The Biosynthesis of Spectinomycin

By L. A. MITSCHER,* L. L. MARTIN and D. R. FELLER
(*College of Pharmacy, The Ohio State University, Columbus, Ohio* 43210)
and J. R. MARTIN and A. W. GOLDSTEIN
(*Abbott Laboratories, Scientific Divisions, North Chicago, Illinois* 60064)

Summary The biosynthesis of spectinomycin has been examined by feeding labelled precursors to *Streptomyces flavopersicus* and specific degradations of the antibiotic performed; the *N*-methyl groups are derived from methionine, the spectinamine moiety comes from D-glucose *via* myoinositol and the remainder of the molecule is derived from glucose.

SPECTINOMYCIN (V) is a broad spectrum antibiotic produced by various strains of the genus *Streptomyces*.[1,2] It is a water soluble aminoglycoside with an unusual structure.[3] Ring A of the molecule appears to be derived from D-glucose *via* an inositol,[4] and ring C from either D-glucose or acetate *via* the polyketide. The results reported here show that both halves are derived eventually from D-glucose and suggest the probable sequence of biochemical events (Scheme 1).

Various compounds (see Table 1) were fed to fermentations of *Streptomyces flavopersicus* (Abbott strain 66-666) actively producing spectinomycin. The organism was grown in a synthetic medium.[5] Samples were analysed by scintillation counting and/or after combustion.[6]

The antibiotic produced was isolated from the fermented liquor by centrifugation, washing of the mycelia, passage of the combined supernatant liquid and washings over an IRC-50(Na^+) ion-exchange column, and elution with 1N hydrochloric acid. Samples were crystallized to constant specific activity and degraded according to Scheme 2.[3] The results are summarized in Table 2.

TABLE 1. *Incorporation of isotopes into spectinomycin following administration of various labelled compounds to* S. Flavopersicus

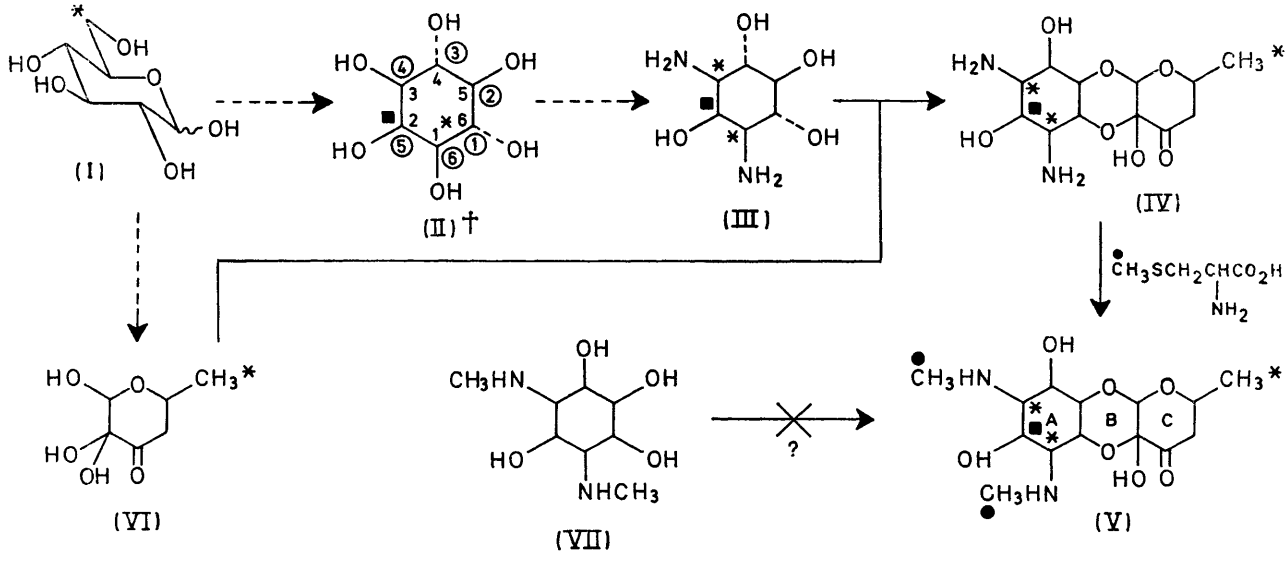
Compound administered[a]	Quantity administered (disintegrations/mm) × 10^6	Isotope incorporated (%)
1 Acetic acid-2-^{14}C (24 h)	22·2	0·26
2 L-Methionine-S-^{14}C-Methyl (48 h)	2·23	38·8
3 D-Glucose-6-^{3}H (48 h)	5·87	3·50
4 D-Galactose-U-^{14}C (24 hr)	1·75	0·01
5 Myoinositol-2-^{14}C (24 h)	6·42	47·0
6 Myoinositol-2-^{14}C (120 h)	6·42	46·8
7 Spectinamine-2-^{14}C (72 h)	1·10	6·60

[a] In all cases the labelled precursors were added after 69 h. Figures in parentheses are the number of hours between the addition of the labelled compound and the isolation of the antibiotic.

