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BIOSYNTHETIC INCORPORATION OF DL-TRYPTOPHAN-(5-^{3}H) INTO ANTHRAMYCIN, SIBIROMYCIN AND TOMAYMYCIN : N.I.H. SHIFT PRODUCED BY ACTINOMYCETES

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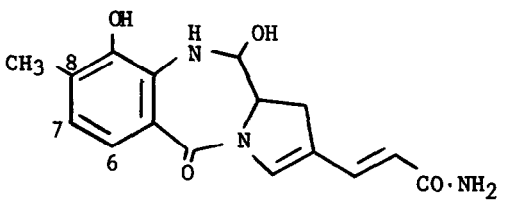
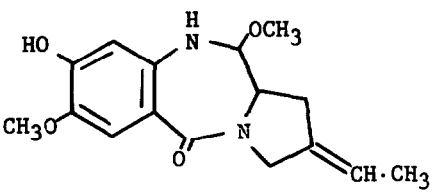
The pyrrolo(1,4)benzodiazepine antitumor antibiotics, anthramycin (I)[1], tomaymycin (II)[2] and sibiromycin (III)[3] are produced by various Actinomycetes. We have previously demonstrated that anthramycin[4], tomaymycin[5] and sibiromycin[6] are biogenetically closely related, the anthranilate part of these molecules being derived from tryptophan probably via the well known kynurenine pathway[7]. Since these antibiotics are hydroxylated in the anthranilate ring it was of interest to determine whether any of these hydroxylation reactions involved an intramolecular migration of aromatic ring substituents (the NIH Shift). Such migrations have been demonstrated in a variety of biological systems including plant, animal and bacterial tissues[8]; however, to our best knowledge observations on the NIH Shift in



I



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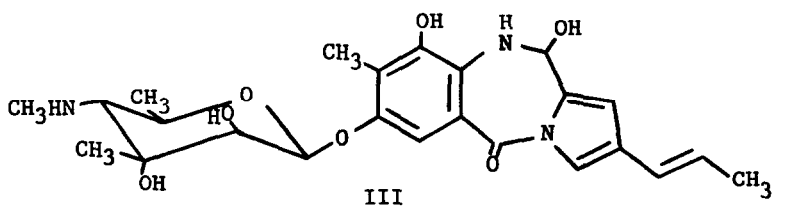


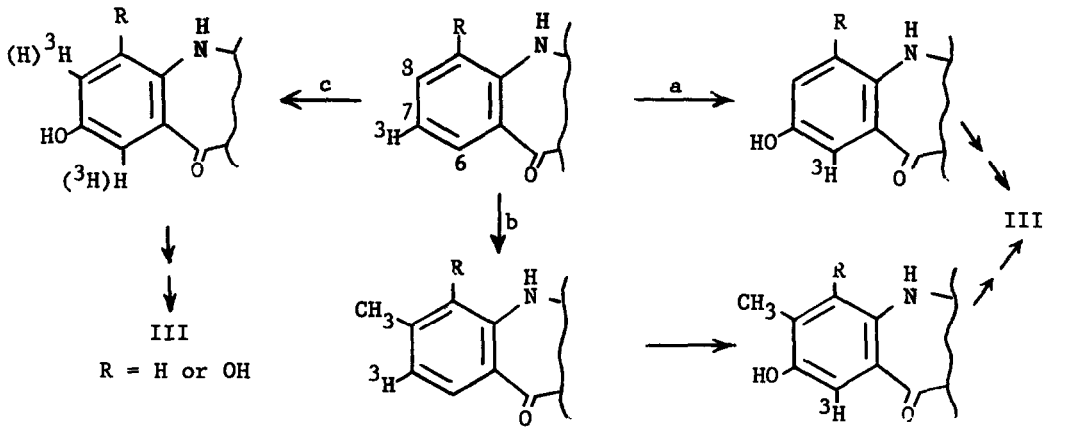
III

Actinomycetes have not been reported. The wealth of knowledge on the NIH Shift has led to the formulation of a number of basic rules concerning the migration or loss of the hydrogen atom from the carbon atom at which hydroxylation has occurred and consequently the order in which substituents are inserted into the aromatic ring[8]. This paper describes the application of these rules in our antibiotic biosynthesizing systems.

The incorporation of DL-tryptophan (5-^{3}H)/(7a-^{14}C) into anthramycin is straightforward. This antibiotic lacks both a substituent at C-7, the expected position for labelling by C-5 of tryptophan, and also hydroxyl groups at adjacent positions and therefore complete retention of tritium is to be expected. And indeed this was found in practice (see table).

