The Biosynthesis of Indolmycin

U. Hornemann,* L. H. Hurley, M. K. Speedie, and H. G. Floss

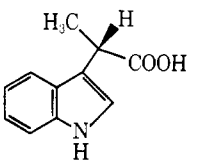
Contribution from the Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, Indiana 47907. *Received July 7, 1970*

Abstract: Indolmycin, an antibiotic produced by a strain of *Streptomyces griseus*, is formed from (*S*)-tryptophan, which loses from its side chain the amino nitrogen atom, the hydrogen atom from C-2, and one of the hydrogen atoms from C-3, two intact methyl groups of (*S*)-methionine, and the guanido carbon atom of (*S*)-arginine. (*R*)-β-Methylindolepyruvate and (2*S*,3*R*)-indolmycenic acid are intermediates in the biosynthesis. The absolute configuration of indolmycin has been determined by chemical correlation with (−)-(*R*)-indoleisopropionic acid. Studies with cell-free extracts of *S. griseus* revealed the presence of a transaminase which converts (*S*)-tryptophan into indolepyruvate and a methyltransferase which C-methylates indolepyruvate.

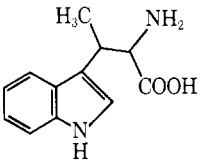
The antibiotic indolmycin (IV) (Scheme I) is produced by *Streptomyces griseus* (ATCC 12648), a strain which was formerly classified as *Streptomyces albus*,[1] and by a strain of *Streptomyces hygroscopicus*.[2] It exhibits antimicrobial activity against gram positive and gram negative bacteria.[3-5] It is apparently well tolerated by mice in doses of up to 500 mg/kg, and it is effective against sepsis caused by polyresistant *Staphylococci* in doses of 50–500 mg/kg.[5] The culture characteristics of the indolmycin-producing strain of *S. griseus* have been described,[4] and a fermentation procedure for the production of indolmycin together with two other antibiotics of unknown structure has been patented.[6] The structure[7] and the relative stereochemistry[5,7] of indolmycin have been determined by a study of the products of its hydrolysis and by spectroscopic methods. Syntheses of indolmycin and of some of its degradation products and analogs have been reported.[5,7] Apparently *C*-desmethyl- and *N*-desmethylindolmycin as well as the enantiomer of the levorotatory natural antibiotic have reduced antibacterial potency.[5]

Our interest in the biosynthesis of indolmycin resulted mainly from the presence of a methyl-branched side chain in this molecule, which could conceivably arise by C-methylation of a tryptophan derivative. A similar structural element is also found in indoleisopropionic acid (I), a metabolite of a *Claviceps* species,[8] in 3-methyltryptophan (II), a constituent of the peptide antibiotic telomycin produced by *Streptomyces canus*,[9] and in 3-methylphenylalanine (III) (see Chart I), which occurs in the peptide antibiotic bottromycin produced by *Streptomyces bottropensis*.[10] This structural similarity might suggest a similarity in the biosynthesis of all these compounds. No information is presently available on the biosynthesis of the amino acids, but the biosynthesis of indoleisopropionic acid has recently been investigated in this laboratory.[11] It was shown that this acid is derived from tryptophan, which provides the indole portion and two of the side-chain carbon atoms, and an intact methyl group from methionine.

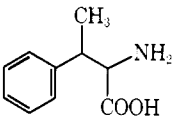
Chart I



I, (*S*)-indoleisopropionic acid



II, 3-methyltryptophan



III, 3-methylphenylalanine

Biological C-methylation reactions have received considerable attention, since they participate in the biosynthesis of compounds of great biochemical importance, *e.g.*, nucleic acids, vitamin K and other quinones, and ergosterol, and because the mechanisms of these reactions pose interesting questions.[12] In this paper we wish to report on the determination of the biosynthetic origin of indolmycin, on the partial elucidation of the biosynthetic pathway, which includes demonstration of a C-methylation reaction, and on the absolute stereochemistry of indolmycin and some of its biosynthetic intermediates. Some of these results have been communicated in preliminary form.[13]

Results

Biosynthetic experiments were carried out with shake cultures of the indolmycin-producing *S. griseus*

(1) J. B. Routien, *J. Bacteriol.*, **91**, 1663 (1966).
(2) Y. Kawaguchi, *et al.* (Bristol Banyu), Japan Patent 10,247 (1964); *cf.*, H. Umezawa, "Index of Antibiotics from Actinomycetes," University Park Press, State College, Pa., 1967, p 743.
(3) K. V. Rao, *Antibiot. Chemother.*, **10**, 312 (1960).
(4) W. S. Marsh, A. L. Garretson, and E. M. Wesel, *ibid.*, **10**, 316 (1960).
(5) M. N. Preobrazhenskaya, E. G. Balashova, K. F. Turchin, E. N. Padeiskaya, N. V. Uvarova, G. N. Pershin, and N. N. Suvorov, *Tetrahedron*, **24**, 6131 (1968).
(6) K. V. Rao and W. S. March (to Chas. Pfizer and Co.), U. S. Patent 3,173,923 (Cl 260-307) (March 16, 1965); *cf. Chem. Abstr.*, **63**, 1198 (1965).
(7) M. Schach v. Wittenau and H. Els, *J. Amer. Chem. Soc.*, **83**, 4678 (1961); **85**, 3425 (1963).
(8) T. Yamano, *J. Agr. Chem. Soc. (Japan)*, **35**, 1284 (1961).
(9) J. C. Sheehan, D. Mania, S. Nakamura, J. K. Stock, and K. Maeda, *J. Amer. Chem. Soc.*, **90**, 462 (1968).
(10) S. Nakamura, T. Yajima, Y. C. Lin, and H. Umezawa, *J. Antibiot., Ser. A*, **20**, 1 (1967); H. Umezawa, "Index of Antibotics from Actinomycetes," University Park Press, State College, Pa., 1967, pp 178–179.
(11) U. Hornemann, M. K. Speedie, K. M. Kelley, L. H. Hurley, and H. G. Floss, *Arch. Biochem. Biophys.*, **131**, 430 (1969).
(12) *Cf.* E. Lederer, *Quart. Rev., Chem. Soc.*, **23**, 453 (1969).
(13) U. Hornemann, L. H. Hurley, M. K. Speedie, H. F. Günther, and H. G. Floss, *Chem. Commun.*, 245 (1969).

