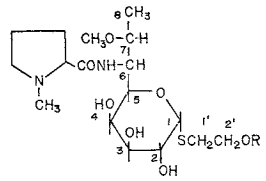
STUDIES WITH *STREPTOMYCES CAELESTIS*
I. NEW CELESTICETINS

Sir:

Celesticetin (Fig. 1, I) is an antibiotic produced by *Streptomyces caelestis*[1,2,3]. We have recently re-examined culture filtrates of *S. caelestis* for antibiotics and we have detected and extracted several bio-active materials in addition to celesticetin. The present preliminary communication describes the properties of four of these antibiotics.

The bioactive materials produced by *S. caelestis* were extracted from the clear filtrates with methylene chloride at alkaline pH (*ca* 8.0). Celesticetin was removed by crystallization as the salicylate salt[1] and the mother liquors were concentrated to an oily residue. Examination of this crude material by tlc* revealed the presence of several materials which inhibited the growth of *Sarcina lutea*. Partial separation of these bioactive components was achieved by counter double current distribution [1-butanol - water (1 : 1 v/v)]. Countercurrent distribution and (or) silica gel chromatography yielded the four bioactive compounds described in this communication.

Fig. 1.



Celesticetin R =  (I)

Desalicetin R = H (II)

Celesticetin B R =  (III)

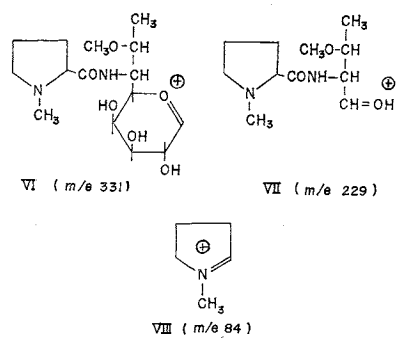
Celesticetin C R =  (IV)

Celesticetin D R =  (V)

The first of these antibacterial agents was isolated as the colorless hydrochloride, $C_{17}H_{32}N_2O_7S \cdot HCl$, Rf^{I} 0.11, Rf^{II} 0.22, Rf^{III} 0.23; $[\alpha]_D^{25}$ +150° (*c* 1, water). The IR spectrum showed the presence of amide I and II bands at 1675 and 1565 cm^{-1} respectively. In addition broad NH and/or OH bands at *ca* 3300 cm^{-1} were also present in the spectrum of this material. The UV spectrum did not show any maxima between 220 and 400 nm. Potentiometric titration in water showed the presence of a basic group, pKa' 7.5. The molecular weight of the free base as determined by mass spectroscopy was 408 (Calcd. for $C_{17}H_{32}N_2O_7S$, 408). These data suggested identity of this antibiotic to desalicetin (Fig. 1, II) which has been obtained by HOEKSEMA[2,3] by alkaline treatment of celesticetin. The nmr spectrum (D_2O) showed the presence of $-CH(-O)-CH_3$ [δ 1.2 (3H, d)], $-NCH_3$ [δ 2.98 (3H, s)], and $-OCH_3$ [δ 3.4 (3H, s)] and also the presence of the anomeric hydrogen [δ 5.54 (1H, d)] in agreement with structure II. Furthermore the mass spectrum showed the molecular ion peak at *m/e*, 408 (Calcd. for desalicetin free base, 408) and a peak at 390 (M^+-H_2O) mass units. Other important ion peaks were observed at 331, 229 and 84 mass units and are assigned to fragments **VI**, **VII** and **VIII** (Fig. 2) respectivly.

The presence of desalicetin in cultures of *S. caelestis* does not appear to be due to degradation of celesticetin under the fermentation conditions, since both celesticetin and

Fig. 2.



VI (*m/e* 331) VII (*m/e* 229)

VIII (*m/e* 84)

* Silica gel G; Rf^{I}, Rf^{II}, Rf^{III}, refer to Rf values in the following systems: 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.

desalicetin were produced* by fermentation. Desalicetin is produced by the organism, most probably, as an intermediate in the biosynthesis of celesticetin.

