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ENANTIOSPECIFIC HYDROLYSIS OF ACETATES OF RACEMIC MONOTERPENIC ALCOHOLS BY *SPIRODELA OLIGORRHIZA**

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Key Word Index—*Spirodela oligorrhiza*; Lemnaceae; duckweed; biotransformation; (±)menthyl acetate; (±)bornyl acetate; (±)*trans*-2-acetoxy-*trans*-dihydropinol; (±) -*cis*-2-acetoxy-*trans*-dihydropinol; (±)-*trans*-2-acetoxy-*cis*-dihydropinol.

Abstract—Acetates of racemic menthol, borneol, *trans*-2-hydroxy-*trans*-dihydropinol, *cis*-2-hydroxy-*trans*-dihydropinol and *trans*-2-hydroxy-*cis*-dihydropinol undergo enantiospecific hydrolysis in cultures of *Spirodela oligorrhiza*. *R* Alcohols are formed faster than *S*. Absence of light inhibits hydrolysis of the first two acetates.

INTRODUCTION

In our previous papers, the reactivity of steroids and some derivatives of shikimic acid in cultures of *Spirodela oligorrhiza* was described. It was shown, that the clone of *S. oligorrhiza* was able to hydrolyse esters of androstane derivatives and esters of aromatic–aliphatic alcohols which underwent subsequent oxidation to their respective ketones. Both reactions were enantiospecific [2, 3]. These results encouraged us to use the clone *S. oligorrhiza* for the transformation of another group of secondary plant metabolites, acetates of selected monoterpenic alcohols.

*Part 25 of the series 'Biotransformations'. For part 24, see ref. [1.]

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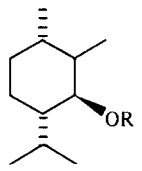
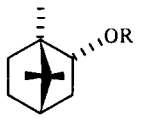
RESULTS AND DISCUSSION

The experiments were carried out according to a procedure previously described elsewhere [2].

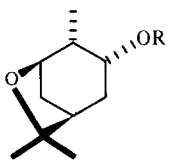
The substrates used for screening were cyclic monoterpenes, some of which were racemic mixtures. The results of screening which were followed by GC, revealed the ability of the clone to transform the selected substrates. We have found the following compounds to undergo transformation, menthyl acetate [(±) **1**], bornyl acetate [(±) **2**], *trans*-2-acetoxy-*trans*-dihydropinol [(±) **3**], *cis*-2-acetoxy-*trans*-dihydropinol [(±) **4**] and *trans*-2-acetoxy-*cis*-dihydropinol [(±) **5**]. Also (−) α-pinene, (−)-1,2-pinol, (±)*trans*-1-hydroxy-2,3-pinol and endo-2,6-acetoxy-1,8-cineol were transformed. The alcohols formed in the hydrolysis as well as the following substrates (−)-1-hydroxy-*cis*-dihydropinol, (±)*endo*-2-hydroxy-1,8-cineol, pinocarveol acetate, (±)-*trans*-1,2-epoxy-*cis*-dihydropinol, 1,8-cineol and (±)-2-oxo-1,8-cineol remained unaffected in the culture of *S. oligrrhiza*.



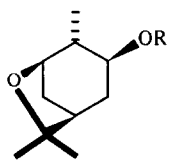
(±) **1** R = COMe
(±) **6** R = H



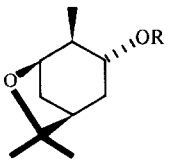
(±) **2** R = COMe
(±) **7** R = H



(±) **3** R = COMe
(±) **8** R = H



(±) **4** R = COMe
(±) **9** R = H



(±) **5** R = COMe
(±)**10** R = H

The extent of transformation was greatest for the first five substrates (±) **1**–(±) **5**. Therefore, transformations of these products were carried out on preparative scale and the products isolated and identified.

Menthyl acetate (**1**) and bornyl acetate (**2**) are commercially available, but 2-hydroxy-dihydropinol acetates (**3**–**5**) were prepared [4–6]. The monoterpenic bicyclic system of dihydropinol containing the tetrahydrofurane ring is known to be present in many compounds of natural origin, such as plant growth hormones and sesquiterpenes, as well as, for example, in the agarofurane group occuring in *Aquilaria agallocha* oil [7].

