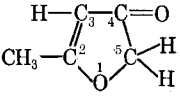
5-Methyl-3(2H)-furanone from Acid-Catalyzed Solvolysis of 2-Deoxy-D-ribose

By JOACHIM K. SEYDEL, EDWARD R. GARRETT*, WERNER DILLER, and KLAUS-JÜRGEN SCHAPER

5-Methyl-3(2H)-furanone (VIII) is produced from 2-deoxy-D-ribose (I) under defined conditions of acid, temperature, and time. Its production is a direct function of sugar concentration and its ultraviolet absorbance serves as a sensitive assay for the sugar. The compound VIII was identified by spectral data (NMR, U.V., I.R.), thin-layer chromatography R_f values in three different solvent systems, and chemical reactivities. The compound VIII obtained from deoxyribose and synthesized by a different route was the same. A mechanism dependent on the direction of water elimination after cyclization, dehydration, and rehydration can explain the formation of VIII and the previously obtained levulinic acid whose production from furfuryl alcohol (III) was explained by a similar mechanism. The spectrally observed kinetic intermediate with a $\lambda_{max.}$ at 225 mμ is consistent with the proposed mechanism.

THE THERMAL degradation of 2-deoxy-D-ribose in acid solution under specifically defined conditions produces an ultraviolet chromophore and the absorbance at $\lambda_{max.}$ 261 mμ is directly proportional to the sugar concentration (1, 2). The chromophore is extractable by chloroform and can easily be destroyed by treatment with mild alkali at room temperature. A direct sensitive spectrophotometric assay method for 2-deoxy-D-ribose in the presence of other sugars and other stable chromophores was developed on the basis of these properties (1). This method was used subsequently by Byvoet (3) for the determination of the deoxyribose content of deoxyribonucleic acid (DNA).

The isolation and identification of the compound which produces the chromophore as a 5-methyl-3(2H)-furanone [2-methyl-Δ^2-furenidone-(4)] (VIII) and its mechanism of formation are described in this paper.



VIII

EXPERIMENTAL

Apparatus—A Cary model 14 recording ultraspectrophotometer, a Varian HA 100 high resolution spectrometer with electronic counter, a Leitz infrared recording spectrometer with accessories for micro-techniques, and an Atlas mass spectrometer were used in these studies.

Materials—2-Deoxy-D-ribose (Carl Roth OHG, Karlsruhe, Germany) and diacetylethylacetate (Theodor Schuchardt G.m.b.H., Munchen, Germany), furfuryl alcohol (Eastman Organic Chemicals, Rochester, N. Y.), and ribitol (Nutritional Biochemical Corp., Cleveland, Ohio). All other chemicals were of analytical reagent grade.

Procedure for the Production, Isolation, and Purification of 5 - Methyl - 3(2H) - furanone [2 - Methyl - Δ^2 - furenidone - (4)] from Acid-Degraded 2 - Deoxy - D - ribose—2 - Deoxy-D-ribose (670 mg.) was dissolved in 1 L. of 1.0 *N* HCl and heated in a thermostated bath at 80.0° for 10 hr. The acid-degraded products were extracted with small portions of chloroform until no absorbance at 261 mμ was observed. The combined chloroform extracts were dried over anhydrous sodium sulfate and concentrated to about 10 ml. The concentrated mixture was purified by thin-layer chromatography.

The plates for thin-layer chromatography were prepared with 0.4-mm. or 1.0-mm. (preparative) layers of Silica Gel GF_{254} (E. Merck AG, Darmstadt). The mixture was spotted, or applied as a line with a syringe in the preparative case, at the origin of the plates. Chromatograms were developed for 12 cm. using as solvent systems: (*a*) chloroform–methanol, 80:15; (*b*) benzene–methanol, 9:1; (*c*) benzene–ether, 9:1. After development and drying, the spots were viewed under short wavelength ultraviolet light (254 mμ) or sprayed with 2,4-dinitrophenylhydrazine. Purification was effected by means of 1-mm. preparative thin-layer plates.

The compounds were scraped after development and extracted with chloroform. The silica gel was separated by centrifugation and filtration. When necessary for refining, the procedure was repeated. The R_f values of 5-methyl-3(2H)-furanone for the respective solvent systems were: (*a*) 0.72, (*b*) 0.37, and (*c*) 0.14. The purified substance was used to obtain the various spectrophotometric data.

