ANTIBIOTICS PRODUCED BY MUTANTS OF *STREPTOMYCES CAELESTIS*. I

7-O-DEMETHYLCELESTICETIN AND ITS DEGRADATION PRODUCTS

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7-O-Demethylcelesticetin is an antibacterial agent produced by *Streptomyces caelestis* strain 22227 a, a mutant of *S. caelestis*. This antibiotic was found to be as active as celesticetin *in vitro*. Treatment of 7-O-demethylcelesticetin with base yielded 7-O-demethyldesalicetin which also has antibacterial properties. Hydrazinolysis of 7-O-demethylcelesticetin gave 2'-hydroxyethyl thiolincosaminide, indicating that *S. caelestis* strain 22227a produces lincosamine, the aminosugar present in the lincosaminide antibiotics.

Celesticetin[1,3] (Fig. 1, I), desalicetin[2,3] (Fig. 1, Ia) and celesticetins B, C and D[4] are related antibiotics produced by *Streptomyces caelestis*. We have recently developed mutants of *S. caelestis* which are capable of producing antibacterial agents different from those produced by the parent culture. The present communication describes the properties of one of these antibiotics produced by a mutant of *S. caelestis* designated strain 22227 a*.

Experimental

Spectroscopic Methods

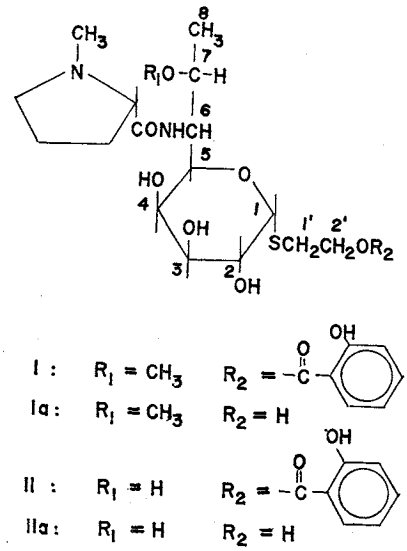
Nuclear magnetic resonance spectra were calibrated downfield from internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate. Spectra were observed with a Varian A-60 spectrometer on solutions (*ca* 0.4 ml, *ca* 0.25 M) of the compounds in deuterium oxide. Infrared spectra were obtained in mineral oil suspension.

Thin-Layer Chromatographic and Assay Procedures.

Thin-layer chromatograms were run on silica gel G using chloroform - methanol (6 : 1 v/v) or ethyl methyl ketone - acetone - water (186 : 52 : 20 v/v) as solvent systems. The antibiotics present in the fermentation were detected by bioautography on *Sarcina lutea* seeded agar.

Antibiotic production was measured by a microbiological disc-plate assay procedure[5] using *Sarcina lutea* as the testing organism.

Fig. 1.



* Taxonomic studies on *S. caelestis* strain 22227a were conducted by Miss ALMA DIETZ of the Upjohn Company.

Production of *Streptomyces caelestis* strain 22227 a

The mutagenic conditions and techniques employed to isolate *S. caelestis* strain 22227 a were in general those described by COATS and ROESER[6] with the only exception that 3 mg/ml of N-methyl-N'-nitro-N-nitrosoguanidine in 0.5 M tris-(hydroxymethyl) aminomethane tris-maleic acid buffer (pH 9.0) was used. Auxotrophic mutants were isolated and tested for antibiotic production.

Fermentation Procedures

Seed cultures of *S. caelestis* strain 22227 a were prepared in a medium consisting of glucose monohydrate, 25 g/liter and Pharmamedia, 25 g/liter (Trader's Oil Mill Co., Fort Worth, Texas, U.S.A.); seed medium presterilization pH 7.2. The cultures were incubated at 28°C for 72 hours on a rotary shaker. Fermentation medium consisting of corn starch 30 g/liter; glucose monohydrate, 10 g/liter; cornsteep liquor, 10 g/liter; ammonium sulfate, 3 g/liter; and sodium chloride 2 g/liter was adjusted to pH 7.2, mixed with calcium carbonate (5 g/liter), and inoculated at a rate of 5 per cent (v/v) with the 72-hour seed culture. Fermentations were incubated at 28°C on a rotary shaker and beers were harvested after total fermentation time of 72~96 hours.

Isolation of 7-O-Demethylcelesticetin (II)

1. Filtration and adsorption on amberlite XAD-2: Fermentation broth (*ca.* 12.5 liters) was filtered with the aid of diatomaceous earth. The filter cake was washed with 2 liters of water and the aqueous wash was combined with the clear filtrate. This solution was passed over a column, prepared from 500 ml of Amberlite XAD-2 (Rohm and Hass Co., Philadelphia, Pa., U.S.A.) packed in water. The spent beer was found bio-inactive and was discarded. The column was washed with water and then eluted with 3 liters of methanol - water (95 : 5 v/v). The methanolic eluate was concentrated to dryness to give 3.3 g of material containing essentially all of the bioactivity present in the fermentation broth. This preparation was found to contain 7-O-demethylcelesticetin by thin-layer chromatography on silica gel G using the solvent systems described earlier.

