(2) Garrett, E. R., Seydel, J. K., and Sharpen, A. J., *J. Org. Chem.*, **31**, 2219(1966).
(3) Byvoet, P., *Anal. Biochem.*, **13**, 170(1965).
(4) Becker, A., *Helv. Chim. Acta*, **32**, 1114(1949).
(5) Eugster, C. H., Allner, K., and Rosenkranz, R. E., *Chemia*, **15**, 516(1961); Rosenkranz, R. E., Allner, K., Good, R., Philipsborn, W. V., and Eugster, C. H., *Helv. Chim. Acta*, **46**, 1259(1963).
(6) Hofmann, A., Philipsborn, W., and Eugster, C. H., *Helv. Chim. Acta*, **48**, 1322(1965).
(7) Olsen, S., and Russwurn, H., *Liebigs Ann. Chem.*, **639**, 1(1961).
(8) Reichstein, T., and Oppenauer, R., *Helv. Chim. Acta*, **16**, 988(1933); **17**, 390(1934).
(9) Levene, P. A., and London, E. S., *J. Biol. Chem.*, **83**, 793(1929).
(10) Deriaz, R. E., Stacey, M., Teece, E. G., and Wiggins, L. F., *J. Chem. Soc.*, **1949**, 1222.
(11) Thiele, J., *Liebigs Ann. Chem.*, **319**, 144(1901).
(12) Stones, W. E., and Tollens, B., *ibid.*, **249**, 227(1888).
(13) Birkofer, L., and Beckmann, F., *Ann.*, **620**, 21(1959).
(14) Anet, E. F. L. J., *Tetrahedron Letters*, **15**, 1649(1966).
(15) Fieser, L. F., and Fieser, M., "Advanced Organic Chemistry," Reinhold Publishing Co., New York, N. Y., 1961, p. 204.

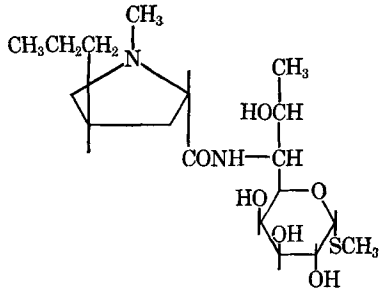
Preparation of Tritium-Labeled Compounds III

Lincomycin by Exposure to Tritium Gas and Partial Determination of Intramolecular Distribution of Tritium

By RICHARD C. THOMAS, GEORGE J. IKEDA, and HARRY HARPOOTLIAN

Lincomycin was tritiated more satisfactorily by its exposure to tritium in the presence of platinum black catalyst than by its exposure alone under conventional Wilzbach conditions. Incorporation of tritium and radiochemical purity, prior to purification, of the catalytically exposed lincomycin were far greater than those of the material exposed without catalyst. The intramolecular distribution of tritium was influenced by the exposure conditions. Catalytically exposed lincomycin contained virtually all its tritium in the propylhygric acid moiety, whereas the noncatalytically exposed sample contained approximately two-thirds of its tritium in this portion of the molecule; the remainder was in the methyl thiolincosaminide moiety.

LINCOMYCIN[1] (I) is a new antibiotic whose discovery and biological properties (1), isolation and characterization (2), and structure (3–7) have been reported.



I

Clinical use has shown it to be an effective agent for treatment of infections in man (8). This paper is concerned with preparation of radioactive lincomycin by two tritium-gas-exposure methods and a comparative determination of the resulting intramolecular distributions of tritium.

