dioxaphospholenes (II, III, and IV), in the amounts of 30, 40, and 25%, respectively.

The residue was distilled under vacuum. The liquid collected at 50–55° (0.5 mm) (10.78 g) was analyzed by H^1 and P^{31} nmr spectrometry and found to consist of the trimethoxyphospholene II (60%), dimethoxybenzoxyphospholene III (10%), trimethyl phosphate (20%), and benzyl alcohol (10%). The residue of this distillation (25.16 g) contained the dimethoxybenzoxyphospholene III (60%) and methoxydibenzoxyphospholene IV (40%).

The residue of the previous distillation (25.16 g) was submitted to fractional short-path distillation. (1) The *first fraction* (10.55 g, 30%) was collected at 95–97° (0.05 mm). Spectral analysis showed that this fraction consisted of at least 95% of the dimethoxybenzoxyphospholene III and of less than 5% of a phosphate ester. The latter was removed as a first fraction in a short-path redistillation. Pure **2,2-dimethoxy-2-benzoxy-4,5-dimethyl-2,2-dihydro-1,3,2-dioxaphospholene (III)** was collected at 96–97° (0.05 mm). The H^1 nmr signals are listed in Table I. The infrared spectrum in CCl_4 had bands at (μ): 3.44 (s), 5.76 (w), 6.69 (w), 6.90 (s), 6.99 (w), 7.21 (s), 7.29 (shoulder), 7.78 (s), 8.04 (s), 8.26 (w), 8.68 (s), 9.1–9.7 (broad and strong), 10.01 (s), and 10.28 (s).

Anal. Calcd for $C_{13}H_{19}O_5P$: C, 54.5; H, 6.7; P, 10.8. Found: C, 54.2; H, 6.4; P, 10.9.

(2) The *second fraction* (1.03 g, 14%) of the above distillation was collected at 96–100° (5×10^{-6} mm) and was **2-methoxy-2,2-dibenzoxy-4,5-dimethyl-2,2-dihydro-1,3,2-dioxaphospholene (IV)**. The H^1 nmr signals are listed in Table I. The infrared spectrum in CCl_4 was very similar to the spectrum of the dimethoxybenzoxyphospholene III except that the relative intensities of some of the bands were different.

Anal. Calcd for $C_{19}H_{23}O_5P$: C, 63.0; H, 6.4; P, 8.5. Found: C, 62.9; H, 6.5; P, 8.5.

Reaction of the 2,2,2-Trimethoxy-4,5-dimethyl-2,2-dihydro-1,3,2-dioxaphospholene with 1.4 Molar Equiv of Benzyl Alcohol. The biacetyl–trimethyl phosphite adduct II and benzyl alcohol (1.4 molar equiv) were mixed under N_2. The mixture was heated 4 hr at 100° (*ca.* 50 mm). The distillate (6.03 g, 1.3 molar equiv) was shown to be methanol. The residue was analyzed and was fractionally distilled as indicated above. The trimethoxy-, dimethoxybenzoxy-, and methoxydibenzoxyphospholenes II, III, and IV were formed in 13, 38, and 41% yields, respectively. There was spectral evidence for the formation of 5% of tribenzyl phosphite, $(C_6H_5CH_2O)_3P$, which could have been formed in the reaction or during the fractional distillation.

Reaction of the 2,2,2-Trimethoxy-4,5-dimethyl-2,2-dihydro-1,3,2-dioxaphospholene with 3.4 Molar Equiv of Benzyl Alcohol. The biacetyl–trimethyl phosphite adduct II (5.80 g) was mixed with benzyl alcohol (10.14 g, 3.4 molar equiv) at 100° under N_2. The mixture was kept 12 hr at 100°, while a slow stream of N_2 was being bubbled through the solution. The gas stream was passed through a trap at −70° to collect the methanol. The residue was analyzed by H^1 and P^{31} nmr spectrometry and was found to consist of methoxydibenzoxyphospholene IV (15%), tribenzoxyphospholene V (60%), and small amounts of a phosphate ester. The identification of these products was based on the signals listed in Table I.

Other Alcoholyses of the Biacetyl–Trimethyl Phosphite Adduct II. (a) Equimolar amounts of the adduct II and benzyl alcohol were heated at 100° (170 mm). Practically all of the methanol evolved in this reaction was collected within 30 min. The residue was analyzed by H^1 and P^{31} nmr spectrometry to confirm the results described in the previous experiment carried out over a 3-hr period.

(b) The adduct II was kept with 3 molar equiv of benzyl alcohol for 2 hr at 40° (165 mm). No methanol was produced under these conditions. The expected amount of methanol was collected when the reaction mixture was heated for 30 min at 100° (165 mm).

