STUDIES OF BINDING C_3-SUBSTITUTE RIFAMYCINS TO HUMAN AND BOVINE SERUM ALBUMIN

ALESSANDRO ASSANDRI, ANTONIO PERAZZI and MARISA BERTI

Gruppo Lepetit S.p.A., Via Durando, 38, Milan, Italy

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The interactions of a series of C_3-substituted rifamycins with human and bovine serum albumins were studied in order to find possible correlations between the degree of binding and the structural features of the various molecules.

The results obtained indicate some of the physicochemical properties and, therefore, of the structural requirements which appear to determine or influence the bonding mechanisms of this series of rifamycins. Two types of interaction were found to exist, ionic and hydrophobic types. The findings suggest that the inhibition by protein of the antibacterial activities of these antibiotics depends on the type of bonding mechanism rather than the degree of binding.

The importance of reversible binding to serum proteins, and in particular to the albumin fraction, in determining the body distribution of a drug has been repeatedly demonstrated[1,2]. Moreover, the influence of protein-binding on the effectiveness of antibiotics against infections *in vivo* has been assessed[3,4].

Our interest in C_3-substituted rifamycins, a class of antibiotics with a broad antibacterial spectrum[5], led us to investigate *in vitro* the characteristics of the reversible binding of a series of these drugs to human and bovine albumin. A correlation has been found between the binding strength and the molecular structure, a result which promises a useful approach in predicting the physiological disposition of new semi-synthetic rifamycins.

Materials and Methods

1. Chemicals

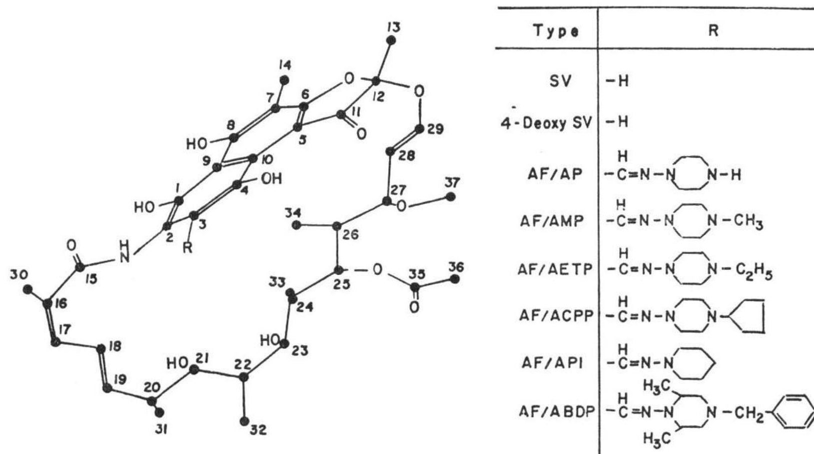
37-^{3}H-SV, (rifamycin SV), spec. act. 0.408 mCi/mmole; 37-^{3}H-4-DSV (4-deoxy-rifamycin-SV), spec. act. 0.471 mCi/mmole (rifamycins SV and DSV were obtained biosynthetically with ^{3}H-methionine); 38-^{14}C-AF/AP (3-(1-piperazinyl)imino methyl rifamycin SV), spec. act. 1.62 mCi/mmole; 38-^{14}C-AF/AMP, (3-[[(4-methyl-1-piperazinyl)imino]methyl]rifamycin SV), spec. act. 10.77 mCi/mmole; 38-^{14}C-AF/AETP, (3-[[(4-ethyl-1-piperazinyl)imino]methyl]rifamycin SV), spec. act. 10.29 mCi/mmole; 38-^{14}C-AF/ACPP, (3-[[(4-cyclopentyl-1-piperazinyl)imino]methyl]rifamycin SV), spec. act. 10.21 mCi/mmole; 38-^{14}C-AF/ABDP, (3-[[(4-cis-aminobenzyl-1-2,6-dimethyl-piperazinyl)imino]methyl]rifamycin SV), spec. act. 12.15 mCi/mmole; 38-^{14}C-AF/API, (3-[[1-piperidinyl)imino]methyl]rifamycin SV), spec. act. 1.79 mCi/mmole. The molecules were synthesized in their hydroquinonic forms (HQ) by G. SARTORI of Gruppo Lepetit, Milan. The quinonic forms (Q) from the same compounds were prepared by the authors by oxidation with MnO_2 in $CHCl_3$. For formulas see Fig. 1.