2. Silica gel chromatography. Isolation of 7-O-demethylcelesticetin hydrochloride: A column was prepared from 600 g of silica gel (Merck-Darmstadt, 7734) packed in the solvent system consisting of chloroform-methanol (6 : 1 v/v). Crude 7-O-demethylcelesticetin (*ca* 3.0 g), obtained by the procedure described above, was dissolved in 100 ml of methanol and 100 ml of the solvent system. This solution was mixed with 50 g of silica gel and the mixture was concentrated to dryness. The obtained powder was added on the top of the column and the column was then eluted with the chloroform - methanol solvent system. Fractions (20 ml each) were analyzed for bioactivity and by uv spectra and thin-layer chromatography. Fractions 98~149 containing 7-O-demethylcelesticetin were combined and the solution was concentrated to dryness. The residue was dissolved in a mixture of 10 ml of methanol and 5 ml of 1 N methanolic hydrogen chloride, and this solution was mixed with 500 ml of ether. The precipitated colorless 7-O-demethylcelesticetin hydrochloride was isolated by filtration and dried; yield 330 mg.

Anal. Calcd for $C_{23}H_{34}N_2O_9S \cdot HCl$: C 50.22, H 6.41, N 5.09, Cl 6.45, S 5.83.
Calcd for $C_{23}H_{34}N_2O_9S \cdot HCl \cdot H_2O$: C 48.63, H 6.57, N 4.93, Cl 6.24, S 5.68.
Found: C 48.52, H 6.29, N 5.03, Cl 6.52, S 5.77.

Thin-layer chromatographic behavior of the isolated material is presented in Figs. 2 and 3. The ir and nmr spectra are presented in Figs. 4 and 5, respectively.

Base Hydrolysis of 7-O-Demethylcelesticetin. Isolation of 7-O-Demethyldesalicetin (IIa).

The procedure described by HOEKSEMA and HINMAN[2] for the degradation of celesticetin was used. Salicylic acid (300 mg), obtained by degradation of 1.5 g of 7-O-demethylcelesticetin hydrochloride, was identified by ir, uv and nmr spectra. 7-O-Demethyldesalicetin hydrochloride (930 mg) was purified by countercurrent distribution (1,000 transfers, K=0.07) using 1-butanol - water (1 : 1 v/v) as the solvent system. The nmr spectrum of 7-O-

demethyldesalicetin is presented in Fig. 8. The mass spectrum showed mol. ion at 394 (Calcd for free base $C_{16}H_{30}N_2O_7S$, 394). The compound forms a trimethylsilyl (TMS) derivative containing five TMS groups (M^+, 754); Calcd for $C_{16}H_{30}N_2O_7S \cdot 5\text{-TMS}$, 754. The ir spectrum showed strong absorption bands at 3500~3300, 1675, 1565, 1350 (broad), 1260, 1180, 1140, 1120, 1005, 900, 860 and 800 cm^{-1}.

Hydrazinolysis of 7-O-Demethylcelesticetin. Isolation of 2'-Hydroxyethyl Thiolincosaminide (VII)

The procedure described by ARGOUDELIS *et al.*[7] was used. 7-O-Demethylcelesticetin hydrochloride (2.0 g) was dissolved in 50 ml of hydrazine hydrate. The solution was kept at reflux for 24 hours. It was then concentrated to dryness *in vacuo*. The residue was purified by countercurrent distribution using 1-butanol - water (1 : 1) as the solvent system. The distribution was analyzed after 500 transfers, by thin-layer chromatography and uv spectra. Tubes 10~40 were combined and the solution was concentrated to dryness to give 800 mg of material which was characterized as 2'-hydroxyethyl thiolincosaminide. Tubes 80~135 contained hygric acid hydrazide and tubes 400~430 were found to contain salicylic acid hydrazide. Hygric acid and salicylic acid hydrazides were characterized by ir, nmr, uv and mass spectra. The nmr spectrum of 2'-hydroxyethyl thiolincosaminide is presented in Fig. 12. The mass spectrum of this material, presented in Fig. 9, shows mol. ion at 283 mass units; Calcd for $C_{10}H_{21}N_6OS$, 283.

Celesticetin (I) Hydrochloride

Celesticetin salicylate[1] was dissolved in 1 N methanolic hydrogen chloride. Addition of ether to the methanolic solution resulted in the formation of celesticetin hydrochloride which was isolated by filtration and dried. Analytical and mass spectral data (M^+, 528) were consistent with the structure of celesticetin hydrochloride. The nmr spectrum of celesticetin hydrochloride is presented in Fig. 5.

Desalicetin (Ia) Hydrochloride

This material was isolated by alkaline degradation of celesticetin as described by HOEKSEMA *et al.*[1] Desalicetin was transformed to the hydrochloride salt which was then purified by countercurrent distribution using 1-butanol - water (1 : 1 v/v). The obtained material, $C_{17}H_{32}N_2O_7S \cdot HCl$ (Calcd mol. weight 408), showed mol. ion peak at 408 and other major fragments at 390, 349, 331, 229 and 84 mass units. The ir spectrum showed absorption bands at 3500~3300, 1672, 1562, 1350 (broad), 1258, 1170, 1140, 1125, 1005, 900, 860 and 805 cm^{-1}.

