NOTES

INCORPORATION OF EXOGENOUS SALICYLIC ACID INTO CELESTICETIN

A.D. ARGOUDELIS and J.H. COATS

Research Laboratories, The Upjohn Company, Kalamazoo, Michigan U.S.A.

(Received for publication June 18, 1974)

Streptomyces caelestis has the ability to produce a series of celestosaminide antibiotics[1,2,3] differing from celesticetin (**I**) in the acid moiety esterified to the primary hydroxyl of desalicetin (**II**). This finding strongly suggests that the enzymatic system responsible for the ester formation is not specific and that other acids might substitute for salicylic acid.

It was, therefore, of interest to examine the ability of *S. caelestis* to incorporate acids, other than salicylic acid, into bioactive compounds. First, however, in order to establish the feasibility of utilization of exogenous acids we decided to examine the incorporation of exogenous radioactive salicylic acid into celesticetin.

[7-^{14}C]-Salicylic acid was added to 24-hour cultures of *S. caelestis*. The fermentations were harvested after a total fermentation time of 48 hours. Thin layer bio-radiograms of culture filtrates showed the presence of one radioactive and bioactive spot with R_f value identical to that of celesticetin produced in the control fermentations.

Crystalline radioactive celesticetin hydrochloride, isolated by adsorption on Amberlite XAD-2 and elution with 95% aqueous methanol, contained *ca* 7.6% of the radioactivity present in the clear filtrate. The crystalline material was identified as pure celesticetin hydrochloride by nmr, IR, UV spectra and tlc procedures. Thin-layer bio-radiograms of the isolated radioactive celesticetin hydrochloride (Fig. 2) show the presence of one bioactive compound containing all radioactivity.

Fig. 1.

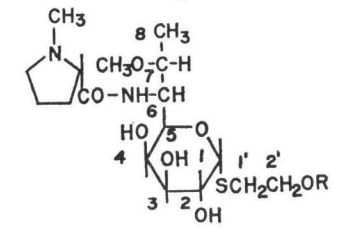


Fig. 2. Thin layer bio-radiogram of radioactive celesticetin hydrochloride.
Peaks indicate radioactivity while the circle shows zone of bioactivity *vs S. lutea*.

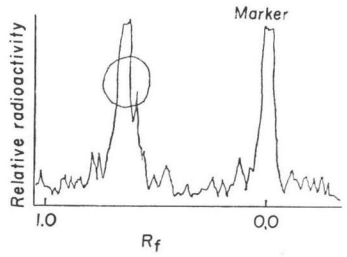
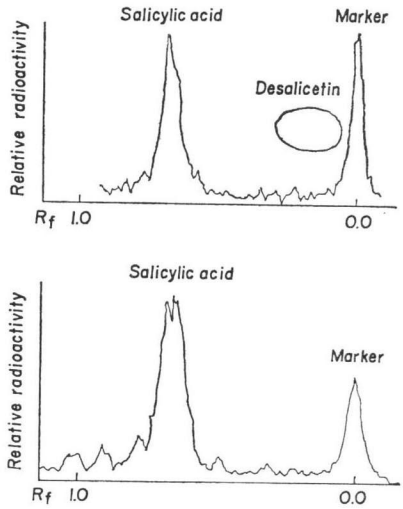


Fig. 3. Thin layer bio-radiogram of mixture of desalicetin hydrochloride and salicylic acid (upper) and of salicylic acid (lower) obtained from alkaline hydrolysis of radioactive celesticetin.
Peaks indicate radioactivity while the circle shows zone of bioactivity *vs S. lutea*.



Alkaline hydrolysis of the radioactive celesticetin, followed by acidification, yielded crystalline salicylic acid separated from the reaction mixture by filtration. This material contained 79% of the radioactivity present in the radioactive celesticetin hydrochloride. The filtrate containing desalicetin (**II**, Fig. 1) and residual salicylic acid, accounted for 21% of the radioactivity. Tlc bio-radiogram (Fig. 3, upper) showed that all the radioactivity of the filtrate was due to salicylic acid. A bioactive spot with an R_f value corresponding to that of desalicetin was not radioactive. Tlc bio-radiograms of the isolated salicylic acid (Fig. 3, lower) showed, as expected, on radioactive peak but no bioactive spot.

