**Communication to the editor**

# STUDIES WITH *STREPTOMYCES CAELESTIS.* III. NEW ANTIBIOTICS CONTAINING LINCOSAMINE OR CELESTOSAMINE

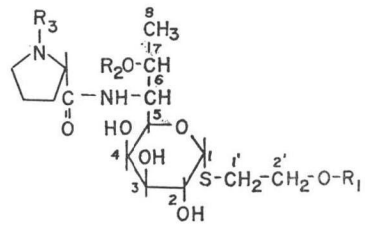
Sir:

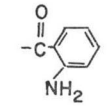
In a previous communication[1] in this series we described a method for the separation and characterization of celestosaminide (celesticetin-related) antibiotics using gas chromatography—mass spectroscopy (GC-MS). This method was applied to the analysis of a mixture of unknown celestosaminides and resulted in the characterization of four new antibiotics (**I** to **IV**, Fig. 1). The present communication describes details of this study.

The bioactive materials produced by *Streptomyces caelestis* were extracted from the clear filtrate with methylene chloride at alkaline pH (*ca* 8.0). Celesticetin[2] was removed by crystallization as the salicylate salt and the mother liquors were concentrated to an oily residue. Examination of this crude material by tlc* revealed the presence of several compounds which inhibited the growth of *Sarcina lutea*. Distribution of the oily residue between 1-butanol - water (1:1 v/v, pH 2.0 adjusted with aqueous HCl), followed by concentration of the aqueous phase to dryness, yielded material containing several antibiotics. Countercurrent distribution [l-butanol-water (1:1 v/v)] separated celesticetin[2,3], celesticetin B[4] and desalicetin[4] from a mixture of celesticetin C[4] and several unknown antibiotics.

Silica gel chromatography of the mixture using chloroform-methanol (6:1 v/v) separated celesticetin C from a mixture of closely related antibacterial agents designated preparation A. The carbonyl region in the IR spectra of prep A indicated the presence of amide and ester

Fig. 1.



| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| Desalicetin | H | $CH_3$ | $CH_3$ |
| 7-O-Demethyldesalicetin | H | H | $CH_3$ |
| I | $-C(=O)-CH_2CH_2CH_3$ | $CH_3$ | $CH_3$ |
| II | $-C(=O)-C_3H_7$ | H | $CH_3$ |
| III | $-C(=O)-C_4H_9$ | $CH_3$ | $CH_3$ |
| IV |  | H | $CH_3$ |

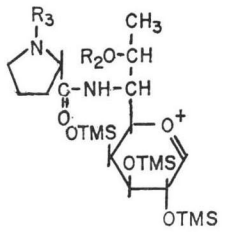
* Silica gel G; solvent I, methyl ethyl ketone-acetone-water (186:52:20); solvent II, 2-pentanone-methyl ethyl ketone-methanol-water (2 : 2 : 1 : 1); solvent III, chloroform-methanol (6 : 1). Bioactive compounds were detected by bioautography on *Sarcina lutea*-seeded trays.

Table 1

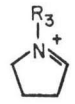
| Compound | $R_1$* | $R_2$* | $R_3$* | TMS Groups | $M^+$ | *m/e* (VI) | *m/e* (VII) | $T_R$(UCW 98)** min. 290°C |
|---|---|---|---|---|---|---|---|---|
| I | $-C(=O)CH_2CH_2CH_3$ | $CH_3$ | $CH_3$ | 3 | 694 | 547 | 84 | 1.60 |
| II | $-C(=O)C_3H_7$ | H | $CH_3$ | 4 | 752 | 605 | 84 | 1.72 |
| III | $-C(=O)C_4H_9$ | $CH_3$ | $CH_3$ | 3 | 708 | 547 | 84 | 2.56 |
| IV |  | H | $CH_3$ | 5 | 872 | 605 | 84 | 8.80 |

* $R_1$, $R_2$, $R_3$ refer to the groups present in the basic celestosaminide structure shown in Fig. 1.
** For experimental details on GC or GC-MS *see* Ref. 1.

Fig. 2



VIa $R_2 = CH_3$ ; $R_3 = CH_3$
VIb $R_2$ = TMS; $R_3 = CH_3$



VII $R_3 = CH_3$

bonds, identical to the absorptions of celestosaminide antibiotics. The NMR spectrum of prep A showed the presence of $-NCH_3$ (2.98; s), $-OCH_3$ ($\delta$, 3.4; s), hygric acid moiety ($\delta$, 2.2; m), $-SCH_2CH_2\overset{O}{\overset{\|}{C}}-$ ($\delta$, 2.8; m), anomeric hydrogen ($\delta$, 5.54; d) and aromatic hydrogens ($\delta$, 6.5 to 8.9; m). These structural features are present in most celestosaminide antibiotics[4].