2'-Hydroxyethyl Thiocelestosaminide (IX) Hydrochloride

The procedure described by ARGOUDELIS *et al.*[7] was used for the hydrolysis of celesticetin salicylate. The obtained 2'-hydroxyethyl thiocelestosaminide was dissolved in methanolic hydrogen chloride (pH *ca* 2.0) and this solution was mixed with ether. The precipitated hydrochloride salt was isolated by filtration and dried. The nmr spectrum of 2'-hydroxyethyl thiocelestosaminide hydrochloride is presented in Fig. 12. Analytical and mass spectral data were consistent with the structure of this material.

Table 1. Molecular rotations of the thioglycosides

Compound	$[M]_D$	$[\alpha]_D^{25}$
Methyl thiolincosaminide·HCl	+551°	+218°
2'-Hydroxyethyl thiolincosaminide·HCl	+557°	+197°

The specific rotations were determined in water.

Methyl Thiolincosaminide (X) Hydrochloride

Methyl thiolincosaminide was obtained by hydrazinolysis of lincomycin[7]. The isolated methyl thiolincosaminide was transformed to its hydrochloride salt by precipitation from a methanolic hydrogen chloride solution with ether.

Discussion and Results

Antibiotics Produced by *Streptomyces caelestis* Strain 22227 a

The major activity produced by *S. caelestis* is celesticetin[1]. Recent studies have

shown the presence of several antibacterial agents in cultures of this organism. In addition to celesticetin and desalicetin, celesticetin B (desalicetin 2'-isobutyrate), celesticetin C (desalicetin 2'-anthranilate) and celesticetin D (desalicetin 2'-acetate) have been isolated from fermentations of *S. caelestis**[4].

Antibiotic production by *S. caelestis* strain 22227 a was followed by thin-layer chromatography. Two bioactivities, one major (Rf, 0.65), and one minor** (Rf, 0.28) were detected in culture filtrates when silica gel and ethyl methyl ketone - acetone - water (186 : 52 : 20) were used. The major bioactivity could not be differentiated from celesticetin in the above tlc system. However, this material separated from celesticetin when chloroform-methanol (6 : 1 v/v) was used as the solvent system. The two antibiotics had Rf values of 0.40 and 0.70 respectively. It should be noted that celesticetin has not been detected in culture filtrates of *S. caelestis* strain 22227 a.

Isolation and Characterization of 7-O-Demethylcelesticetin

The major bioactivity produced by strain 22227 a was extracted from the clear broth by adsorption on Amberlite XAD-2 followed by elution with aqueous methanol. Purification by silica gel chromatography afforded the pure antibiotic which was isolated as the colorless hydrochloride. Since strain 22227 a which produces the antibiotic under discussion is a mutant of *S. caelestis*, we suspected that this material was a member of the celesticetin family of antibiotics. As described in this section, this antibiotic was identified as being 7-O-demethylcelesticetin (II). Therefore, the latter designation will be used for the remainder of the paper.

The paper chromatographic pattern of 7-O-demethylcelesticetin in several systems is almost identical to that of celesticetin. The tlc mobility of this antibiotic relative to the mobilities of celesticetin, celesticetin B and desalicetin is presented in Figs. 2 and 3. Both tlc systems are necessary for differentiation of 7-O-demethylcelesticetin from the above-mentioned antibiotics produced by *S. caelestis*. 7-O-Demethylcelesticetin hydrochloride $[\alpha]_D^{25}+115°$ (*c* 0.85, water) is very soluble in water, soluble in methanol and ethanol and relatively insoluble in less polar organic solvents. Poten-

Fig. 2. Thin-layer chromatographic comparison of : 7-O-demethylcelesticetin (I), celesticetin (II, Rf 0.70), celesticetin B (II, Rf 0.60) and desalicetin (II, Rf 0.30)
Support : Silica Gel G
Solvent : Methyl ethyl ketone - acetone - water (186 : 52 : 20)
Bioactivities were detected by biautography on *Sarcina lutea* seeded agar.

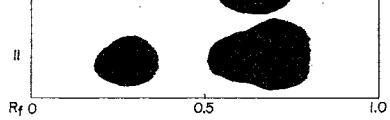
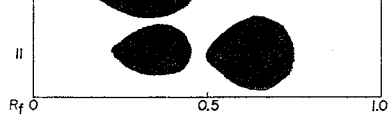


Fig. 3. Thin-layer chromatographic comparison of 7-O-demethylcelesticetin (I), celesticetin (II, Rf 0.70), celesticetin B (II, Rf 0.70) and desalicetin (II, Rf 0.40)
Support : Silica Gel G
Solvent : Chloroform-methanol (6 : 1)
Bioactivities were detected by biautography on *Sarcina lutea* seeded agar.



* Additional antibiotics produced by *S. caelestis* have been isolated. Work on these compounds will be reported when completed.

