Letters in Applied Microbiology 1993, **17**, 212–214

Transformation of alpha-santonin via two independent pathways by *Pseudomonas* strain S ATCC 43388

D. COLACO†, I. FURTADO†, U.P. NAIK‡, S. MAVINKURVE*† & S.K. PAKNIKAR§ *Departments of †Microbiology and §Chemistry, Goa University, Taleigao Plateau, Goa, India 403 202, and ‡Department of Biochemistry, State University of Brooklyn, Brooklyn, NY, USA*

GWG/195: received 11 April 1993 and accepted 26 April 1993

COLACO, D., FURTADO, I., NAIK, U.P., MAVINKURVE, S. & PAKNIKAR, S.K. 1993. Transformation of alpha-santonin via two independent pathways by *Pseudomonas* strain S ATCC 43388. *Letters in Applied Microbiology* **17**, 212–214.

Pseudomonas strain S (ATCC 43388) transforms alpha-santonin in the presence of dicyclohexylcarbodiimide (DCCD) to 1,2-dihydrosantonin (DHS) and two more products, D1 and D2. D2 was identified as 4,5-dihydroxysantonin (DHXS). Cells incubated with semicarbazide (SC) formed DHXS but not DHS. Simultaneous formation of DHS and DHXS is indicative of the presence of two distinct pathways of alpha-santonin biotransformation.

Various products of alpha-santonin (Fig. 1a) reported so far, both by Japanese workers (Hikino *et al.* 1970; Fujimoto *et al.* 1978; Iida *et al.* 1981; Sato *et al.* 1984) as well as from this laboratory using *Pseudomonas* strains S ATCC 43388 (Sangodkar and Mavinkurve 1982; Naik *et al.* 1982; Furtado *et al.* 1988) involved saturation of the parent molecule at the 1–2 position. Formation of 1,2-dihydrosantonin (DHS) (Fig. 1b) as the first product during growth of *Pseudomonas* strain S, involving an NADP-dependent 1,2-reductase, has been reported (Naik and Mavinkurve 1987). The present investigation reports formation of a 4,5-hydroxylation product with the 1–2 unsaturation intact (Fig. 1c) via yet another pathway, independent of DHS.

Materials and Methods

STRAIN AND MAINTENANCE

Pseudomonas strain S (ATCC 43388) grown on mineral medium (Sangodkar and Mavinkurve 1982, 1984) was maintained at room temperature (RT; 27–30°C) and subcultured fortnightly.

* Corresponding author.

PRODUCTION AND PURIFICATION OF TRANSFORMATION PRODUCT

Five litres of 0·4% (w/v) glucose medium, were inoculated with 5% of a 48 h culture and incubated at RT on a rotary shaker at 150 rev min^{-1} for 24 h. The pH of the culture broth was then adjusted to 8 and dicyclohexyldicarbodiimide DCCD (1 mmol l^{-1}) or semicarbazide SC (2 mmol l^{-1}) was added. After 10 min, 0·025% (w/v) of alpha-santonin was added and the flasks incubated as above for another 24 h. The transformation products were extracted, concentrated and separated using a silica gel column as described earlier (Furtado *et al.* 1988). Fractions showing single products were pooled and crystallized using petroleum ether (40–60°C) and characterized spectrophotometrically.

Results and Discussion

Inhibitors are known to cause the accumulation of products during microbial transformation (Shukla and Kaul 1974; Hieda *et al.* 1982). Since the transformation products of alpha-santonin were low in yield and transient, the ATPase