Human serum albumin (HSA), bovine serum albumin (BSA) were purchased from Sigma Chem. Co. All calculations are reported in terms of a molecular weight (Mw) of 69,000 for HSA and 67,000 for BSA.

2. Binding assay

Because of the high lipophilicity of many of the rifamycins under investigation, the binding could not be determined by those traditional methods utilizing membranes, *e.g.*, equilibrium dialysis. In-

Fig. 1. Rifamycins studied, basic structure and type of substitutions



Type	R
SV	-H
4-Deoxy SV	-H
AF/AP	H -C=N-N N-H
AF/AMP	H -C=N-N N-CH$_3$
AF/AETP	H -C=N-N N-C$_2$H$_5$
AF/ACPP	H -C=N-N N-
AF/API	H -C=N-N
AF/ABDP	H H$_3$C -C=N-N N-CH$_2$- H$_3$C

stead, a gel filtration technique, the "large zone-small zone elution method", was used[6]: Sephadex G-25 (medium type, from Pharmacia Fine Chem.) swollen in 0.05 M sodium phosphate buffer, at the stated pH values, with or without 10^{-3} M ascorbic acid was packed in an LKB glass column (0.9×30 cm, with thermostatic jacket). Once the partition coefficients of the different rifamycins had been determined, in the absence of the macromolecular component, the columns were pre-equilibrated with the buffered protein solutions, and loaded with the ligands (10 μg) dissolved in 0.3 ml of the same medium containing 0.025 ml of ethanol. Elution was performed with the same buffer solutions, at 4° and 37°C, and 0.5-ml fractions were collected.

The "large zone-small zone elution method" permits calculation of the Bound/Free ratio (B/F) of the small molecular weight ligands by means of the following equation:

$$\frac{{}^{0}V_e - V_o}{V_e - V_o} - 1 = \frac{{}^{0}\sigma}{\sigma} - 1 = \frac{B}{F} K_a \times n(P_t - P_b) \qquad (1)$$

where V_e and ${}^{0}V_e$ are the elution volumes of the small Mw ligands in the presence or the absence of the macromolecules; σ and ${}^{0}\sigma$ are the corresponding chromatographic partition coefficients; V_o is the void volume of the column. B and F refer to the bound and free fractions of small Mw ligands, K_a is the association constant, and n is the number of identical, non-interacting, binding sites of the macromolecule. P_t and P_b are the total and bound protein concentrations.

From equation (1) once the B/F ratio has been calculated and P_t known, and when $P_t \gg B$, the product $K_a \times n$, defined as the "combining affinity" is given by:

$$K_a \times n = \frac{B}{F \times P_t} \text{(liters mole}^{-1}\text{)} \qquad (2)$$

Since in our binding assays the assumption above ($P_t \gg B$) met the experimental conditions it was convenient to use the "combining affinity" as the reference parameter of the binding strength.

3. Thermodynamic parameters

ΔG^0, ΔH^0 were calculated from the following equations:

$$\Delta G^0 = RT \ln K_a \times n \qquad \text{(cal/mole ligand bound)} \qquad (3)$$

$$\Delta H^0 = \frac{\ln (K_{a1} \times n_1 / K_{a2} \times n_2)}{(1/T_2 - 1/T_1)} \qquad \text{(cal/mole ligand bound)} \qquad (4)$$

$$\Delta S^0 = \frac{\Delta H - \Delta G}{T} \qquad (\text{cal} \cdot \text{mole}^{-1} \text{ degree}^{-1}) \qquad (5)$$

4. Determination of minimal inhibitory concentration (MIC)

MIC's were determined by the serial dilution technique published by ARIOLI *et al.*[7] in the presence

or absence of 30% bovine serum corresponding to a final concentration of 12% BSA. The test strain was *Staphylococcus aureus* ATCC 6538.