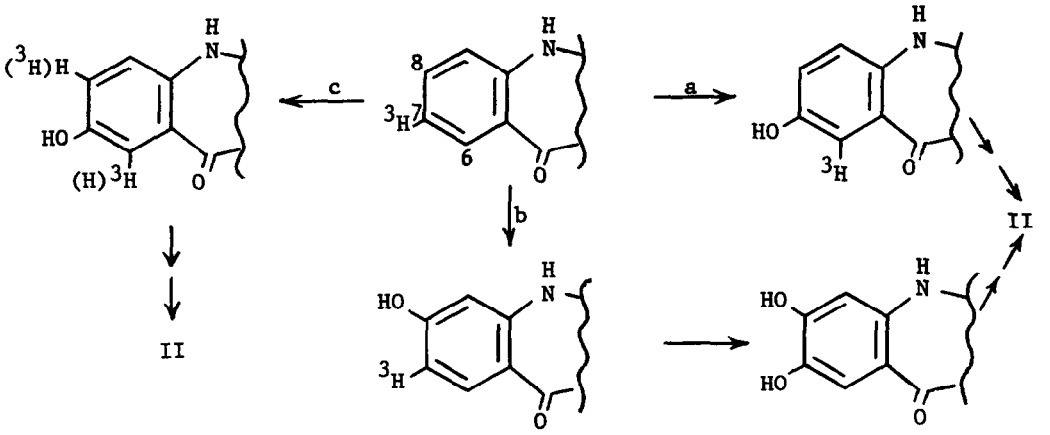
In the case of sibiromycin the three alternative pathways are as shown in scheme 1. Pathways "a" or "b" would both result in tritium retentions from tryptophan (5-^{3}H) of 85% or greater in sibiromycin, while pathway "c" involving non-selective migration of displaced tritium would result in a tritium retention of about one half this percentage in this antibiotic. The observed retention of 91% is only in accord with selective migration of the displaced tritium to C-6 (pathway "a") or methylation at C-8 prior to hydroxylation at C-7 (pathway "b").

The possible pathways for conversion of tryptophan (5-^{3}H) into tomaymycin are as shown in scheme 2. Selective migration of displaced tritium to C-6 during hydroxylation at C-7 but prior to hydroxylation at C-8 would result in 85% greater retention of tritium in tomay-



Scheme 1

mycin (pathway "a"). Nonselective migration of tritium equally to C-6 and C-8 during hydroxylation at C-7 but again prior to hydroxylation at C-8 would result in about 42 to 47% retention in tomaymycin (pathway "c"). Hydroxylation at C-8 prior to hydroxylation at C-7 would result in complete loss of tritium during the conversion of tryptophan (5-^{3}H) to tomaymycin (pathway "b"). The experimentally determined retention of 16% can be explained in two ways. First it is suggested that "b" is the major pathway, with "a" or "c" being minor alternated pathways or second that a modification of pathway "c" is operative such that an 84% migration to C-8 and a 16% migration to C-6 occurs during the first hydroxylation reaction. The tryptophan (5-^{3}H) used in this experiment was prepared by catalytic dehaloge-



Scheme 2

nation of 5-bromo-DL-tryptophan with tritium gas (Research Products International). This procedure has been demonstrated to produce tryptophan with greater than 90% of the tritium in the 5-position[9] and therefore our 16% retention cannot adequately be explained by non-specific labelling. Finally, the possibility that 5-hydroxytryptophan is an intermediate on the pathway to either sibiromycin or tomaymycin has been excluded since this substrate fed in labelled form, with tritium predominately in the aromatic ring, was not detectably incorporated (i.e. less than 0.1%) in either case.

TABLE - Incorporation of DL-Tryptophan-(5-^{3}H)/(7a-^{14}C), ($^{3}H/^{14}C$ ratio = 12.34/1) into Anthramycin, Sibiromycin and Tomaymycin

Antibiotic	% Incorp.[a]	$^{3}H/^{14}C$ ratio			Average ^{3}H retention (%)
		After TLC[b]	DNA Complex[c]	Recrystallization[d]	
Anthramycin	18.0	12.1	12.9	12.4	101
Sibiromycin	8.1	11.1	11.3	nd[e]	91
Tomaymycin	4.0	2.1	1.93	1.74	16

[a] Based on carbon-14.

[b] Purification by T.L.C. and rechromatography to ensure radiochemical purity.

[c] Purification by complexation with DNA and dialysis against two changes of buffer.[10]

[d] Recrystallization to constant $^{3}H/^{14}C$ ratio.

[e] Not determined.

Acknowledgments

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References and Notes

1. M.D. Tendler and S. Korman, Nature, 199:501 (1963).
2. K. Airma, M. Kohsaka, G. Tamura, H. Imanaka and H. Sakai. J. Antibiotics. 25:437 (1972).
3. A. S. Mesentsev, V. V. Kuljaeva and L. M. Rubasheva, J. Antibiotics. 27:866 (1974).
4. L. H. Hurley, M. Zmijewski and C-J Chang. J. Amer. Chem. Soc. 97:4372 (1975).
   L. H. Hurley and M. Zmijewski. J. Chem. Soc. (Chem. Comm.) 337 (1974).
5. L. H. Hurley, C. Gairola and M. Zmijewski. J. Chem. Soc. (Chem. Comm.) 120 (1975).
   L. H. Hurley, C. Gairola and N. Das. Biochemistry. Submitted for publication.
6. L. H. Hurley, N. Das and C. Gairola. Manuscript in preparation.
7. D. M. Greenberg in "Metabolic Pathways" Vol. 1. D. M. Greenberg, Ed., Academic Press, New York, N. Y. 1969, p153.
8. J. W. Daly, D. M. Jerina and B. Witkop, Experientia, 28:1129 (1972).
9. J. Renson, J. Daly, H. Weissbach, B. Witkop and S. Undenfriend. Biochem. Biophy. Res. Commun. 25:504 (1966).
10. All three antibiotics form covalent linkages with DNA when incubated in buffer at neutral pH. Non-covalently bound material can then be selectively removed by dialysis.