studies of aurodox were of particular interest. In this report we describe the incorporation of ^{14}C-labelled precursors into aurodox.

The carbon skeleton of aurodox suggests that the antibiotic is in part derived from coupling of acetate units *via* the polyketide biosynthetic route[10]. The C-methyl groups could arise through transmethylations from suitable C_1-donors[11] or by the insertion of propionate[12] or isoprenoid units. The C-ethyl group could be generated by the incorporation of a butyrate unit into the polyketide chain[13], whereas the pyridone ring of aurodox could be derived *via* the nicotinic acid pathway[14] or, alternatively, from cadaverine. These possibilities were examined experimentally by measuring the incorporation of various ^{14}C-labelled precursors into aurodox (Table 1).

Radioactively labelled substrates at 10~100 μCi per 100 ml broth were individually added to fermentation cultures of *S. goldiniensis* 2 days after inoculation. After a further 5-day incubation period, aurodox was isolated according to procedures previously described[3].

According to the data in Table 1 the labels of methionine, glycine, acetate, propionate, butyrate, and β-hydroxybutyrate were incorporated into aurodox. The pattern of these incorporations (Table 2) was determined by measuring the radioactivity in fragments of aurodox derived by the degradation scheme[15,16] shown in Fig. 1.

The distribution of radioactivity in acetate-derived aurodox reveals little about the assembly of the polyketide chain. In contrast, the incorporation patterns of propionate and butyrate clearly show that an intact unit of each of these precursors is incorporated into aurodox. Localization of all three propionate carbons in fragment **8** suggests that propionate is incorporated into C-41 and C-8 as well as either C-7 or C-9. Butyrate labelling of goldinono-1,4-lactone-3,7-hemiacetal (**5**) may be due to incorporation into the C-ethyl carbons 45 and 46, and the two adjacent carbons 27 and 28. β-Hydroxybutyrate, a known precursor of butyrate, was also incorporated primarily into **5**. Perhaps the most interesting result is the extent of incorporation of methionine-methyl-^{14}C (9.7%) and the locali-



1 Aurodox (X-5108) R = CH_3; R' = H

2 Mocimycin (Kirromycin) R = R' = H

3 Efrotomycin R = CH_3; R' = Disaccharide ($C_{15}H_{27}O_8$)

zation of radioactivity in **5** (33%). These observations suggest that the geminal C-methyl groups at C-32 are derived from methionine-methyl; the lack of any incorporation of mevalonate-2-^{14}C and of incorporation of propionate into **5** are consistent with this proposal. The incorporation pattern of methionine methyl-^{14}C also suggests that the N-methyl (C-40), O-methyl (C-43), and two of the three C-methyls, (C-42 and C-44) are also derived from methionine. Glycine-2-^{14}C incorporation follows the same patterns as methionine-methyl-^{14}C, presumably because the methylene carbon of glycine serves as a C_1-donor.

Table 1. Incorporation of ^{14}C-labelled substrates into aurodox

Substrate (sodium salts)	Percentage incorporation
Acetate-1-^{14}C	2.6
Acetate-2-^{14}C	2.1
Propionate-1-^{14}C	2.2
Propionate-2-^{14}C	1.6
Propionate-3-^{14}C	0.9
Butyrate-1-^{14}C	2.2
Butyrate-2-^{14}C	2.6
Butyrate-3,4-^{14}C	2.2
β-Hydroxybutyrate-3-^{14}C	2.5
Methionine-methyl-^{14}C	9.7
Glycine-1-^{14}C	0.8
Glycine-2-^{14}C	2.9
Cadaverine-1,5-^{14}C	0.6
Mevalonate-2-^{14}C	0
Formate-^{14}C	0
Nicotinic acid-7-^{14}C	0
Quinolinic acid-6-^{14}C	0

The origin of the pyridone moiety of aurodox remains to be determined. The nicotinic acid pathway is apparently not involved since nicotinic acid-7-^{14}C and quinolinic acid-6-^{14}C were not incorporated. The possibility that cadaverine plays a role in the synthesis of the pyridone group was ruled out by the observation that the total radioactivity in the goldinamine part isolated as **6**, was lower than that contained in the goldinonic acid portion, isolated as **5**.