** The minor component has not been isolated pure. It appears to be 7-O-demethyldesalicetin (IIa) (*see* later discussion).

tiometric titration in water indicated the antibiotic to be amphoteric. A basic group, pKa′ 7.5, and a weak acidic group with pKa′ of 9.6 were found to be present in the molecule. The molecular weight found by titration of 7-O-demethylcelesticetin hydrochloride was 534 (calcd. 550). Analytical values, combined with potentiometric titration data, suggested the molecular formula $C_{23}H_{34}N_2O_9S \cdot HCl \cdot H_2O$ for 7-O-

Fig. 4. Infrared spectrum of 7-O-demethylcelesticetin hydrochloride (Nujol mull)

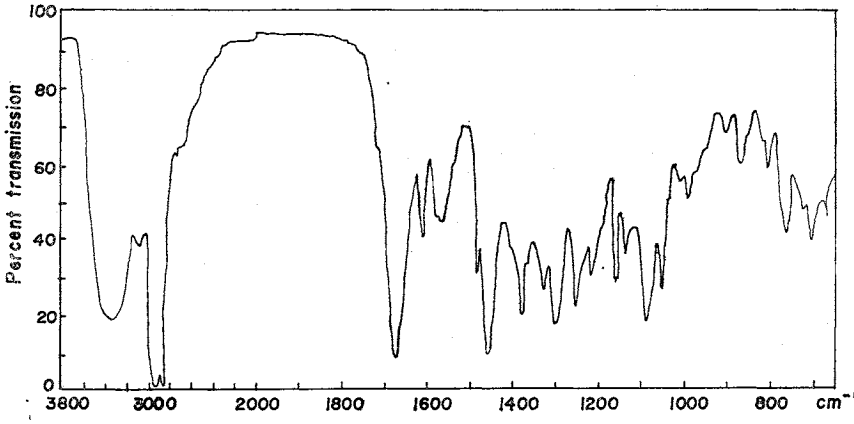
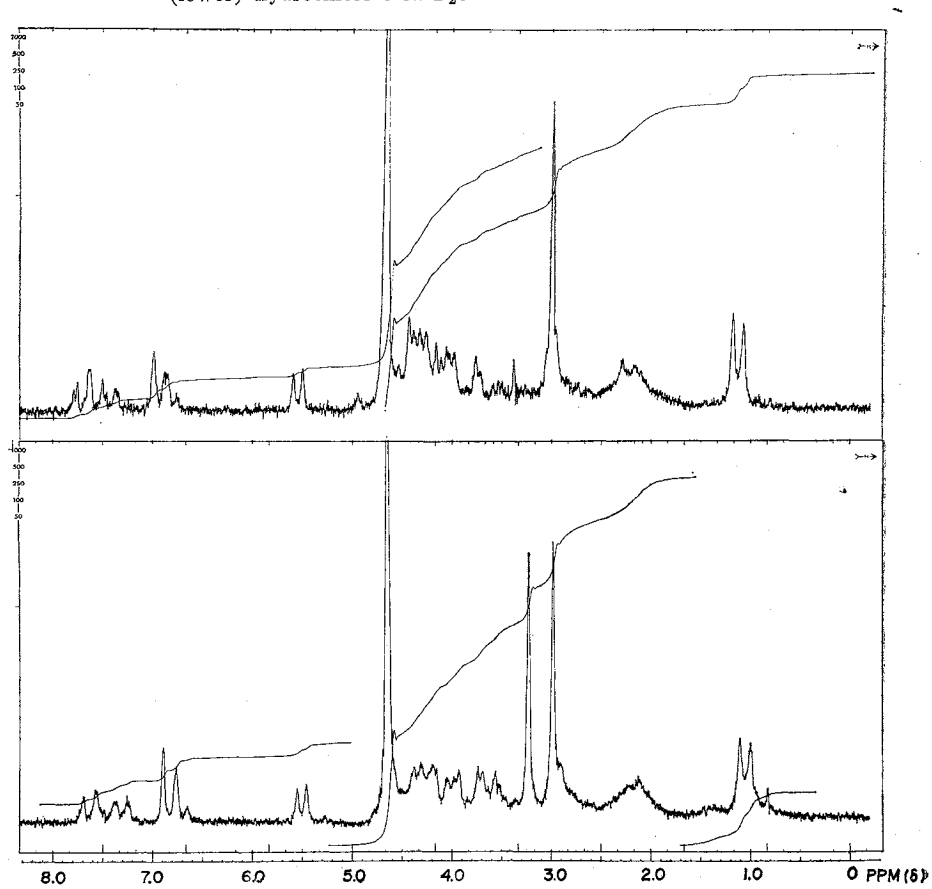
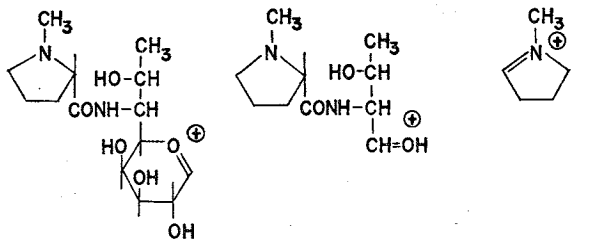