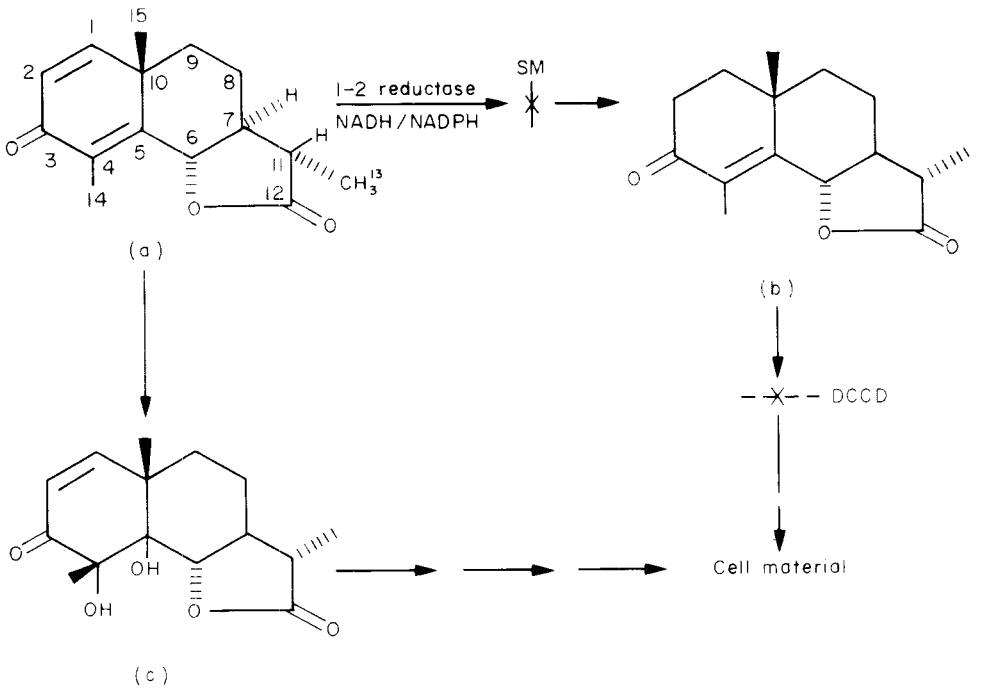

Fig. 1. Two independent metabolic pathways of metabolism of alpha-santonin (a) via the intermediates 1,2-dihydrosantonin (b) and 4,5-dihydroxysantonin (c).

inhibitor, DCCD was used with a view to accumulate products. DHS has been reported as the early product of transformation in growing as well as cometabolic conversions (Fujimoto *et al.* 1978; Naik *et al.* 1988).

Although the transformation of alpha-santonin in the presence of DCCD at pH 6 was confined to accumulation of DHS (Naik *et al.* 1988), two more products designated as D1 and D2 with Rf 0·2 and 0·22 were accumulated at pH 8. The product D2 eluted in 90% v/v of ether in the petroleum ether solvent system in the gas chromatogram, with a retention time of 46·83 min from a DB-1 column at a temperature range of 50–250°C using N_2 as the carrier gas. The compound formed white shiny crystals at RT, melted at 220°C and had M+ 220 and u.v.(EtoH) 226 nm: i.r.(nujol): 3530, 3478 (hydroxyl), 1775 (γ-lactone) and 1678 cm^{-1} (conjugated carbonyl), PMR: ($CDCl_3$: 1·28 (3H, d, J = 6 Hz), 1·39 (3H, *s*), 4·02 (1H, S, exchangeable with D_2O), 4·16 (1H, S, exchangeable with D_2O), 4·20 (1H, *d*, J = 12 Hz) 6·02 and 6·38 (1H, each *d*, J = 10 Hz). On the basis of these physical and spectral features, the compound was identified as 4,5-dihydroxysantonin (DHXS) (Fig. 1c) with a molecular formula of $C_{15}H_{20}O_5$. Preliminary studies of D1 suggests the compound to be derived from DHXS. Further investigations are in progress.

The cells incubated with alpha-santonin and DCCD at pH 8 accumulated DHXS, perhaps by an oxygenase (Trudgill 1986) which appears to be very specific for alpha-santonin with 1,2-unsaturation, since incubation with DHS, with 1,2-saturation failed to give the corresponding dihydrodihydroxy derivative, thereby indicating that DHXS is formed via an independent pathway and not through DHS.

