and Mr. P. F. Stokely for the equivalent weight determination.

(10) Alfred P. Sloan Research Fellow.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALIFORNIA
RIVERSIDE, CALIFORNIA

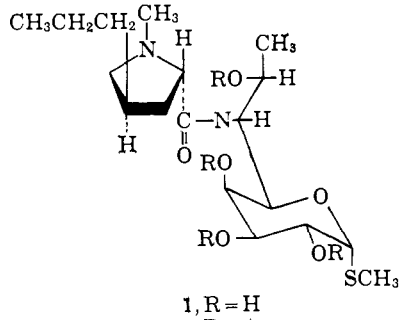
FRED TEBBE
PHILIP M. GARRETT
M. FREDERICK HAWTHORNE[10]

RECEIVED AUGUST 19, 1964

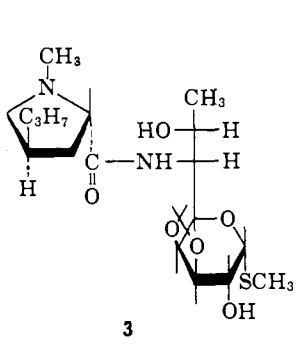
Chemical Studies on Lincomycin. I. The Structure of Lincomycin

Sir:

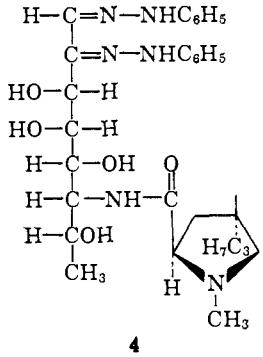
The antibiotic lincomycin[1-5] has been found to have structure **1** as shown by the following data. Lincomycin, $C_{18}H_{34}N_2O_6S$, contains one N-methyl, two C-methyls, one basic function, $pK_a' = 7.5$, and gives a negative iodoform test.[6] The infrared absorption is indicative of a monosubstituted amide (1530 and 1640 cm.$^{-1}$). A tetraacetate, **2**, and isopropylidene lincomycin, **3**, could be prepared. Aqueous 2 *N* acid liberated methanethiol and another fragment isolated as the phenylosazone **4**, $C_{29}H_{42}N_6O_5$.



1, R = H
2, R = Ac

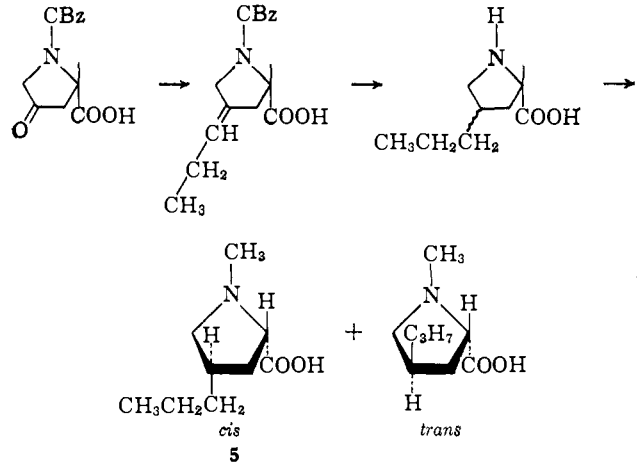


3



4

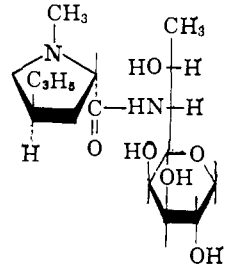
A vigorous acid hydrolysis freed an amino acid, **5**, $C_9H_{17}NO_2$. Preliminary comparisons with L-hygric acid and n.m.r. studies suggested an *n*-propylhygric acid. Rotational shifts on acidification suggested the L-series.[7] Synthesis from 4-keto-L-proline[8] placed the *n*-propyl group at C-4 and confirmed the L- configuration. The amide of one of the two diastereomers so formed was found to be identical with that obtained from lincomycin. Oxidation[9] of **5** to (*R*)(+)-propylsuccinic acid showed it to be *trans*-L-4-*n*-propylhygric acid.



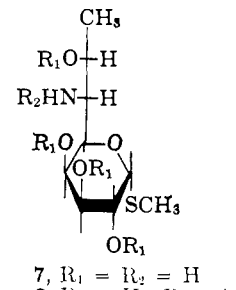
5

Lincomycin and its acetonide (**3**) took up 4 and 2 moles of periodate, respectively. Its nickel desulfurization product, $C_{17}H_{32}N_2O_6$, **6**, consumed 2 moles, liberating 1 mole of formic acid.

