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TRANSFORMATION OF ANDROSTANE DERIVATIVES BY *SPIRODELA OLIGORRHIZA**

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Key Word Index—*Spirodela oligorrhiza*; Lemnaceae; duckweed; biotransformation; testosterone acetate; androstenolone acetate; testosterone; androstenolone; androstenedione; 5α-androstanedione.

Abstract—*Spirodela oligorrhiza* (duckweed) is capable of transforming some steroids of the androstane series. Hydrolysis of the acetates of testosterone and of 3β-hydroxyandrost-5-en-17-one by this species yielded the corresponding alcohols. Further transformation of testosterone and reduction of androst-4-ene-3,17-dione indicated the interconversions of the hydroxyl-ketone function on C-17 and reduction of the Δ^4-double bond to the *trans-A/B* system. Only a trace amount of 3β-hydroxyandrost-5-en-17-one underwent further transformations.

INTRODUCTION

Several reports have appeared concerning the isolation of androstane derivatives from higher plants [1–3]. These reports provide evidence for the ability of some plant species to synthesize these compounds and suggest the use of the plants for studies on the transformations of a series of C_{19}-steroids. Suspension cultures of *Dioscorea deltoidea* [2] were able to reduce the carbonyl group on C-3 and the double bond in androst-4-ene-3,17-dione. Also a culture of *Nicotiana tabacum* exhibits similar capabilities [3]. The transformation of [4-^{14}C]androst-4-ene-3,17-dione by *Pisum sativum* at different stages of its growth was also described [4]. The labelled substrate underwent a reduction to yield testosterone.

The above mentioned papers exemplify the ability of higher plants to metabolise steroids. This subject has been reviewed by Reinhard and Alfermann [5].

RESULTS

In the present paper, the application of a clone‖ from *Spirodela oligorrhiza* for the controlled biotransformation of organic compounds is described. Some preliminary results have been reported [6]. In the aqueous media the culture of *S. oligorrhiza* provides convenient conditions for carrying out such biotransformations. The cultures were grown under standard conditions on Bollard's [7] nutrient medium with continuous illumination and under aseptic conditions. To cultures of *S. oligorrhiza* steroid substrates were added and after 2 weeks of shaking, the mixture of products was extracted and analysed chromatographically. TLC and GLC analyses indicated that steroids from the C_{19} group underwent transformation (Table 1). Under the experimental conditions, the presence of trace amounts of metabolites, originating from the *S. oligorrhiza* culture were found in the chloroform extracts.

As both chromatographic methods used indicated, the transformation of testosterone acetate (1), and androstenolone acetate (2) proceeded to the greatest extent. Therefore, the metabolism of these two steroids was investigated in detail. The transformations of both steroid acetates by *S. oligorrhiza* were carried out in several

Table 1. Screening of selected steroids transformed by *Spirodela oligorrhiza*

Substrate	Reactivity*
Testosterone acetate (1)	+ (a, b)†
3β-Hydroxyandrost-5-en-17-one acetate (2)	+ (a, b)
Testosterone propionate	+ (a, b)
Testosterone (3)	+ (a, b)
3β-Hydroxyandrost-5-en-17-one (4)	+ (a, b)
Androst-4-ene-3,17-dione (5)	+ (a, b)
5α-Androstane-3,17-dione (6)	+ (a, b)
19-Hydroxyandrost-4-ene-3,17-dione	+ (a)
5α-Dihydrotestosterone	+ (a)
5β-Dihydrotestosterone	+ (a)
19-Nor-testosterone	+ (a)
17α-Methylandrost-4-ene-3β,17β-diol	+ (a, b)
Progesterone	+ (a)
Cholesterol	− (a)
Sitosterol	− (a)

* + denotes the presence of transformation products along with the starting material, − denotes the presence of the starting material only.

†a = analysis by TLC. b = analysis by GLC.

* Part 18 of the series "Biotransformations". For Part 17 see *J. Basic Microb.* (1985) 25, 1.

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‖The clone originated from the collection of the Department of Botany and Physiology of Plants, Agricultural University of Wroclaw.

parallel runs to obtain sufficient quantities of products for their structural analysis. The composition of the products was analysed by GLC according to Schöller and Jayle [8]; the error of the GLC analyses did not exceed 10%. Individual compounds were isolated by chromatography on silica gel columns [9]. The identification of individual products was made by comparing their retention times with those of authentic compounds and also confirmed by spectroscopic analysis of pure isolated compounds.

It was found that testosterone (3), androst-4-ene-3,17-dione (5), and 5α-androstane-3,17-dione (6) were formed from steroid acetate 1, while 3β-hydroxyandrost-5-en-17-one (4), and traces of 3 and 5 were formed from steroid acetate 2. The gas chromatograms of the reaction products obtained from 1 and 2 showed the presence of other compounds which could not be isolated in pure state and were not identified. Some spectral data of one of them (compound 7) obtained from acetate 2 are given in the Experimental section.

The results obtained for steroid acetates 1 and 2 indicated that further transformation of products formed after the hydrolysis of the ester bond had occurred. For this reason, the products of the first reaction (3, 4 and 5) were also subjected to transformation in the same manner. The presence of compounds 3, 5 and 6, as shown in Table 2, was demonstrated in the products of transformation of both 3 and 5. The transformation of 4 yielded trace amounts of 3 and 5. Compound 7 appeared again accompanied by other unidentified products.