5. Lipophilic indices

R_m were determined by reversed-phase thin-layer chromatography by PELIZZA *et al.*[8], according to the formulation of BOYCE and MILBORROW[9]:

$$R_m = \log\left(\frac{1}{R_f} - 1\right) \tag{6}$$

6. Ionization constants

(pKa_1 and pKa_2) of the different rifamycins were determined by spectrophotometric analysis, by RADAELLI *et al.* and PASQUALUCCI *et al.*[10,11,12].

Results

1. Preliminary

In a previous work[6] the authors demonstrated the binding of HQ-AF/AMP (rifampicin) to the albumin fraction of bovine serum. Since rifamycins can exist in two forms, *i.e.*, quinones and hydroquinones, which might have different affinities for the different serum fractions, the investigation was then repeated with oxidized rifampicin, with the same results. Because of this and because the albumin fraction is present in the largest relative amount in plasma, albumin is pharmacokinetically the most important component. Therefore, we have limited our investigations to the albumin fractions from human and bovine serum proteins. This does not exclude the possibility that among the different rifamycins studied some of them might bind to protein fractions other than albumin.

2. Binding of HQ and Q-Rifamycins to HSA and BSA

The "combining affinities" of the complexes between the rifamycins SV, AF/AP, AF/AMP, AF/AETP, AF/ACPP, AF/ABDP, AF/API and HSA or BSA were measured by the "large zone-small zone elution method" at 37°C and pH 7.4 in phosphate buffer in presence (hydroquinones) or

Fig. 2. Interactions between HQ rifamycins and BSA or HSA: graphical estimation of the combining affinities ($K_a \times n$ products).

On the ordinate are the Bound/Free ratios, on the abscissa the albumin concentrations.

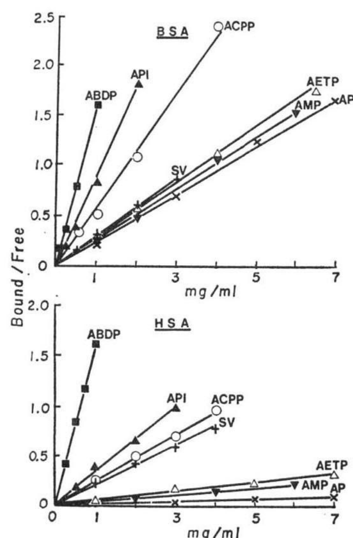


Fig. 3. Interactions between Q rifamycins and BSA or HSA; graphical estimation of the combining affinities ($K_a \times n$ products).

On the ordinate are the Bound/Free ratios, on the abscissa the albumin concentrations.

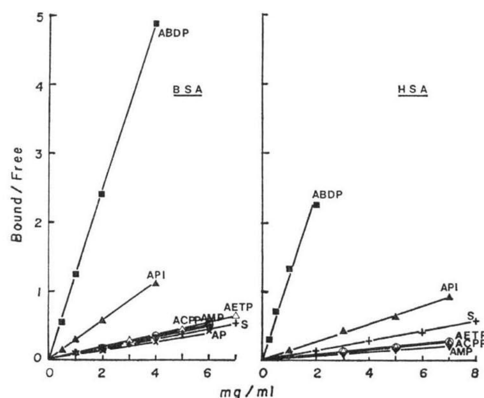


Table 1. Combining affinities-values of Q and HQ rifamycins to bovine and human serum albumin 37°C, pH 7.4.

