ANTIBIOTICS PRODUCED BY *S. CAELESTIS*

II. SEPARATION AND CHARACTERIZATION OF CELESTOSAMINIDE ANTIBIOTICS BY GAS CHROMATOGRAPHY-MASS SPECTROSCOPY

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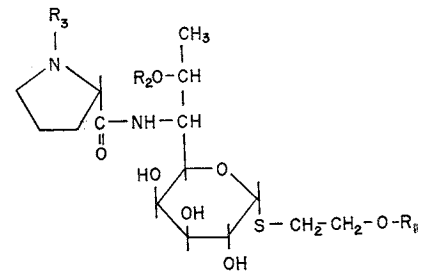
(Received for publication November 30, 1972)

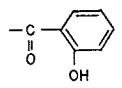
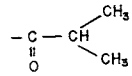
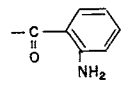
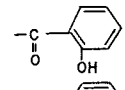
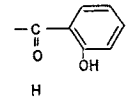
The streptomycete *S. caelestis* produces a number of antibiotics related to celesticetin (celestosaminides). GC of TMS-derivatized celestosaminides indicates the type of acid moiety present in the antibiotic (aromatic or alkyl) while GS-MS data lead to the molecular weight and other specific structural features. The six celesticetins described here provide data which can be applied to the characterization of new celestosaminides.

The isolation of celesticetin (I, Fig. 1) was reported by HOEKSEMA *et al.*[1] Continued investigations of the antibiotics produced by *Streptomyces caelestis* resulted in the isolation of desalicetin (VI, Fig. 1) and celesticetins B (II, Fig. 1) and C (III, Fig. 1).[2] Mutants of *S. caelestis* have also been reported to produce celesticetin related antibiotics. For example, strains 22227a and 22218a produce 7-O-demethylcelesticetin (IV, Fig. 1)[3] and N-demethyl-7-O-demethylcelesticetin (V, Fig. 1)[4] respectively.

Recent investigations revealed the presence of small amounts of other antibiotics in cultures of *S. caelestis* and its mutants which could not be adequately separated by conventional chromatographic (paper or tlc) procedures. The present communication describes the use of combined gas chromatography-mass spectroscopy for both the separation and characterization of the above celestosaminide antibiotics. Application of this technique resulted in the detection and characterization of several other celesticetin related antibiotics and these studies will be the subject of subsequent communications.

Fig. 1.



	R_1	R_2	R_3
I Celesticetin		CH_3	CH_3
II Celesticetin B		CH_3	CH_3
III Celesticetin C		CH_3	CH_3
IV 7-O-Demethyl-celesticetin		H	CH_3
V N-Demethyl-7-O-demethyl-celesticetin		H	H
VI Desalicetin	H	CH_3	H

Experimental

Materials: Celesticetin (I) was obtained by procedures described by HOEKSEMA *et al.*[1] and by ARGOUDELIS *et al.*[3] Celesticetin B (II), celesticetin C (III), and desalicetin (VI) were identical to materials described by ARGOUDELIS and BRODASKY.[2] 7-O-Demethylcelesticetin (IV) was obtained by fermentation of *S. caelestis* strain 22227a[3] and N-demethyl-7-O-demethylcelesticetin (V) was isolated from fermentations of *S. caelestis* strain 22218a[4].

Preparation of Trimethylsilyl Ethers: Approximately 5 mg of the compound under investigation was sealed in a 0.3 ml Reactivial (Pierce Chemical Company, Illinois) which was then evacuated (3 mm/Hg) through the septum using a hypodermic needle connected to the vacuum line. Either Supelco silylating reagent or Powersil (Dupont Company) both having the same composition (*bis*-trimethylsilylacetamide 3 parts; trimethylsilylimidazole 3 parts; and trimethylchlorosilane 2 parts) were used for derivatization. After evacuation of the sample in the Reactivial, 0.3 ml of the reagent was added by injection through the septum. The vial was heated 5~10 minutes at 70°C or untill the solute dissolved. These solutions of antibiotic derivative were stable for 1~2 weeks at −10°C.

Gas Chromatography: Conditions for GC-MS investigations were established using a Hewlett-Packard 402 Biomedical GC unit. A 4′ by $^1/_4$″ glass column packed with Hewlett-Packard prepared packing (3.8% UCW 98 on 80~100 mesh Diatoport S) operating isothermally at 280 or 290°C was monitored with a flame ionization detector. Sample volumes of 0.2~1 mcl were used.

Gas Chromatography-Mass Spectroscopy (GC-MS): The mass spectra were recorded on a LKB 9000 instrument using a column containing the same packing described above. Temperatures were adjusted to obtain similar retention times or resolution of peaks as obtained with the Hewlett-Packard 402 Unit. The mass spectrometer was operated at 70 electron volts and all other conditions were as indicated in the instrument manual specifications.

Results and Discussion

The procedures used for the separation and identification of members of the celesticetin family of antibiotics have been mainly paper or thin-layer chromatography or combinations of the two.[2] However, as the number of antibiotics discovered in fermentations of either *S. caelestis* or its mutants increases, resolution

Table 1.