Spectrophotometric Data—U.V.: $\lambda_{max.}$ 258 mμ, ϵ = 14,100, chloroform; $\lambda_{max.}$ 258 mμ, ϵ = 12,400, ethanol; $\lambda_{max.}$ 261 mμ, HCl; $\lambda_{max.}$ 295 mμ, NaOH.

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I.R. (film): 2930, 2870, 2720, 1750, 1710, 1608, and 1150 cm.$^{-1}$. NMR: chemical shift δ-values are given in parts per million (p.p.m.) from an internal tetramethylsilane standard; coupling constants are in Hz; spectra were run at room temperature in $CDCl_3$ as solvent. NMR data were H-(3), 5.48 (Q), J_{3,CH_3} 0.8; H-(5), 4.50 (Q), J_{5,CH_3} 0.9; =C—CH_3, 2.24 (D), J 0.8.

Synthesis of 5-Methyl-3(2H)-furanone (VIII)—The compound was synthesized in a four-step reaction sequence in accordance with the methods of Becker (4) and Rosenkranz *et al.* (5).

Diacetylethylacetate, b.p. 103°, n_D 1.4663, was prepared from acetylacetate. γ,γ'-Dibromo-diacetyl-ethylacetate, m.p. 49–50° corrected, yield 88%, was prepared from diacetylethylacetate. [Lit. values: 38%, m.p. 54–55°, 52–54° (4).] 4-Carbethoxy-5-methyl-3(2H)-furanone was prepared subsequently by a method used by Becker (4), yield 37%, and purified by vacuum sublimation, m.p. 72–73°. (Lit. values: 55%, m.p. 74–76°.) The I.R., U.V., and NMR data were identical with those reported by Rosenkranz *et al.* (5) and are consistent with their structure assignments. The data do not support the structure assigned by Becker (4).

5-Methyl-3(2H)-furanone, b.p. about 60° (12 mm.), was prepared from 4-carbethoxy-5-methyl-3(2H)-furanone in accordance with the literature (5, 6). The final compound was purified by preparative thin-layer chromatography [plate 1.0 mm. Silica Gel GF_{254} (E. Merck AG), solvent: benzene–methanol 9:1] R_f: 0.37. The NMR data were H-(3), 548 [5, 41] (Q), J_{3,CH_3}0.8 [about 1]; H-(5), 4.50 [4.60] (Q), J_{5,CH_3}0.9 [0.9]; =C—CH_3, 2.24 [2.24] (D), J 0.8. Figures in brackets are the data reported by Hofmann *et al.* (6).

Degradation of Furfuryl Alcohol and Ribitol—Furfuryl alcohol and ribitol, 1×10^{-4} M, in 1.00 N HCl were maintained at 80.0° and aliquots analyzed on the Cary recording ultraviolet spectrophotometer as a function of time.

RESULTS AND DISCUSSION

When an aqueous solution of 2-deoxy-D-ribose is heated, a chromophore appears with a U.V. absorption at 261 mμ. The rate of appearance of this chromophore is not strictly first order as shown by preliminary kinetic studies (1). It is similar to an autocatalytic reaction with an initial lag before the first-order decay in the appearance of the chromophore. It is probable that the 261 mμ chromophore is associated with a final product of a sequential series of reactions. Prior to the 261 mμ chromophore a band, $\lambda_{max.}$ 225 mμ, appears by an apparent first-order rate process, which is proportional to hydrogen ion activity at a constant temperature. It is possible that this 225 mμ chromophore is a precursor to the compound responsible for the 261 mμ chromophore. The rate of appearance of the 261 mμ chromophore is proportional to the hydrogen ion activity at a constant temperature. At 80° and in 1 N HCl the final absorbance at 261 mμ is reached after 5 hr. and remains constant for at least 24 hr. The 261 mμ absorbance was found to be directly proportional to the deoxyribose concentration and serves as an assay method for deoxyribose (1, 3).