Table I. Incorporation of Labeled Substrates into Indolmycin by *S. griseus*

Expt	Precursor	Quantity fed, μmol	Radioactivity fed, dpm	Radioactivity in neutral extract, % of total fed	% radioactivity of neutral extract in indolmycin	Incorporation into indolmycin, %
1	Anthranilic acid-*g*-^{3}H	4	5.90×10^{7}	14.0	83	11.6
2	Acetic acid-*1*-^{14}C sodium salt	7	2.07×10^{7}	1.6	*a*	<0.05
3	(*S*)-Alanine-*U*-^{14}C	11	1.50×10^{7}	1.5	*a*	<0.07
4	(*S*)-Arginine-*guanido*-^{14}C	5	1.40×10^{7}	2.7	79	2.1
5	(*S*)-Arginine-*guanido*-^{14}C	8	1.32×10^{7}	5.6	88	4.9
6	(*S*)-Arginine-*guanido*-^{14}C	8	2.20×10^{7}	1.2	84	1.0
7	Formic acid-^{14}C sodium salt	14	2.50×10^{7}	0.05	*a*	<0.05
8	(*R*)-Glucose-*U*-^{14}C	3	2.20×10^{7}	0.8	*a*	<0.05
9	Indole-*2*-^{14}C	2	2.66×10^{7}	7.4	90	6.7
10	(*S*)-Methionine-CH_3-^{14}C	7	2.61×10^{7}	12.3	71	8.7
11	(*S*)-Methionine-CH_3-^{14}C	7	1.06×10^{7}	5.3	70	3.7
12	(*S*)-Methionine-CH_3-^{14}C	7	1.75×10^{7}	2.0	65	1.3
13	(*S*)-Tryptophan-*indole*-^{3}H	2	3.80×10^{7}	28.0	66	18.5
14	(*S*)-Tryptophan-*alanine-3*-^{14}C	10	1.09×10^{7}	16.7	43	7.2
15	(*R,S*)-Tryptophan-*alanine-1*-^{14}C	5	6.20×10^{6}	29.0	33	9.6
16	(*R,S*)-Tryptophan-*alanine-1*-^{14}C	5	1.07×10^{7}	18.5	95	17.6
17	(*R,S*)-Tryptophan-*alanine-2*-^{14}C	20	2.03×10^{7}	7.8	59	4.6
18	(*R,S*)-Tryptophan-*alanine-3*-^{14}C	18	2.97×10^{7}	10.0	50	5.0
19	(*R,S*)-Tryptophan-*alanine-3*-^{14}C	10	5.52×10^{6}	9.0	100	9.0
20	(*S*)-Threonine-*U*-^{14}C	8	2.40×10^{7}	2.0	*a*	<0.04
21	Urea-^{14}C	20	3.00×10^{7}	0.7	*a*	<0.03

[a] Below limit of detection.

strain ATCC 12648 grown in a complex medium[6] containing dextrose, distiller's solubles, soybean meal, and salts. The amount of indolmycin produced at the time of harvest was about 2 mg/100 ml of culture as determined by semiquantitative chromatographic comparison with known amounts of authentic indolmycin. The antibiotic was isolated from the culture filtrate by extraction with ethyl acetate and purified by successive tlc in two systems (A and B). Besides indolmycin, two other van Urk positive compounds were detected upon chromatography of the ethyl acetate extract in system A. The compound of lowest R_f was extractable with Na_2CO_3 and was later shown to be identical with indolmycenic acid (V).[14] The other compound (substance "X") migrated between indolmycin and indolmycenic acid. It was labeled by tryptophan, but not by the other precursors of indolmycin. It therefore did not seem to be an intermediate of indolmycin biosynthesis and was not further investigated.

The time course of indolmycin formation was investigated by measuring the incorporation of radioactive tryptophan into the antibiotic at different times during the culture period. As shown in Figure 1, maximal incorporation into indolmycin occurred between 36 and 60 hr after inoculation of the culture.

An incubation period of 12 hr seemed to be sufficient, because a more prolonged exposure to the radioactive precursor (24 hr) did not result in higher incorporation of radioactivity. Since the production of substance "X" lags behind indolmycin formation by about 0.5 day, precursor feeding to 36-hr old cultures for 12 hr was chosen as the standard condition for further experiments to avoid possible interference by the formation of this unrelated indolic compound. The efficiency of incorporation of potential radioactive precursors into indolmycin was evaluated as follows. The thin-layer chromatograms (system A) of the carbonate-washed ethyl acetate extracts were scanned for radioactivity, and the areas under the peaks were integrated to obtain the percentage of the radioactivity of the extract residing in indolmycin. From this value and from the total radioactivity of the ethyl acetate extract, the total radioactivity incorporated into indolmycin was calculated, which was related to the total radioactivity of the precursor fed to give the percentage incorporation. The radiochemical purity of the indolmycin samples was established by elution of the antibiotic from the plates and rechromatography in system B.

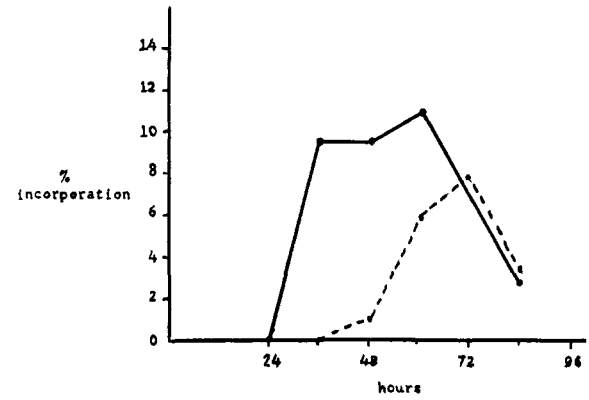


Figure 1. Time course of the incorporation of (*S*)-tryptophan into indolmycin and substance "X" by *S. griseus*. At the times indicated, the cultures were incubated with 1.5×10^{8} dpm of (*S*)-tryptophan-*indole*-^{3}H for a period of 12 hr: •—•, indolmycin; •---•, substance "X".

Using these conditions a number of precursors were fed to *S. griseus* cultures and the incorporations given in Table I were obtained. It is apparent that the indole moiety and the side-chain carbon atoms of tryptophan are efficiently incorporated into indolmycin,

(14) U. Hornemann, L. H. Hurley, M. K. Speedie, and H. G. Floss, *Tetrahedron Lett.*, 2255 (1970).

Table II. Degradation of Indolmycin

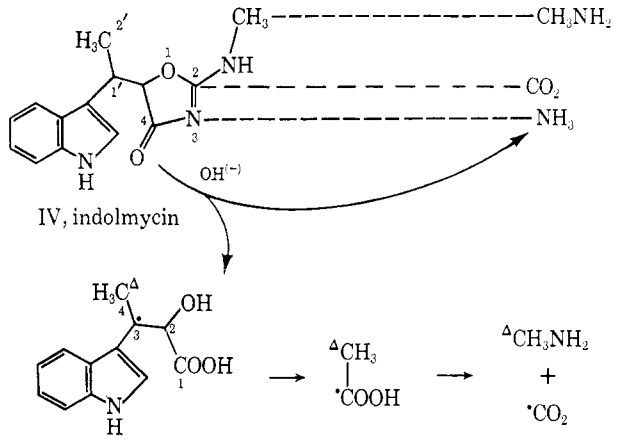
Precursor	% radioactivity of indolmycin recovered in: CH_3NH_2 (from hydrolysis)	CO_2 (from hydrolysis)	IMA[c]	CH_3COOH	CO_2 (from CH_3COOH)	CH_3NH_2 (from CH_3COOH)
(*S*)-Tryptophan-*alanine-3*-^{14}C	6.5[a]	2.0[a]	71.0	71.0	70.0	1.0
(*S*)-Methionine-*methyl*-^{14}C	55.0[a]	1.0[a]	31.0	31.0	0.3	30.6
(*S*)-Arginine-*guanido*-^{14}C	<0.1	102.0	<0.1	NE[b]	NE[b]	NE[b]

[a] By difference. [b] Not examined. [c] IMA = indolmycenic acid.

as are the tryptophan precursors anthranilic acid and indole. Likewise, the guanido carbon atom of arginine and the methyl group of methionine are utilized efficiently for indolmycin formation. As shown by the various repetitions of experiments, there is considerable biological variation within the system. A number of other compounds were fed to the organism (Table I, experiments 2, 3, 7, 8, 20, and 21) but were not detectably incorporated. The metabolic fate of these compounds has not been further examined and thus, the possibility cannot be excluded that at least in some cases the lack of incorporation is due to permeability problems.