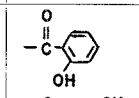
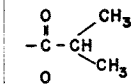
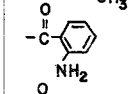
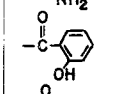
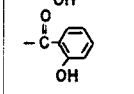
Compound	R_1	R_2	R_3	TMS groups	M^+	*m/e* (VII)	*m/e* (VIII)	T_R (UCW 98) (min.) 290°C	T_R (UCW 98) (min.) 280°C	T_R (PPE) (min.) 268°C
Celesticetin		CH_3	CH_3	4	816	547	84	10.2	16.7	1.2
Celesticetin B		CH_3	CH_3	3	694	547	84	2.6	3.8	—
Celesticetin C		CH_3	CH_3	4	815	547	84	11.9	17.9	—
7-O-Demethyl celesticetin		H	CH_3	5	874	605	84	—	16.7	1.6
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	—

Fig. 2. GLC of celesticetin (I), celesticetin B (II), celesticetin D (III) on UCW 98 at 290°C.

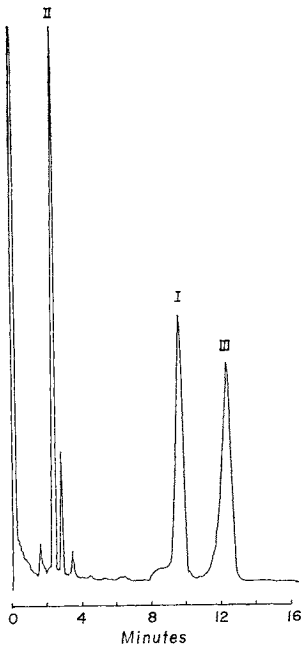


Fig. 3. GLC of celesticetin (I), 7-O-demethylcelesticetin (IV) on PPE at 268°C.

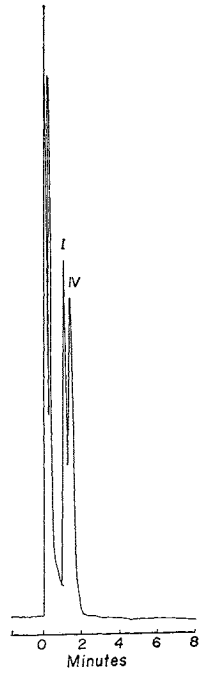
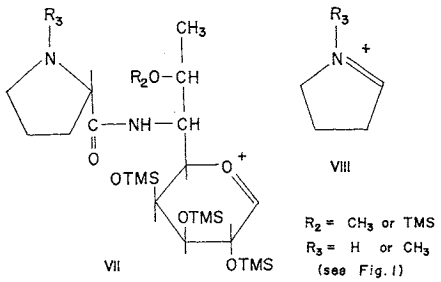


Fig. 4. R_2 is either CH_3 or TMS; R_3 is H or CH_3 (see Fig. 1).



of this group of antibacterial agents decreases and the identification of the components by Rf alone is less than satisfactory.

Celesticetin and the related lincosaminide antibiotics are not amenable to direct gas chromatography[5]. However the celestosaminides like the lincosaminides[6] were easily transformed to their trimethylsilyl ethers which are sufficiently volatile for application of gas chromatography-mass spectroscopy to the isolation and characterization this class of antibiotics. We have generally employed the most reactive silylating reagents since very short reaction times are realized and anhydrous samples are not required. These reagents also have the advantage of reacting with even the most hindered hydroxyl groups. Whenever possible, the reaction mixtures were prepared with moderately high antibiotic concentrations allowing the use of low injection volumes in the LKB to reduce source fouling.

Gas chromatographic retention times and pertinent mass spectral data for the six compounds used in this study are given in Table 1.

The data for this series of celestosaminides on UCW 98 indicate that compounds in which R_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 very low retention times. The lack of alkyl substitution at R_2 and/or R_3 does not seem to effect retention times significantly. Celesticetin (I) and 7-O-demethylcelesticetin (IV) were not resolved on the UCW 98 column. The two antibiotics were separated, however, using a 3% PPE column. A typical GC trace of a mixture of celesticetin (I), celesticetin B (II) and celesticetin C (III) is shown in Fig. 2. Separation of celesticetin (I) and 7-O-demethylcelesticen (IV) using the PPE column is shown in Fig. 3.

Having established the conditions necessary for separating the TMS derivatives of the compounds under investigation by GC, they were subsequently characterized by application of combined GC-MS. Three major *m/e* peaks sufficient to identify the

individual compounds, were observed in the MS. The high mass value in each series (Table 1) is the molecular ion and reflects the variation in the number of TMS groups in the derivative. The second value represents the silylated portion of the molecule (**VII**, Fig. 4) remaining after loss of the $S\text{-}CH_2\text{-}CH_2\text{-}O\text{-}R_1$ group.

The low mass peak is due to the fragment **VIII** (Fig. 4) resulting from the proline moiety of the celestosaminide molecule. As shown in Table 1, N-demethyl-7-O-demethylcelesticetin afforded a penta-TMS derivative indicating the absence of silylation at the imino group of the proline ring. This is supported by the presence of the base peak at 70 mass units due to fragment **VIII** (Fig. 4, $R_3=H$).

The data reported in Table 1 indicate that gas chromatographic retention times can by themselves be useful in identifying the type of ester present in the celestosaminide under investigation. This information together with the mass spectral data can lead to the determination of the structures of these compounds.

References

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