Type	$K_a \times n \times 10^{-4} \pm$S.E. (liters mole^{-1})			
	BSA		HSA	
	HQ	Q	HQ	Q
4-DSV	2.76±0.11	—	—	—
SV	2.05±0.08	0.56±0.02	1.46±0.06	0.51±0.01
AF/AP	1.60±0.05	0.49±0.08	0.11±0.02	n.d.*
AF/AMP	1.75±0.06	0.61±0.03	0.28±0.02	0.21±0.04
AF/AETP	2.01±0.04	0.63±0.09	0.36±0.03	0.28±0.02
AF/ACPP	3.59±0.10	0.54±0.05	1.71±0.04	0.27±0.04
AF/ABDP	10.31±0.37	8.31±0.13	11.35±0.24	8.73±0.42
AF/API	5.52±0.27	1.99±0.03	2.50±0.14	0.95±0.05

* n.d.: non detectable.

in absence (quinones) of ascorbic acid. The results, as reported in Figs. 2 and 3 where the calculated B/F ratio is plotted against the protein concentration, show for the series of ligands considered that affinity values range between 10^3 and 10^5 (see Table 1). Higher binding strengths were found for BSA, as compared with HSA, and it is very apparent that the affinity values are much lower for the Q-forms. There is however, an exception to the general behaviour: rifamycin AF/ABDP seems to bind with about the same strength to both BSA and HSA, in either its Q or HQ form.

3. Approaches to the Bonding Mechanism

In order to clarify some of the structural features affecting the interactions between these rifamycins and bovine albumin the following experiments were carried out.

(1) Binding of 4-deoxy-rifamycin SV to BSA

The large difference in binding strength between Q and HQ rifamycins suggested that the hydroxyl groups at C_1 and/or C_4 are involved in the bonding mechanism, and it was of interest to verify whether and to what extent 4-DSV would bind to BSA. Actually, the "combining affinity" measured for 4-DSV proved to be even higher than that for SV (see Table 1), indicating that only the C_1-hydroxy group is relevant to the bonding reaction.

(2) Temperature dependence

In evaluating the forces involved in the binding of a ligand by protein, it is often useful to determine whether the free energy change comes about from a change in the heat content (ΔH) or in the entropy of the system (ΔS). In effect, no variation of the binding affinities could be observed for the rifamycins studied within the range of temperature between 4° and 37°C, with the sole exception of SV for which the enthalpy change accounts for about one third of the total free energy change (Table 2).

(3) pH dependence

All the investigated molecules possess, in their hydroquinonic form, an acid function (C_1-C_8 dihydroxy), however, while rifamycin SV has no other ionizing groups, all the others have one basic nitrogen function.

Moreover the pK_{a_1}-values of the rifamycins studied are with the sole exception of AF/API, comparable to that of SV (pK_{a_1}=1.8). For AF/API (pK_{a_1}=4.8), the presence of a hydrazonic group causes a weakening of the C_1–C_8 dihydroxy acid function; this effect is, on the contrary, neutralized in

Table 2. Thermodynamic parameters of the interactions between HQ-rifamycins and BSA. The lipophilic indices of the ligands are also reported.

Type	ΔG^0	ΔH^0	ΔS^0	Rm*
SV	−6114	−2077	+13.02	−0.930
AF/AP	−5964	0.0	+19.24	−0.293
AF/AMP	−6018	0.0	+19.41	−0.277
AF/AETP	−6103	0.0	+19.69	−0.244
AF/ACPP	−6461	0.0	+20.84	−0.138
AF/ABDP	−7111	0.0	+22.94	+0.340
AP/API	−6726	0.0	+21.70	−0.086

* Data from PELIZZA *et al.*[8].

Table 3. pH Dependence of the interaction between HQ-rifamycins and BSA

Type	pK_{a_1}	pK_{a_2}	$K_a \times n \times 10^{-4} \pm$S.E.	
			pH 5.8	pH 7.4
SV	1.8	—	7.96±0.28	2.05±0.08
AF/AP	1.9	8.2	0.85±0.10	1.60±0.05
AF/AMP	1.7	6.7	1.02±0.13	1.75±0.06
AF/AETP	1.7	6.7	1.25±0.12	2.01±0.04
AF/ACPP	2.4	6.5	1.52±0.05	3.59±0.10
AF/ABDP	2.6	6.0	3.90±0.16	10.31±0.37
AF/API	4.8	—	6.56±0.23*	5.52±0.27

pK_{a_1} refer to the C_1-C_8 dihydroxy group (estimated in water), pK_{a_2} refer to the N_4 of the piperazine moiety (estimated in 80% of methylcellosolve).