The role of tryptophan, methionine, and arginine as indolmycin precursors was substantiated by degradation of the labeled samples of the antibiotic. As outlined in Scheme I, alkaline hydrolysis yields indol-

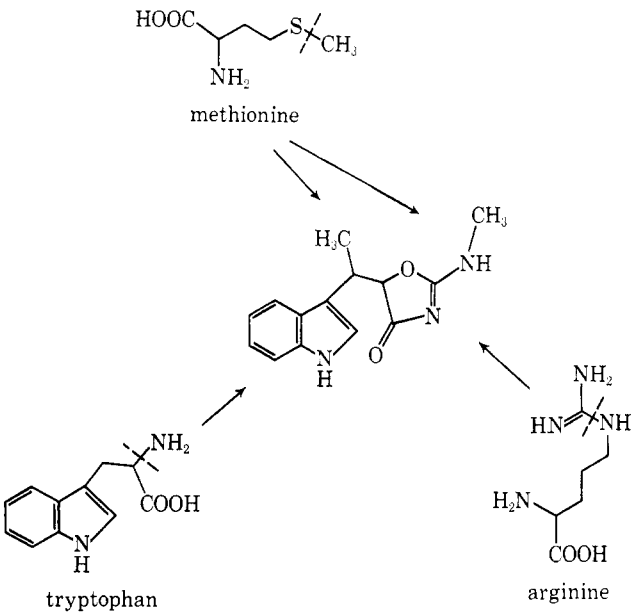
Scheme I. Degradation of Indolmycin



V, indolmycenic acid

mycenic acid as well as methylamine, CO_2, and ammonia.[6] Because of a shortage of carrier material, this hydrolysis of indolmycin was carried out in a milligram scale, and the total radioactivity recovered in the various degradation products was determined rather than their specific radioactivities.[15] The purity of indolmycenic acid was checked by chromatography in systems C and D. After dilution with carrier material it was degraded further by Kuhn–Roth oxidation,[16] and the resulting acetic acid was subjected to Schmidt degradation.[17] The data obtained are presented in Table II. They show that the guanido carbon atom of arginine-*guanido*-^{14}C labels exclusively C-2 of the oxazolinone ring, and that most of the radioactivity from C-3 of the tryptophan side chain appears at C-1′ of indolmycin, the carbon atom bearing the *C*-methyl group. The methyl group of methionine is specifically incorporated into the *N*-methyl group and also the *C*-methyl group of the antibiotic. These experiments thus establish the building blocks of indolmycin as shown in Scheme II. The backbone of the molecule

Scheme II. Building Blocks of Indolmycin



is provided by tryptophan, which undergoes C-methylation at the side chain. The additional carbon atom of the oxazolinone ring is derived from the guanido group of arginine, and the *N*-methyl group is also provided by methionine. No information is as yet available about the origin of the two nitrogen atoms of the oxazolinone portion of the molecule, particularly whether these are also provided by arginine.

The results so far do not shed any light on the sequence and the mechanisms of the reactions involved in indolmycin formation. Considering plausible mechanisms it would seem likely that indolepyruvate (VI) is the substrate for the C-methylation reaction, leading to a hypothetical pathway of indolmycin formation as shown in Scheme III. The subsequent experiments were carried out in order to examine the validity of this postulated pathway. This scheme implies that tryptophan should lose the hydrogen from C-2 of its side chain and one of the two hydrogens from C-3 during its transformation into indolmycin, and that an intact methyl group from methionine, rather than the methyl carbon atom with only two of its hydrogen atoms, should be incorporated in the C-methylation reaction. These assumptions were tested by suitable double-labeling experiments with tritium and ^{14}C. The various tritiated precursors, labeled in the hydro-

(15) The radioactivities of the hydrolytic degradation products do not always add up to 100%, because the hydrolysis was not always complete.

(16) H. Simon and H. G. Floss, "Bestimmung der Isotopenverteilung in markierten Verbindungen," Springer-Verlag, West Berlin and Heidelberg, 1967, p 12.

(17) Reference 16, p 23.

Table III. Incorporation of Double-Labeled Substrates into Indolmycin and Indolmycenic Acid

Precursor	$^3H/^{14}C$ ratio of substrate fed	Indolmycin		Indolmycenic acid	
		$^3H/^{14}C$	Tritium retention,[a] %	$^3H/^{14}C$ ratio	Tritium retention,[a] %
(*R,S*)-Tryptophan-*alanine-3*-^{14}C,2-^3H	6.33	0.1	1.6	0.03	0.5
(*R,S*)-Tryptophan-*alanine-3*-^{14}C,3-^3H	4.10	2.13	52	2.22	54
(*S*)-Methionine-*methyl*-^{14}C,^3H	3.65	3.65	100	3.50	96

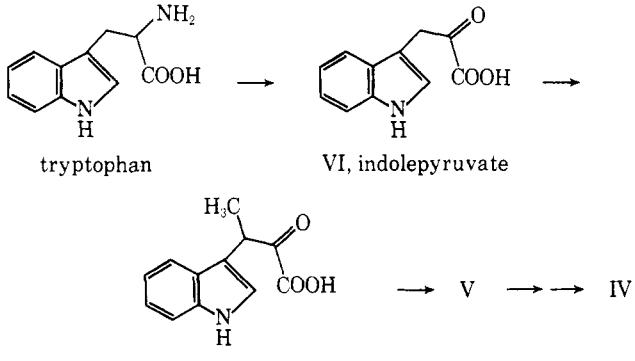
[a] Tritium retention = ($^3H/^{14}C$ of product)/($^3H/^{14}C$ of substrate) × 100 (%).

Table IV. Incorporation of Labeled Potential Precursors into Indolmycin by *S. griseus*