Thermal Stability of the Biacetyl–Trimethyl Phosphite Adduct II. The adduct II was heated for 3.5 hr at 110° (165 mm). No volatile materials were collected in a trap fitted to the system and cooled at −70°. The residue was shown to be unreacted adduct II by H^1 nmr spectrometry.

Preparation of Tribenzyl Phosphite from the Reaction of Tris(dimethylamino)phosphine with Benzyl Alcohol. Tris(dimethylamino)phosphine (4.63 g) was mixed with benzyl alcohol (9.86 g, 3.2 molar equiv) at 20°. The mixture was kept 5 hr at *ca.* 100° while a show stream of N_2 was being bubbled through the solution. The gas stream was passed through a trap at −70° giving the dimethylamine. The residue (9.6 g, 96%) consisted of tribenzyl phosphite of 90% purity by H^1 nmr spectrometry. Short-path distillation gave tribenzyl phosphite, bp 142–148° (0.02 mm), in 60% yield; $\delta P^{31} = -138.6$ ppm (septet). The H^1 nmr spectrum in CCl_4 had a 15 H^1 signal at τ 2.8 and a 6 H^1 doublet at τ 5.26, $J_{HP} = 7.9$ cps. The infrared spectrum in CCl_4 had bands at (μ): 3.38, 3.49, 3.56, 6.25, 6.72, 6.92, 7.33, 8.00, 8.30, 8.40, 9.6–10.4 (broad and very strong), and 10.95.

Anal. Calcd for $C_{21}H_{21}O_3P$: C, 71.5; H, 6.0; P, 8.8. Found: C, 71.2; H, 6.3; P, 9.1.

Preparation of the Biacetyl–Tribenzyl Phosphite Adduct V. A solution of biacetyl (0.50 g) in anhydrous benzene (5 ml) was added dropwise to a solution of tribenzyl phosphite (2.11 g, 1.03 molar equiv) in benzene (3 ml) at 0° with stirring under N_2. The solution was kept 10 hr at 25° and was evaporated at 25°, first at 15 mm and then at 0.05 mm. The residue (2.54 g) was a colorless, thick oil that could not be distilled or crystallized. The spectral analysis disclosed that this residue consisted of 2,2,2-tribenzoxy-4,5-dimethyl-2,2-dihydro-1,3,2-dioxaphospholene (V) of at least 98% purity. The H^1 nmr signals are listed in Table I. The infrared spectrum in CCl_4 had bands at (μ): 3.37 (s), 3.48 (s), 5.78 (w), 6.70 (w), 6.90 (s), 7.00 (w), 7.22 (s), 7.30 (shoulder), 7.80 (s), 8.05 (s), 8.28 (s), 8.70 (s), 9.1–9.8 (broad and strong), and 10.3 (s). The crude material was submitted for analysis.

Anal. Calcd for $C_{25}H_{27}O_5P$: C, 68.5; H, 6.2; P, 7.0. Found: C, 67.5; H, 6.3; P, 6.5.

Celesticetin. V. The Structure of Celesticetin

Herman Hoeksema

Contribution from the Biochemical Research Division, The Upjohn Company, Kalamazoo, Michigan. *Received September 1, 1967*

Abstract: The structure of celesticetin is β-salicyloyloxyethyl 6,8-dideoxy-6-(1-methyl-L-pyrrolidine-2-carboxamido)-7-O-methyl-1-α-thio-D-*erythro*-D-*galacto*-octopyranoside.

A partial structure[1] of celesticetin[2] (**1**) was incomplete as to questions regarding branching and stereoisomerism of the aminooctose as well as the relative locations of the methoxyl and amino groups on this sugar. Recent studies[3] on lincomycin (**2**) produced by

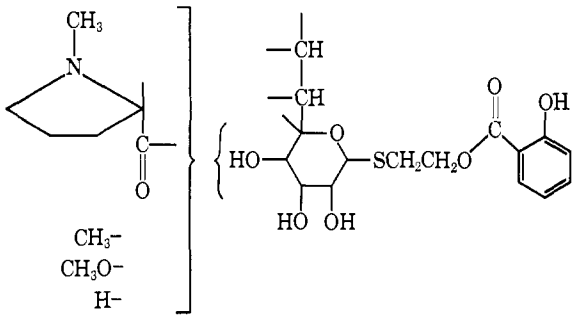
(1) H. Hoeksema and J. W. Hinman, *J. Amer. Chem. Soc.*, 86, 4979 (1964).