* The AF/API pH-dependence was measured at pH 8.5 instead of 5.8 because it was strongly retained by the gel matrix at the lower pH-value.

Table 4. Influence of 30% bovine serum (≃12% BSA) on the MIC's of HQ-rifamycins against *Staphylococcus aureus*

Type/AF	MIC (μg/ml)		MIC (+BSA) / MIC (−BSA)
	0% BSA	12% BSA	
SV	0.012	0.012	1
AF/AP	0.005	0.01	2
AF/AMP	0.0025	0.005	2
AF/AETP	0.005	0.005	1
AF/ACPP	0.005	0.01	2
AF/ABDP$_{cis}$	0.002	0.2	100
AF/API	0.002	0.1	50

Table 5. Free energies of formation of the complexes between HQ or Q rifamycins and BSA 37°C, pH 7.4.

Type/AF	$\Delta G^0_{(HQ)}$	$\Delta G^0_{(Q)}$	$\Delta G^0_{(HQ)} - \Delta G^0_{(Q)}$
SV	−6114	−5316	− 799
AP	−5964	−5234	− 730
AMP	−6018	−5369	− 649
AETP	−6103	−5389	− 714
ACPP	−6461	−5293	−1167
ABDP	−7111	−6978	− 133
API	−6726	−6097	− 629

the piperazine derivatives by the presence of the electrophilic aminic group (N_4) giving the internal salt[8,13].

The binding affinity of the complex SV-BSA rises with lowering of the pH (from 7.4 to 5.8) while the opposite occurs for all the other rifamycins (Table 3). The pH-dependence of the binding between AF/API and BSA could not be verified at pH 5.8 because of its physico-chemical properties, the investigation was instead carried out at pH 8.5.

(4) Binding strength and biological activity

It is known that the antibiotic effectiveness may be affected by the interaction with serum protein and that it is sometimes correlated with the concentration of the unbound fraction only[3].

For this reason, we compared the antibacterial activities of the rifamycins in the presence and absence of 30% bovine serum (≃12% BSA). The data, reported in Table 4, showed that BSA strongly inhibited only the activities of AF/API and AF/ABDP.

Discussion

All the rifamycins studied, in their reduced form (4-DSV, SV, AF/AP, AF/AMP, AF/AETP, AF/ACPP, AF/ABDP, and AF/API) were found to interact with BSA and HSA with "combining affinity-values" ($K_a \times n$) between 10^3 and 10^5 liters per mole. With the exception of AF/ABDP the binding strength to BSA was greater than to HSA. Lack of temperature-dependence suggests for all the C_3-substituted rifamycins under investigation the formation of apolar or hydrophobic bonds which are essentially athermal entropy-driven interactions ($\Delta H=0$).

As to the interaction between SV and BSA, it is primarily entropically driven, but also shows temperature-dependence, the bonding mechanism involved appears to be of an electrostatic type in which the enthalpy change contributes to a minor extent to the free energy change[14]. However, it is well known that thermodynamic parameters alone do not provide a diagnostic criterion for choosing between the types of forces involved in ligand binding by proteins. Indeed the molecular mechanism must be consistent with thermodynamics, but it is also clear that thermodynamic studies in themselves cannot select between alternative, consistent molecular explanations. Actually, in agreement with the hypothesis of an ionic interaction, the lowering of pH, which causes a decrease of the net charge on the albumin molecule without affecting the dissociation of the C_1–C_8 dihydroxyl group, leads to an increased binding strength of rifamycin SV. On the contrary, for the other C_3-substituted rifamycins, lowering the pH causes a weakening of the binding strength. This last finding is consistent with two possible mechanisms: (1) uncovering of new binding sites on the albumin molecule, which may result from a reversible swelling induced by the strong electrostatic repulsions that develop in a protein molecule with an increase in negative charge[15~18], (2) an increased dissociation of the cationic nitrogen of the piperazine moiety, causing in itself or through the formation of an internal salt a noncompetitive inhibition of the ionic site(s) of the albumin molecules. In order to avoid a misleading interpretation of the data on the pH-dependence, we want to emphasize that the results obtained with the C_3-substituted rifamycins, according to the last hypothesis, are consistent also with ionic interactions.