The antibiotics present in prep A were transformed to their trimethylsilyl (TMS) ether

Table 2

| Compounds | $R_1$* | $R_2$* | $R_3$* | TMS Group | $M^+$ | *m/e* (VI) | *m/e* (VII) | $T_R$(UCW 98)** min. 290°C | $T_R$(UCW 98)** min. 280°C |
|---|---|---|---|---|---|---|---|---|---|
| Celesticetin |  | $CH_3$ | $CH_3$ | 4 | 816 | 547 | 84 | 8.72 | |
| Celesticetin B |  | $CH_3$ | $CH_3$ | 3 | 694 | 547 | 84 | 2.16 | |
| Celesticetin C |  | $CH_3$ | $CH_3$ | 4 | 815 | 547 | 84 | 10.96 | |
| 7-O-Demethyl celesticetin |  | H | $CH_3$ | 5 | 874 | 605 | 84 | — | 16.7 |
| N-Demethyl-7-O-demethylcelesticetin |  | H | H | 5 | 860 | 591 | 70 | — | 18.2 |
| Desalicetin | H | $CH_3$ | $CH_3$ | 4 | 696 | 547 | 84 | — | 2.6 |

* $R_1$, $R_2$, $R_3$ refer to the groups present in the basic celestosaminide structure shown in Fig. 1.
** For experimental details on GC or GC-MS *see* Ref. 1.

derivatives[1] and this mixture was analyzed by GC-MS as described by BRODASKY and ARGOUDELIS[1]. Results are presented in Table 1. Four compounds were separated and designated **I** to **IV** in order of increasing retention time ($T_R$). TMS-derivatives of known celestosaminides were run as controls and the results obtained with these compounds are listed in Table 2.

As shown previously[1] (see also Tables 1 and 2) celestosaminides in which $R_1$ (Fig. 1) is an aromatic ester display high retention times while the presence of aliphatic esters or the absence of an ester group at this position results in low retention times. Based on this correlation three of the four celestosaminide antibiotics present in preparation A, namely **I, II,** and **III** were considered to be aliphatic esters and **IV** an aromatic (celesticetin-like) ester. These conclusions were confirmed by examination of the structure of these antibiotics by GC-MS.

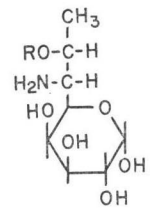
The mass spectra of TMS-derivatives of celestosaminide antibiotics are characterized by the presence of three major *m/e* peaks sufficient to identify the individual compounds. The high mass value (Tables 1 and 2) is the molecular ion and reflects the number of TMS groups in the derivative. The second value represents the silylated portion of the molecule (**VI**, Fig. 2) remaining after loss of the $-SCH_2 \cdot CH_2OR_1$ group (see Fig. 1). The low mass peak is due to fragment **VII** (Fig. 2) resulting from the hygric acid moiety of the celestosaminide molecule.

The mass spectra of the TMS-derivatives of **I, II, III** and **IV** contained the low mass peak at *m/e*, 84 (fragment **VII**, Fig. 2) indicating the presence of hygric acid in the molecules of all four antibiotics.

The mass spectra of the TMS-derivatives of **I** and **III** (3TMS) contained peaks at *m/e* 547 (fragment **VIa**, Fig. 2) similar to the mass spectra of the TMS-derivatives of celesticetin, celesticetins B, C and desalicetin (Table 2). This indicates that **I** and **III** are derivatives of celestosamine (Fig. 3), the aminosugar produced by *S. caelestis*.

The mass spectrum of the TMS-derivative of **I**, including the molecular ion peak, is identical to that of celesticetin B. **I** and celesticetin B are easily separated by GC (Tables 1 and 2) indicating that these antibiotics are esters of isomeric acids at C-2′ of desalicetin. Since celesticetin B is desalicetin 2′-isobutyrate, we conclude that **I** is desalicetin-2′-butyrate (Fig. 1).

Fig. 3



Celestosamine, R = $CH_3$
Lincosamine, R = H

The mass spectra data indicate that **III** differs from the known celestosaminides in the $-SCH_2 \cdot CH_2OR_1$ part of the molecule. Specifically the molecular ion peak (708) suggests that $R_1 = \overset{O}{\overset{\|}{C}}-C_4H_9$ (Fig. 1). No data, available at present, indicate which of the possible pentanoic acids is present in **III**.