The first series of transformations was made by using 20 mg of substrates (±) **1**–(±) **5** per 100 ml of standard culture.

The only reaction observed was the hydrolysis of ester links of the substrates. Menthyl acetate hydrolysed enantiospecifically; (−) menthol [(−) **6**] was found to be the major product. Under the same conditions, bornyl acetate hydrolysed non-enantiospecifically; isolated borneol [(±) **7**] had no optical activity.

The three racemic 2-hydroxy-dihydropinol acetates (±) **3**– (±) **5** hydrolysed in *S. oligorrhiza* culture in the following way. Acetate (±) **3** hydrolysed enantiospecifically yielding within 14 days only (−)-*trans*-2-hydroxy-dihydropinol, [(−)**8**] and unreacted (+) **3**. Acetate (±) **4** reacted similarly as (±) **3** to yield (−)*cis*-2-hydroxy-*trans*-dihydropinol, [(−) **9**] and unreacted (+) **4**. Finally, acetate (±) **5** hydrolysed non-entiospecifically to form optically inactive (±)-*trans*-2-hydroxy-*cis*-dihydropinol, [(±) **10**], isolated together with unreacted (±) **5**.

The compounds were identified by comparing their spectra with those of authentic compounds. The entiospecificity of hydrolysis was observed for substrates (±) **1**, (±) **3** and (±) **4**. Only for (−)-menthol [(−) **6**], however, could the absolute configuration of the carbon atom with hydroxy group be concluded directly from the sign of optical rotation. It was the *R* configuration. (−) Menthol, the natural product, was formed faster than its enantiomer. For alcohols (−) **8** and (−) **9** formed from acetates (±) **3** and (±) **4**, respectively, one cannot deduce the configuration of their asymmetric carbon atom directly from the sign of optical rotation.

However, our earlier observation of the biotransformability of *S. oligorrhiza* [3] lead us to conclude, that alcohols (−) **8** and (−) **9** formed in excess have the *R* configuration. This suggestion is based on the hydrolysis of racemic aromatic–aliphatic esters [3] and on the hydrolysis of (±) menthyl acetate [(±) **1**], where *R* alcohols were formed.

In further experiments, hydrolysis of acetates (±) **1** and (±) **2** was carried out at two different concentration of substrates in cultures of *S. oligorrhiza* (Figs 1 and 2). Both acetates hydrolysed completely within 14 days, irrespective of whether 20 or 100 mg of acetate was used per 100 ml of standard culture, but the courses of hydrolysis, however, were different. The plant lost its green colour after nine days at the lower concentration of both substrates or after three days at the higher one. After a prolonged transformation experiment, the plant died. The effect of illumination upon the degree of hydrolysis was also studied. Acetates (±) **1** and (±) **2** were introduced into cultures and kept under illumination or in darkness. At a concentration of 100 mg of (±) **1** per 100 ml culture, 23% hydrolysis was found after 10 days

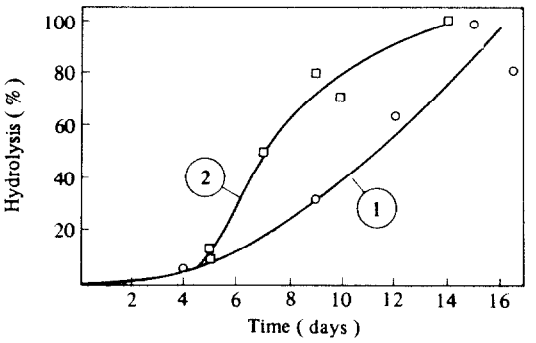


Fig. 1. Degree of hydrolysis of menthyl acetate (**1**) (○) and bornyl acetate (**2**) (□) with time. The concentration of substrate was 1 g/dm³.