Hydrazinolysis cleaved **1** into **5** as the hydrazide and compound **7**, $C_9H_{18}NO_5S$, methyl α-thiolincosaminide. The base **7**, $pK_a' = 7.5$, containing no amide, gave a negative iodoform test[6] and consumed 5 moles of periodate. Acylation of **7** with the propyl carbonate mixed anhydride of **5**, both natural and synthetic, yielded crystalline **1** as the hydrochloride. Two acetyl derivatives, **8** and **9**, were obtained. The n.m.r. spectrum of **7**, in addition to suggesting galactose stereochemistry and an axial methylthio function,[10] displayed a distinct doublet of doublets centered at δ 3.3, $J = 10.5$ and 3.0 c.p.s., suggesting, in contradiction to the earlier iodoform data, that the hydrogen on the carbon bearing nitrogen was split by only two hydrogens. Therefore, the amine was not adjacent to the terminal methyl group.



6



7, $R_1 = R_2 = H$
8, $R_1 = H$; $R_2 = Ac$
9, $R_1 = R_2 = Ac$

Conversion of **8** to acetonide (**10**), hydrazinolysis to **11**, followed by oxidation, first with periodate, then with nitric acid, afforded mucic acid, confirming the galactopyranose configuration. Borohydride reduction of the above periodate product (not isolated), hydrolysis with

(1) D. J. Mason, A. Dietz, and C. De Boer, *Antimicrobial Agents Chemotherapy*, 554 (1962).

(2) R. R. Herr and M. E. Bergy, *ibid.*, 560 (1962).

(3) L. J. Hanka, D. J. Mason, M. R. Burch, and R. W. Treick, *ibid.*, 565 (1962).

(4) C. N. Lewis, H. W. Clapp, and J. E. Grady, *ibid.*, 570 (1962).

(5) G. K. Daikos, *et al.*, *ibid.*, 197 (1963); W. J. Holloway, *et al.*, *ibid.*, 200 (1963); J. Harnecker, *et al.*, *ibid.*, 204 (1963); E. W. Walters, *et al.*, *ibid.*, 210 (1963); J. C. Trakas and H. E. Lind, *ibid.*, 216 (1963).

(6) Although the negative iodoform test denied the presence of a methylcarbinol (*cf.* ref. 1), subsequent n.m.r. and chemical studies proved the iodoform test to be anomalous.

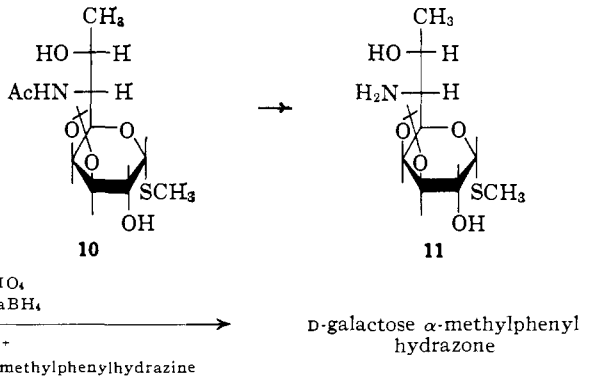
(7) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 1, John Wiley and Sons, Inc., New York, N. Y., 1961, p. 83.

(8) A. A. Patchett and B. Witkop, *J. Am. Chem. Soc.*, **79**, 185 (1957).

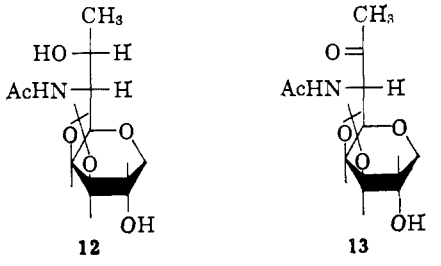
(9) A. Neuberger, *J. Chem. Soc.*, 429 (1945); G. W. Kenner, J. S. Dalby, and R. C. Sheppard, *ibid.*, 4387 (1962).

(10) A complete discussion of the n.m.r. spectrum will be the subject of a later communication.

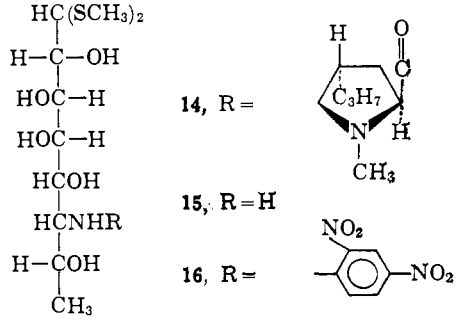
acid, and isolation as D-galactose α-methylphenylhydrazone established the D- series.



Nickel desulfurization of **10** gave **12**, $C_{13}H_{23}NO_6$. Chromic acid oxidation of **12** afforded **13**, $C_{13}H_{21}NO_6$, which gave a positive iodoform test and displayed a new n.m.r. peak, intensity 3H at δ 2.3, consistent with a methyl group adjacent to a carbonyl function. The product of borohydride reduction of **13** again gave a negative iodoform test. These data located the side-chain substituents.