Expt	Precursor	Quantity fed, μmol	Radioactivity fed, dpm	Radioactivity in neutral extract, % of total fed	Incorporation into indolmycin, %
1	(*S*)-3-Methylindolepyruvate-*ring*-^3H	1.5	1.70×10^7	6.4	<0.3
2	(*R*)-3-Methylindolepyruvate-*ring*-^3H	1.0	3.60×10^6	13.0	9.0
3	(*R,S*)-Tryptophan-*alanine-3*-^{14}C	5.0	1.30×10^7	13.5	10.0
4	(2*RS*,3*SR*)-3-Methyltryptophan-*ring*-^3H	5.0	4.02×10^7	8.3	5.5
5	(2*RS*,3*RS*)-3-Methyltryptophan-*ring*-^3H	5.0	2.26×10^7	1.3	<0.3
6	(*S*)-Tryptophan-*alanine-3*-^{14}C	10.0	1.09×10^7	19.3	5.8
7	Natural indolmycenic acid-*3*-^{14}C	1.0	4.16×10^5	34.0	28.0
8	Indolmycenic acid-*3*-^{14}C from hydrolysis of indolmycin	1.0	2.30×10^6	12.0	12.0
9	(2*S*,3*R*)-Indolmycenic acid-*3*-^{14}C	1.0	3.60×10^5	17.0	17.0
10	(2*R*,3*R*)-Indolmycenic acid-*3*-^{14}C	1.0	1.27×10^5	2.2	<0.1
11	(2*S*,3*S*)-Indolmycenic acid-*ring*-^3H	1.0	8.0×10^5	12.5	<0.1
12	(2*R*,3*S*)-Indolmycenic acid-*ring*-^3H	1.0	8.4×10^5	3.4	<0.1
13	(*R,S*)-Tryptophan-*alanine-3*-^{14}C	5.0	1.13×10^6	8.5	6.6

gens to be examined, were mixed with the corresponding ^{14}C compounds, fed to *S. griseus* cultures, and the $^3H/^{14}C$ ratios of the products were compared to those of the starting materials. Besides indolmycin, indolmycenic acid was isolated and analyzed in each of these experiments. The data are summarized in Table III. In addition, the indolmycin obtained from

Scheme III. Hypothetical Pathway of Indolmycin Formation



the experiment with double-labeled methionine was hydrolyzed to give methylamine ($^3H/^{14}C$ = 3.65) and indolmycenic acid ($^3H/^{14}C$ = 3.38). The results of these experiments are in agreement with the above assumptions and thus support the hypothetical pathway outlined in Scheme III. Attention is drawn to the finding that in all these experiments the isotope ratio of indolmycenic acid is very nearly the same as that of indolmycin.

Preliminary investigations into the enzymology of indolmycin formation have resulted in the detection of two enzyme activities which are assumed to be related to the pathway. Cell-free extracts of *S. griseus* were obtained which catalyze the α-ketoglutarate-dependent transamination of (*S*)-tryptophan to give indolepyruvate,[18] and the transfer of the methyl group of *S*-adenosylmethionine to indolepyruvate,[19] respectively. Tryptophan cannot substitute for indolepyruvate in the latter reaction. The product of this reaction has been identified as 3-methylindolepyruvate (VII) and, by chemical correlation with (*R*)-indoleisopropionic acid, has been shown to have the R configuration.[19,20] To evaluate their role as indolmycin precursors, (*R*)- and (*S*)-3-methylindolepyruvate-*ring*-^3H were prepared from the appropriate isomers of ring-tritiated 3-methyltryptophan.[11] (*S*)-3-Methylindolepyruvate was obtained from (2*RS*,3*RS*)-3-methyltryptophan (β-methyltryptophan isomer B)[11,21] by treatment with L-amino acid oxidase and excess catalase, while (*R*)-3-methylindolepyruvate was obtained from (2*RS*,3*SR*)-3-methyltryptophan (isomer A) by transamination with α-ketoglutarate catalyzed by the transaminase preparation from *S. griseus*. Both enantiomers were fed to separate *S. griseus* cultures in a parallel feeding experiment, but only the *R* isomer was utilized for indolmycin biosynthesis (Table IV, experiments 1 and 2). The efficiency of its incorporation was comparable to that of (*R,S*)-tryptophan, which had

(18) U. Hornemann, M. K. Speedie, and H. G. Floss, unpublished results.

(19) U. Hornemann, M. K. Speedie, L. H. Hurley, and H. G. Floss, *Biochem. Biophys. Res. Commun.*, **39**, 594 (1970).

(20) In our previous publications[11,14,19] the levorotatory isomer of indoleisopropionic acid was erroneously assumed to have *S* rather than *R* configuration. As a consequence, the configurational assignments for 3-methylindolepyruvate, indolmycenic acid, indolmycin, and 3-methyltryptophan given in these papers are also incorrect.

(21) H. R. Snyder and D. S. Matteson, *J. Amer. Chem. Soc.*, **79**, 2217 (1957).

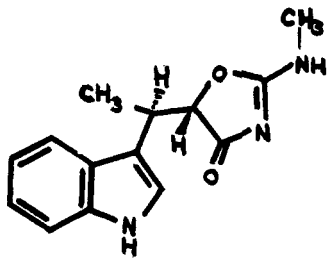


Figure 2. Absolute configuration of indolmycin.

been fed to a parallel culture (Table IV, experiment 3). That the incorporation of (*R*)-3-methylindolepyruvate into the antibiotic is specific has been confirmed by degradation of a sample of indolmycin obtained by feeding (*R*)-3-methylindolepyruvate-*methyl*-^{14}C prepared enzymatically[19] from methyl-labeled *S*-adenosylmethionine. The purified indolmycin was subjected to Kuhn–Roth oxidation using indolmycenic acid as carrier material. Assuming that the yield of acetic acid from indolmycin and indolmycenic acid is the same, the resulting acetic acid (65% yield) accounted for 102% of the radioactivity of the antibiotic.

In another set of experiments, the two diastereomers of 3-methyltryptophan, both as racemates, were fed to cultures of *S. griseus*. The results closely parallel those with (*R*)- and (*S*)-3-methylindolepyruvate, *i.e.*, the 2*RS*,3*RS* isomer of the amino acid is biologically inactive whereas the 2*RS*,3*SR* isomer is utilized for indolmycin formation about as efficiently as tryptophan (Table IV, experiments 4–6).

As mentioned earlier, indolmycenic acid was detected as a natural constituent of the indolmycin-producing strain of *S. griseus*. The compound was identified by tlc and by mass spectrometry of its methyl ester, and its absolute stereochemistry was shown by chemical correlation with (*R*)-indoleisopropionic acid to be 2*S*,3*R*.[14,20] Natural indolmycenic acid-*3*-^{14}C, prepared biosynthetically from tryptophan-*alanine*-*3*-^{14}C, was very efficiently incorporated into indolmycin (Table IV, experiment 7). By comparison, a sample of indolmycenic acid-*3*-^{14}C obtained by hydrolysis of ^{14}C-labeled indolmycin was incorporated only half as efficiently into the antibiotic (Table IV, experiment 8). The hydrolysis of indolmycin proceeds with epimerization at C-5 of the oxazolinone ring, yielding a mixture of diastereomeric indolmycenic acids (α- and β-indolmycenic acids).[7] Their absolute configuration at C-3 was determined by correlation with (−)-(*R*)-indoleisopropionic acid, the absolute configuration of which had been established by Sjöberg using the quasi-racemate method.[22] A sample of indolmycenic acid-*3*-^{14}C, obtained by hydrolysis of indolmycin-*1'*-^{14}C, was reduced with $LiAlH_4$, the diol was cleaved with periodate, and the aldehyde was oxidized with moist silver oxide. The resulting indoleisopropionic acid was purified by tlc in system H and then cocrystallized with an excess of the cinchonine salt of (−)-(*R*)-indoleisopropionic acid from hot isopropyl alcohol.[23] As shown in Table V, the specific radioactivity of the salt remained constant, whereas in the cocrystallization of a sample of labeled (*S*)-indoleisopropionic acid with the salt of the *R* isomer, the radioactivity was completely lost. This result establishes the absolute configuration at C-1′ of indolmycin and at C-3 of the indolmycenic acids derived from it as *R*. Since the relative stereochemistry of indolmycin had already been determined by nmr studies and by stereospecific synthesis as 5*R*,1′*S* or 5*S*,1′*R*,[5,7] its absolute configuration must be 5*S*,1′*R* as shown in Figure 2, which is the same as that of natural indolmycenic acid. The same correlation was independently established by Chan and Hill[24] and these workers in addition correlated indolmycenic acid with (−)-(2*R*,3*S*)-2,3-epoxybutyric acid.

