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MICROBIOLOGICAL HYDROXYLATION OF (+)- and (-)-BORNYL ACETATE WITH *HELMINTHOSPORIUM SATIVUM* AND *FUSARIUM CULMORUM*.

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Abstract The regiospecificity of hydroxylation of (+)- and (-)-bornyl acetate by cultures of *Helminthosporium sativum* and *Fusarium culmorum* has been determined.

Previous studies in our laboratory have shown that (-)-bornyl acetate (1) is converted to a mixture of 2,5-, 2,3- and 2,6-bornanediol in 40-60% yield by cultures of *H. sativum* grown on Czapek-Dox medium[1]. The ratio of these diols was ∿ 5:2:1 respectively and the major product (2,5-bornanediol) was shown to be a mixture (2.4:1 - 3.6:1) of 5-*exo* and 5-*endo* isomers (2a,b).

We have recently investigated the microbial transformation of (+)-bornyl acetate (5) under identical conditions[1] and have found that the hydroxylation process in this case is more efficient and regiospecific. Thus addition of (+)-bornyl acetate to 3-day-old cultures of *H. sativum* followed by the usual workup after 7 days produced a mixture of 5-*exo*-hydroxyborneol (6a) and 5-*endo*-hydroxyborneol (6b) in 53-64%. The only other significant product was a minor amount (∿ 2%) of 3-*exo*-hydroxyborneol (7). The ratio of 5-*exo*- to 5-*endo*-hydroxyborneol was estimated by g.l.c. and n.m.r. to be 2.5:1 - 7.1 and therefore the C(5) hydroxylation process had occurred with a variable degree of stereoselectivity.

Inevitably these investigations led us to consider the possibility that the final step (?) in the biosynthesis of culmorin (11) by *Fusarium culmorum* could involve 5-*endo*-hydroxylation of longiborneol (10) (or a suitable derivative) and the results of recent biosynthetic investigations[2] are consistent with this proposal. As in the case of *H. sativum*[1] we assumed that *F. culmorum* contained a C(5)-hydroxylase system which would also be capable of functionalising monoterpenoid analogs of longiborneol (10). Support for this prediction was obtained by feeding (-)-bornyl acetate (1) to 7-day-old cultures of *F. culmorum* grown on Raulin-Thom medium. After 18 days, ether extraction of the broth provided starting material and a mixture in which the major product[3] (∿ 12% overall yield) was 5-*exo*-hydroxybornyl *acetate* (8). When (+)-bornyl acetate (5) was used as substrate the major product (∿ 12% yield) was also the 5-*exo*-hydroxy derivative (9). Thus *F. culmorum* had functionalised each enantiomer in a similar fashion but with much less efficiency than *H. sativum*.

The efficiency, regiospecificity, and stereoselectivity of the microbiological hydroxylation of bornyl acetate compares favourably with the microbial transformations of other terpenoids[4] and provides a further example of the oxidative vulnerability of the C(5) position in this compound[1]. In addition the production of 5-hydroxybornyl acetate[5] by

F. culmorum cultures provides a means of producing bornane derivatives with a variety of functional groups at the C(2), C(3), C(5) and C(6) positions.

The extension of these investigations to the sesquiterpenoid area is part of current research in our laboratory.

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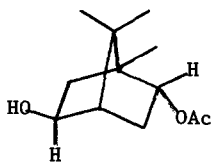
1. M.S. Allen, N. Darby, P. Salisbury and T. Money. *J.C.S. Chem. Comm.*, 358 (1977).
2. J. R. Hanson and R. Nyfeler, *J.C.S. Perkin I*, 2471, (1976) and references cited.
3. Several minor products present in the reaction mixture have not yet been identified.
4. G. A. Fonken and R. A. Johnson, "Chemical Oxidations with Microorganisms", Marcel Dekker, New York, p. 23, (1972): K. Kieslich, "Microbial Transformations of Non-Steroid Cyclic Compounds", Wiley, p. 56, (1976).
5. Preliminary observations indicate that *F. culmorum* converts isobornyl acetate to 5-hydroxyisobornyl acetate. Hence 5-ketoisobornyl acetate is accessible by a microbiological route which complements the laboratory synthesis involving remote oxidation cf. N. J. Toivonen and A. Halonen, *Suomen Kemi, 19B*, 1, (1946). D. H. Hunter, M.Sc. thesis, U.B.C. Vancouver (1974).



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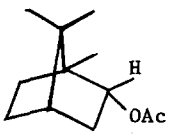

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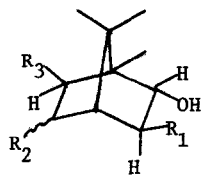
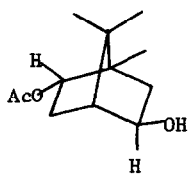


2a,b; $R_1=R_3=H$, $R_2=OH$

3 ; $R_1=R_2=H$, $R_3=OH$

4 ; $R_1=OH$, $R_2=R_3=H$



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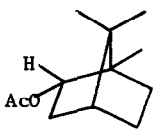

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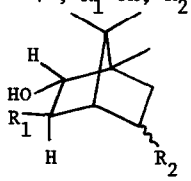
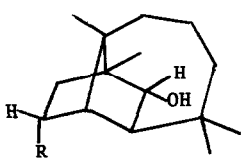


6a,b; $R_1=H$, $R_2=OH$

7 ; $R_1=OH$, $R_2=H$



10; R=H

11; R=OH

i, Helminthosporium sativum
ii, Fusarium culmorum