Biotransformation of *endo*-Bicyclo[2.2.1]heptan-2-ols and *endo*-Bicyclo[3.2.0]-hept-2-en-6-ol into the Corresponding Lactones

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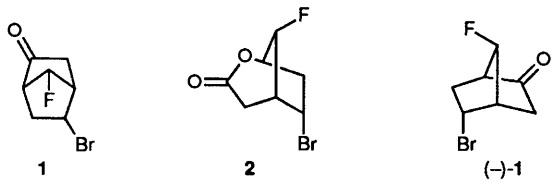
The secondary alcohols **3**, **4** and **10** have been converted into the corresponding lactones using *Acinetobacter calcoaceticus* NCIMB 9871: the transformations (*endo*-**4**) ⇒ **6** + **11** and **10** ⇒ **8** + **9** can be conducted *in vitro* using a coupled enzyme system (*Thermoanaerobium brockii* dehydrogenase and *Acinetobacter calcoaceticus* monooxygenase) with *in situ* recycling of $NADPH/NADP^+$.

Recently there has been considerable interest in the bio-oxidation of cyclic ketones into lactones.[1–5] For example, the racemic dihalogenonorbornanone **1** is oxidised enantioselectively by resting whole cells of *A. calcoaceticus* to give the lactone **2** (e.e. > 95%) and the recovered ketone (−)-**1** (e.e. > 95%). The latter compound was used to prepare an optically pure sample of an anti-HIV agent.[6] Such enantioselective Baeyer–Villiger oxidations are impossible to emulate



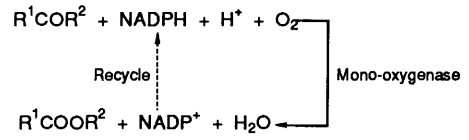


Fig. 1

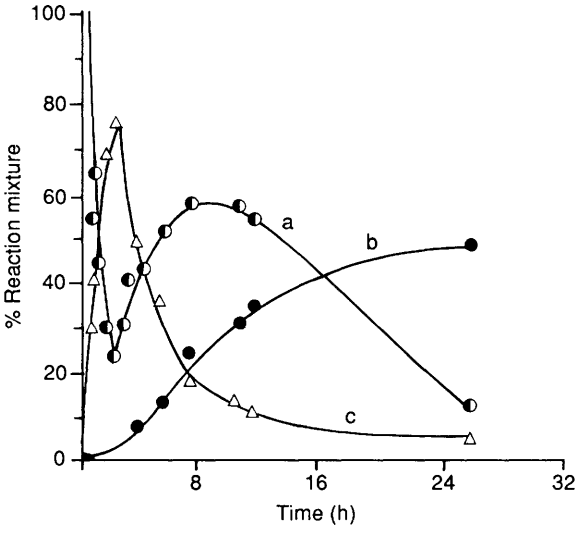


Fig. 2 a = ketone **1**, b = lactone **2**, c = alcohol **3**

using conventional chemical oxidants: however the appeal of the bio-oxidation system to the majority of organic chemists is not great, because of the need to grow up and then to emulate an organism such as *A. calcoaceticus*.

A monooxygenase enzyme in the microorganism is responsible for the Baeyer–Villiger oxidation. The enzyme has been isolated and purified[7] and this purified protein can be used to convert cyclic ketones into lactones.[8] The drawback to the use of the isolated enzyme is that a stoichiometric amount of the expensive co-factor [NADPH] must be used or the cofactor needs to be recycled (Fig. 1). NADPH recycling is not straightforward and is not the sort of protocol that is readily undertaken by organic chemists.

As a result of more detailed studies on the process **1** ⇒ **2** and similar oxidations, we believe we have now developed a process that may, in due course, prove to be more useful for the non-expert inasmuch as whole cells are not required for the bio-oxidation and co-factor recycling is accommodated within the new scheme.