From these results, the following conclusions can be drawn: (1) Only one propionate unit and one butyrate unit are utilized by *S. goldiniensis* in the assembly of the antibiotic carbon skeleton; (2) six methyl groups are most likely derived *via* transfer of one-carbon units; (3) the pyridone group is not derived from nicotinic acid or cadaverine. Spectroscopic studies with ^{13}C-enriched aurodox derived from ^{13}C-labelled precursors should permit more specific determination of the biosynthetic origin and are now in progress.

References and notes

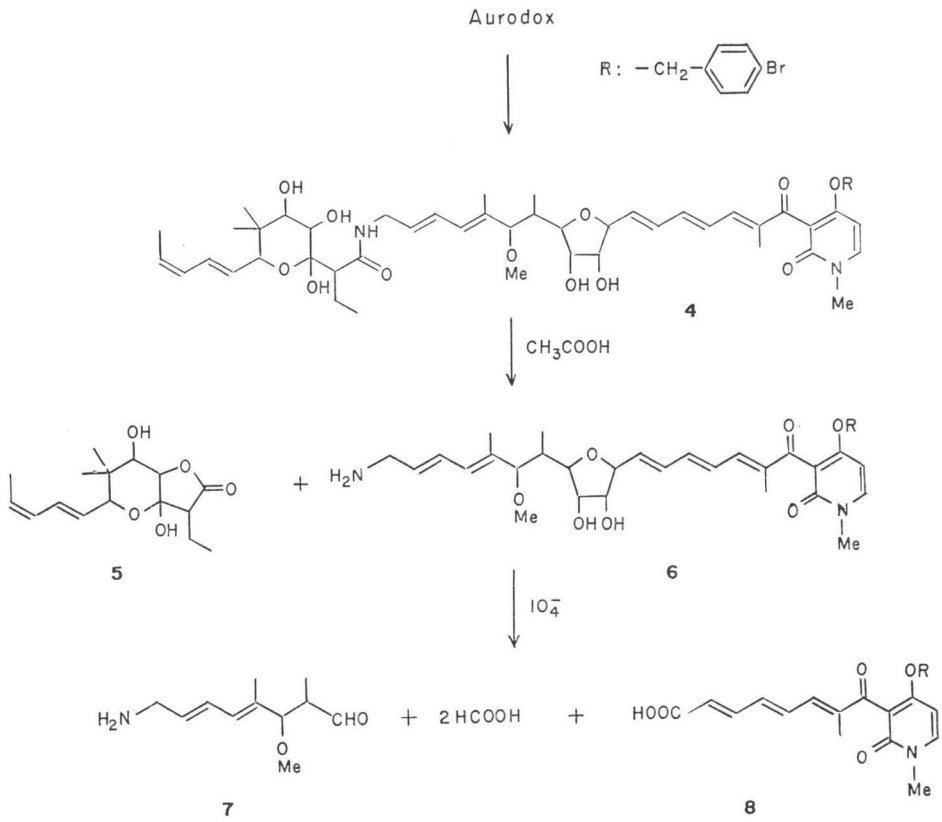
1) A preliminary account of this study was presented at the Annual Meeting of the American Society for Microbiology, 1974. (Abstr. Ann. Meeting American Society for Microbiology,

Table 2. Distribution of label radioactivity in aurodox derived from various ^{14}C-labelled precursors

Labelled precursor	Molar radioactivity, percent of aurodox			
	5	**6**	**7**	**8**
Acetate-1-^{14}C	49.6	50.5	38.9	11.5
Acetate-2-^{14}C	42.6	57.4	41.4	16.0
Propionate-1-^{14}C	1.0	98.7	1.2	89.1
Propionate-2-^{14}C	5.1	99.2	4.5	87.6
Propionate-3-^{14}C	5.7	92.7	5.4	88.4
Butyrate-1-^{14}C	73.8	29.5	3.5	22.5
Butyrate-2-^{14}C	73.2	37.4	12.1	23.3
Butyrate-3,4-^{14}C	62.7	44.1	8.5	33.8
β-Hydroxybutyrate-3-^{14}C	79.1	20.9	ND*	ND
Methionine-methyl-^{14}C	33.8	67.3	41.4	26.0
Glycine-2-^{14}C	32.4	67.2	52.8	12.0
Cadaverine-1,5-^{14}C	63.0	37.0	ND	ND

* ND=not determined

Fig. 1. Degradation of aurodox bromobenzyl ether



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2) Aurodox is the nonproprietary designation for antibiotic X-5108 and has been selected by the U. S. Adopted Names Council. The previously proposed name "goldinodox" has been withdrawn.
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