These results indicate that exogenous radioactive salicylic acid is incorporated into the celesticetin molecule without randomization to form the ester at C-2′ of desalicetin. It is not known whether a precursor of desalicetin or desalicetin itself participates in the esterification reaction leading to celesticetin. Whatever the exact sequence of the enzymatic esterification, the results of the present study, combined with the previous observations on antibiotics produced by *S. caelestis*[1,2,3], strongly suggest that other acids could be utilized by *S. caelestis* for production of new celestosaminides.

Experimental

General

Radioactivity was determined by procedures described by ARGOUDELIS, *et al.*[4]

Thin-layer chromatography was run on silica gel using 2-pentanone-methyl ethyl ketone-methanol-water (2:2:1:1) as solvent system. The antibiotics present in the fermentation were detected by bioautography on *Sarcina lutea* (UC 130) seeded agar medium.

Fermentation Procedures

Seed cultures of *S. caelestis* (UC 2011) 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 presterilization pH 7.2. The cultures were incubated at 28°C for 72 hours on a rotary shaker (250 rpm). Fermentation medium consisting of glucose monohydrate, 25.0 g/liter; Brewer's yeast 2.5 g/liter; $(NH_4)_2SO_4$, 5.0 g/liter; $CaCO_3$, 8.0 g/liter; NaCl, 4.0 g/liter; soy flour, 7.0 g/liter and KH_2PO_4, 0.4 g/liter was adjusted to pH 7.2 and inoculated at a rate of 5% (v/v) with the 72 hour seed culture.

Fifty μC of [7-^{14}C] salicylic acid (New England Nuclear; specific activity, 4.8 mC/mM; weight 1.4 mg) was mixed with 1.4 mg of radioinactive salicylic acid. The mixture was dissolved in 2 ml of water and the solution was added to the fermentation broth 24 hours after inoculation, at a concentration of 25 μC (1.4 mg of salicylic acid) per 100 ml of fermentation medium. Fermentations containing 100 ml of medium per 500 ml Erlenmeyer flask, were incubated at 28°C on a rotary shaker (250 rpm) and harvested after a total fermentation time of 48 hours. Antibiotic titers were measured by disc plate assay using *Sarcina lutea* as an assay organism[5].

Isolation of Radioactive Celesticetin

Fermentation broth (*ca* 200 ml) was filtered with the aid of diatomaceous earth. The fllter cake was washed with 100 ml of water and the aqueous wash was combined with the clear filtrate. This solution was passed over a column prepared from 20 ml of Amberlite XAD-2 (Rohm and Haas Co., Philadelphia, Pa., U.S.A.) packed in water. The spent beer was bioinactive and was discarded. The column was washed with 100 ml of water which was collected as one fraction. This solution was also bioinactive and was discarded. The column was then eluted with 95% aqueous methanol. Fractions containing celesticetin were combined and the solution was concentrated to dryness. The residue obtained was triturated with 10 ml of absolute methanol and 20 ml of ether. Insoluble material was bioinactive and was discarded. The filtrate was mixed with 100 mg of radionactive celesticetin, the mixture was concentrated to dryness and the new residue was dissolved in 3 ml of 1 N methanolic hydrogen chloride. Addition of ether to the methanolic solution yielded colorless crystalline radioactive celesticetin hydrochloride. Thin layer bio-radiogram of the radioactive celesticetin hydrochloride is shown in Fig. 2.

Degradation of Radioactive Celesticetin to Desalicetin Hydrochloride and Salicylic Acid

Twenty mg of radioactive and 180 mg of radioinactive celesticetin hydrochloride were mixed and dissolved in 2 ml of 1 N aqueous sodium hydroxide. The solution was allowed to stand at room temperature for 24 hours and was then mixed with 2.5 ml of 1 N aqueous hydrochloric acid. Crystalline salicylic acid was isolated by filtration (32.8 mg). The filtrate containing desalicetin hydrochloride was adjusted to a volume of 10 ml. Both the salicylic acid and the solution containing desalicetin hydrochloride were analyzed by tlc. Results are shown in Fig. 3. The

radioactivity present in both preparations was also determined.

Acknowledgement

The authors wish to thank Dr. T.F. BRODASKY for helpful discussions and Messrs. W.A. MURPHY and K.J. GEIPEL for technical assistance.

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