Results and Discussion

A time-course experiment on the transformation (±)-**1** ⇒ **2** + (–)-**1** using fractured cells of *A. calcoaceticus* gave the following results (Fig. 2). Over the first 2.5 h period of the bio-transformation the concentration of the ketone dropped to *ca.* 20% of the initial value (by GC analysis) and the first formed product was found to be the corresponding alcohol **3** (by comparison with an authentic sample). The reduction is

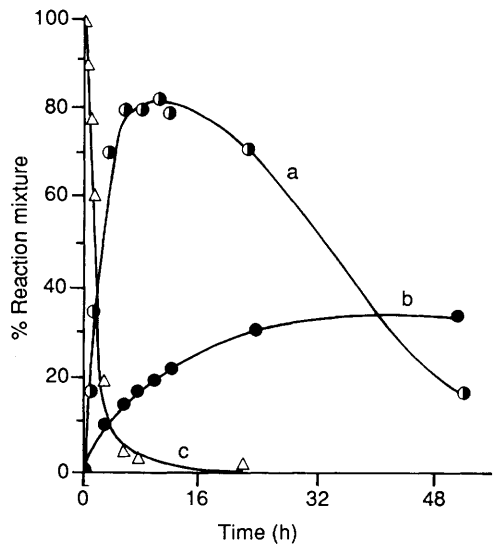
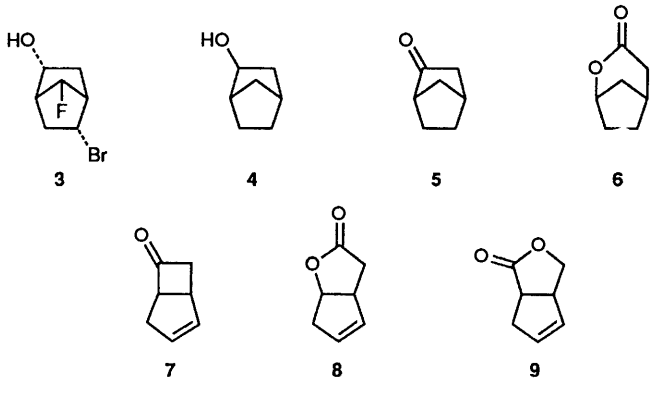


Fig. 3 a = ketone **1**, b = lactone **2**, c = alcohol **3**

seemingly non-enantiospecific since an 80% conversion of ketone to alcohol is observed. Over the next 3 h period the alcohol concentration decreased, the amount of ketone rose and the production of lactone commenced. Thereafter, until *ca.* 12 h the concentration of ketone remained at a fairly constant level (50–60%) as lactone was steadily produced. The only products observed to any extent after 16 h, as initially observed,[6] were the ketone (–)-**1** and the lactone **2**. The total concentration of identifiable material then decreases, as further metabolism of the product takes place.



Incubation of the alcohol **3** with fractured cells of the microorganism NCIMB 9871 followed a course (Fig. 3) very similar to that seen in Fig. 2 at time 150 min and thereafter. Thus, the alcohol was converted into ketone (*ca.* 80% over 6 h), lactone production steadily increases, and as a consequence the concentration of ketone diminishes after *ca.* 10 h.

The bio-oxidations of *exo*- and *endo*-bicyclo[2.2.1]heptan-2-ols *exo*-**4** and *endo*-**4** with fractured cells of the *Acinetobacter* sp. followed a similar pattern; that is, the alcohol concentration dropped as ketone **5** was produced and lactone **6** was observed in increasing concentrations during the course of the reactions.

As documented previously,[9] whole cell oxidation of the racemic bicyclo[3.2.0]heptenone **7** gave the corresponding oxabicyclo[3.3.0]heptenone system **8** and **9**. With *Acinetobacter* sp. NCIMB 9871 lactone production was complete after 3 h. Oxidation of 6*endo*-bicyclo[3.2.0]hept-2-en-6-ol **10** to the lactones **8** and **9** by fractured cells of *A. calcoaceticus* NCIMB 9871 was also very rapid, complete reaction being observed