Fig. 5. NMR spectra of 7-O-demethylcelesticetin (upper) and celesticetin (lower) hydrochloride in D_2O



demethylcelesticetin hydrochloride. Mass spectral data agreed with the formula $C_{23}H_{34}N_2O_9S$ (mol. weight 514) for the free antibiotic. The infrared spectrum (Fig. 4) of 7-O-demethylcelesticetin hydrochloride is almost identical to the spectrum of celesticetin hydrochloride in all regions, and specifically in the carbonyl region (1670, 1578 cm^{-1}). The 1670 cm^{-1} peak is due to co-absorptions of amide carbonyl and a hydrogen-bonded ester grouping, while the 1578 cm^{-1} peak indicates the amide II group. In addition, absorption at 1608 cm^{-1} indicates the presence of aromatic C=C. The uv spectrum of 7-O-demethylcelesticetin hydrchloride in methanol showed maxima at 238 (a=18) and 304(a=7.3)nm. The maxima shifted to 242 (a=13.4) and 335 (a=8.7) nm in alkaline methanol, indicating the presence of the salicyloyl moiety in 7-O-demethylcelesticetin[1]. This conclusion is supported by the titration data discussed above (pKa′ of phenolic group, 9.6). The nmr spectra of 7-O-demethylcelesticetin and celesticetin hydrochlorides (Fig. 5) are very similar. The only distinct difference is that the sharp singlet at δ 3.35 (3 H) due to the $-OCH_3$ group of celesticetin is not present in the spectrum of 7-O-demethylcelesticetin. Otherwise both spectra show the presence of $-\overset{\overset{O}{\|}}{C}HCH_3$ [δ 1.15 (3 H, d)], $-SCH_2CH_2O\overset{\overset{O}{\|}}{C}-$ (δ, *ca* 2.8), hygric acid methylene hydrogens (δ 2.2), $-NCH_3$ [δ, 2.98 (3 H, s)], anomeric hydrogen [δ, 5.6, 1 H, d (J=6.0 c.p.s.)], and the aromatic absorption at δ, 6.8 to 7.8 (4 H) due to the hydrogens of the salicyloyl moiety.

Fig. 6.



III, m/e 317 IV, m/e 215 V, m/e 84

Table 2. *In vitro* antibacterial spectrum* of 7-O-demethyl-celesticetin and celesticetin hydrochlorides

Test organism		Minimum inhibitory concentration (mcg/ml)	
		Celesticetin HCl	7-O-Demethyl-celesticetin HCl
S. aureus	UC 80	2.0	2.0
St. hemolyticus	UC 152	0.5±0.25	1.0±0.5
St. faecalis	UC 3235	4.0	4.0
E. coli	UC 51	>500	>500
P. vulgaris	UC 93	>500	>500
K. pneumoniae	UC 57	62.5	62.5
Ps. aeruginosa	UC 95	>500	>500
D. pneumoniae	UC 41	0.5	1.0

* Test method : Two fold dilution endpoints in brain-heart infusion broth ; incubate at 37℃ for 20 hours.

The mass spectrum of 7-O-demethylcelesticetin showed the molecular ion peak at *m/e* 514 (calcd. for $C_{23}H_{34}N_2O_9S$, 514) and a peak at 496 (M^+-H_2O) mass units. Other major ion peaks were observed at 317, assigned to fragment III, at 299 (due to fragment III-H_2O), and at 215 and 84 mass units, assigned to fragments IV and V respectively (Fig. 6).

The ion peak at *m/e* 84 is the most intense peak in the spectrum of all celestosaminide antibiotics[4]. In addition the mass spectrum of 7-O-demethylcelesticetin showed peaks at 138 and 120 mass units. These ion peaks are also present in the spectrum of celesticetin and are due to the salicylate part of the molecule.

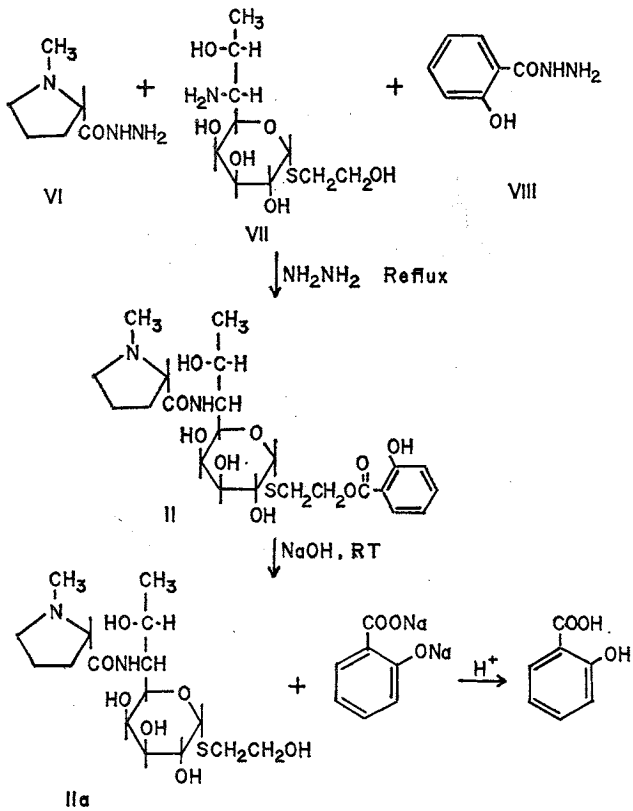
The physical and spectral data discussed support structure II (exclusive of stereochemistry) as the structure for the major activity produced by *S. caelestis* strain