Cells incubated with yet another inhibitor, SC, and alpha-santonin show a very long lag phase of about 36–40 h followed by normal growth and formation of products more polar than alpha-santonin, the prominent and early one being DHXS. Interestingly, DHS is not detected throughout the incubation of this system.

Under normal conditions and in the absence of any inhibitor *Pseudomonas* cells formed DHS within 3–4 h, whereas the dihydroxy products appeared only during the late exponential phase, much after DHS has been formed and

depleted (unpublished results). Our results with DCCD and SC reveal the new pathway via DHXS, which indicates that the utilization of alpha-santonin via DHS is much favoured under normal conditions and that the pathway via dihydroxy products, though operative, comes into prominence under stress conditions.

The authors thank Dr G. Schmaus of Holzminden, for providing the spectral data. Financial assistance by UGC (India) is acknowledged.

References

Fujimoto, Y., Shimizu, T., Ishimoto, T. and Tatsuno, T. 1978 Studies on the microbiological transformation of alpha-santonin and its analogues. *Yakugaku Zasshi* **98**, 230–233.

Furtado, I., Mavinkurve, S. and Paknikar, S.K. 1988 Microbiological transformation of alpha-santonin to 11-demethyl-eudesm-4-ene-3, 6-dione. *Letters in Applied Microbiology* **6**, 27–30.

Hieda, T., Mikami, Y., Obi, Y. and Kisaki, T. 1982 Microbial transformation of sclareol. *Agricultural and Biological Chemistry* **46**(10), 2477–2484.

Hikino, H., Tokuoka, Y. and Takemoto, T. 1970. Biochemical synthesis VIII: Microbial transformation of alpha-santonin to 1,2-dihydrosantonin. *Chemical and Pharmaceutical Bulletin (Tokyo)* **18**, 2127–2128.

Iida, M., Hatori, Y., Iisuka, H. and Yamakawa, K. 1981 Microbial reduction of carbon 1 and 2 double bond in 1-alpha-santonin (microbial transformation XVI). *Advances in Biotechnology* **3**, 507–512.

Naik, U.P. and Mavinkurve, S. 1987 Alpha-santonin 1,2-reductase and its role in the formation of dihydro-santonin and lumisantonin by *Pseudomonas cichorii* S. *Canadian Journal of Microbiology* **33**, 658–663.

Naik, U.P., Paknikar, S.K. and Mavinkurve, S. 1982 Transformation of alpha-santonin to lumisantonin by *Pseudomonas cichorii* S. *Indian Journal of Chemistry* **21B**, 501.

Naik, U.P., Marinkurve, S., Naik, U.S. and Paknikar, S.K. 1988 Microbial transformation of alpha-santonin by *Pseudomonas cichorii* S. Identification of products. *Indian Journal of Chemistry* **27B**, 381–382.

Sangodkar, U.M.X. and Mavinkurve, S. 1982 Isolation and characterisation of alpha-santonin assimilating *Pseudomonas*. *Journal of Biosciences (India)* **4**, 79–84.

Sangodkar, U.M.X. and Mavinkurve, S. 1984 Environmental and nutritional requirements for optimal growth and transformation of alpha-santonin by *Pseudomonas cichorii* strain S. *Indian Journal of Microbiology* **2**, 202–206.

Sato, Y., Oda, T., Inove, J., Kunugi, M. and Suzuki, K.T. 1984 Steriochemistry of microbial hydrogenation of (−)alpha-santonin to (+) 1,2-dihydro-alpha-santonin by *Streptomyces cinereocrocatus* NRRL 3443. *Chemical and Pharmaceutical Bulletin (Tokyo)* **32**, 504–509.

Shukla, O.P. and Kaul, S.M. 1974 A constitutive pyridine degrading system in corynebacterium sp. *Indian Journal of Biochemistry and Biophysics* **11**, 201–207.

Trudgill, P.W. 1986 Terpenoid metabolism by *Pseudomonas*. In *The Bacteria*, Vol. 10, ed. Sokatch, J.R. and Ornston, N.L. pp. 463–525. New York: Academic Press.