Table V. Cocrystallization of (*S*)-Indoleisopropionic Acid-*1,2*-^{14}C and Indoleisopropionic Acid-*2*-^{14}C Obtained from the Degradation of Indolmycin-*1'*-^{14}C with the Cinchonine Salt of (−)-(*R*)-Indoleisopropionic Acid

	(*S*)-Indoleisopropionic acid-*1,2*-^{14}C,[a] dpm/mg	Indoleisopropionic acid-*2*-^{14}C from indolmycin-*1'*-^{14}C, dpm/mg
Calcd	2900[b]	246[c]
1st crystalln	356	271
2nd crystalln	62	238
3rd crystalln		246

[a] Obtained biosynthetically from tryptophan-*alanine*-*2,3*-^{14}C with a strain of *Claviceps*.[11] [b] 2.22×10^5 dpm of radioactive material + 76.4 mg of carrier. [c] 3.69×10^4 dpm of radioactive material + 150 mg of carrier.

To evaluate more completely the specificity of the biological system, all four stereoisomers of indolmycenic acid were prepared in labeled form and fed to parallel cultures of *S. griseus*. The (2*R*,3*R*)- and (2*S*,3*R*)-indolmycenic acids were obtained by resolution of the diastereomeric mixture of indolmycenic acids resulting from indolmycin hydrolysis by tlc in system E. The 2*R*,3*S* and 2*S*,3*S* isomers were prepared by reduction of (*S*)-3-methylindolepyruvate with $NaBH_4$ followed by tlc separation in the same system. The results of the feeding experiments (Table IV, experiments 9–13) show that only the stereoisomer having the absolute configuration of natural indolmycenic acid is biologically active. It is utilized considerably more efficiently for indolmycin biosynthesis than tryptophan.

Some attempts were made to test for possible degradation of indolmycin by *S. griseus*. The antibiotic is apparently not degraded to indolmycenic acid, because upon feeding indolmycin labeled biosynthetically from tryptophan-*alanine*-*3*-^{14}C to a culture, only a very small amount of radioactivity (<1.5%) appeared in the acid fraction. To examine for N-demethylation and remethylation of the resulting *N*-desmethylindolmycin, a sample of indolmycin labeled with ^{14}C at C-1′ and with tritium in the *C*- and *N*-methyl groups ($^3H/^{14}C$ = 4.51) was incubated with a 36-hr old *S. griseus* culture. Twelve hours later the indolmycin was reisolated (70% recovery) and was found to have a $^3H/^{14}C$ ratio of 4.55 corresponding to 101% tritium retention. Furthermore, chromatography did not indicate the presence of any new radioactive compound at the R_f of *N*-desmethylindolmycin. Thus, N-demethylation either did not occur, or could not be demonstrated under the conditions employed.

Discussion

The results of this study firmly establish the origin of the carbon skeleton of indolmycin from tryptophan,

(22) B. Sjöberg, *Ark. Kemi*, **13**, 1 (1958).
(23) F. Kögl and B. Verkaaik, *Z. Phys. Chem.*, **280**, 167 (1944).
(24) T. H. Chan and R. K. Hill, *J. Org. Chem.*, **35**, 3519 (1970).

methionine, and arginine. Furthermore, they leave no doubt that 3-methylindolepyruvate and indolmycenic acid are intermediates of the biosynthesis, as postulated in the hypothetical biosynthetic pathway outlined in Scheme III. In particular, the results of the double-labeling experiments and the stereochemical data strongly support this postulate. The results of the feeding experiments with the 3-methyltryptophans (Table IV) *per se* could be taken as evidence that the C-methylation occurs at the tryptophan stage. However, the finding that cell-free extracts of the organism catalyze the C-methylation of indolepyruvate, but not of tryptophan, seems to rule out this possibility, and the results of the feeding experiments can be adequately explained by the specificity of the α-ketoglutarate-dependent transaminase of *S. griseus* for the 2*S*,3*R* isomer of 3-methyltryptophan and by the specificity of the system for only (*R*)-3-methylindolepyruvate. Thus, it seems extremely likely that the *C*-methyl group of indolmycin is introduced into indolepyruvate as substrate. This report provides the second example of a biological C-methylation at the side chain of a tryptophan derivative, the first one being indoleisopropionic acid, a metabolite of the fungus *Claviceps*. In both cases, experiments with methionine labeled in the methyl group with ^{14}C and tritium indicate that the methyl group is transferred with all three hydrogen atoms, although in the absence of experiments with methyl-deuterated methionine, the possibility of an isotope effect cannot be completely excluded. It seems reasonable to assume that the C-methylation reaction proceeds through the enol form of indolepyruvate, and the data from the double-labeling experiments reported in the present study are in agreement with this assumption. Notably, the C-methylation in these two cases leads to products of opposite configuration: indoleisopropionic acid from *Claviceps* has the *S* configuration,[8,20] whereas the methyltransferase of *S. griseus* produces (*R*)-3-methylindolepyruvate. It will be interesting to examine the steric course of the reaction in these two organisms. If the reaction proceeded through an enol, it would be nonconcerted and could thus involve either retention or inversion of configuration. In this connection, it would also be interesting to examine the biosynthesis of 3-methylphenylalanine and 3-methyltryptophan, which are constituents of the Streptomycete antibiotics bottromycin[10] and telomycin,[9] respectively. While the configuration of 3-methylphenylalanine is unknown, 3-methyltryptophan from telomycin has been identified[9] with the isomer A of Snyder and Matteson[20] which we have shown[11,20] to be the 2*RS*,-3*SR* isomer. By analogy with the stereochemistry of indolmycin, it is tempting to predict that the constituent of telomycin will turn out to have the 2*S*,3*R* configuration.

The later stages of indolmycin biosynthesis are as yet largely unresolved. As the most likely possibility, it is assumed that an intact amidino group is transferred from arginine to indolmycenic acid. This has analogies in the biosynthesis of streptomycin,[25] although in that case the amidino group is attached to a nitrogen atom. Subsequent cyclization would complete the oxazolinone ring. The N-methylation could be the terminal step of the sequence or alternatively, arginine could be methylated to ω-*N*-methylarginine, a naturally occurring compound[26] followed by transfer of a methylated amidino group. These questions, as well as the enzymology and eventually the regulation of indolmycin biosynthesis, are the subject of further studies.

(25) J. B. Walker and M. S. Walker, *Biochim. Biophys. Acta*, **93**, 201 (1964).