The second celesticetin-like compound has been named celesticetin B and assigned structure **III** (Fig. 1). This antibiotic was also isolated as the crystalline hydrochloride, $C_{21}H_{38}N_2O_8S \cdot HCl$; Rf^{I} 0.41, Rf^{II} 0.54, Rf^{III} 0.52; $[\alpha]_D^{25}+146°$ (*c* 1, water). Potentiometric titration in water showed the presence of one basic group pKa' 7.4. The IR spectrum showed absorption bands at 1734 (ester carbonyl), 1680 and 1560 (amide **I** and **II** respectively) and 3340 cm^{-1} (broad absorption due to -NH-, -OH). Celesticetin B did not show maxima between 220 and 400 nm in the UV spectrum. The mass spectrum of celesticetin B showed molecular ion peak at *m/e* 478 (Calcd. mol. weight for $C_{21}H_{38} \cdot N_2O_8S$, 478) and another ion peak at 460 mass units assigned to M^+-H_2O. Other major ion peaks were observed at 331, 229 and 84 mass units and are assigned to fragments **VI**, **VII** and **VIII** (Fig. 2) respectively. The nmr spectrum of celesticetin B differs from that of celesticetin in only two areas. First, the absorption due to the aromatic hydrogens of celesticetin is not present in the spectrum of celesticetin B. Second, the spectrum of celesticetin B shows the presence of three -C-CH_3 groups [δ 1.1~1.4 (9H, d)] while the celesticetin molecule contains one -C-CH_3 group. Otherwise the spectrum of celesticetin B is identical to that of celesticetin showing the presence of -OCH_3 [δ 3.35 (3H, s)], -NCH_3 [δ 2.98, (3H, s)], -S$CH_2 \cdot CH_2$OC- (δ *ca* 2.8), hygric acid methylene

hydrogens (δ 2.2) and the anomeric hydrogen [δ 5.6, 1H, d(j=6.0 c.p.s.)] The data indicate that the difference between celesticetin and celesticetin B is in the acyl group at C-2' of desalicetin. Consideration of the molecular formula and of the mass spectra of celesticetin B suggest C_3H_7CO- as the formula of the acyl group in this antibiotic. Since the nmr spectrum showed the presence of three C-CH_3 groups in the celesticetin B molecule, one of which is in the aminosugar moiety, it is concluded that the acyl group

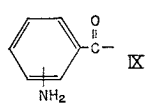
is $(CH_3)_2CHC$- and therefore celesticetin B has the structure presented by **III**. The stereochemistry of celesticetin B is expected to be identical to that of celesticetin since the two antibiotics have similar molecular rotations ($[M]_D$, +697° for celesticetin B and +655° for celesticetin). Furthermore the stereochemistry at C-1 of the respective molecules is identical because the anomeric hydrogen appears as a doublet in the nmr spectra of both antibiotics with identical coupling constants (J=6.0 c.p.s.).

Celesticetin C (**IV**) is the designation given to the third bioactive material described in this communication. This antibiotic was also isolated as hydrochloride, $C_{24}H_{37}N_3O_8S \cdot HCl$; Rf^{I} 0.43, Rf^{II} 0.66, Rf^{III} 0.59; $[\alpha]_D^{25}$ +123° (*c* 1.0, water). Potentiometric titration in water showed the presence of a basic group, pKa' 7.6. The IR spectrum showed main absorption bands at 3440 to 3350 cm^{-1} and at 1680, 1612 and 1586 cm^{-1}. Celesticetin C hydrochloride showed UV maxima at 243 (a=11.6), 328 (a=6.3) nm in water; 247 (a=12.3), 338 (a=8.5) nm in 95 % ethanol; 228 (a=18.8), 270 (a=1.6), 280 (sh) (a=1.2) nm in 0.5 N aqueous hydrochloric acid; and 242 (a=12.0), 321 (a=5.4) nm in 0.5 N aqueous sodium hydroxide. The mass spectrum of celesticetin C showed the molecular ion peak at *m/e* 527 (Calcd. mol. weight for $C_{24}H_{37} \cdot N_3O_8S$, 527) and a peak at 509 (M^+-H_2O) mass units. Other major ion peaks were observed at 331, 229 and 84 mass units assigned to fragments **VI**, **VII** and **VIII** (Fig. 2) respectively. The nmr spectrum of celesticetin C is identical to that of celesticetin in all areas except the area of the absorptions due to the aromatic hydrogens. This fact and the UV spectral data suggest that celesticetin and celesticetin C differ in the acyl group attached at C-2' of desalicetin. Consideration of the mol. formula and of the mass spectra of celesticetin C suggest C_6H_6NC- as the formula of the acyl group