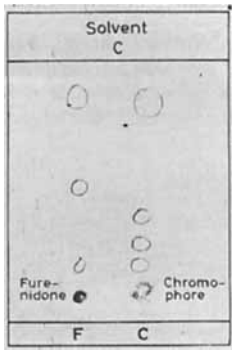
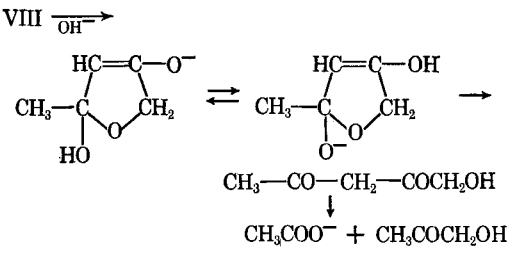


Fig. 1—Typical thin-layer chromatograms of a crude reaction mixture of synthesized 5-methyl-3(2H)-furanone, F, *and a chloroform extract of the acid-degraded 2-deoxy-*D*-ribose,* C. *The solvent system was benzene–ether, 9:1.*



Scheme I

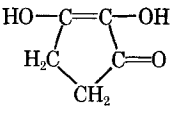
The chromophore-containing compound is extractable from acid solutions with chloroform and is unstable in alkaline solutions (1). The half-life at 28° in NaOH ($8.5 \times 10^{-4}M$) is 61 min. The compound reduces Fehling's solution and changes the color of a $FeCl_3$ solution to a red-brown. The oily colorless compound yellows and resinifies on standing in air.

Only a small fraction of the deoxyribose degrades to form the 261 mμ chromophoric compound. On the assumption that this compound is VIII, a maximum yield of 15% can be calculated from ultraviolet absorbance. Since many other compounds are present, isolation and purification were difficult. A characteristic thin-layer chromatogram of the chloroform extract of degraded 2-deoxy-D-ribose as compared with VIII is shown in Fig. 1. The spots were observed by spraying with 2,4-dinitrophenylhydrazine. The spots closest to the origin are VIII and the chromophoric compound, respectively. The hydrazones of both were red in color, indicative of compounds with unsaturated ketones.

The infrared spectrum of the purified isolates of degraded deoxyribose containing the ultraviolet chromophore had strong absorption bands at 1740, 1710, and 1608 cm.$^{-1}$. No hydroxyl group was indicated. Although the hydroxyl group absence and the presence of a conjugated ketone with a five carbon skeleton derived from a pentose would be consistent with an "angelica-type" lactone [X, XI (Scheme II)], the infrared spectra of X denied the hypothesis of such 4-hydroxy pentenic acid lactones which are formed on heating levulinic acid (7).

Several other substances are known to be products of the acid degradation of sugars. Reichstein and Oppenauer (8) reported the isolation of reductic acid (XIII) from strenuous acid treatment of pen-

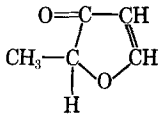
toses. The presence of hydroxyl groups in this compound denies this structure as our compound. The known products of sugar degradation such as levulinic acid (XII) (9) and ω-oxylevulinic aldehyde (10) are denied from their recorded ultraviolet absorption spectra.



XIII

The consideration of α-pyrone and furfural (12) as possible degradation products can be excluded by the wavelengths of their ultraviolet absorption and by the results of NMR and mass spectra.

The possible structures from the considered information are VIII and its structural isomer, XIV.



XIV

The three signals observed in the NMR are at 5.48, 4.50, and 2.24 p.p.m. and the intensities are in the ratio of 1:2:3 protons (Fig. 3). These signals can be assigned to a methyl group attached to a double bond, a methylene group attached to an ether oxygen, and a vinyl proton. They are inconsistent with XI and exclude XIV. The mass spectrum showed a peak consistent with a molecular weight of 98.

The 5-methyl-3(2H)-furanone (VIII) has been described in the literature (5, 6) and the NMR data were consistent. The comparison of I.R. (Fig. 2), U.V., NMR (Fig. 3), and thin-layer chromatograms in three different solvent systems (Fig. 4) demonstrated that VIII synthesized for comparative purposes and the isolated and characterized compound which gives rise to a 261 mμ chromophore from the thermal acid degradation of deoxyribose were identical. The chemical properties of structure VIII (5, 6) are the same as those of the acid degradation product of deoxyribose. Both compounds reduced Fehling's solution, gave a red-brown color with $FeCl_3$, formed phenylhydrazones, were stable in acidic solutions, and unstable in alkali with a shift of the 261 mμ chromophore to 295 mμ. The probable structures of compound VIII in alkali and its subsequent breakdown in analogy with the literature (5) are as shown in Scheme I.

Mechanism—Supportive evidence of the mechanism for the formation of VIII from deoxyribose through the pathway I–VIII (Scheme II) is available from the literature.