Radioactive lincomycin was required for metabolism studies in animals and man. Tritium labeling by the gas-exposure method of Wilzbach (9) was undertaken as the most attractive route to radioactive lincomycin. Incorporation of tritium, however, was poor and radiation-induced degradation was extensive; so, even though a usable product was obtained, attention was shifted to modifications of Wilzbach's method. The use of an electric discharge, as well as other sources of radiation, to promote ionization and excitation of the tritium gas was not particularly attractive since the authors had previously observed increased radiation-induced decomposition in applying this method to sulfonylurea compounds (10). Meshi and Takahashi (11), however, reported a modification of the Wilzbach method in which the compound, intimately mixed with platinum black catalyst, is exposed to tritium gas. Although this technique appears to have been little used since its introduction, it is reported to give better tritium incorporation than the conventional Wilzbach method. This was indeed found to be the case when the catalytic technique was applied to lincomycin.

Meshi and Sato (12) have shown that, in the cases of several compounds, the catalytic modification gives a different intramolecular distribution of tritium than does the Wilzbach method. A partial determination of the intramolecular distribution of tritium in lincomycin, labeled by

Received January 23, 1967, from The Upjohn Co., Kalamazoo, MI 49001
Accepted for publication March 13, 1967.
The authors thank the members of the Physical and Analytical Chemistry Unit, The Upjohn Co., for the analytical results.
Previous paper: Thomas, R. C., Buhler, D. R., and Ikeda, G. J., *J. Pharm. Sci.*, **56**, 507(1967).
[1] Lincocin is The Upjohn Co. trademark for lincomycin hydrochloride.

the two methods, was therefore made. Virtually all the tritium was accounted for in the propylhygric acid moiety of the catalytically tritiated sample whereas only two-thirds was in this moiety in the sample tritiated without catalyst.

EXPERIMENTAL

Determination of Radioactivity—All counting was performed with a Tri-Carb[2] model 314X or 314-EX2A liquid scintillation spectrometer at −8° under conditions suitable for measuring tritium. Appropriate aliquots of samples were dissolved in 15 ml. of diotol scintillator (13) [toluene–dioxane–methanol (350:350:210 by volume) containing 73 Gm. naphthalene, 4.6 Gm. 2,5-diphenyloxazole, and 0.08 Gm. 1,4-bis-2-(5-phenyl-oxazolyl) benzene per liter]. The absolute counting efficiency for each sample was determined by recounting following addition of an internal standard of tritium-labeled toluene and results then expressed as microcuries (μc.).

Paper chromatograms were scanned for radioactivity with a Vanguard[3] model 880 radiochromatogram scanner. Thin-layer chromatograms were scanned for radioactivity by scraping sequential 1-cm. sections of the silica gel support into individual vials and counting in the usual manner with a liquid scintillation spectrometer.

Paper and Thin-Layer Chromatography—Paper chromatograms were developed by the descending method using Whatman No. 2 paper in the following systems: (*a*) 1-butanol–water (84:16 by volume), (*b*) 1-butanol–acetic acid–water (2:1:1 by volume), (*c*) 5% citric acid in water made to pH 7.0–7.5 with ammonia, and (*d*) 2-propanol–2 *N* HCl (65:35 by volume). Thin-layer chromatography was carried out on 250-μ films of Silica Gel GF[4] using the following systems: (*a*) methanol and (*b*) methyl ethyl ketone–acetone–water (15:5:5 by volume). Chemical detection was accomplished by spraying the developed paper sheets or thin-layer plates with a mixture of four parts 2% aqueous $NaIO_4$ and one part 1% $KMnO_4$ in 2% aqueous Na_2CO_3. Yellow spots appear on a purple background.

Column Chromatography—A 2.8-cm. diameter column was packed to a height of 55 cm. with 200–400 mesh acidic cationic exchange resin.[5] The column was washed consecutively with 4 *N* HCl, saturated NaCl solution, 4 *N* NaOH solution, 1 *N* HCl, and 0.1 *N* HCl prior to each use. The lincomycin sample to be purified was added to the column in 25 ml. of 0.1 *N* HCl. The column was then washed with 1 L. of 0.1 *N* HCl followed by 1 L. of water. Lincomycin was eluted by passing 1.5 L. of 2 *N* NH_4OH through the column at a flow rate of approximately 100 ml./hr. while collecting 20-ml. fractions.