(2) (a) C. DeBoer, A. Dietz, J. R. Wilkins, C. N. Lewis, and G. M. Savage, "Antibiotics Annual (1954–1955)," Medical Encyclopedia, New York, N. Y., p 831; (b) H. Hoeksema, G. F. Crum, and W. H. DeVries, *ibid.*, p 837.

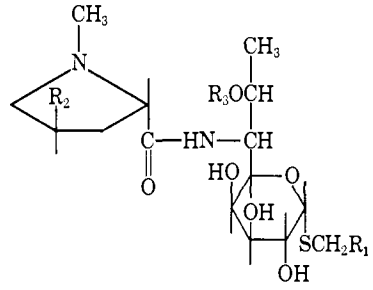
(3) H. Hoeksema, B. Bannister, R. D. Birkenmeyer, F. Kagan, B. J. Magerlein, F. A. MacKellar, W. Schroeder, G. Slomp, and R. R. Herr, *J. Amer. Chem. Soc.*, 86, 4223 (1964).

a different organism, *S. lincolnensis*, demonstrated its relationship to celesticetin. Subsequent investigations have disclosed a whole family of antibiotics in this class, produced by *S. lincolnensis* under various fermentation conditions.[4]

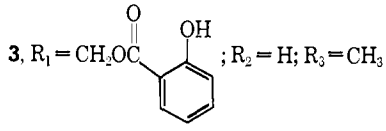
The lincomycin structure determination opened a new avenue into the complete elucidation of the celesticetin molecule. Results of this approach, in which an assumption of the identity of the lincomycin and celesticetin octosamines was verified, have appeared in a preliminary communication.[5] Structures **3** and **4** were thus established for celesticetin and desalicetin, respectively.



1



2, $R_1 = R_3 = H$; $R_2 = n\text{-}C_3H_7$

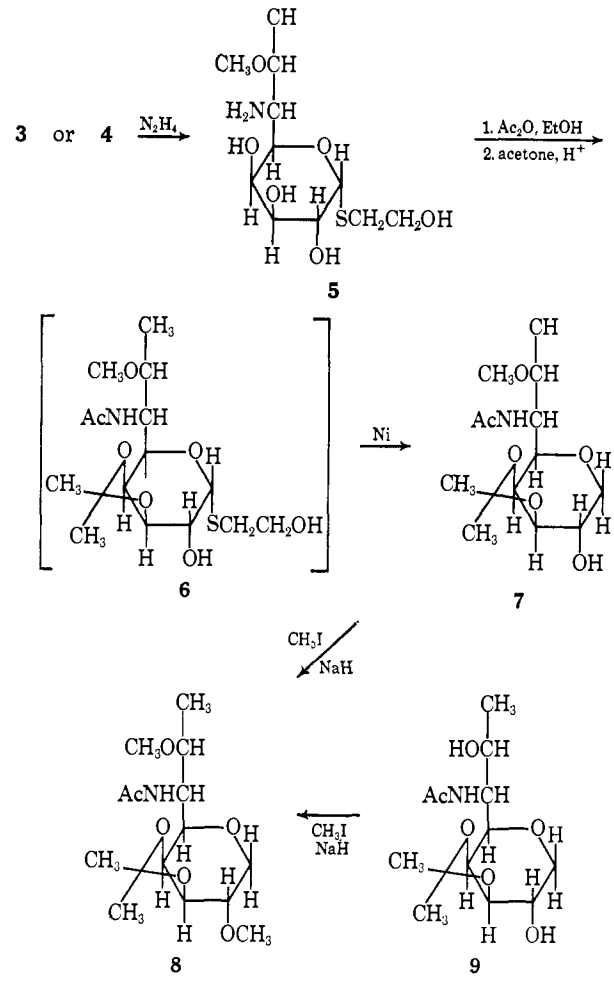


4, $R_1 = CH_2OH$; $R_2 = H$; $R_3 = CH_3$

Hydrazine hydrate, under reflux, cleaved both the amide and ester bonds of celesticetin, affording β-hydroxyethyl thiocelestosaminide (**5**) isolated as the crystalline monosolvate of hydrazine. Desalicetin (**4**) likewise yielded **5** under these conditions. Three sequential transformations were then carried out with only a cursory purification of each intermediate. N-Acetylation of **5** readily occurred with acetic anhydride in ethanol. The amorphous acylate reacted with acetone, catalyzed by sulfuric acid, yielding crude β-hydroxyethyl 3,4-O-isopropylidenethiocelestosaminide (**6**). Crystalline **7** was finally obtained by countercurrent distribution following nickel desulfurization of acetonide **6**. Methylation of the remaining free hydroxyl in **7** constituted the fourth step. For this, methyl iodide and sodium hydride in purified dimethylformamide were employed, and crystalline **8** was obtained after a countercurrent distribution.