Birkofer and Beckmann (13) have demonstrated

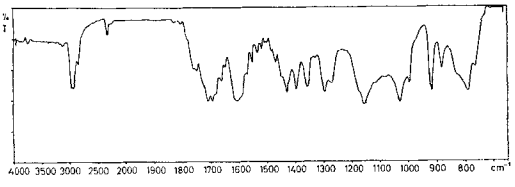


Fig. 2—Infrared spectrum of the isolated 261 mμ chromophoric compound obtained from the acid degradation of 2-deoxy-D-ribose which is the same as the spectrum of synthesized 5-methyl-3(2H)-furanone (film with microtechnique accessories).

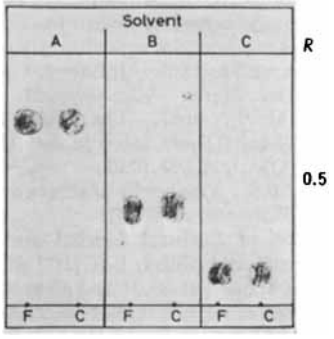


Fig. 4—Typical thin-layer chromatograms of synthesized 5-methyl-3(2H)-furanone, F, and the purified isolated 261 mμ chromophoric compound from degraded 2-deoxy-D-ribose, C, in several solvent systems. Key: A, chloroform–methanol, 80:15; B, benzene–methanol, 9:1; C, benzene–ether, 9:1.

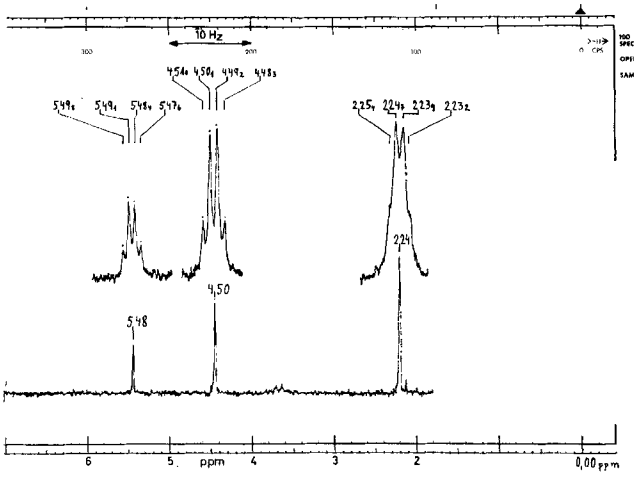
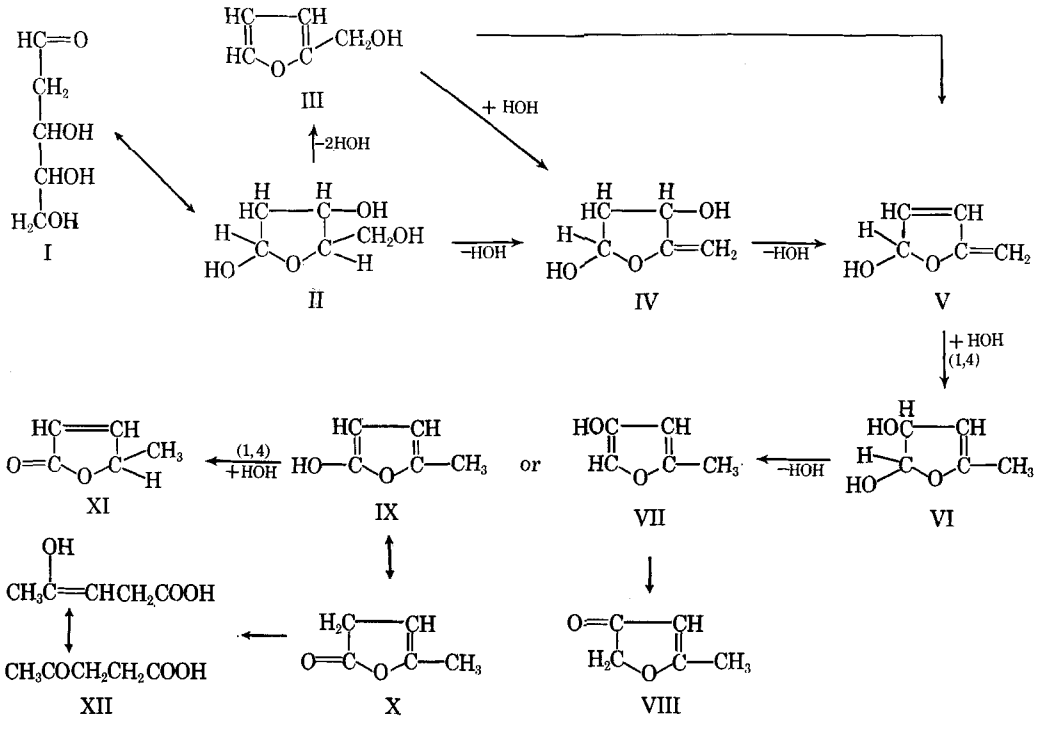