Exposure of Lincomycin to Tritium Gas—(*a*) A 1.0-Gm. sample of finely pulverized lincomycin base was exposed to 4 c. of carrier-free tritium gas at room temperature in the dark for 8 days under approximately 0.2 Atm. tritium pressure. Following removal of the tritium gas, the crude sample was dissolved in 20 ml. of water and the resulting solution was lyophilized. Labile tritium was completely removed by repeating this procedure three times. The crude sample had a specific activity of approximately 11 μc./mg. and was grossly impure, radiochemically, as shown by the previously mentioned paper and thin-layer chromatography systems.

(*b*) Another 1.0-Gm. sample of finely pulverized lincomycin base was thoroughly mixed with 1.0 Gm. of platinum black[6] and exposed to 4 c. of carrier-free tritium gas under the conditions mentioned previously. In this case, however, the tritium gas was entirely adsorbed on the catalyst so the pressure was approximately 10^{-5} mm. At the end of the 8-day exposure period, the mixture was added to 20 ml. of water and the catalyst was removed by filtration through diatomaceous earth.[7] The filtrate, containing the lincomycin, was lyophilized and labile tritium was completely removed as previously described. The crude sample had a specific activity of approximately 15 μc./mg. Its radiochemical purity was approximately 80% as determined by paper and thin-layer chromatography.

Purification of Tritiated Lincomycin—In each case the crude lincomycin was taken up in 25 ml. of 0.1 *N* HCl and subjected to ion-exchange chromatography as previously described. Fractions containing lincomycin (as determined by counting individual column fractions and subjecting selected ones to paper chromatography and scanning for radioactivity) were combined and lyophilized to a residue *in vacuo*. The residue was recrystallized as the hydrochloride salt by taking it up in 3 ml. of water, adding 30 ml. of acetone, and adjusting the pH to 2 with 12 *N* HCl. This procedure was repeated until constant specific activity was attained.

In the case of lincomycin subjected to conventional Wilzbach tritiation, a constant specific activity of 0.60 μc./mg. was attained in three crystallizations with a yield of 0.317 Gm. (as the hydrochloride salt). A constant specific activity of 10.5 μc./mg. with a yield of 0.629 Gm. was obtained for the catalytically exposed material after two crystallizations. Both samples were shown to be radiochemically pure by paper and thin-layer chromatography, followed by scanning for radioactivity, in the previously mentioned systems. In each case the radioactive zone corresponded to the colored zone, produced by the spray reagent, which in turn corresponded to the migration of authentic lincomycin hydrochloride.

Both tritiated samples had infrared spectra corresponding to that of authentic lincomycin hydrochloride.

Anal.[8]—Calcd. for $C_{18}H_{34}N_2O_6S \cdot HCl \cdot H_2O$: C, 46.90; H, 7.87; Cl, 7.69; N, 6.08; S, 6.96; eq. wt., 461. Found (noncatalytically tritiated sample): C, 46.86; H, 8.11; Cl, 7.89; N, 6.10; S, 7.09; eq. wt., 458. Found (catalytically tritiated sample): C, 47.09; H, 8.09; Cl, 7.78; N, 6.32; S, 7.21; eq. wt., 462.

Acid Hydrolysis of Tritiated Lincomycin (4)—(*a*)

[2] Packard Instrument Co., Downers Grove, Ill.
[3] Vanguard Instruments, Division of Technical Measurements Corp., North Haven, Conn.
[4] Brinkmann Instrument Co., Great Neck, N. Y.
[5] Marketed as Dowex 50X8 by the Dow Chemical Co., Midland, Mich.
[6] Engelhard Industries, Inc., Newark, N. J.
[7] Marketed as Celite by the Johns-Manville Corp., New York, N. Y.
[8] Another and larger sample of tritiated lincomycin hydrochloride, purified, recrystallized, and dried by the methods described, contained 1 mole of water as determined by Karl Fischer titration.

