number of cells for immunological and biochemical studies. It also indicates the importance of studying nucleic acid metabolism of this pathogen for developing a defined medium, understanding its biology and for defining a possible new target for chemotherapy.

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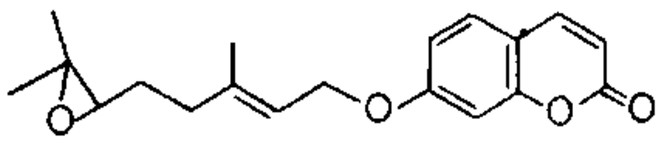
MICROBIAL HYDROLYSIS OF 7-OXYGENATED COUMARINS

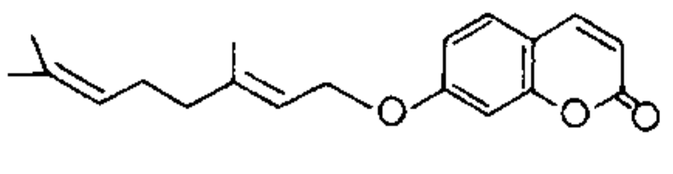
P. BHATTACHARYYA, S. S. JASH and P. K. CHAKRABARTTY

Department of Chemistry and Microbiology, Bose Institute, 93/1, A. P. C. Road, Calcutta 700 009, India.

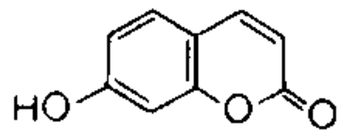
REACTIONS with microbial enzymes have been carried out for long in the case of steroids and alkaloids[1,2]. We are now exploring the possibility of using microbial enzymatic reactions with other class of compounds and we now report the hydrolysis of 6′, 7′-epoxy auraptene (I) and auraptene (II) to 7-hydroxy coumarin (III).



(I)



(II)



(III)

Rhizobium meliloti SU47 (obtained from CSIRO, Canberra, Australia) was grown in a medium containing $(NH_4)_2\ SO_4$, 1 g; $MgSO_4$, 0.5 g; $Na_2\ HPO_4$; 1 g; yeast extract, 0.2 g; glucose, 20 g (autoclaved separately) $FeCl_3$ trace, $MnSO_4$ trace per litre. The pH of the medium after sterilization and addition of glucose was 7.0. The organism was allowed to grow for 24 hr at 28°C on a rotary shaker. The compound (I) dissolved in 90% ethanol (stock solution 4 μg/ml) was then added to it to a final concentration of 100 μg/ml and incubated for another 48 hr. The culture filtrate was extracted with chloroform and washed with water, dried over anhydrous sodium sulphate and finally evaporated. The semisolid mass was then chromatographed over silica gel. The column was eluated (100 ml each time) successively with petroleum ether, petroleum ether : benzene (1 : 1), benzene, benzene : chloroform (1 : 1) and chloroform. Chloroform eluate furnished a crystalline compound m.p. 228°C. The homogeneity of the compound was tested on silica gel G plate which showed a single spot different from that of the starting compound. The compound was soluble in alkali giving a yellow solution from which it could be regenerated by acidification indicating the presence of a δ-lactone. The strong fluorescence and the UV spectrum (λ_{max} 300, 305 and 325 nm log ε 3.9, 3.95 and 4.14) suggest it as a coumarin with an oxygen atom at 7-position. The IR absorption bands at 3500, 1710 and 1630 cm^{-1} also indicate it to be a coumarin with hydroxyl function. The ^{1}H NMR spectrum of the compound showed signals at δ 10.05 (s, 1H, phenolic OH, exchangeable with D_2O) δ 6.21 (d, 1H, J = 9.5 Hz, H-3) δ 7.71 (d, 1H, J = 9.5 Hz, H-4) characteristic of coumarin compounds and δ 7.42 (d, 1H, J = 9.0 Hz, H-5) and δ 6.89 (d, 1H, H-6) δ 6.80 (s, 1H, H-8). The upfield shift (6.84 of the H-6 proton is

characteristic of an aromatic proton adjacent to an oxygen substitutent.