22227 a. 7-O-Demethylcelesticetin has antibacterial activity comparable to that of celesticetin (Table 2). This fact coupled with biogenetic considerations and experience of the effect of chemical alterations on activities of the lincosaminide* antibiotics, requires stereochemistry at C-1, C-2, C-3 and C-4 as shown in II. The α-anomeric linkage of the thioglycosidic group is supported by the observed coupling constant of the doublet at δ, 5.5 which is due to the anomeric hydrogen.

Degradation of 7-O-Demethylcelesticetin

Degradative studies also agree with assignment of structure II for the main antibiotic produced by *S. caelestis* strain 22227 a. As shown in Fig. 7, treatment of 7-O-demethylcelesticetin with 1 N aqueous sodium hydroxide at room temperature for 20 hours yielded, after acidification, IIa and salicylic acid. IIa, isolated as the amorphous hydrochloride, was purified by countercurrent distribution using 1-butanol - water (1 : 1 v/v) as the solvent system. The highly purified material, $[\alpha]_D^{25}+134°$ (c 1, water) was easily characterized as 7-O-demethyldesalicetin hydrochloride. Specifically the ir of this material was identical to that of desalicetin (Ia) hydrochloride with amide I and II carbonyl bands at 1675 and 1565 cm^{-1}. 7-O-Demethyldesalicetin did not show uv absorption indicating the absence of the salicyloyl moiety present in 7-O-demethylcelesticetin. The nmr spectrum (D_2O) (Fig. 8) showed the presence of $-\underset{\underset{O}{|}}{C}HCH_3$ [δ, 1.2 (3 H, d)], $-NCH_3$ [δ, 2.98, (3 H, s)] and anomeric hydrogen [δ, 5.54 (1 H, d)]. The sharp singlet at δ, 3.4 (3 H) in the spectrum of desalicetin (assigned to the $-OCH_3$ group) is not present in the spectrum of 7-O-demethyldesalicetin in agreement with structure IIa. Finally the mass spectrum of 7-O-demethyldesalicetin showed the molecular ion peak at m/e 394 (calcd for 7-O-demethyldesalicetin free base, 394) and a peak at 376 (M^+-H_2O) mass units. Other important peaks were observed at 317, 215 and 84 mass units and are assigned to fragments III, IV and V (Fig. 6) respectively.

Fig. 7.



7-O-Demethyldesalicetin

* Dr. B. BANNISTER of The Upjohn Company has shown[8] that alterations of the stereochemistry at C-2, C-3, or C-4 of the aminosugar present in lincomycin[9] result in complete loss of activity. This aminosugar has identical stereochemistry with the aminosugar present in celesticetin (*see* further discussion and Reference 2).

Fig. 8. NMR spectrum of 7-O-demethyldesalicetin hydrochloride in D_2O

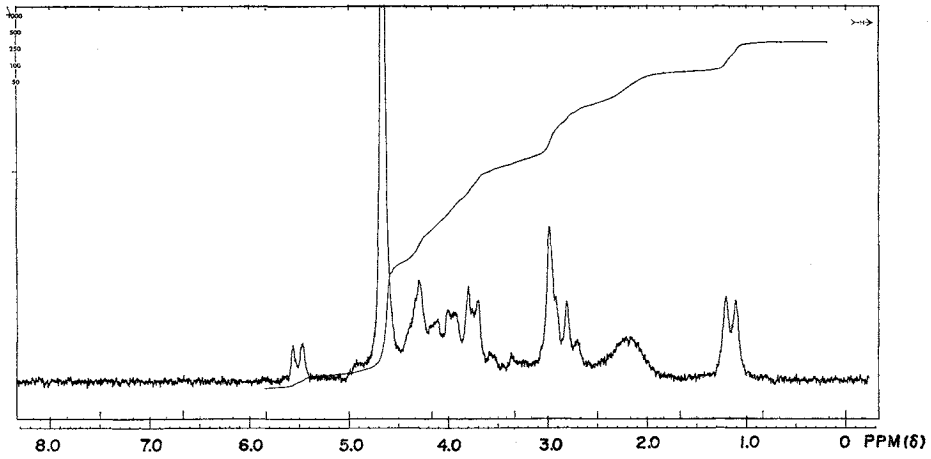
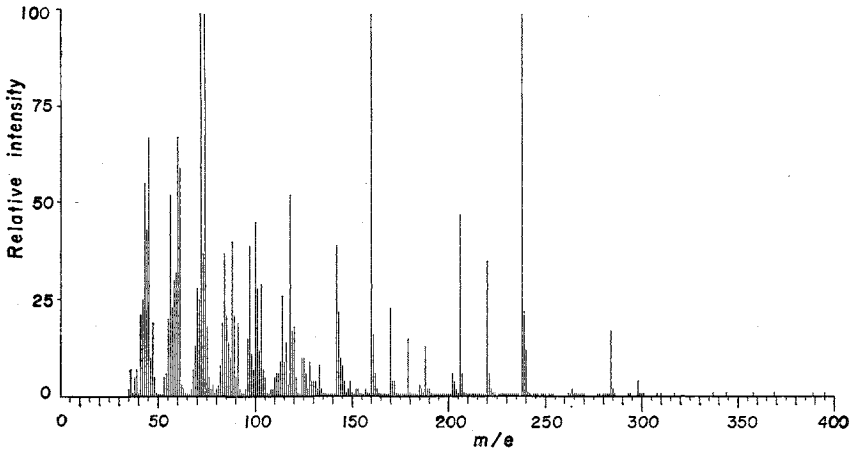