TABLE I—INTRAMOLECULAR DISTRIBUTION OF TRITIUM IN TRITIATED LINCOMYCIN

Component Counted	from Noncatalytically Tritiated Lincomycin		from Catalytically Tritiated Lincomycin	
	μc./m*M*	%	μc./m*M*	%
Lincomycin·HCl[a]	2.48	100	12.5	100
Propylhygric acid·HCl	1.53	62	11.9	95
Methyl thiolincosaminide	0.83	33.4	0.23	1.85
HCl distillate[b]	0.01	0.40	0.10	0.99
Methyl mercaptan[b]	0.08	3.22	0	0
Hydrazine distillate[b]	0.34	13.7	3.06	24.5

[a] The lincomycin hydrochloride was diluted with nonradioactive carrier for degradation. (See *Footnote 9.*) [b] Based on total lincomycin hydrochloride subjected to HCl hydrolysis or hydrazinolysis.

A solution of 1.252 Gm. of noncatalytically tritiated lincomycin hydrochloride[9] in 15 ml. of 1 *N* HCl was refluxed for 20 hr. while passing the evolved gases through a trap containing 10 *N* NaOH to trap methyl mercaptan liberated during hydrolysis. The reaction mixture was evaporated to dryness *in vacuo* and the distillate was collected for radioactivity determination. The residue was leached with boiling acetonitrile and the resulting solution was treated with charcoal[10] and evaporated to a small volume. The propylhygric acid hydrochloride was allowed to crystallize in the refrigerator overnight, filtered, and dried at room temperature *in vacuo*. Yield, 0.163 Gm.; m.p. 184–185° (capillary, uncorrected). The infrared spectrum was identical to that of anhydrous authentic propylhygric acid hydrochloride.

Anal.—Calcd. for $C_9H_{17}NO_3 \cdot HCl$: C, 52.05; H, 8.74. Found: C, 51.82; H, 8.90.

The radioactivities of the lincomycin hydrochloride, the contents of the NaOH trap, the distillate, and the propylhygric acid hydrochloride were determined and are listed in Table I.

(*b*) A 1.268-Gm. sample of catalytically tritiated lincomycin hydrochloride[9] was hydrolyzed as described above to yield 0.030 Gm. of propylhygric acid hydrochloride after three recrystallizations, m. p. 188–189° (capillary, uncorrected). The infrared spectrum of the sample corresponded to that of anhydrous authentic propylhygric acid hydrochloride.

Anal.—Calcd. for $C_9H_{17}NO_2 \cdot HCl$: C, 52.05; H, 8.74. Found: C, 51.74; H, 8.88.

The radioactivities of the various fractions are presented in Table I.

Hydrazinolysis of Tritiated Lincomycin (5)—(*a*) A solution of 0.400 Gm. of noncatalytically tritiated lincomycin hydrochloride[9] in 2 ml. of hydrazine hydrate was refluxed for 70 hr. The reaction mixture was evaporated to dryness *in vacuo* and the distillate was collected for radioactivity determination. The residue was triturated with hot acetonitrile, cooled, and filtered. The crystalline material (0.214 Gm.) was recrystallized from dimethylformamide, filtered, washed with cold methanol, and dried at room temperature *in vacuo* to yield 0.092 mg. of methyl thiolincosaminide, m.p. 222–223° (capillary, uncorrected). Its infrared and mass spectra corresponded to those of the authentic compound.

The radioactivities of the distillate and the methyl thiolincosaminide are presented in Table I.

[9] The tritiated lincomycin hydrochloride samples were diluted with nonradioactive carrier lincomycin hydrochloride by recrystallization from water–acetone prior to degradation.

[10] Darco G60.