Additional information about the binding mechanism was obtained from the data showing that the strength of the bond of the hydroquinone form was greater than that of the quinone form (Table 1), and those showing that of the two hydroquinonic hydroxyl groups the one in position 1 is the only one involved, directly or indirectly, either favouring or inhibiting the formation of the rifamycin-albumin complex (see 4-DSV). Apropos of this effect, it is to be emphasized that in the quinonic form the keto group at C_1 forms a hydrogen bridge with the phenolic hydroxyl at C_8, thus diminishing its acid strength. Effectively, the free-energies of formation of the rifamycin-BSA complexes for the quinonic forms of SV, AF/AP, AF/AMP, AF/AETP, and AF/ACPP, are practically identical, ranging between 5,234 and 5,389 cal./mole, while those of Q AF/API (6,097 cal./mole) and Q AF/ABDP (6,978 cal./mole) are higher. These data, together with the [$\Delta G^0_{(HQ)}$ - $\Delta G^0_{(Q)}$]-differences, are reported in Table 5, and appear to differentiate the binding mechanism of AF/API and AF/ABDP from that of the other rifamycins.

Although it is not clear whether the apparently different nature of the binding of AF/API and AF/ABDP to BSA bears any relation to the fact that these two rifamycins are the only ones whose antibacterial activity is strongly inhibited by the presence of albumin, undoubtedly this remains an attractive hypothesis. To summarize: a) all rifamycins may probably interact with albumin by a low affinity nonspecific binding, which involves the basic molecular structure either in the reduced or oxidized form (*e.g.* rifamycin S).

b) Rifamycin SV forms an ionic bond through the C_1–C_8 dihydroxy acid function. The same bonding seems to be retained also by rifamycins AF/AP, AF/AMP, AF/AETP, AF/ACPP, AF/ABDP, and AF/API in their hydroquinone forms, even taking into account a probable inhibiting action of the piperazine cation*.

c) Very probably, rifamycins AF/ABDP and AF/API, either as hydroquinones or quinones,

* The stronger ionic bonding of AF/ACPP, probably rises from the structural features, the lower dissociation of the anionic function, and the steric masking by the cyclopentyl ring on the basic nitrogen of the piperazine moiety.

through the piperazine or piperidine lipophilic side chains, undergo also hydrophobic interactions.

d) The number of binding sites to BSA should be nearly 1 for SV, AF/AP, AF/AMP, AF/AETP and AF/ACPP, >1 for AF/API and AF/ABDP[19].

e) The binding mechanisms do not have to be correlated in any way with the lipophilic indices of the different rifamycins even though they seem to be on casual scrutiny.

Although the factors which completely characterize formation of these complexes are probably diverse and more complicated than indicated, in any event, the authors suggest that the mechanism deduced, even though hypothetical, may prove to be of use in a research program for new semi-synthetic rifamycins substituted in position C_3, in so far as studies in progress have demonstrated the influence of binding to serum albumin on the pharmacological and pharmacokinetic behaviour of these antibiotics.

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NOTES

BIOSYNTHESIS OF AURODOX (ANTIBIOTIC X-5108).

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

CHAO-MIN LIU, HUBERT MAEHR, MICHAEL LEACH, MARK LIU and PHILIP A. MILLER

Chemical Research Department
Hoffmann-La Roche Inc.
Nutley, New Jersey 07110, U.S.A.