Fig. 3—NMR spectrum of the isolated 261 mμ chromophoric compound obtained from the acid degradation of 2-deoxy-D-ribose which is the same as the spectrum of synthesized 5-methyl-3(2H)-furanone.



Scheme II

that levulinic acid (XII) is a product of acid treatment of furfuryl alcohol (III) and passes through the isolated intermediate, 4,5-dihydroxy-4,5-dihydrosilvan (VI). They postulated the formation of this intermediate (VI) from 1,4-hydration of the precursor (V) which was produced from acid catalyzed rearrangement of furfuryl alcohol (III). This could occur just as readily from dehydration and rehydration of furfuryl alcohol (III) to form the postulated product (IV) of deoxyribose dehydration. This product (IV) can also form V by a subsequent dehydration.

On further dehydration, the 4,5-dihydroxy-4,5-dihydrosilvan (VI) will produce either the enolic form of the lactone of levulinic acid (XII) or VIII. Since the yield favors levulinic acid (XII) [about 50% (13)] rather than VIII [about 15% (1)], removal of the hydroxyl from the 3 position from VI is favored over the removal of the hydroxyl from the 2 position.

Recently, Anet (14) has shown that the methyl ether of the furanose form of aldoses produces a 5-substituted -3(2H)-furanone, on treatment with 0.1 *N* HCl at 70° in 30 min. These are conditions approximately equivalent to those used in the formation of VIII from deoxyribose. It was also reported that VIII rapidly polymerized in nonaqueous solvents containing traces of acid (5, 14) consistent with our observed resinification of chloroform-extracted VIII from deoxyribose degradation. This polymerization may be the explanation of the slow decrease in absorbance of the developed 261 mμ chromophore of degraded deoxyribose when held in acidic solution for long periods of time (1). The reported shifting (13) of the $\lambda_{max.}$ 261 mμ (acid solution) to 296 mμ in 0.01 *N* NaOH is the same as reported for the compound from degraded deoxyribose.

The sequence of reactions from deoxyribose through the pathway I–VI to the products VIII and levulinic acid (XII) is a plausible one consistent with all available information and identifications. It is also consistent with the observed kinetics of the formation of VIII by acid treatment of deoxyribose (1). The S-shaped curve for the formation of VIII implies that the reaction proceeds through a relatively stable intermediate or intermediates. It is possible that the 225 mμ absorbance which precedes the 261 mμ absorbance assigned to VIII represents an intermediate. In Scheme II, a plausible assignment of the 225 mμ $\lambda_{max.}$ by Woodward's rules is structure V, calculated $\lambda_{max.}$ 224 mμ (15).

The degradation of furfuryl alcohol (III) in 1.00 *N* HCl at 80.0° initially produced a 225 mμ absorbance that disappeared in 1 hr. while a 261 mμ absorbance appeared. The 261 mμ absorbance reached its maximum value, 0.62, after 5 hr. and was constant for 24 hr. Addition of alkali at room temperature shifted the $\lambda_{max.}$ to 293 mμ and a rapid loss of this new chromophore was observed. As predicted by Scheme II, furfuryl alcohol showed degradative properties similar to deoxyribose and produced the same chromophore with the same properties under the same conditions which were consistent with VIII. When ribitol was treated in the same manner, no significant chromophore was observed.

REFERENCES

(1) Seydel, J. K., and Garrett, E. R., *Anal. Chem.*, **37**, 271 (1965).

(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.

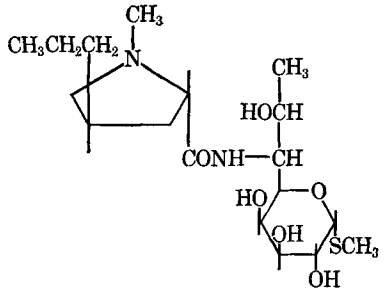
Preparation of Tritium-Labeled Compounds III

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

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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.