(*b*) A 1.27-Gm. sample of catalytically tritiated lincomycin hydrochloride[9] was subjected to hydrazinolysis as described above. The crystalline residue (0.734 Gm.) from trituration was recrystallized to yield 0.296 Gm. of methyl thiolincosaminide, m.p. 223–224° (capillary, uncorrected). Its infrared and mass spectra corresponded to those of the authentic compound.

The radioactivities of the distillate and the methyl thiolincosaminide are presented in Table I.

RESULTS AND DISCUSSION

The radiochemical purity of the crude, catalytically tritiated lincomycin was far greater than that of lincomycin tritiated by the conventional Wilzbach method; 80% and 6%, respectively. It appears that either less radiation decomposition occurred under the catalytic conditions or that if high specific activity impurities were formed they were largely adsorbed on the platinum-black catalyst and not removed in the washing operation. In either event, the catalytic sample was easier to purify and radiochemically pure product was obtained in greater yield.

The incorporation of stably bound tritium in lincomycin by the catalytic method was 17 times that obtained by the conventional method. This effect of platinum black catalyst was found and reported by Meshi and Takahashi (11) in the cases of salicylic acid, valine, prednisolone, benzoic acid, inositol, and thiamine and by Meshi and Sato (12) in the cases of benzene, naphthalene, dinitrobenzene, phthalic anhydride, and benzoyl valine. They ascribe this increased incorporation to catalytic exchange of hydrogen rather than to increased absorption of decay energy by the system.

The intramolecular distributions of tritium in lincomycin prepared by the two methods were quite different. As shown in Table I, the noncatalytic sample contained 62% of its tritium in the propylhygric acid moiety and 33.4% in the methyl thiolincosaminide portion. Corresponding values for the catalytic sample were 95% and 1.84%, respectively. The origin of the small amount of tritium lost to the solvent during HCl hydrolysis is uncertain. The methyl mercaptan, cleaved from methyl thiolincosaminide during acid hydrolysis, contained 3.22% of the molecular tritium in the case of the noncatalytic sample and no tritium in the case of the catalytic sample. The rather large amount of tritium lost to the solvent during hydrazinolysis had to originate predominantly in the propylhygric acid moiety in the case of the catalytic sample and most likely did in the case of the noncatalytic sample. It has been shown (6) that during

hydrazinolysis the number 2 position of propylhygric acid is completely racemized. It, therefore, is likely that the tritium lost to the hydrazine solvent originated in this position of the lincomycin. Thus, 13.7% and 24.5% of the molecular tritium can be assigned to the number 2 position of the propylhygric acid moiety in the cases of the noncatalytic and catalytic samples, respectively.

In regard to ease of purification, yield of product, and incorporation of stable tritium, the catalytic method was superior to the conventional Wilzbach method for tritiating lincomycin. On the other hand, the noncatalytically tritiated lincomycin had a more uniform distribution of tritium making it more suitable for drug metabolism studies. If metabolism resulted in hydrolysis of lincomycin at the amide linkage, the methyl thiolincosaminide moiety could not be traced in the case of the catalytic sample because of its lack of tritium.