The TMS-derivatives of **II** and **IV** contained 4 and 5 TMS groups, respectively. The mass spectra of these compounds are characterized by the presence of a peak at *m/e* 605 assigned to fragment **VIb** (Fig. 2). In this respect, **I** and **IV** behave like 7-O-demethyl celesticetin[5] (Table 2). This indicates that **II** and **IV** are derivatives of lincosamine (Fig.3), the aminosugar produced by *Streptomyces lincolnensis*[6] and by mutants of *S. caelestis*[5,7].

Mass spectral data ($M^+$, 752) combined with the low retention time of the TMS derivative of II indicate that it is a butyrate or isobutyrate ester at C-2′ of 7-O-demethyldesalicetin (Fig. 1). Information available at present cannot distinguish between the two possible isomers.

The high retention time of the TMS-derivative of **IV** suggested an aromatic ester (like celesticetin or celesticetin C) at C-2′ of 7-O-demethyldesalicetin. The molecular ion peak at 873 suggested the structure of 7-O-demethyldesalicetin 2′-anthranilate (Fig. 1) for **IV**. This is supported by the UV and mass spectra of preparation A. Of the four antibiotics present in preparation A, **I, II** and **III**, like celesticetin B[4], are expected to show end absorption only. The UV spectrum of prep

A was identical to that of celesticetin C[4] indicating the presence of the same chromophoric system (anthranilate ester) in both **IV** and celesticetin C. Furthermore, aromatic hydrogen absorption in the nmr spectrum of preparation A is due to **IV**. The absorption pattern of the aromatic hydrogens is identical to that of celesticetin C[4] and ethyl anthranilate[8] in agreement with the postulated structure for **IV** (Fig. 1).

The production of several celestosamine or lincosamine-containing antibiotics (esters at C-2′ of desalicetin or 7-O-demethyldesalicetin) by *S. caelestis* suggests that the enzymes responsible for the ester bond have low substrate specificity. Furthermore, this work as well as the previous studies[2,4,5,6] on antibiotic production by *S. caelestis* and its mutants indicated the feasibility of directing the production of new celestosaminides by incorporation of appropriate acid precursors in the culture medium. This work will be reported in a subsequent communication. It is important to note the production of the two lincosamine-containing antibiotics, **II** and **IV**, by *S. caelestis*. The significance of this observation in regard to the biosynthesis of celestosaminides and specifically the sequence of methylation at the C-7 hydroxyl group is not known at this time.

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## References

1) BRODASKY, T. F. & A. D. ARGOUDELIS: Antibiotics produced by *Streptomyces caelestis*. II. Separation and characterization of celestosaminide antibiotics by gas chromatography-mass spectroscopy. J. Antibiotics 26:131～134, 1973
2) HOEKSEMA, H.; G.F. CRUM & W.H. DEVRIES: Isolation and purification of celesticetin. Antibiot. Ann. 1954/1955:837～841, 1955
3) HOEKSEMA, H.: Celesticetin. V. The structure of celesticetin. J. Am. Chem. Soc. 90:755～757, 1968
4) ARGOUDELIS, A.D. & T.F. BRODASKY: Studies with *Streptomyces caelestis*. I. New celesticetins. J. Antibiotics 25:194～196, 1972
5) ARGOUDELIS, A.D.; J.H. COATS, P.G. LEMAUX & O.K. SEBEK: Antibiotics produced by mutants of *Streptomyces caelestis*. I. 7-O-Demethylcelesticetin acid and its degradation products. J. Antibiotics 25:445～455, 1972
6) HOEKSEMA, H.; B. BANNISTER, R. D. BIRKENMEYER, F. KAGAN, B. J. MAGERLEIN, F. A. MACKELLAR, W. SCHROEDER, G. SLOMP & R.R. HERR: Chemical studies in lincomycin. I. The structure of lincomycin. J. Am. Chem. Soc. 86:4223～4224, 1964
7) ARGOUDELIS, A.D.; J.H. COATS, P.G. LEMAUX & O. K. SEBEK: Antibiotics produced by mutants of *Streptomyces caelestis*. II. N-Demethylcelesticetin and N-demethyl-7-O-demethylcelesticetin. J. Antibiotics 26:7～14, 1973
8) Sadtler Research Laboratories, Inc.: 1969 Catalog of NMR Spectra, No. 6540M.