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Microbiological Transformations. 21. An Expedient Route to Both Enantiomers of Marmin and Epoxyauraptens *via* Microbiological Dihydroxylation of 7-Geranyloxycoumarin

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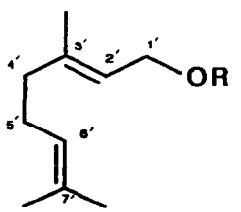
Abstract : The expedient synthesis of either enantiomers of Marmin or Epoxyauraptens of high enantiomeric purity is described. This was achieved *via* a stereospecific dihydroxylation of the remote double bond of a geraniol derivative using the fungus *Aspergillus niger*.

In the course of our work related to microbiologically mediated biooxygenations, we have described recently the stereoselective oxidation of the 6-7 double bond of geranyl N-phenylcarbamate 1 by the fungus *Aspergillus niger*[1]. We have shown in particular that, depending on the pH conditions, this can lead to either (6S) or (6R) enantiomer of 2 with ees as high as 95%. This bioconversion provides a remarkable short-cut to the synthesis of diol 2, a valuable chiral synthon which has been used for the synthesis of various natural products in optically active form[2]. We here describe that, using this new technique, it is possible to achieve a two-step synthesis of either enantiomer of marmin 3, a member of the umbelliferone family which has been extracted from various plants such as Indian *Aegle marmelos* Correa, Floridian Grapefruit *Citrus paradisi* peel oil, Californian *Geijera parvisflora*, *Aster praealtus* (Louisiana) or *Baecharis pteronioides* (Texas)[3].

This also allows formal synthesis of the biogenetically related 6',7'-epoxyaurapten 4 and of the bridged bicyclic 3',6'-epoxyaurapten 5, which have been prepared previously from marmin[4]. Both of these products have been isolated from plants[5] and were shown to possess attractive biological properties such as convulsion inhibition[6] as well as spasmolitic[7] or cytosolic hydrolase inhibition activities[8]. Some syntheses of these compounds have been previously published. However, these suffer from the fact that they either lead to racemic compounds[9] or necessitate lengthy routes to introduce the C(6') stereogenic center[4,10].

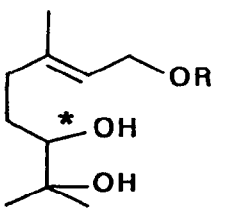
RESULTS AND DISCUSSION

We have noticed previously that the presence of the carbamate moiety of 1 is necessary for its biooxygenation into 2 to proceed, since this presumably plays the role of an anchoring group which assures positioning of the substrate into the enzymatic active site[11]. The synthesis of marmin enantiomers from 2 would necessitate several further steps to replace this urethane moiety by a coumarin entity. Obviously this drawback could be circumvented starting from 7-geranyloxycoumarin 6, provided the coumarin could be accepted as the anchoring moiety by the oxygenating enzyme, and would also confer enough lipophilic character to the substrate in order to allow the bioconversion to proceed[12]. Our results show that this is the case indeed. Thus, bioconversion of 6, following the previously described procedure (1b), led to a 60% yield of (6'S)-(-)-3 at pH 2, whereas (6'R)-(+)-3 is obtained at pH 6 in 43% yield. In both cases, the ee (determined using HPLC analysis of the diastereoisomers formed by reaction of 3 with (-)-camphanic acid chloride) is about 95%. This thus affords a *very expedient and efficient route to both enantiomers of marmin*, as well as the straightforward synthesis of either enantiomer of both 6',7'-epoxyaurapten 4 and 3',6'-epoxyaurapten 5 since it has been shown previously that (6'S) 3 leads to (6'R)-4 which itself affords (6'R)-5 without loss of optical purity[4]. Obviously (6'R)-marmin would lead to the (6'S)-epoxyaurapten 4 and 5 enantiomers. As in the case of 1, this bioconversion can be conveniently carried out on a several gram scale.