REFERENCES

(1) Mason, D. J., Dietz, A., and DeBoer, C., *Antimicrobial Agents Chemotherapy-1962*, **1962**, 554.
(2) Herr, R. R., and Bergy, M. E., *ibid.*, **1962**, 560.
(3) Hoeksema, H., Bannister, B., Birkenmeyer, R. D., Kagan, F., Magerlein, B. J., MacKellar, F. A., Schroeder, W., Slomp, G., and Herr, R. R., *J. Am. Chem. Soc.*, **84**, 4223 (1964).
(4) Herr, R. R., and Slomp, G., *ibid.*, to be published.
(5) Schroeder, W., Bannister, B., and Hoeksema, H., *ibid.*, to be published.
(6) Magerlein, B. J., Birkenmeyer, R. D., Herr, R. R., and Kagan, F., *ibid.*, to be published.
(7) Slomp, G., and MacKellar, F. A., *ibid.*, to be published.
(8) Daikos, G. K., Kontomichalou, P., Papadoyannakis, N., and Tourkantonis, A., *Antimicrobial Agents Chemotherapy-1963*, **1964**, 197; Holloway, W. J., Kahlbaugh, R. A., and Scott, E. G., *ibid.*, **1964**, 200; Harnecker, J., Contreras, J., Gilabert, B., and Ubilla, V., *ibid.*, **1964**, 205; Walters, E. W., Romansky, M. J., and Johnson, A. C., *ibid.*, **1964**, 210; Trakas, J. C., and Lind, H. E., *ibid.*, **1964**, 216.
(9) Wilzbach, K. E., *J. Am. Chem. Soc.*, **79**, 1013(1957).
(10) Thomas, R. C., and Ikeda, G. J., *J. Pharm. Sci.*, **55**, 112(1966).
(11) Meshi, T., and Takahashi, T., *Bull. Chem. Soc. Japan*, **35**, 1510(1962).
(12) Meshi, T., and Sato, Y., *ibid.*, **37**, 683(1964).
(13) Herberg, R. J., *Anal. Chem.*, **32**, 42(1960).

Synthesis of Tetrose Nucleosides I

Adenine Nucleosides of Erythrose and Threose

By DANIEL H. MURRAY and JOHN PROKOP

D- and L-Erythrose and D- and L-threose were individually converted to their triacetates which were condensed with chloromercuri-6-benzamidopurine in the presence of titanium tetrachloride. After deacylation, the four crude mixtures of anomeric nucleosides were each resolved on a strong anion exchange resin, leading to the isolation of all eight possible 9-tetrafuranosyladenines. The anomeric configurational assignments were made by consideration of the mechanism of nucleoside condensation (Baker's *trans* rule) and by Hudson's rules of isorotation. Preliminary results of tests for biological activity with *Streptococcus faecalis* 8043 and with adenosine deaminase are reported.

In recent years substantial attention has been directed toward the synthesis of potential inhibitors of nucleic acid metabolism. This has led to the preparation of a wide variety of 5- and 6-carbon sugar nucleosides, of which several of the compounds containing pentose sugar moieties have exhibited antibacterial and/or antitumor activity, for example, cordycepin, xylofuranosyladenine, and spongoadenosine. The majority of such nucleosides have been shown to act as nucleotides *via* phosphorylation at the 5′-position. On the other hand, some compounds have been shown to act in their nucleoside form, for example, decoyinine (1) and 5′-deoxyxylofuranosyladenine (2). In these, lack of a hydroxyl group at a terminal position removes the possibility of nucleotide formation. The 9-tetrofuranosyladenines are further examples of this class of nucleosides. Only the 9-β-D-erythrofuranosyladenine (III) has been prepared previously (3). It has been of interest in the present study to prepare all eight of the possible 9-tetrosyladenines. The biological activity of these is reported in a separate communication (2).

Received January 4, 1967, from the Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214
Accepted for publication April 24 1967.

DISCUSSION

The general method of synthesis involved coupling of an acetylated sugar with chloromercuri-6-benzamidopurine (II) (Scheme I) in the presence of titanium tetrachloride. Previous experience with this method (4, 5) has indicated that higher yields of nucleoside are frequently obtained as compared with the more usual procedure of condensation in which the acylated sugar is first converted to the corresponding halide. Furthermore, it has been reported that the use of titanium tetrachloride has led to the formation of significant quantities of the *cis* nucleoside[1] (6, 7). It was anticipated that at least one of the *cis* nucleosides in the present series, 9-β-D-threofuranosyladenine (X), might have significant biological activity. Hence, the authors

[1] The terminology *cis* or *trans* nucleoside refers to the configuration of substituents on carbons 1 and 2 of the sugar moiety.