(4) A. D. Argoudelis, J. A. Fox, D. J. Mason, and T. E. Eble, *J. Am. Chem. Soc.*, **86**, 5044 (1964).
(5) H. Hoeksema, *ibid.*, **86**, 4224 (1964).

A similar set of transformations was then used in the lincomycin series. Here the intermediate N-acetyl-3,4-O-isopropylidene-1,5-anhydrolincosaminol (**9**) had already been reported.[3] Dimethylation resulted from treatment of this with methyl iodide and sodium hydride in dimethylformamide and again purification was accomplished by countercurrent distribution. The N-acetyl-2,7-di-O-methyl-3,4-O-isopropylidene-1,5-anhydrolincosaminol (**8**) so obtained was found to be



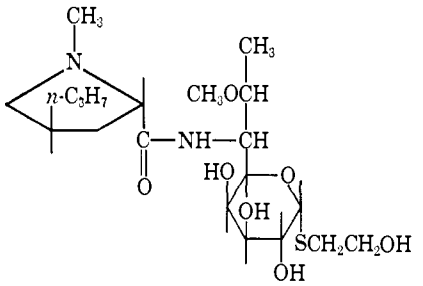
identical with the corresponding product from celesticetin, establishing the identity of the two octoses.

The stereochemistry at carbon 1 has been described[6] as the α configuration by applying the same reasoning to the nmr spectrum of **5** as that used for the methyl thiolincosaminide from lincomycin. The conformation of the carbohydrate moiety in deuterium oxide has likewise been described[6] as being similar to that for lincomycin.

The relationship between these antibiotics suggested possibilities for modifications at position 4′ (in the pyrrolidine ring), carbon 7 of the octose, and on the sulfur substituent. Such interest was stimulated by the greatly enhanced activity of the lincomycins over the celesticetins. Thus it was of interest to prepare the 4′-*n*-propyl homolog of desalicetin, creating a partial crossover into the lincomycin series. Intermediate **5** reacted with the mixed anhydride of propyl carbonic acid and *trans*-1-methyl-4-*n*-propyl-L-proline obtained

(6) G. Slomp and F. A. MacKellar, *ibid.*, **89**, 2454 (1967).

from the hydrolysis of lincomycin. The compound (**10**) so obtained, crystalline as the hydrochloride, displayed antibacterial activity midway between those observed for lincomycin and celesticetin.[7] Further modi-



10

fication studies will be reported elsewhere.

Hydrazinolysis of Celesticetin. β-Hydroxyethyl Thiocelestosaminide (5). A solution containing 5.0 g (9.4 mmol) of celesticetin and 25 ml of hydrazine hydrate was refluxed for 21 hr. The hydrazine was removed by distillation *in vacuo*, and the residue was crystallized from 35 ml of ethanol to give 1.2 g of white crystals melting at 98–108°. Recrystallization from ethanol afforded 0.65 g, melting at 103–106°, $[\alpha]^{25}D$ +243° (*c* 0.8, water).

Anal. Calcd for $C_{11}H_{23}NO_6S \cdot N_2H_4$: C, 40.11; H, 8.26; N, 12.76; S, 9.73. Found: C, 40.15; H, 8.04; N, 11.69, 11.62; S, 9.56.

A solution containing 2.0 g of the above solvate in 30 ml of dimethylformamide was evaporated by boiling to a 10-ml volume, then diluted with 10 ml of dimethylformamide and sufficient ether to cloud. Recovery of 500 mg of crystals, melting at 167°, $[\alpha]^{25}D$ +262° (*c* 1, water), was thus effected.

Anal. Calcd for $C_{11}H_{23}NO_6S$: C, 44.43; H, 7.80; N, 4.71; S, 10.78. Found: C, 44.20; H, 7.78; N, 4.97; S, 10.68.

Preparation of N-Acetyl-3,4-O-isopropylidene-1,5-anhydrocelestosaminol (7). A 14.0-g (47.0 mmol) quantity of **5** in 150 ml of ethanol was stirred at room temperature with 14 ml (excess) of acetic anhydride 0.5 hr. The resulting solution was refrigerated overnight, then evaporated to dryness *in vacuo*. After trituration with ether and drying *in vacuo*, the residue was dissolved in 1500 ml of acetone and 15 ml of concentrated sulfuric acid and stirred 2 hr. Neutralization with dry ammonia and filtration followed. After evaporation of the filtrate an oil (**6**) remained.

