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ENANTIOSELECTIVE HYDROLYSIS OF ESTERS AND THE OXIDATION OF AROMATIC-ALIPHATIC ALCOHOLS OBTAINED THEREFROM BY *SPIRODELA OLIGORRHIZA**

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Key Word Index—*Spirodela oligorrhiza*; Lemnaceae; duckweed; biotransformation; benzyl-acetate; (±)-1-phenyl-ethyl-acetate; (±)-1-(1-naphthyl)-ethyl-acetate; (±)-1-(2-naphthyl)-ethyl-acetate; (±)-2-phenyl-butyl-acetate; acetophenone; 2-acetyl-naphthalene.

Abstract—*Spirodela oligorrhiza* hydrolyses benzyl acetate and enantiospecifically hydrolyses the acetates of racemic mixtures of 1-phenyl-ethanol, 1-(1-naphthyl)-ethanol, 1-(2-naphthyl)-ethanol, and 2-phenyl-butanol. The *R* alcohols are formed faster. Two of the resulting alcohols, 1-phenyl-ethanol and 1-(2-naphthyl)-ethanol, undergo an enantiospecific oxidation. Here, the *S* enantiomers react faster.

INTRODUCTION

In a previous paper [2], we reported that *Spirodela oligorrhiza* (Kurz.) Hegelm is capable of hydrolysing testosterone and androstenolone acetates. Further transformations of testosterone yielded androstenedione.

Since the hydrolysis of the two steroid esters occurred in relatively high yield, we next turned our attention towards the transformation of esters of other structures.

RESULTS AND DISCUSSION

All the transformations were carried out in sterile conditions under continuous illumination as described elsewhere [2, 3]. The substrates were added to 2-week-old cultures. The amounts of substrates ranged from 5–120 mg [standard sample: 20 mg/100 ml of culture containing on average 230 plants (1.04 g fresh wt)]. The experiments lasted for 1–18 days. Higher concentrations of the esters caused the plants to die.

In a series of screening tests, it was shown by GC analysis that the clone of *S. oligorrhiza* used in these studies was able to hydrolyse citronellyl acetate (23% conversion), benzyl acetate (**1**) and the acetates of racemic mixtures of 1-phenyl-ethanol (**2**), 1-(1-naphthyl)-ethanol (**3**), 1-(2-naphthyl)-ethanol (**4**) and 2-phenyl-butanol (**5**). It was, however, unable to effect any transformations on the long chain aliphatic esters, methyl palmitate, methyl stearate, cetyl acetate and dodecyl acetate, and methyl-2-phenyl butyrate, an ester in which the aromatic fragment contains the acidic moiety. In the cases of compounds **2** and **4** and citronellyl acetate, the alcohols produced in the reactions underwent further transformations. It is noteworthy that esters **1**–**5** as well as some of the products of their transformations (see below) can either be found among derivatives of shikimic acid [benzyl alcohol and acetophenone (**7**)] or they constitute structurally extended analogues of those derivatives (**3**, **4** or **5**) [4, 5].

The transformation of esters **1**–**5** was studied in detail in a series of preparative experiments.

Benzyl alcohol was the only product formed from benzyl acetate (**1**), which was the simplest ester studied. At a concentration of 20 mg/100 ml, the ester was hydrolysed completely within 14 days. At higher concentrations, the degree of hydrolysis decreased until at 120 mg/100 ml only about 10% of the ester was hydrolysed (Fig. 1). The higher concentrations of the ester caused the death of the plants.

Biotransformations become practically useful at substrate concentrations of about 100 mg/100 ml, i.e. 1 g/l. At this concentration the degree of hydrolysis of **1** reaches about 15% after 17 days (Fig. 2).

In the experiments involving the racemic esters **2**–**5**, attention was focused on the enantioselectivity of the transformation. The experiments were carried out for 1–7 days and were repeated several times in order to obtain sufficient amounts of the products for their identification whilst at the same time preserving some unreacted esters to detect changes in their optical activity. All the substrates were hydrolysed (Table 1). In two cases (**5** and **9**), however, the resultant alcohols were then subject to oxidation (Table 1).

The transformation products were separated chromatographically and identified by comparing their properties with those of the original compounds (see Experimental). The absolute configurations of optically active alcohols as well as those of unreacted esters were established by comparing their specific rotations with literature data [6].