On the basis of these observations the product was identified as 7-hydroxy coumarin which was finally confirmed by direct comparison with an authentic sample (m.p., m.m.p., UV, IR, NMR).

In control experiment the compound (I) was added to medium without bacterial inocculum and incubated similarly. After 48 hr of incubation the medium was extracted with chloroform and evaporated as described above when a solid mass was obtained which was found to be the original compound by direct comparison.

Similar experiment with compound (II) furnished compound (III).

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RUTHENIUM(III)-CATALYZED EPOXIDATION OF OLEFINS BY N-METHYLMORPHOLINE N-OXIDE

G. CAROLING, J. RAJARAM and
J. C. KURIACOSE
Department of Chemistry, Indian Institute of Technology, Madras 600 036, India.

ALKYLHYDROPEROXIDES in combination with complexes of molybdenum, vanadium, tungsten and titanium have been used for the epoxidation of olefins[1]. The kinetics, mechanism and factors governing catalytic activity in such epoxidations have been investigated[2, 3]. In the presence of catalytic amounts of (tetraphenyl-porphinato) manganese(III), p-cyano-N,N-dimethylaniline-N-oxide epoxidises cyclohexene[4]. However, olefins are converted into the corresponding glycols by tertiary amine-N-oxides like trimethylamine-N-oxide and N-methylmorpholine-N-oxide (NMO) in the presence of catalytic amounts of OsO_4[5–7]. Vijayasri *et al*[8] reported that in the presence of Ru(III) as catalyst in DMF, NMO oxidizes secondary alcohols to the corresponding ketones. Studies on the use of $RuCl_3$–NMO combination in DMF as solvent to epoxidize olefinic substrates like cyclohexene, styrene and terminal alkenes are reported in this communication.

The kinetic investigations were carried out at 35 ± 0.1°C. Oxygen-free nitrogen was bubbled into the reaction mixture to provide an inert atmosphere. The concentration of NMO remaining at any instant was determined titanometrically[9] as reported earlier[8]. In the case of non-aromatic substrates the progress of the reaction, especially at low concentrations of the substrate was followed iodometrically. The reaction mixture was quenched with a known excess of bromine in acetic acid and the unreacted bromine was treated with excess of KI. The iodine liberated was titrated against standard sodium thiosulphate.

The stoichiometry of the reaction is in agreement with the equation $\text{Alkene} + \text{NMO} \xrightarrow{\text{catalyst}} \text{epoxide} + \text{N-methylmorpholine}$. There was no reaction between substrate and NMO in the absence of a catalyst. All the alkenes investigated so far are found to give rise to the corresponding epoxides. This was confirmed by gas chromatographic analysis using an FFAP column and comparison of the retention times with those of authentic samples.

The reactions were carried out under pseudo first order conditions by maintaining [substrate] $\gg$ [NMO]. The reaction was first order in NMO and catalyst. However, the order with respect to the substrate, as determined from the pseudo first order plots, varied depending on the concentration of substrate. Vijayasri *et al*[10] established the formation of Ru(V) species by spectral and cyclic voltametric studies. Accordingly the following mechanism could be proposed for the epoxidation of olefins.

$$\text{Ru(III)} + \text{NMO} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Ru(V) oxo species},$$

$$\text{Ru(V) oxo species} + \text{S} \xrightarrow[\text{slow}]{k_2} \text{product}.$$

Assuming the Ru(V) oxo species to be in steady state concentrations one can deduce the following rate expression.

$$\text{Rate} = \frac{-\text{d[NMO]}}{\text{d}t} = \frac{k_1 k_2 [\text{Ru(III)}]\,[\text{NMO}]\,[\text{S}]}{k_{-1} + k_2[\text{S}]},$$

$$= \frac{k_1[\text{Ru(III)}]\,[\text{NMO}]\,[\text{S}]}{(k_{-1}/k_2) + [\text{S}]}. \qquad (1)$$