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Microbial Transformation of α-Santonin by *Pseudomonas cichorii* S: Identification of Products

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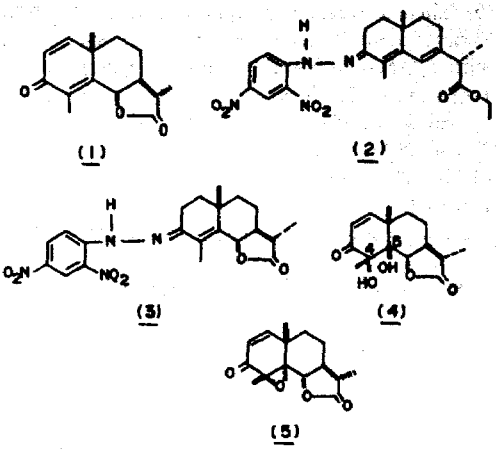
4α, 5α-Dihydroxysantonin (4) and ethyl 3-oxoeudesma-4, 6-diene-12-oate (2, 4-DNP derivative 3) have been characterised as microbial transformation products of α-santonin (1) by *Pseudomonas cichorii* S. The assigned structures have been confirmed by their partial chemical synthesis from α-santonin.



For some time we have been interested in the identification of products obtained during microbial transformation of α-santonin by microorganisms[1,2]. In continuation of the earlier work from our laboratory, herein we report the isolation and characterisation of products obtained during microbial transformation of α-santonin[1] by *Pseudomonas cichorii* s in the presence or absence of metabolic inhibitors.

Since the majority of transformation products contain carbonyl group, we considered the possibility of isolating the metabolites as their 2,4-dinitrophenylhydrazone derivatives[3]. Indeed, using, 2,4-DNPH as a trapping agent, we could isolate three 2,4-DNP derivatives in pure form.

First of these, m.p. 126°, was identified as acetone 2,4-dinitrophenylhydrazone. The second derivative, m.p. 188°, analysed for $C_{10}H_{10}O_4N_4$ (M^+ 250) and exhibited in its PMR spectrum in $CDCl_3$ the following signals: δ 1.96 (3H, *d*, *J* =6.0 Hz), 6,36 (2H,*m*), 7.80(1H, *d*, *J*=9.0, Hz), 7.96(1H, *d*, *J*=9.0 Hz), 8.34-(1H,*dd*, *J*=9.0, 3.0 Hz), 9.16 (1H, *d*, *J*=30 Hz) and 11.08 (1H, *s*). The spectral data and m.p. permitted the identification of this compound as crotonaldehyde 2,4-dinitrophenylhydrazone.

The third 2,4-DNP derivative, m.p. 150°, was obtained in insufficient amount (5 mg) It analysed for $C_{23}H_{28}O_8N_4$(M^+456) and exhibited in its PMR spectrum in $CDCl_3$ signals at δ 1.01 (3H, *s*), 1.25 (3H, *t*, *J* =6 Hz), 1.36 (3H, *d*, *J*=6 Hz), 2.08 (3H, *s*), 3.28 (1H, *q*, *J* =6 Hz), 4.15 (2H, *q*, *J* =6 Hz), 6.51 (1H, *bs*) 8.03 (1H, *d*, *J* =9 Hz), 8.33 (1H, *dd*, *J* =9.0, 3.0 Hz), 9.12 (1H, *d*, *J* =3.0 Hz) and 11.35 (1H, *bs*). Structure 2 which could be derived for this on spectral evidences was unambiguously confirmed by its partial synthesis from α-santonin[1]. Treatment of dihydrosantonin 2,4-DNP (3), m.p. 224° with ethanolic sulphuric acid under refluxing conditions furnished after purification a purple 2,4-DNP derivative in 80% yield identical in all respects with 2.

The use of inhibitors for the accumulation of transformation products in the culture broth is well-known[3-6]. 1,2 Dihydrosantonin, one of the early transformation products of α-santonin[1] (1) was found to be the major product along with trace quantities of an unidentified polar product when dicyclohexylcarbodiimide (DCC) was used as an inhibitor. On the other hand, five products, all more polar than (1) could be detected in the culture broth when semicarbazide was used as an inhibitor. One of these could be isolated in pure form (m.p. 220°) to which we have assigned structure (4). Its mass spectrum displayed the molecular ion at *m/z* 280 consistent with molecular formula $C_{15}H_{20}O_5$. The other spectral data for this compound are: UV(EtOH) : 226 nm; IR(nujol) : 3530, 3478 (hydroxyl), 1775 (γ-lactone) and 1678 cm^{-1} (conjugated carbonyl), PMR ($CDCl_3$:δ 1.28(3H,*d*,*J* =6Hz), 1.39(3H,*s*), 1.56(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(1H,*d*, *J*= 10 Hz) and 6.38 (1H,*d*, *J*= 10 Hz). These spectral data clearly showed that this transformation product is 4,5-dihydroxysantonin (4) (excluding stereochemistry at C-4 and C-5). Although it is previously suggested[7] that the 4,5-dihydroxysantonin, m.p. 261° obtained by $KMnO_4$ oxidation of 1 should be 4α, 5α-dihydroxysantonin (4) we could not find any report confirming this assignment. There are also no reports on the preparation of

4α, 5β or 4β, 5α -diols from α -santonin. The preparation of these diols was therefore, essential for determining the stereochemistry of the newly created hydroxyl groups at C-4 and C-5. Santonin α -epoxide[7] (5) failed to undergo oxirane ring cleavage under a variety of conditions. However, oxidation of santonin with $KMnO_4$/pyridine led to 4α, 5α-dihydroxysantonin, m.p. 220° identical in all respects with the microbial transformation product, which is therefore 4. It should be noted, however, that the m.p. of *cis*-diol (4) is 220° and not 261° (ref. 87) as previously reported.

Identification of other transformation products is in progress.

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