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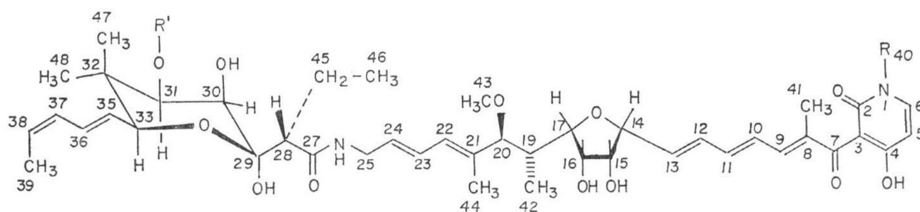
Streptomyces goldiniensis produces aurodox[3] (**1**) and small amounts of its N-demethyl homolog mocimycin (**2**)[4] which is probably identical[5] with kirromycin[6,7]. These antibiotics and a recently discovered disaccharide derivative of aurodox (**3**)[8] are primarily active against Gram-positive 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.

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

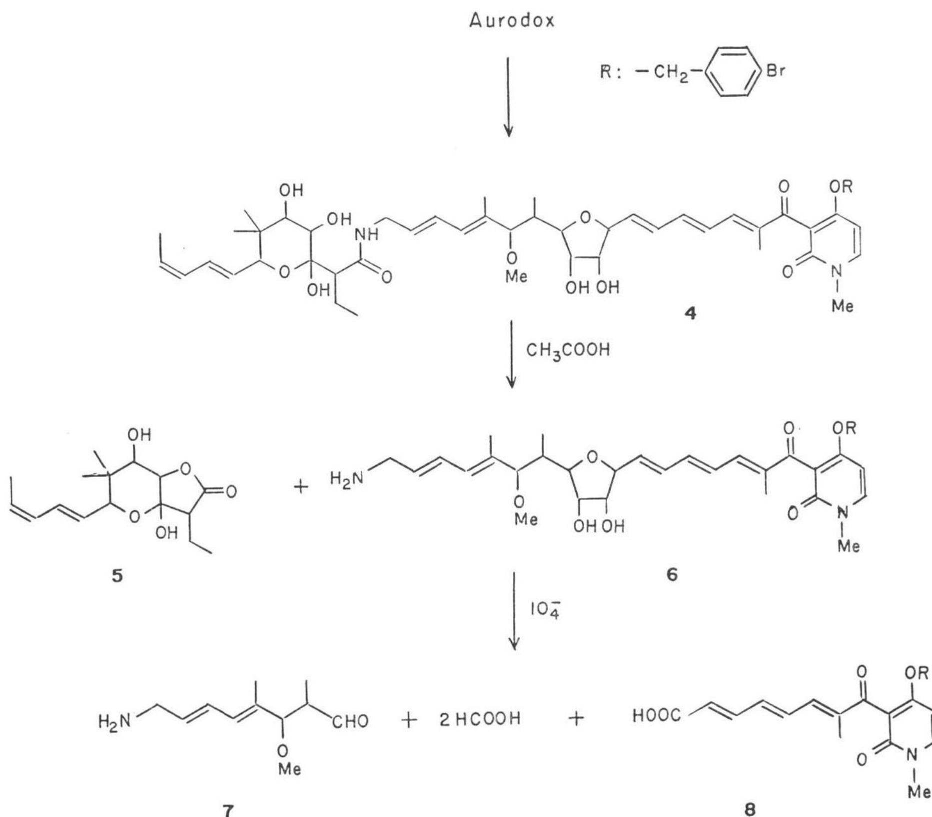
Labelled precursor	Molar radioactivity, percent of aurodox			
	5	6	7	8
Acetate-1-^{14}C	49.6	50.5	38.9	11.5
Acetate-2-^{14}C	42.6	57.4	41.4	16.0
Propionate-1-^{14}C	1.0	98.7	1.2	89.1
Propionate-2-^{14}C	5.1	99.2	4.5	87.6
Propionate-3-^{14}C	5.7	92.7	5.4	88.4
Butyrate-1-^{14}C	73.8	29.5	3.5	22.5
Butyrate-2-^{14}C	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

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,

Fig. 1. Degradation of aurodox bromobenzyl ether



p. 19, 1974).

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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