1 : R = -CONHC$_6$H$_5$

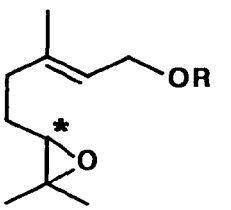
6 : R = Coumarin



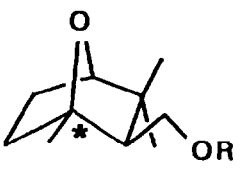
2 : R = -CONHC$_6$H$_5$

3 : R = Coumarin

Coumarin =



4 : R = Coumarin



5 : R = Coumarin

Aside from this very effective short-cut for the synthesis of compounds 3, 4 and 5 of high optical purity, we emphasize the fact that a coumarin moiety can advantageously be used instead of the carbamate moiety in this type of bioconversion. This observation is quite noticeable since it allows to broaden the range of the possible substrates for this type of microbiological transformations.

In conclusion, the biooxygenation of 7-geranyloxycoumarin 6 allows a two-step synthesis of marmin 3 which can be transformed into epoxyauraptens 4 and 5. Either enantiomer of these natural compounds, showing high optical purity, can be prepared very expediently and conveniently simply by controlling the pH of the bioconversion medium. This is without doubt by far the shortest route to these biologically active products.

EXPERIMENTAL PART

The main procedure used to carry out these bioconversions has been described previously (1b). However, the following further modifications have been used. The incubation was made by adding a piece of gelose supporting the mycelium and black spores aged of about 60 h. After 32 h growth at 29°C in a 7 L fermentor, 2.2 L of the 4 L culture was filtered off. The mycelium was suspended in 2 L of water and filtered again. The fungal cake was then separated into two equal parts and added to two 2 L fermentors, one containing 1 L of phosphate buffer (0.05 M; pH 6), the other one containing 1 L of HCl (0.013 M) - KCl (0.05 M) buffer (pH 2). A solution of 500 mg of substrate in 15 mL of ethanol was added immediately. The work-up was achieved as described previously.

(+)-Marmin : (6'R)-7-[(6',7'-dihydroxy-3',7'-dimethyl-2'-octenyl) oxy]coumarin 3.
After 40 h incubation at pH 6, 296 mg (yld 43%) of (+)-marmin 3 was obtained as a white solid after flash chromatography purification (ether/hexane = 0 to 100%). Mp 122-123°C [lit. 121°[4b]; 124-125°[3b]]; $[\alpha]^{26}_{D}$+16.3 (c 0.6, $CHCl_3$) [lit. $[\alpha]^{26}_{D}$+22.9 (c 0.6, $CHCl_3$)[4b]; $[\alpha]^{28}_{D}$+27 [3b]; $[\alpha]^{30}_{D}$+25 [3a]]. The ee was determined using HPLC analysis of the derivatives obtained by reaction of 3 with (-)-camphanic acid chloride and showed a value of 94%. Anal. Calcd : C, 68.66; H, 7.28; O, 24.03. Found : C, 68.68; H, 7.38; O, 24.03. ^{1}H NMR IR and mass spectra were identical with those described previously[3b,4b].

(-)-Marmin : (6S)-7-[(6',7'-dihydroxy-3',7'-dimethyl-2'-octenyl) oxy]coumarin 3.
Using the same procedure carried out at pH 2, 330 mg of (-)-marmin was obtained (yld 60%); Mp 120-122°C; $[\alpha]^{26}_{D}$-15.9 (c 0.6, $CHCl_3$); ee 93%. This compound can be recristallized to optical purity (hexane, ethyl acetate) and affords a product of $[\alpha]^{26}_{D}$-17.1 (c 0.6, $CHCl_3$)[4b]. Measured at λ = 436 nm, in order to compare it with the reported value : $[\alpha]^{26}_{436}$-81 (c 0.8, $CHCl_3$) we find a value of $[\alpha]^{26}_{436}$-31.9 (c 0.6, $CHCl_3$). No trace of the minor enantiomer is observed in the ee determination of our sample, showing a value

higher than 98%. Anal. Found : C, 68.74; H, 7.62; O, 23.67. ^{1}H NMR IR and mass spectra were identical with those of (+)-marmin 3 obtained at pH 6.

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