Experimental Section

Fermentations. *Streptomyces griseus* ATCC 12648 was maintained at 24° on slants of Emerson's agar. To prepare seed cultures, spores were transferred aseptically to 500-ml Erlenmeyer flasks containing 100 ml of culture medium, which were incubated for 5–6 days at 24° on a New Brunswick Model VS gyrotory shaker. Two-milliliter samples of the seed cultures were used to inoculate 500-ml Erlenmeyer flasks containing 100 ml of the same medium and these were incubated with shaking as before. After 36 hr, the radioactive precursors, dissolved in 1 ml of water and sterilized by passage through a millipore filter, were added to the cultures and these were incubated for another 12 hr. Cells for the isolation of enzymes were produced by incubation of the cultures for 48 hr. In all feeding experiments, the culture medium used[6] consisted of (grams/liter): dextrose (10.0), distiller's solubles (2.5), soybean meal (1.5), K_2HPO_4 (5.0), NaCl (2.0), and $CaCO_3$ (2.0) in distilled water, pH 7.5. At the end of the culture period the pH of the medium is 7. This medium contains insoluble particles and is therefore unsuitable for the production of cells for enzyme experiments. For this purpose, a medium was used which consisted of (grams/liter): peptone (Difco) (20.0), yeast extract (Difco) (2.0), $FeSO_4 \cdot 7H_2O$ (0.001), and a trace element solution[27] (1 ml/l.) in distilled water, pH 7. The pH of the cultures is 8–9 after 48 hr of incubation.

Chromatography. Thin-layer chromatography on silica gel was used throughout this study. Analytical separations were carried out on 5 × 20 cm plates precoated with silica gel G (Merck), whereas for preparative isolations 20 × 20 cm plates coated with 0.25–0.5 mm thick layers of silica gel G (Merck) were used. Indolic compounds were visualized by spraying the plates with van Urk's reagent[28] (1 g of *p*-dimethylaminobenzaldehyde dissolved in a mixture of 20 ml of concentrated HCl and 10 ml of water). Suitable conditions for the elution of radioactive compounds from the silica gel were determined by monitoring the recovery of radioactivity in the eluate. The following chromatography systems were used (development time and R_f values refer to precoated plates): system A, chloroform–dimethylformamide–ethyl acetate, 3:1:1, 2.5 hr, indolmycin R_f 0.75; system B, *n*-hexane–isopropyl alcohol–ethanol, 2:2:1, 3 hr, indolmycin R_f 0.45; system C, ethanol–isopropyl alcohol–concentrated ammonia, 9:7:4, 2.5 hr, indolmycenic acid R_f 0.45, indoleisopropionic acid R_f 0.33; system D, ethyl acetate–isopropyl alcohol–concentrated ammonia, 9:7:4, 2.5 hr, indolmycenic acid R_f 0.27; system E, chloroform–acetic acid, 19:1, 1 hr, rerun six times, (2*RS*,3*SR*)-indolmycenic acid R_f 0.27, (2*RS*,3*RS*)-indolmycenic acid R_f 0.37; system F, chloroform–ethanol, 5:1, 3 hr, indolmycenic acid methyl ester R_f 0.55; system G, ligroin (100–115°)–isopropyl alcohol, 9:1, 1.5 hr, rerun four times, (2*RS*,3*SR*)-indolmycenic acid methyl ester R_f 0.32, (2*RS*,3*RS*)-indolmycenic acid methyl ester R_f 0.38; system H, *n*-octanol–ligroin–acetone, 5:5:2, 3 hr, indolmycenic acid R_f 0.08, 3-(β-indolyl)butane-1,2-diol R_f 0.46, 2-(β-indolyl)propionaldehyde R_f 0.95, indoleisopropionic acid R_f 0.54; system I, ligroin–isopropyl alcohol, 84:16, 1.5 hr, indoleisopropionic acid methyl ester R_f 0.53.

Nonlabeled Compounds. The diastereomeric mixture of racemic indolmycenic acids was synthesized from *N*-[1-(β-indolyl)ethyl]-*N*-isopropylamine and diethyl acetoxymalonate as described by Preobrazhenskaya, *et al.*[5] A portion of this mixture was separated into the two diastereomers by esterification with diazomethane, fractional crystallization of the methyl esters from benzene, and alkaline hydrolysis, as described by the same authors.[5] The (2*RS*,3*SR*)-indolmycenic acid was obtained chromatographically pure by this procedure, whereas the methyl ester of (2*RS*,3*RS*)-indolmycenic acid still contained substantial amounts of the 2*RS*,-3*SR* isomer. Indoleisopropionic acid was synthesized from indole and lactic acid by the method of Johnson and Crosby.[29] The race-

(26) W. K. Paik and S. Kim, *J. Biol. Chem.*, **245**, 88 (1970).
(27) E. J. Kirsch and J. D. Korshalla, *J. Bacteriol.*, **87**, 247 (1964).
(28) H. W. van Urk, *Pharm. Weekblad.*, **66**, 473 (1929).
(29) H. E. Johnson and D. G. Crosby, *J. Org. Chem.*, **28**, 1246 (1963).

mate was resolved by crystallization of the cinchonine salt to a rotation of $[\alpha]^{22}D$ +110° (*c* 1, ethanol) (lit. $[\alpha]^{22}D$ +105° (*c* 1, ethanol) as described by Kögl and Verkaaik[23] to give the salt of (*R*)-indole-isopropionic acid. (2*RS*,3*RS*)- and (2*RS*,3*SR*)-3-methyltryptophan[21] were a gift from Dr. H. R. Snyder, Urbana. Authentic indolmycin was provided by Chas. Pfizer and Co.

Labeled Compounds. (*S*)-Arginine-*guanido*-^{14}C, (*R*)-glucose-*U*-^{14}C, (*S*)-methionine-*methyl*-^{14}C and (*R*,*S*)- and (*S*)-tryptophan-*alanine*-3-^{14}C were purchased from Amersham/Searle, (*S*)-alanine-*U*-^{14}C and indole-2-^{14}C picrate from New England Nuclear, sodium formate-^{14}C, (*S*)-threonine-*U*-^{14}C and urea-^{14}C from Volk Radiochemicals, (*R*,*S*)-tryptophan-*alanine*-2-^{14}C from Tracerlab and (*R*,*S*)-tryptophan-*alanine*-1-^{14}C from Calbiochem. Indole-2-^{14}C was liberated from its picrate as previously described.[11] Sodium acetate-1-^{14}C, anthranilic acid-*G*-^{3}H,[11] (*S*)-methionine-*methyl*-^{3}H,[11] (2*RS*,3*RS*)- and (2*RS*,3*SR*)-3-methyltryptophan-*ring*-^{3}H,[11] (*S*)-tryptophan-*ring*-^{3}H,[11] and (*R*,*S*)-tryptophan-*alanine*-2-^{3}H[30] were preparations synthesized earlier in this laboratory. (*R*,*S*)-Tryptophan-*alanine*-3-^{3}H was synthesized from formaldehyde-^{3}H by the method of Weygand and Linden.[31]

