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Biosynthesis of the Antibiotic Indolmycin by *Streptomyces griseus*. C-Methylation at the β-Carbon Atom of the Tryptophan Side-chain

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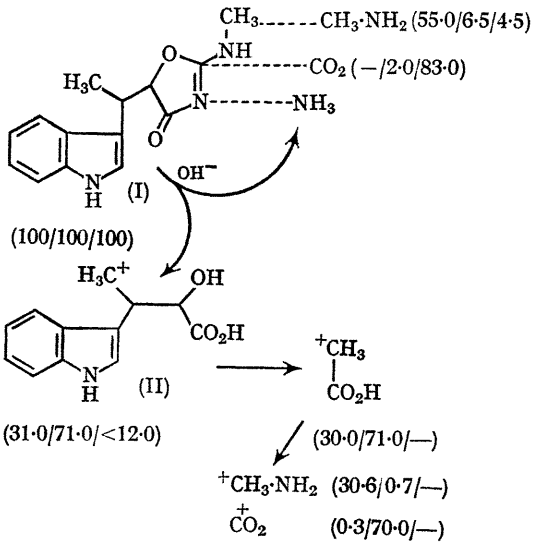
Indolmycin (I) is an antibiotic produced by a strain of *Streptomyces griseus*.[1] The presence of an extra methyl group (attached to a presumably tryptophan-related skeleton) and an oxazolinone ring[2] prompted us to study its biosynthesis.

Streptomyces griseus (ATCC 12648) was grown in 100 ml. shake cultures at 24° in a medium[3] containing soybean meal and distillers' solubles for 2 days. The radioactive precursors were then added, and after incubation for another 18 hr. the cultures were extracted with ethyl acetate. The extract containing the indolymcin was washed with sodium carbonate solution and subjected to t.l.c. (silica gel G, chloroform–dimethylformamide–ethyl acetate 3 : 1 : 1, R_F 0·74). The chromatogram was scanned for radioactivity and the percentage of the total radioactivity present in indolmycin was determined by integration. The incorporation of precursors into indolmycin was calculated by multiplying this percentage by the total activity in the ethyl acetate extract as determined independently by liquid scintillation counting. To check for radiochemical homogeneity, a portion of the indolymcin was re-chromatographed (silica gel G, dioxan–ether–water 2 : 2 : 1, R_F 0·50).

Sodium [^{14}C]formate, [^{14}C]urea, sodium [1-^{14}C]acetate, [*u*-^{14}C]-L-alanine, [*u*-^{14}C]-L-threonine, and [*u*-^{14}C]-D-glucose all gave incorporations below 0·2%. Although there is considerable variation in the percentages of incorporation in the various experiments, it is clear that both the indole moiety and all three carbon atoms of the tryptophan side-chain are efficiently incorporated into indolymcin, as are the tryptophan precursors indole and anthranilic acid. Likewise, the methyl group of methionine and the guanidino-group of arginine were efficiently incorporated into the antibiotic. Further evidence for the specific precursor role of these three compounds was obtained by degradation. Alkaline hydrolysis of indolymcin gave CO_2, methylamine, ammonia, and indolmycenic acid (II), which after chromatographic purification and dilution with carrier[4] was further degraded by Kuhn–Roth oxidation and Schmidt degradation of the resulting acetic acid. Because of lack of carrier material, the hydrolysis of indolmycin was carried out on a milligram scale and total activities were determined rather than specific activities of the various products. The data (Scheme)



Scheme. *Degradation of indolmycin. Given in parentheses* (A/B/C) *are the relative total radioactivities of indolmycin and its degradation products from feeding experiments with* (A) [*Me*-^{14}C]-L-*methionine*; (B) [alanine-3-^{14}C]-DL-*tryptophan*; and (C) [guanidino-^{14}C]-L-*arginine*.

show that the guanidino-group of arginine labels exclusively C-2 of the oxazolinone ring and that the radioactivity of C-3 of the tryptophan side-chain appears in the carbon atom next to the indole grouping of indolmycin. The methyl group of methionine is not only incorporated into the *N*-methyl group, but also specifically into the *C*-methyl group of

indolmycin. The backbone of this antibiotic is thus provided by tryptophan, which undergoes *C*-methylation. The extra carbon atom of the oxazolinone ring is derived from the guanidino-group of arginine. This result does not shed any light on the mechanism of the formation of the oxazolinone ring nor does it necessarily imply that the entire guanidino-group is transferred. We can exclude, however, the possibility that the guanidino-group is incorporated *via* urea.

In order to learn more about the sequence of the steps involved in this biosynthesis, we prepared labelled indolmycenic acid by hydrolysis of indolmycin obtained from feeding experiments with [^{3}H]- and [^{14}C]-tryptophan, respectively. Both of these samples, after purification, were fed to the organism and, as shown in the Table, the radioactivity was efficiently incorporated into indolmycin. This indicates that the *C*-methylation precedes the formation of the oxazolinone ring. We consider it likely that β-methylindolepyruvate is another intermediate in the pathway immediately preceding indolmycenic acid. This compound could be formed from tryptophan by transamination and methylation.

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Incorporation of labelled substrates into indolmycin by Streptomyces griseus

Precursor	Incorporation (%)
[^{3}H]Anthranilic acid	11·8
[2-^{14}C]Indole	7·0
[^{3}H]-L-Tryptophan	18·4
[*alanine*-3-^{14}C]-DL-Tryptophan	2·4
[*alanine*-2-^{14}C]-DL-Tryptophan	4·6
[*alanine*-1-^{14}C]-DL-Tryptophan	9·5
[*alanine*-3-^{14}C]-L-Tryptophan	7·5; 5·8; 1·5
[*guanidino*-^{14}C]-L-Arginine	1·0; 2·0; 5·0
[*Me*-^{14}C]-L-Methionine	1·3; 3·7; 8·7
[^{3}H]Indolymcenic acid	7·5
[^{14}C]Indolmycenic acid	12·0

0·5—1 mg. of precursor with 10^7 to $1·5 \times 10^8$ d.p.m. fed to 2-day-old 100 ml. shake cultures for 18 hr.

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