present in this antibiotic. Since the nmr spectrum indicates the presence of four

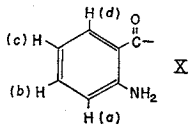
* Mutants of *S. caelestis* have been found to produce significant amounts of desalicetin (J. H. COATS, personal communication).

Fig. 3.



IX

Fig. 4.



X

aromatic hydrogens the structure of the acyl group can be represented by **IX** (Fig. 3).

The IR spectrum of celesticetin C indicates hydrogen-bonded ester carbonyl at 1680 cm^{-1} (as in celesticetin). This would require the amino group of **IX** to be ortho to the ester grouping, an assignment which agrees with the UV data. Celesticetin C shows maxima at 243 and 328 nm in water and at 228 and 270 nm in acid which are in excellent agreement with the reported values for anthranilic acid[4]. The nmr spectrum of celesticetin C also suggests for the acyl moiety of this antibiotic the structure **X**. The absorption pattern* of the aromatic hydrogens is identical to that of anthranilic acid ethyl ester[5]. A doublet (1H) at δ 7.95 is assigned to Hd (Fig. 4, **X**), a triplet (1H) at δ 7.05~7.45 is due to H_b, a doublet (1H) at δ 6.8 is assigned to H_a and a triplet (1H) centered at δ 6.5 is assigned to H_c. Since celesticetin C has a molecular rotation, $[M]_D$ of +648°, almost identical to that of celesticetin (+655°), it is assumed that both antibiotics have identical stereochemistry at all assymetric centers.

The fourth compound described in this communication has been designated celesticetin D and assigned structure **V**. Since only small amounts of this antibiotic were isolated, its nature was determined by IR and mass spectroscopy and comparison with an authentic sample of desalicetin-2′-acetate. The IR spectrum of celesticetin D was similar to the spectra of the celesticetin-like antibiotics and specifically to celesticetin B (**III**). The mass spectrum showed the molecular ion peak at *m/e*, 450 (Calcd. for $C_{19}H_{23}N_2O_8S$, 450) and a peak at 432 (M^+-H_2O) mass units. Other ion peaks were observed at 331, 229 and 84 mass units and are assigned, as in the previous cases, to fragments **VI**, **VII** and **VIII** (Fig. 2). The IR and mass spectral data indicate that celesticetin D is most probably desalicetin acetate ester at the primary hydroxyl group (C-2′). Therefore this compound was compared by tlc to authentic desalicetin-2′-acetate (prepared by R. D. BIRKENEMEYER of The Upjohn Company), and both compounds were found to have identical tlc (silica gel) behavior, Rf^{I} 0.34, Rf^{II} 0.39, Rf^{III} 0.53.

Celesticetin, desalicetin and celesticetins B, C and D have similar *in vitro* antibacterial spectra being mainly active against Gram positive microorganisms. Celesticetin C was as active as celesticetin in the *in vitro* system used. Celesticetin B had 25% of the celesticetin activity while desalicetin was the least active. *In vivo* evaluation of these antibiotics is incomplete.

Celesticetin, desalicetin and celesticetin B, C and D are not the only antibiotics produced by *S. caelestis*. MEYER[6] has reported the isolation of desdanine, desdamethine and ethesdanine from cultures of this organism growing in the presence of methionine or ethionine. We are presently working on additional antibiotics produced by this culture which will be the subject of future communications.

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* D_6-Dimethylsulfoxide was used as solvent for the nmr spectrum of celesticetin C hydrochloride.