For the preparation of (*S*)-3-methylindolepyruvate-*ring*-^{3}H, 0.5 mg of (2*RS*,3*RS*)-3-methyltryptophan-*ring*-^{3}H (4.0×10^{7} dpm) was dissolved in 1 ml of 10^{-2} *M* phosphate buffer, pH 7.0, and incubated with 1 mg of L-amino acid oxidase, excess catalase, and a trace of *n*-octanol for 3 hr at 37° while a slow stream of oxygen was passed through the solution. The reaction mixture was acidified to pH 3–4 with tartaric acid, and after addition of 5 ml of water and 1 mg of indolepyruvic acid as carrier material, the radioactive 3-methylindolepyruvate was extracted in three portions into 20 ml of ethyl acetate. The solvent was evaporated in a vacuum at room temperature, the residue taken up in 5 ml of water, and the solution adjusted to pH 7 with 0.02 *N* NaOH (yield 1.7×10^{7} dpm, radiochemical purity >95% as determined by radiochromatography in system G after conversion into indolmycenic acid methyl ester). (*R*)-3-Methylindolepyruvate-*ring*-^{3}H was prepared from (2*RS*,3*SR*)-3-methyltryptophan-*ring*-^{3}H (10 μmol, nominally 1×10^{8} dpm) by incubation with 10 μmol of α-ketoglutarate, 0.03 μmol of pyridoxal phosphate, and 1 ml of a dialyzed cell-free extract from *S. griseus*[18] for 2 hr at 30°. The reaction was stopped by addition of tartaric acid and worked up and analyzed as described for the *S* isomer to give 4.0×10^{6} dpm (*R*)-3-methylindolepyruvate-*ring*-^{3}H of >95% radiochemical purity.

For the preparation of (2*R*,3*S*)- and (2*S*,3*S*)-indolmycenic acid-*ring*-^{3}H, 1.1×10^{7} dpm of (2*RS*,3*RS*)-3-methyltryptophan was converted into (*S*)-3-methylindolepyruvate as described above, which was isolated without the addition of carrier indolepyruvate. Excess $NaBH_4$ was added to the solution of the sodium salt to a final pH of 9. After acidification with tartaric acid to pH 3, the solution was extracted with three 2-ml portions of ethyl acetate to give 5×10^{6} dpm of indolmycenic acids. These were resolved by tlc in system E, and the bands of the two isomers were located by inspection under uv light and scanning for radioactivity, scraped out, and eluted separately with a 10% solution of Na_2CO_3. The eluates were acidified with tartaric acid and extracted with ether, and the ether solutions were dried and evaporated. The residues were each taken up in 2 ml of water and adjusted to pH 7 with 0.02 *N* NaOH (yield 2.64×10^{6} dpm 2*S*,3*S* isomer and 8.64×10^{5} dpm 2*R*,3*S* isomer, radiochemical purity >95%).

(2*R*,3*R*)- and (2*S*,3*R*)-indolmycenic acid-3-^{14}C were prepared by hydrolysis of indolymycin biosynthetically labeled from tryptophan-*alanine*-3-^{14}C as described below, followed by chromatographic resolution of the mixture of diastereomers as above.

All doubly labeled compounds used in this work were mixtures of the corresponding singly labeled species.

Isotope Analysis. All radioactivity measurements were carried out in a Beckman LS 100 liquid scintillation counter. A solution (10 ml) containing 7 g of PPO and 0.3 g of dimethyl-POPOP in 1 l. of toluene was used as the scintillator solution for most samples. Toluene-insoluble samples were dissolved in 2 ml of methanol before addition of the scintillator solution, or were counted in Bray's solution.[32] Samples were counted to at least 2% statistical error and counting efficiencies were determined by recounting the samples after addition of a known amount of toluene-^{14}C or -^{3}H as internal standard. Radioactivity on chromatograms was detected using a Packard Model 7201 radiochromatogram scanner. At the setting used (time constant 30, linear range 300, scanning speed 1 cm/min), the instrument permitted the detection of 10^{3} dpm ^{14}C or 5×10^{3} dpm ^{3}H in one particular band on the chromatogram.

Isolation of Indolmycin from Cultures and Determination of Percentage Incorporation of Precursors. At the end of the fermentation period, the mycelium and the medium were separated by vacuum filtration. The mycelium was washed and discarded, and the filtrate and washings were combined, acidified to pH 3–4, and extracted with three 150-ml portions of ethyl acetate. To remove the acids, the organic phase was extracted with three 5-ml portions of 10% Na_2CO_3 solution and the soda solution was retained for the isolation of indolmycenic acid. The ethyl acetate phase, which contains the neutral substances including indolmycin, was dried over Na_2SO_4, concentrated in a vacuum, and made up to 5 ml with ethyl acetate. An aliquot of this solution, usually 10 μl, was counted to determine the total radioactivity in the neutral fraction. Another aliquot, usually 0.5 ml, was chromatographed on a 5 × 20 cm tlc plate in system A together with reference spots of authentic indolmycin. The plate was then scanned for radioactivity and the indolmycin band was located by brief inspection under uv light and spraying of the reference spots with van Urk's reagent. To determine the percentage of the radioactivity of the neutral fraction residing in indolmycin, the area under the recorder tracing from the radiochromatogram scanner was integrated by cutting out and weighing the paper. This percentage figure multiplied by the total radioactivity of the neutral fraction gave the total radioactivity incorporated into indolmycin, which was divided by the total radioactivity fed and multiplied by 100 to give the percentage incorporation. If no radioactivity was detected at the position of indolmycin, it was assumed on the basis of the limits of detection by the scanner that less than 10^{4} dpm ^{14}C or 5×10^{4} dpm ^{3}H had been incorporated and these figures were used to calculate upper limits of the percentage of incorporation. Indolmycin samples which gave a radioactive peak were eluted with hot ethyl acetate and rechromatographed in system B. In each case, a single peak of radioactivity was observed which coincided with the indolmycin reference spot. For the preparative isolation of indolmycin, the neutral fraction was chromatographed on 20 × 20 cm plates in system A, the indolmycin eluted with hot ethyl acetate, and an aliquot used to establish radiochemical purity by chromatography in system B.

Isolation of Indolmycenic Acid from Cultures. The soda solution containing the extractable acids from the culture was acidified with tartaric acid to pH 3–4 and extracted with three 10-ml portions of ethyl acetate. The organic phase was dried over Na_2SO_4, the solvent evaporated in a vacuum, and the residue dissolved in 5 ml of ethyl acetate. The percentage incorporation of radioactivity into indolmycenic acid was determined by the procedure described for indolmycin, using chromatography systems C and E. System C was also used for the preparative isolation of indolmycenic acid. In some experiments, purification of indolmycenic acid was achieved by esterification of the ethyl acetate solution with excess diazomethane at 0° for 20 min, followed by chromatography of the indolmycenic acid methyl ester in systems F and G.

Degradation of Indolmycin.[7] The radiochemically pure sample of labeled indolmycin and 1 mg of nonlabeled carrier indolmycin were placed in a round-bottomed flask equipped with a gas inlet and a reflux condenser. Two counting vials adapted as traps, each containing 20 mg of citric acid, 2 ml of methanol, and 10 ml of scintillation fluid, were connected to the system through the condenser outlet. Two milliliters of a 10% NaOH solution were added to the flask, and the reaction mixture was heated for 45 min in an oil bath of 120°, while a stream of nitrogen was passed through the apparatus. At the end of the reaction the counting vials were analyzed for radioactivity which was attributed to methylamine. Half of the remaining reaction mixture was acidified to pH 2–3 with 2 *N* HCl and extracted with ethyl acetate. This extract was washed with 10% Na_2CO_3 solution, which was then acidified with tartaric acid and reextracted with ethyl acetate. An aliquot of this extract was counted for radioactivity, which was attributed to indolmycenic acids. Two new vials containing 1 ml of ethanolamine, 2 ml of methanol, and 10 ml of scintillation solution were attached to the apparatus in which the hydrolysis had been carried out. The remaining alkaline reaction mixture was acidified with 2 *N* HCl and heated in an oil bath of 80° for 2 hr while the CO_2 was flushed into the counting vials in a stream of nitrogen. The vials were then analyzed for radioactivity which was attributed to CO_2 from C-2 of indolmycin.