All the transformations were found to be enantioselective with the *R* enantiomer reacting faster than the *S* enantiomer (Table 1). The *R*:*S* ratios quoted in Table 1 for the alcohols obtained by the hydrolysis of their acetates by *S. oligorrhiza* indicate that, when taking into

*Part 20 of the series "Biotransformations"; for part 19 see ref. [1].

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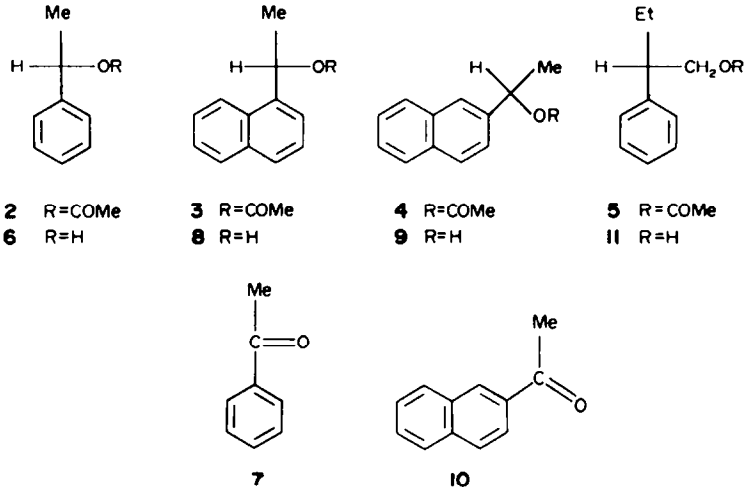

2 R=COMe **6** R=H

3 R=COMe **8** R=H

4 R=COMe **9** R=H

5 R=COMe **11** R=H

7

10

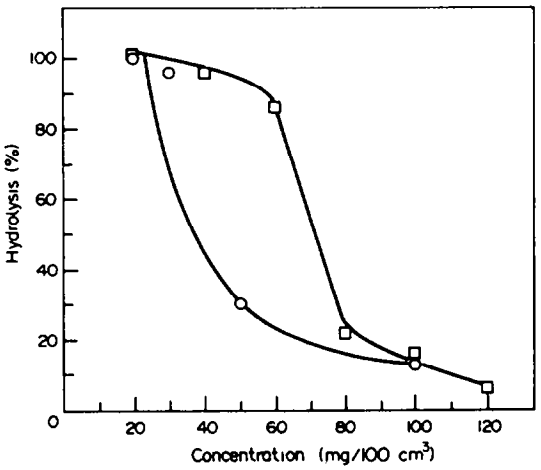

Fig. 1. The degree of hydrolysis after 14 days of different concentrations of benzyl acetate (**1**) (O——O), and of 2-phenyl-butyl acetate (**5**) (□——□).

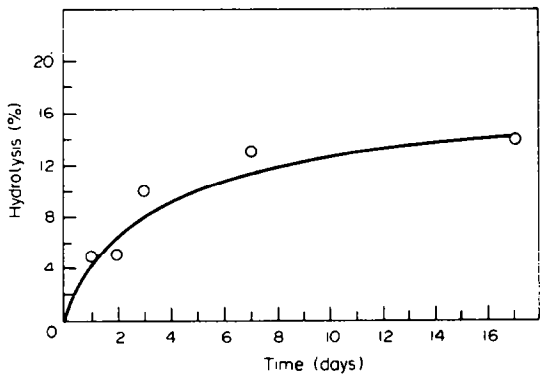

Fig. 2. The degree of hydrolysis of benzyl acetate (**1**) with time. The concentration of the substrate was 1 g/l.

account the extent of reaction, the highest enantioselectivity occurred in the transformation of the racemic mixture of **2**. It decreased for the racemic mixtures of **3** and **4** and was smallest for the racemic mixture of **5**. The absolute configuration of alcohol (−)-**11** formed from (±)-**5** could not be determined.

As mentioned above, alcohols **6** and **9** obtained from **2** and **4**, respectively, were subsequently oxidized to the corresponding ketones **7** and **10**, respectively (Table 1). Therefore, in additional experiments, the racemic mixtures of the alcohols **6** and **9** were subjected to transformation by the plant under the same conditions as for their acetates. The products were separated from unreacted alcohols and identified spectrally. The oxidation was enantioselective, too (Table 1). The alcohols (−)-**6** and (−)-**9** with *S* configuration were oxidized faster than their enantiomers yielding ketones **7** and **10**, respectively.