Further reactions confirmed these assignments and established side-chain stereochemistry. Lincomycin was mercaptolyzed to methylthiolincomycose, **14**, $C_{19}H_{36}N_2O_6S_2$. Hydrazinolysis of **14** afforded lincosamine dimethyl dithioacetal, $C_{10}H_{23}NO_5S_2$, **15**, which was converted to the 2,4-dinitrophenyl derivative, $C_{16}H_{25}N_3O_9S_2$, **16**.



Periodate–permanganate[11] oxidation of **16** afforded 2,4-dinitrophenyl-D-allothreonine, an amorphous solid isolated by countercurrent distribution and identified by analyses and optical rotations.[12] Thus, lincomycin appears to be chemically related to celesticetin,[13,14] for which the partial structure has been published.[15]

(11) R. U. Lemieux and E. von Rudloff, *Can. J. Chem.*, **33**, 1701 (1955).
(12) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 11, John Wiley and Sons, Inc., New York, N. Y., 1961, p. 1564.
(13) C. De Boer, A. Dietz, J. R. Wilkins, C. N., Lewis, and G. M. Savage, *Antibiotics Ann.*, 831 (1955).
(14) H. Hoeksema, G. F. Crum, and W. H. Devries, *ibid.*, 837 (1955).
(15) J. W. Hinman and H. Hoeksema, 129th National Meeting of the American Chemical Society, Dallas, Texas, April, 1956.

Acknowledgment.—We gratefully acknowledge the analytical work of Dr. W. A. Struck and associates, and the advice and encouragement of Dr. T. E. Eble.

BIOCHEMICAL RESEARCH DIVISION
THE UPJOHN COMPANY
KALAMAZOO, MICHIGAN

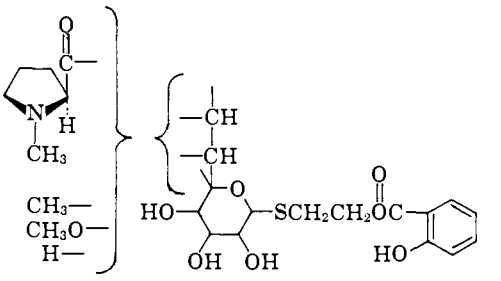
H. HOEKSEMA
B. BANNISTER
R. D. BIRKENMEYER
F. KAGAN
B. J. MAGERLEIN
F. A. MACKELLAR
W. SCHROEDER
G. SLOMP
R. R. HERR

RECEIVED JULY 27, 1964

Celesticetin. IV. The Structure of Celesticetin

Sir:

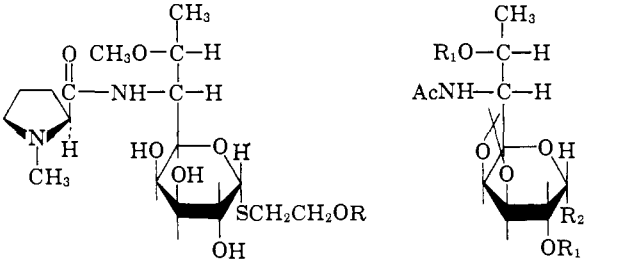
The partial structure of the antibiotic celesticetin has been reported[1] to be **1**.



1

Structural studies on lincomycin[2] have shown that it is chemically related to celesticetin. The present studies now extend the comparison between the two materials and celesticetin is shown to have structure **2** and desalicetin to be **3**.

The earlier work on celesticetin did not resolve the question as to whether the octose consisted of a straight or a branched chain. The exact locations of the hygric amide and methoxyl functions were also in doubt, and the stereochemistry of the sugar was unknown. The preparation of the identical compound, **4**, from both lincomycin and celesticetin resolves these questions. Celesticetin is found to have the same carbon chain, order of substitution, and stereochemistry as lincomycin.



2, R = salicyloyl; celesticetin
3, R = H; desalicetin
4, R_1 = Me; R_2 = H
5, R_1 = H; R_2 = —SCH_3
6, R_1 = R_2 = H

N-Acetylmethylthiolincosaminide, **5**, has previously been prepared from lincomycin.[2] Treatment with Raney nickel desulfurized **5**, giving **6** as a crystalline

(1) H. Hoeksema and J. W. Hinman, 129th National Meeting of the American Chemical Society, Dallas, Texas, 1956; *J. Am. Chem. Soc.*, in press.
(2) H. Hoeksema, B. Bannister, R. D. Birkenmeyer, F. Kagan, B. J. Magerlein, F. A. MacKellar, W. Schroeder, G. Slomp, and R. R. Herr, *ibid.*, **86**, 4223 (1964).