Fig. 9. Mass spectrum of 2'-hydroxyethyl thiolincosaminide



could not be separated by tlc from the minor component produced by *S. caelestis* strain 22227 a. It is assumed, therefore, that this organism produces both 7-O-demethylcelesticetin and 7-O-demethyldesalicetin. However, the degradation of the former antibiotic to the latter under the fermentation conditions cannot be excluded.

Hydrazinolysis of 7-O-demethylcelesticetin hydrochloride (Fig. 7) followed by countercurrent distribution of the reaction mixture yielded the hydrazides of hygric acid (**VI**) and salicylic acid (**VIII**). In addition, the aminosugar moiety of 7-O-demethylcelesticetin was isolated as the hydrochloride salt. The mass spectrum (Fig. 9) showed molecular ion peak at *m/e* 283 (Calcd. for $C_{10}H_{21}NO_6S$, 283). The fragmentation pattern is identical to that of methyl thiolincosaminide* (**X**, Fig. 10), the aminosugar present in lincomycin[8].

The major fragments observed are presented in Fig. 11. The molecular ion gives ion fragments **XI** (*m/e* 206) and **XII** (*m/e* 170) by loss of $HSCH_2CH_2OH$ followed

* The fragmentation patterns of both lincomycin and methyl thiolincosaminide have been elucidated by Drs. FRED KAGAN and MARVIN F. GROSTIC of The Upjohn Company. This work has been submitted for publication.

Fig. 11.

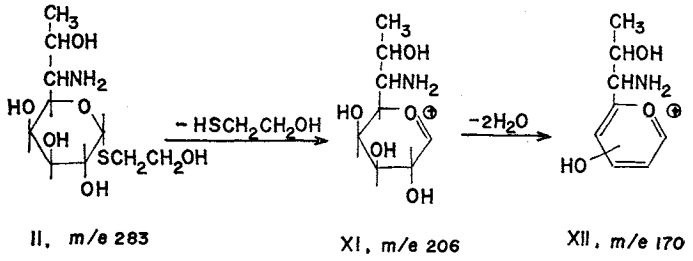
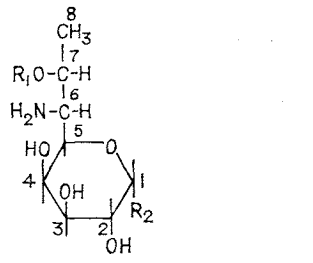


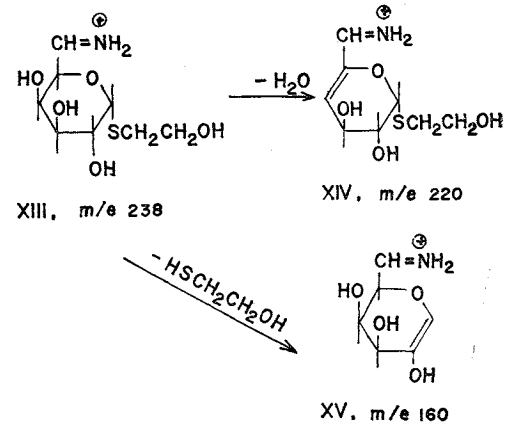
Fig. 10.