In similar experiments, racemic mixtures of 1-(1-naphthyl)-ethanol (**8**), 2-phenyl-butanol (**11**), and 1-(2,4-dimethyl-phenyl)-ethanol remained unchanged.

The oxidation of (±)-1-phenyl-ethanol (**6**) to acetophenone (**7**) is a reversible reaction in which the ketone is reduced back to optically inactive starting alcohol **6**. The degree of the reduction measured in a separate experiment was 37%.

Of all the racemic esters, 2-phenyl-butyl acetate (**5**) was hydrolysed at the highest rate. At a concentration of 20 mg per 100 ml of culture, the hydrolysis was completed after 14 days (Fig. 1). A four-fold increase in the ester concentration reduced the degree of hydrolysis to nearly 13%, but the plants died at this concentration.

The survival of the plants was measured as a function of the amount of **5** added (Fig. 3). The plants survived for up to 18 days at a substrate concentration of 5 mg per 100 ml of culture. The culture preserved its green colour and was capable of reproduction. At the highest concentration (30 mg/100 ml) the culture survived for 4 days. The degree of hydrolysis was not measured in the latter experiment, but can be deduced from Fig. 1.

In conclusion, it is suggested that *S. oligorrhiza* is capable of hydrolysing low M_r esters in a similar way to microorganisms [7]. In some cases, secondary oxidation of the resulting alcohols occurs. Both reactions are

Table 1. Transformation of compounds **2**–**5** and oxidation of racemic mixtures of alcohols **6** and **9**

Substrate	Time of transformation (days)	Degree of hydrolysis (%)	Products: Major product	Products: Unreacted substrate	Products: Products of further transformation
Esters			*Alcohols*		
(±)-**2**	4	50	(+)-**6** $R:S = 87:17$	(−)-**2**	7 traces
(±)-**3**	7	33	(+)-**8** $R:S = 73:27$	(−)-**3**	None
(±)-**4**	7	15	(+)-**9** $R:S = 68:32$	(−)-**4**	**10** traces
(±)-**5**	1	43	(−)-**11**	(+)-**5**	None
Alcohols			*Ketones*		
(±)-**6**	14	64	7	(+)-**6** $R:S = 85:15$	None
(±)-**9**	14	11	**10**	(+)-**9** $R:S = 83:17$	None

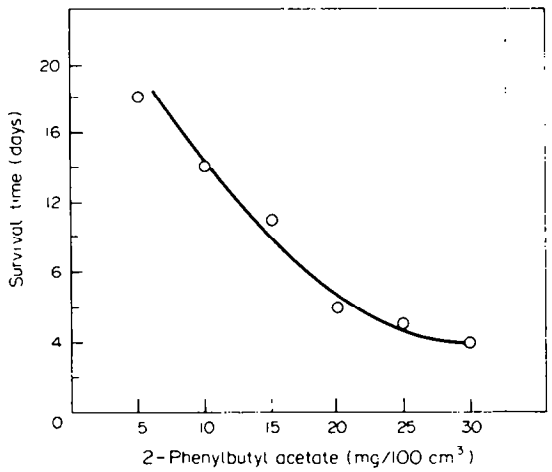

Fig. 3. The survival time of *S. oligorrhiza* in the presence of different amounts of 2-phenyl-butyl acetate.

enantioselective with respect to the substrates. The structure of a substrate has a marked effect upon the rate of hydrolysis. Thus: (±)-**5** is easily hydrolysed whereas (±)-methyl-2-phenyl-butyrate, which differs from **5** in that it has the acidic group in its aromatic fragment, is not hydrolysed; alcohols (±)-**6** and (±)-**9** are oxidized whereas structurally similar alcohols either do not react at all, e.g. (±)-1-(2,4-dimethyl-phenyl)-ethanol or react, very slowly, e.g. (±)-**8**. It should also be pointed out that the esters of primary alcohols, e.g. **1** and **5**, are hydrolysed even at high concentrations (1 g/l.).