TABLE 2. *Distribution of isotopes in spectinomycin labelled by bioincorporation*

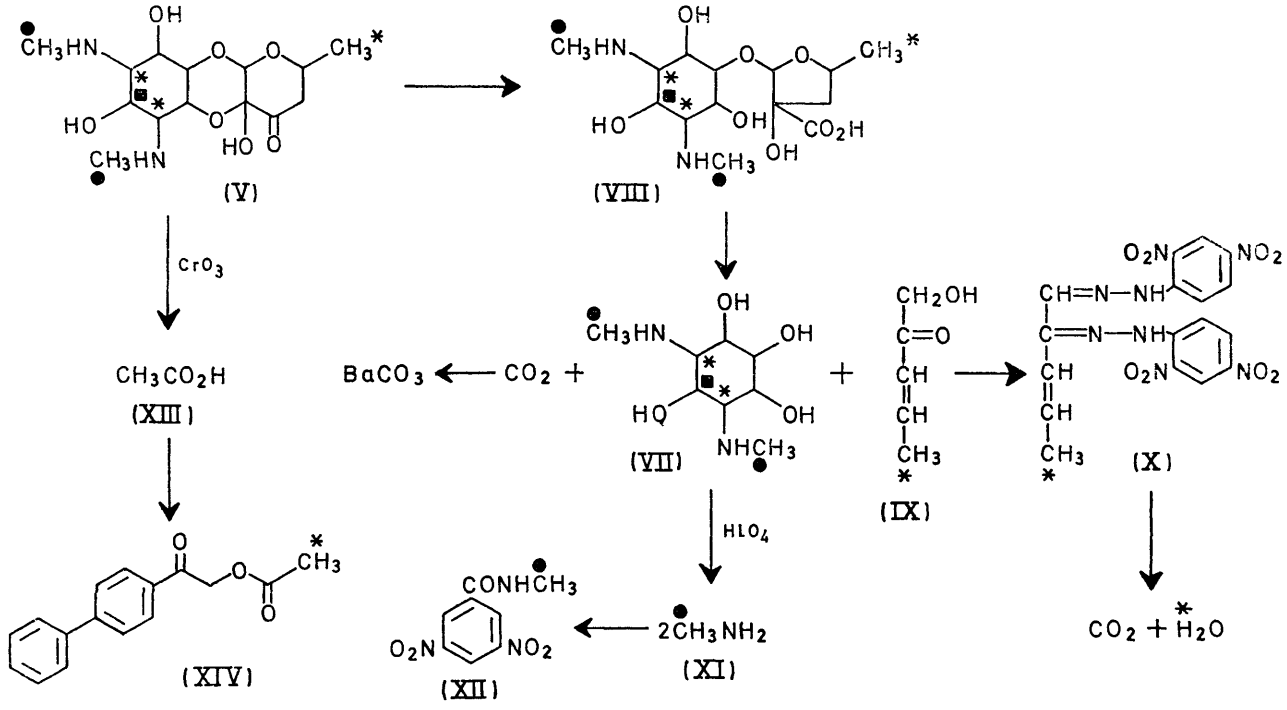
Precursor	Spectinomycin[a] (μCi/mM)	Recovery of label (%)	Total recovery (%)
2	(i) 0·0828	(VII) = 94·16; (XII) = 92·10; (X) = 0; $BaCO_3$ = 0	94·16
	(ii) 0·1062	(VII) = 91·05; (X) = 0; $BaCO_3$ = 0	91·05
3	(i) 0·4192	(VII) = 49·45; (X) = 37·45; $BaCO_3$ = 0	86·90
	(ii) 0·5088	(VII) = 46·11; (X) = 36·91; $BaCO_3$ = 0	83·02
5	(i) 0·1631	(VII) = 90·55; (X) = 0; $BaCO_3$ = 0	90·55
	(ii) 0·1523	(VII) = 89·20; $BaCO_3$ = 0	89·20
6	(i) 0·1319	(VII) = 91·05; (X) = 0; $BaCO_3$ = 0	91·05
	(ii) 0·1195	(VII) = 88·03; (X) = 0; $BaCO_3$ = 0	88·03

[a] (i) and (ii) represent individual experiments. The values refer to the specific activity of the spectimonycin after dilution and crystallization to constant specific activity.



SCHEME 1. *Suggested pathway of spectinomycin biosynthesis in* Streptomyces flavopersicus.

† Encircled numbers refer to glucose, the others are myoinositol numbers.



SCHEME 2.

The incorporation data in Table 1 show that acetate and D-galactose play no significant role in spectinomycin biosynthesis. The latter finding is especially interesting because addition of galactose to the medium greatly stimulates spectinomycin production.[5] Excellent incorporation was obtained when myoinositol-2-^{14}C and methionine-*S*-^{14}C-methyl were fed to the organism. After degradation the label was found exclusively in the spectinamine moiety (VII). D-glucose-6-^{3}H was also an excellent precursor considering the anticipated dilution with a sizable exogenous pool. Degradation showed nearly equal incorporation of radioactivity in rings A and C of the molecule. In particular, essentially all the ^{3}H in ring C was associated with the lone C-methyl group of spectinomycin, as required by Scheme 1, and as shown by Kuhn-Roth degradation.[7] Thus, spectinomycin is biochemically closely related to the other members of the aminoglycoside family, despite its structural divergence, in that D-glucose provides almost all the atoms of rings A and C.[4]

Radioactive spectinamine (VII) was prepared by cleavage of radioactive spectinomycin formed biosynthetically from myoinositol-2-^{14}C and was re-fed to the organism. Incorportaion of about 6·6% was achieved. This level is considerably below that of myoinositol itself and reflects either degradation and resynthesis, or the operation of a minor pathway. It seems likely, by analogy with the biosynthesis of streptomycin,[8] *inter alia*, that *N*-methylation takes place after assembly of the ABC ring system (Scheme 1). The possibility of selective permiability and the detailed sequence of biochemical events between glucose and spectinomycin are now being studied.

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