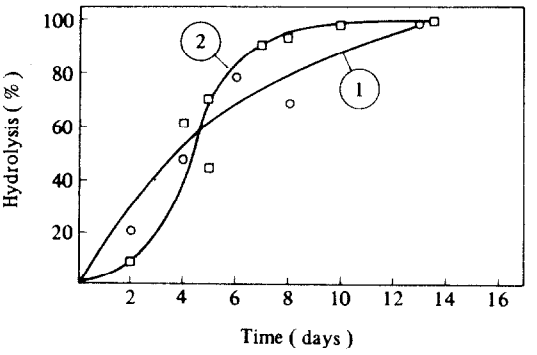


Fig. 2. Degree of hydrolysis of menthyl acetate (**1**) (○) and bornyl acetate (**2**) (□) with time. The concentration of substrate was 0.2 g/dm³.

in light but only 7% in darkness. Similar experiments made with 20 mg of (±) **2** after five days: 75% hydrolysis occurred with light and 9% in darkness.

The results presented above, as well as those published before [2, 3], show that *S. oligorrhiza* species are capable of hydrolysing esters of alcohols belonging to three different groups of natural compounds, viz. steroids, shikimic acid derivatives and monoterpenes.

EXPERIMENTAL

Cultivation of the *S. oligorrhiza* clone and preparation of cultures is described elsewhere [2]. Substrates (20–100 mg) were introduced directly into the cultures. Flasks containing cultures and substrates were shaken for 1–14 days; products were then extracted with $CHCl_3$ and analysed.

TLC was carried out on silica gel (Merck) with hexane–Me_2CO mixts (10:1 or 3:1) as eluents. GC was performed on an FID instrument on the following columns: 3% OV-17 on Gas-Chrom Z, 80–100 mesh; 10% DEGA on Chromosorb W AW DMCS, 80–100 mesh; 10% Carbowax 20 M on Chromosorb W AW, 80–100 mesh; 10% SE 30 on Chromosorb W AW DMCS 80–100 mesh. Temps were 120–180°. N_2 (50 ml/min) was used as carrier.

Preparative sepns were made on silica gel (Merck) columns using hexane–Me_2CO mixts (10:1 and 4:1).

Transformation of menthyl acetate [(±) **1**]. Substrate (120 mg) was divided among 6 flasks containing *S. oligorrhiza* cultures.

The reaction was carried out for 3 days. A mixture (97 mg) was isolated and sepd chromatographically to yield 50.2 mg of menthol $[\alpha]_{Hg}^{20} = -2.0°$ and 32 mg of unreacted substrate **1**. For the time course of hydrolysis, the transformation of (±) **1** as described above was carried out for 2, 3, 4, 6, 8, and 13 days with the amount of substrate at 20 mg per 100 ml of culture and for 4, 7, 12, 15, and 16 days with 100 mg of substrate. The effect of illumination was analysed using 6 flasks with standard cultures to each of which 100 mg of (±) **1** was added. Three of these flasks were shaken in darkness, the other 3 kept under illumination. After 10 days, the products of transformation were isolated.

Transformation of bornyl acetate [(±) **2**] was carried out as described above.

After five days 87 mg of a mixt was isolated from an initial dose of 120 mg and sepd chromatographically to yield 11 mg of borneol (**7**) and 20 mg of unreacted substrate **2**. Both compounds were optically inactive. Similar expts were performed with 20 or 100 mg of the substrate per 100 ml culture. Transformations were sampled after 2, 3, 4, 6, 8, and 13 days. The effect of illumination was analysed in two simultaneous expts with three samples each with 20 mg of substrate (±) **2** in 100 ml of culture in light or in darkness. After 5 days, the products were analysed.

Transformations of trans-2-*acetoxy*-trans-*dihydropinol* [(±) **3**], cis-2-*acetoxy*-trans-*dihydropinol* [(±) **4**] *and* trans-2-*acetoxy*-cis-*dihydropinol* [(±) **5**] were carried out as described above using 120 mg of substrate. From product mixts the following compounds were isolated; from (±) **3**: 13 mg of alcohol (−) **8**; $[\alpha]_{Hg}^{20} -69.6°$, 22 mg of ester (−) **3**; $[\alpha]_{Hg}^{20} +56.8°$; from (±) **4**: 17 mg of alcohol (−) **9**: $[\alpha]_{Hg}^{20} -11°$, 14 mg of ester (+) **4**; $[\alpha]_{Hg}^{20} +27°$; from (±) **5**: 18 mg of alcohol **10**, 21 mg of ester **5**; both compounds were optically inactive.

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