The above oil, dissolved in 500 ml of ethanol, was refluxed in a suspension containing 500 ml of ethanol and 150 ml (loosely packed) of Raney nickel for 10 hr. The catalyst was separated and thoroughly washed, and the combined solutions were evaporated to dryness. An oil remained and this was distributed countercurrently in the system 1-butanol–water for 500 transfers. The peak fraction, K = 0.82, was recovered from tubes 200–250, 4.6 g, 33%. This crystallized on drying, mp 198–205°, $[\alpha]^{25}D$ +71° (*c* 1, 50% ethanol).

Anal. Calcd for $C_{14}H_{25}NO_6$: C, 55.43; H, 8.31; N, 4.62; methoxyl, 10.63. Found: C, 55.03; H, 8.28; N, 4.70; methoxyl, 10.43.

(7) D. J. Mason and C. N. Lewis, private communications.

Preparation of N-Acetyl-2,7-di-O-methyl-3,4-isopropylidene-1,5-anhydrolincosaminol (8). A. From 7. A solution containing 1.0 g (3.3 mmol) of **7** in 50 ml of dried dimethylformamide was prepared. To it 2.0 ml of methyl iodide and 200 mg (4.3 mmol) of sodium hydride (53% in mineral oil) were added, and this was stirred overnight. Evaporation to dryness followed and the residue was washed with pentane, then subjected to a 500-transfer countercurrent distribution in the system 1-butanol–water. A component, K = 1.6, consisting of 300 mg of crystals, was isolated by evaporation. Recrystallization from acetone and Skellysolve B gave an analytical sample, mp 149–150°, $[\alpha]^{25}D$ +65° (*c* 0.85, 50% ethanol).

Anal. Calcd for $C_{15}H_{27}NO_6$: C, 56.76; H, 8.58; N, 4.41. Found: C, 56.86; H, 8.79; N, 4.41.

B. From 9. To a solution containing 1.9 g (6.6 mmol) of **9** and 50 ml of dried redistilled dimethylformamide was added 0.6 g (13.3 mmol) of sodium hydride (53% in mineral oil) and 2.0 g (14 mmol) of methyl iodide. After 1 hr of vigorous stirring, a second 2.0-g quantity was added and at equal intervals subsequent third and fourth 2.0-g quantities were supplied. After stirring overnight, a few drops of water were added. The mixture was evaporated to dryness, and the residue was washed with pentane. The residue was then distributed countercurrently for 1000 transfers in the system 1-butanol–water and the material under two peaks of K = 1.6 and 1.85 was recombined and isolated, then redistributed in the same system for 2000 transfers. This time a single broad peak, covering tubes 25–138 of the 1000-tube Craig apparatus, was obtained, and it was found that the residue from tubes 25–90 could be crystallized from acetone and Skellysolve B. The product **8** was so isolated, 30 mg, mp 150–151°. The mixture melting point with **8** from celesticetin (mp 149–150°) was 148–150°. The infrared spectra and nmr curves indicated identity.

Preparation of N-(4-*trans*-Propyl-L-hygroyl)-β-hydroxyethyl Thiocelestosaminide Hydrochloride (10). A 2.3-g (20 mmol) quantity of propyl chloroformate was slowly added to a 225-ml acetone suspension containing 4.16 g (20 mmol) of *trans*-1-methyl-4-*n*-propyl-L-proline and 11.2 ml (80 mmol) of triethylamine. Vigorous stirring was carried out for 1 hr, and following filtration the filtrate was added at 5° to 5.94 g (20 mmol) of β-hydroxyethylthiocelestosaminide (**5**) in 50 ml of water. Following 1 hr of storage at 0°, the solution was evaporated to an aqueous concentrate *in vacuo* and this was extracted three times with 20 ml of chloroform. The extract was washed with water and dried. The chloroform solution was evaporated *in vacuo*. A residual oil crystallized on refrigerated storage. Crystallization as the hydrochloride was effected by dissolving 2.5 g of this oil in 25.2 ml of 6 *N* hydrochloric acid and adding 960 ml of acetone and 230 ml of ether to yield 0.9 g of white crystals.

Anal. Calcd for $C_{20}H_{38}N_2O_7 \cdot HCl$: C, 49.32; H, 8.07; S, 6.58; Cl, 7.28. Found: C, 49.39; H, 8.16; S, 6.75; Cl, 7.84.

Acknowledgment. The author wishes to thank Mr. James A. Shiley for valuable technical assistance and Mr. Raymond Anderson and his associates for the analytical results.