VII : $R_1 = H$; $R_2 = -SCH_2CH_2OH$

IX : $R_1 = OCH_3$; $R_2 = -SCH_2CH_2OH$

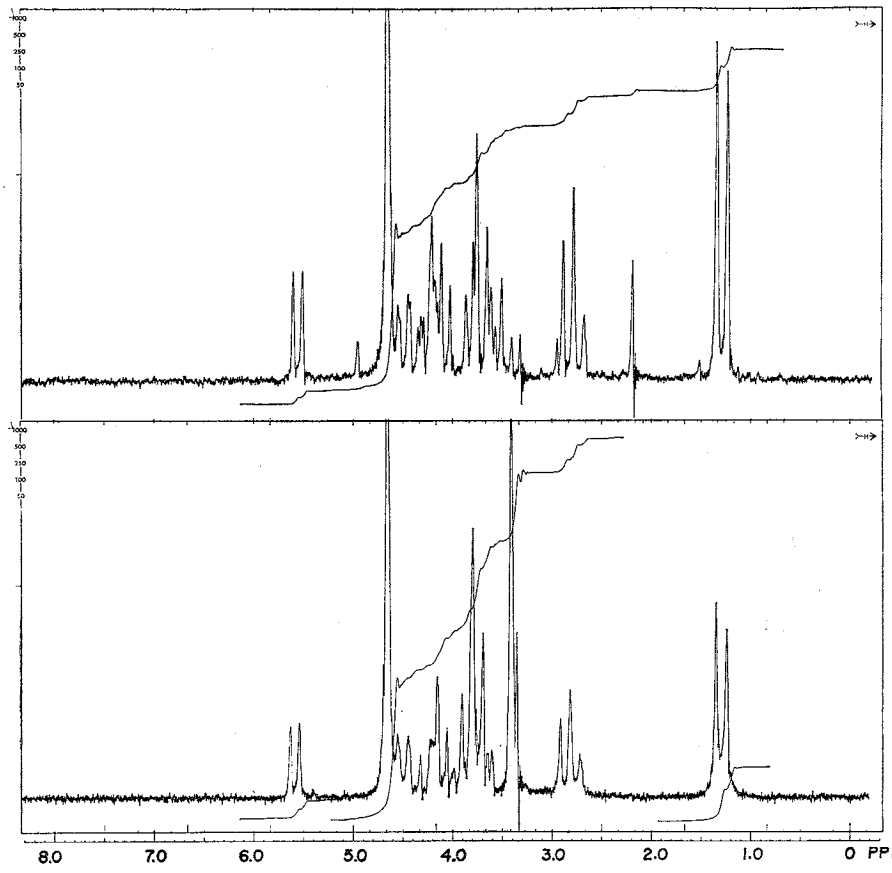
X : $R_1 = H$; $R_2 = -SCH_3$



by loss of H_2O. An alternative cleavage of the mol. ion gives ion fragment **XIII** (*m/e* 238), the base peak. Loss of water from **XIII** affords **XIV** (*m/e* 220) while loss of $HSCH_2CH_2OH$ gives **XV** (*m/e* 160). In addition to these ion peaks, the mass spectrum contains significant peaks at 74, 100 and 118 mass units. All three ion peaks are also present in the spectrum of methyl thiolincosaminide. The nmr spectra of 2′-hydroxyethyl thiolincosaminide (**VII**) and of 2′-hydroxyethyl thiocelestosaminide (**IX**) hydrochlorides (Fig. 12) differ only in that the sharp singlet at δ, 3.4 (3 H) of the spectrum of 2′-hydroxyethyl thiocelestosaminide (**IX**) (assigned to the $-OCH_3$ group) is not present in the spectrum of 2′-hydroxyethyl thiolincosaminide. Otherwise both spectra indicated the presence of $-\underset{\underset{O}{|}}{C}HCH_3$ [δ, 1.1 (3 H, d)], $-SCH_2CH_2O-$ [δ, 2.9 (4 H)] and anomeric hydrogen [δ, 5.57 (1 H, d)].

The mass spectral data clearly indicated the identity of the basic structures of methyl thiolincosaminide (**X**) and 2′-hydroxyethyl thiolincosaminide (**VII**), the amino-sugar moiety of 7-O-demethylcelesticetin. On the other hand, the nmr spectra established identity of the basic structures of **VII** and 2′-hydroxyethyl thiocelestosaminide (**IX**). Those finding are in agreement with HOEKSEMA's studies[3] which established the identity of the stereochemistry of the amino-octoses present in celesticetin and lincomycin. It is assumed that 2′-hydroxyethyl thiolincosaminide (**VII**) has the stereochemistry of methyl thiolincosaminide and 2′-hydroxyethyl thiocelestosaminide. This assumption which appears reasonable on the basis of biosynthetic considerations is supported by molecular rotation measurements.

Fig. 12. NMR spectra of 2′-hydroxyethyl thiolincosaminide (upper) and 2′-hydroxyethyl thiocelestosaminide hydrochlorides in D_2O



As shown in Table 1, the two related aminosugar thioglycosides have identical molecular rotations indicating identical stereochemistry in their asymmetric centers. Furthermore, the assignment of an α-thioglycosidic group is supported by the observed coupling constant (J=6.0 cps) of the doublet (1 H) at *ca* δ, 5.57 which is due to the anomeric hydrogen of 2′-hydroxyethyl thiolincosaminide.

The discussion regarding the chemical nature of 7-O-demethylcelesticetin and its degradation products indicates that *S. caelestis* strain 22227 a has the ability to biosynthesize lincosamine (Fig. 10, R_1=H ; R=OH), the aminosugar moiety of lincomycin, *S. caelestis* strain 22227 a and *S. lincolnensis*, therefore, have the ability to form thioglycosides **VII** and **X** and couple them to hygric and propylhygric acids yielding the corresponding antibiotics **IIa** and lincomycin respectively.

Bioactivity of 7-O-Demethylcelesticetin

The *in vitro* antibacterial spectra of 7-O-demethylcelesticetin and celesticetin hydrochloride are presented in Table 2. Both antibiotics have the same antibacterial spectrum and are equally active against the organisms tested. Similarly, 7-O-demethyldesalicetin is as active as desalicetin *in vitro*. *In vivo* evaluation of these antibiotics is incomplete.

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