* The clone was taken from the collection of the Department of Botany and Physiology of Plants, Agricultural University, Wroclaw

EXPERIMENTAL

The selected clone* of *S. oligorrhiza* was cultured on a Bollard nutrient [3]. A 3% soln of TSB (Difco, U.S.A.) nutrient was used to control the sterility of the cultures. Solid substrates were added in Me_2CO or EtOH soln, liquid substrates were added directly with a microsyringe. After each transformation experiment the cultures were extracted with $CHCl_3$ and the extracts analysed.

TLC was carried out on silica gel (Merck, Darmstadt) developed with petrol–Me_2CO (5:1). GC was carried out as follows: 2 m × 4 mm (i.d.) columns, filled with 3% OV-17 on Gas-Chrom Z (80–100 mesh), 10% OV-17 on Chromosorb WAW DMCS (80–100 mesh), 10% SE-30 on Chromosorb WAW DMCS (60–80 mesh) or 10% DEGA on Chromosorb WAW DMCS (80–100 mesh), N_2 50 ml/min, FID 3.2×10^{-10} AFS. Preparative CC was carried out according to [8] using silica gel (Merck, Darmstadt) (230–400 mesh) developed with petrol–Me_2CO (5:1 or 8:1) as eluents

Preparation of the acetates **1**–**5**. These were prepared by acetylation of the appropriate parent alcohols with Ac_2O in C_5H_5N. The products were characterized by their IR and NMR spectra (data not shown).

Transformation of benzyl acetate (**1**). This was carried out for 14 days after introducing 20–120 mg of the ester to 100 ml of culture. The products were separated and analysed chromatographically. Ester **1** and benzyl alcohol were found (Fig. 1).

The time dependence of the degree of hydrolysis of **1** was measured in five separate 100 ml cultures to each of which 100 mg of the esters was introduced. The cultures were extracted at predetermined times and the extracts analysed chromatographically (Fig. 2).

Transformation of (±)-1-*phenyl-ethyl acetate* (**2**). 120 mg of the substrate was transformed for 4 days, 83 mg of products was obtained. The crude mixture was analysed by GC and gave the results listed in Table 1. Unreacted ester **2** and alcohol **6** were isolated by CC. $[\alpha]_{20}^{Hg} = +33.3°$; optical purity: 66.7%.

Transformation of (±)-1-(1-*naphthyl*)-*ethyl acetate* (**3**). This was carried out for 7 days with 120 mg of (±)-**3**. 94 mg (78%) of products was extracted. Unreacted **3** and pure alcohol **8** were isolated. **8**: $[\alpha]_{20}^{Hg} = +35.1°$; optical purity: 45.7%.

Transformation of (±)-1-(2-*naphthyl*)-*ethyl acetate* (**4**). This was

carried out for 7 days with 120 mg of (±)-**4**. Unreacted **4** and pure alcohol **9** were isolated by CC. $[\alpha]_{20}^{Hg} = +14°$; optical purity: 36.3%.

Transformation of (±)-*2-phenylbutyl acetate* (**5**). This was carried out for 1 day. Unreacted **5** and pure alcohol **11** were isolated by CC. To establish the optimal concentration of the ester, 20, 30, 50 or 100 mg of (±)-**5** was added to 100 ml of culture. Results are shown in Fig. 1.

Transformation of (±)-*1-phenylethanol* (**6**). The substrate was obtained by reduction of acetophenone with $NaBH_4$ [9]. The transformation was carried out for 14 days with 120 mg of (±)-**6**. 50.3 mg of a mixture was obtained from which alcohol **6**, $[\alpha]_{20}^{Hg} = +35.3°$; optical purity: 71% and acetophenone (**7**) were isolated.

Transformation of (±)-*1-(2-naphthyl)-ethanol* (**9**). The substrate was obtained by reduction of 2-acetyl-naphthalene, under the same condition to those just described. The transformation was carried out for 14 days. CC yielded pure **9** $[\alpha]_{20}^{Hg} = +25.3°$; (optical purity: 67%) and **10**.

Transformation of acetophenone (**7**). Acetophenone was transformed for 9 days in standard conditions. Unreacted **7** and alcohol **6** (yield, 37%) were isolated by CC.

Toxicity test. To 100 ml samples of *S. oligorrhiza* culture, 5, 10, 15, 20, 25 or 30 mg of ester (±)-**5** was added and the time taken for the plants to die measured (Fig. 3). Control samples showed no changes during the time of experiment.

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