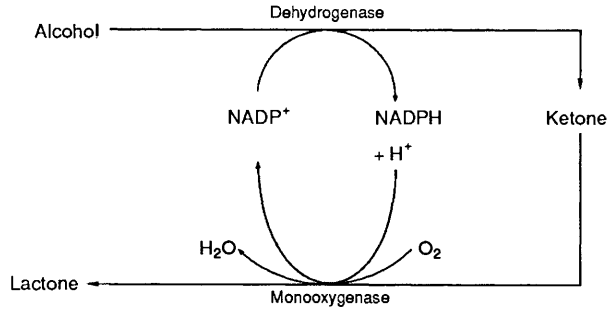
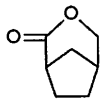


Fig. 4

within 60 min. The mixture of lactones was contaminated with another compound (*ca.* 15% by GC) which, on the basis of GC retention time, we believe to be 6*exo*-bicyclo[3.2.0]hept-2-en-6-ol.

endo-**10**



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The cell-based work obviously showed that the enzyme catalysed two-step conversion of a secondary alcohol into a lactone was feasible. We were interested to see if this biotransformation could be conducted with equal ease *in vitro*. We were excited to find that *endo*-bicyclo[2.2.1]heptan-2-ol (*endo*-**4**) and 6*endo*-bicyclo[3.2.0]hept-2-en-6-ol **10** were converted into the corresponding lactones using a coupled enzyme system and closed-loop recycling of the required $NADP^+$ cofactor (Fig. 4). Thus the alcohol dehydrogenase from *Thermoanaerobium brockii*[10,11] was used in conjunction with the purified monooxygenase from *Acinetobacter* sp. NCIMB 9871. *endo*-Bicyclo-[2.2.1]heptan-2-ol (*endo*-**4**) (15 mmol dm^{-3}) was transformed using 100 units of the alcohol dehydrogenase, catalytic $NADP^+$ (5 mmol dm^{-3}) and 40 units of the monooxygenase to give lactones **6** and **11** (67% yield; 79% conversion after 5.5 h by GC). The lactone **6** was the major component in the mixture of lactones (>95% pure, e.e. = 11%).

Under essentially the same conditions 6*endo*-bicyclo[3.2.0]-hept-2-en-6-ol **10** gave the lactones **8** and **9** in a 2:1 ratio (41% isolated yield; 95% conversion after 8.5h by GC); the (1*S*,5*R*)-lactone **8** was present in 86% e.e. Reduction of the amount of $NADP^+$ by a factor of 10 (to 0.5 mmol dm^{-3}) slowed the rate of reaction involving *endo*-bicyclo[2.2.1]heptan-2-ol (*endo*-**4**); after 6 h (29% conversion by GC) the reaction was quenched and the lactone **6** was obtained in 16% yield (e.e. = 56%).

The above-described conversions of the secondary alcohols *endo*-**4** and **10** into the corresponding lactones using a commercially available $NADP^+$ dependent dehydrogenase, a readily obtained mono-oxygenase, and an inexpensive protocol (*i.e.* catalytic in $NADP^+$) auger well for the invention of regio- and stereo-controlled transformations of use in organic synthesis.

Experimental

Oxidation of the Bicyclic Alcohol **10**.—6*endo*-Bicyclo[3.2.0]-hept-2-en-6-ol **10** (66.1 mg/0.6 mmol/15 mmol dm^{-3}), alcohol dehydrogenase (100 units), the sodium salt of nicotinamide adenine dinucleotide phosphate (153.2 mg/0.2 mmol/5 mmol dm^{-3}) and the monooxygenase (40 units) in 0.1 mol dm^{-3}-Tris/HCl buffer (40 ml) at pH 9.0 was shaken in an orbital incubator (200 rpm) at 30 °C: the reaction was followed by gas chromatography. After 8.5 h, 95% conversion into lactone was observed. The reaction mixture was frozen overnight and then extracted into dichloromethane (5 × 50 ml). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. The residue was chromatographed over silica gel using dichloromethane as the eluant giving, as a colourless oil, a mixture of the two lactones **8** and **9** in a 2:1 ratio (30.7 mg). Lactone **8** was present in 86% e.e. as judged by NMR spectroscopy using a chiral europium shift reagent.

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