The indolmycenic acid samples obtained in the degradation of indolmycin from (*S*)-tryptophan-*alanine*-3-^{14}C and (*S*)-methionine-

(30) H. G. Floss, U. Mothes, and H. F. Günther, *Z. Naturforsch. B*, **19**, 784 (1964).
(31) F. Weygand and H. Linden, *ibid.*, *B*, **9**, 682 (1954).
(32) A. Bray, *Anal. Biochem.*, **1**, 279 (1960).

methyl-^{14}C were radiochemically pure as evidenced by chromatography in systems C and D. The samples were mixed with known amounts of synthetic carrier indolmycenic acids and subjected to Kuhn–Roth oxidation.[16] The resulting acetic acid samples were further degraded by the Schmidt procedure.[11,17]

Degradation of Indolmycenic Acid to Indoleisopropionic Acid. Radiochemically pure indolmycenic acid-*3*-^{14}C (1.1×10^6 dpm), obtained by hydrolysis of indolmycin, and 5 mg of racemic synthetic indolmycenic acid were dissolved in 5 ml of dry ether. $LiAlH_4$ (10 mg) was added with stirring and the reaction was allowed to proceed for 15 min at room temperature. Excess $LiAlH_4$ was destroyed by the addition of 0.5 ml of water and the solution was acidified with 2 *N* H_2SO_4. The aqueous phase was extracted three times with ether, and any residual indolmycenic acid was removed from the ether solution by washing with a 10% Na_2CO_3 solution. The ether phase was dried over Na_2SO_4 and evaporated; yield 5.0×10^5 dpm of 3-(β-indolyl)butane-1,2-diol-*3*-^{14}C, 85% radiochemically pure as judged by tlc in system H. The diol dissolved in 1 ml of methanol was added to a stirred mixture of 0.5 ml of 0.1 *N* aqueous sodium periodate solution, 2 ml of petroleum ether, and 2 ml of ether at about 14° under nitrogen. After 12 min, the phases were separated and the aqueous layer was extracted twice with 5 ml of ether. The ether extract was dried over Na_2SO_4 and evaporated to give 3.88×10^5 dpm of 2-(β-indolyl)-propionaldehyde-*2*-^{14}C of 90% radiochemical purity (system H). Freshly prepared Ag_2O (20 μmol) and 1 ml of water were added to the aldehyde (3.5×10^5 dpm). The reaction mixture was allowed to stand at room temperature for 15 min with occasional shaking. After acidification to pH 3 with 1 *N* HCl the solution was extracted three times with ether and the ether phase was extracted with 10% Na_2CO_3 solution. The soda solution was acidified with tartaric acid and reextracted with ether. This ether extract was dried and evaporated to give 1.25×10^5 dpm of indoleisopropionic acid, which was shown by esterification with diazomethane and chromatography in system I to be 81% radiochemically pure. This material (3.69×10^4 dpm indoleisopropionic acid) was recrystallized repeatedly from isopropyl alcohol with 150 mg of (*R*)-indoleisopropionic acid cinchonine salt, and after each crystallization about 10 mg was used to measure the specific radioactivity of the salt. The recovery in each crystallization was about 60%.

Acknowledgments. We are indebted to Chas. Pfizer and Co. for providing us with an indolmycin-producing strain of *S. griseus* and a sample of indolmycin, to Dr. H. R. Snyder, Urbana, for a gift of the two isomers of 3-methyltryptophan, and to Dr. R. K. Hill, Athens, Georgia, for providing us with a copy of his manuscript prior to publication. H. G. Floss is the recipient of U. S. Public Health Service Research Career Development Award No. GM 42-389. Marilyn K. Speedie is the recipient of a Mead Johnson Undergraduate Research Award and a National Science Foundation Undergraduate Summer Research Participant. This work was supported by National Institutes of Health Research Grant No. NB 07646 and by grants from Eli Lilly and Company and the Purdue Research Foundation.

A Purine Nucleoside in Syn Conformation. Molecular and Crystal Structures of 5′-Methylammonium-5′-deoxyadenosine Iodide Monohydrate

Wolfram Saenger

Contribution from the Max-Planck-Institut für experimentelle Medizin, Abteilung Chemie, 34 Göttingen, Germany. *Received July 28, 1970*

Abstract: 5′-Methylammonium-5′-deoxyadenosine (Figure 1), a poor substrate for adenosine desaminase, could be crystallized as its iodide monohydrate in space group $P2_1$. The structure was solved from three-dimensional X-ray data and refined to $R = 4.1\%$ for the 1415 significant data. The nucleoside exists in the unusual syn conformation, atom N-3 of the adenine residue being in intramolecular hydrogen bonding contact with the ammonium nitrogen N-5′. The conformation of the ribose is C-2′ endo, C-3′ exo. Atom N-5′ is in the trans,gauche position with respect to C-3′ and O-1′ and coplanar with the adenine heterocycle. The packing of the molecules is such that the heterocycles are not stacked but arranged in fishbone manner at 120° to each other. The iodide ions are located above and below the adenine heterocycles at only 3.82 Å distance. The water of hydration is fourfold disordered and forms a linear hydrophilic region within the crystal structure.

The deduction of the reaction mechanism of an enzyme is possible only if the structural properties of the substrates which are accepted by the enzyme are known. When 5′-methylammonium-5′-deoxyadenosine (Figure 1) was subjected to the reaction with the enzyme adenosine desaminase, it was observed that the conversion of this substrate to the corresponding inosine derivative was about two orders of magnitude slower than with adenosine itself.[1] Since 5′-methylammonium-5′-deoxyadenosine could be crystallized as its iodide, the protonated species being the same as the one present in the enzymatic assay, it became worthwhile to investigate its structural properties.

Experimental Section

5′-Methylammonium-5′-deoxyadenosine[2] was crystallized as its iodide from aqueous methanol at 4° by Dr. H. Hettler. One of the colorless rhomb-shaped crystals of approximate dimensions $0.1 \times 0.15 \times 0.1$ mm was used for all subsequent X-ray crystallographic investigations. The crystal lattice parameters (Table I) were derived from precession photographs and diffractometer measurements. The density of the crystals has been determined by the flotation technique and is in agreement with the calculated density but one water molecule of hydration per asymmetric unit must be included.

The intensity data were collected by Dr. H. A. Paulus, Darmstadt, on a Stoe four-circle automatic diffractometer equipped with a graphite monochromator in vertical position and a Philips microfocus Mo X-ray tube. The 1597 data were gathered up to a glancing

(1) B. Jastorff, Thesis Universität Braunschweig, 1970.

(2) A. Murayama, Göttingen, unpublished results, 1969.