DISCUSSION

The application of whole plants for carrying out controlled chemical reactions on a preparative scale encounters difficulties connected with the adaptation of standard cultivation to the requirements of the chemical laboratory. The use of tissue cultures of plants considerably facilitates investigations, but the methods for their use require special techniques and often the results obtained do not reflect the metabolic possibilities of the whole plant from which the culture was prepared [5]. The application of plants, the growth of which in an aqueous medium becomes similar to that of microorganisms and tissue cultures, should help to avoid these difficulties.

The results presented here are an attempt to utilize such a water plant, *Spirodela oligorrhiza*, the culture of which is suitable for carrying out controlled transformations of organic compounds [6].

Table 2. The composition of the transformation products as determined by GLC

Transformed substrate	Composition of transformation products (%)						
	1	2	3	4	5	6	u*
Testosterone acetate (1)	58		16		8	7	12
3β-Hydroxyandrost-5-en-17-one acetate (2)		40	1	41	1		19
Testosterone (3)			87		8	5	
3β-Hydroxyandrost-5-en-17-one (4)			1	64	1		35
Androst-4-ene-3,17-dione (5)			13		82	5	

* Unidentified compounds.

The Lemnaceae are widespread all over the world [10] and members have been the subject of some chemical investigations. Among other compounds, the presence of sterols in some species of *Spirodela* was reported and these included campesterol, sitosterol and stigmasterol [11, 12]. A positive effect of the addition of estradiol and progesterone on florescence of *Lemna minor* was observed [13]. These studies provided some basis for the expectation that *S. oligorrhiza*, which belongs to the Lemnaceae family, would be able to transform steroid substrates.

The screening of the clone used by us proved that indeed it is able to transform some steroids, including practically all the androstane derivatives examined (Table 1).

The hydrolysis of the ester bond was the main reaction observed in the transformations of the steroid acetates 1 and 2. The amounts of identified products and unreacted substrates 1 and 2 are indicated in Table 2. The product of hydrolysis of acetate 1, i.e. testosterone (3), was metabolized further, whereas 3β-hydroxyandrost-5-en-17-one (4), formed from acetate 2, was virtually unmetabolized. In the first case, the oxidation of the hydroxy group at C-17 to a carbonyl group was observed and the androstenedione (5) formed was then reduced to 5α-androstane-3,17-dione (6). These reactions, however, occurred with lower yields than the hydrolysis of the esters (Table 2).

The second alcohol (4) was resistant to oxidation at C-3 and isomerization of the Δ^5-double bond. These reactions proceeded to a minimal extent.

The investigations on the course of the transformations of substrates 3 and 5 confirmed the results obtained from the transformation experiments performed for the acetate 1. A selective ability was observed to reduce the Δ^4-bond of androstenedione (5) to a 5α-system only (diketone 6 was formed). This reaction proceeded only in the presence of a carbonyl group on C-17. Testosterone (3) did not undergo a similar reduction of the double bond. The second reaction observed was the mutual oxidation and reduction of an oxygen function on C-17 in both substrates (3 and 5).

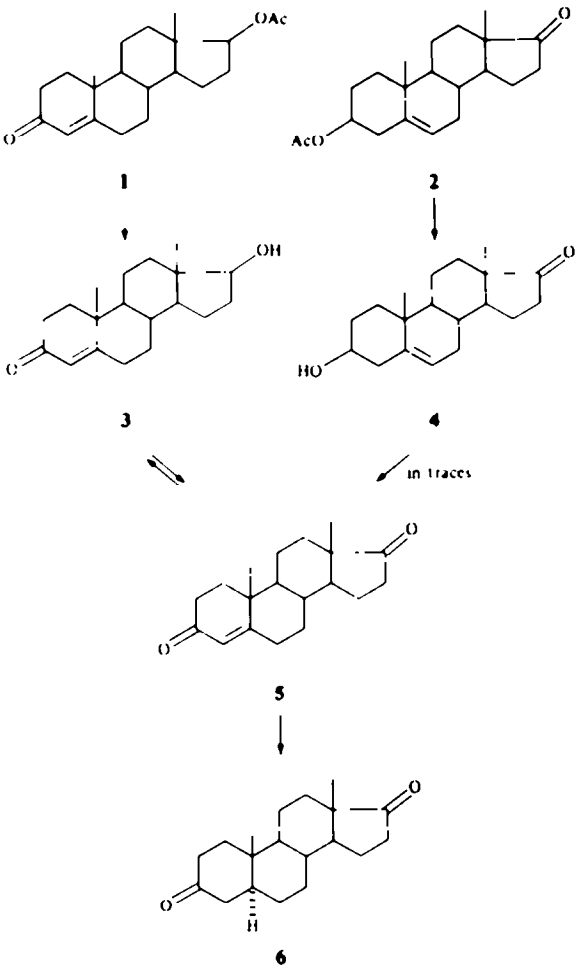
The results of the incubation of substrate 4 with *S. oligorrhiza* confirmed its resistance to metabolism by the plant.

Small amounts of unidentified compounds with GLC retention times lying outside the range of those for steroids (Table 2) were also detected as the products of transformation of 1, 2 and 4. These compounds will be the subject of further investigations.

The transformations of compounds 3 and 5 were found to be similar to the metabolism of testosterone in mammals [14]. The same process was also observed in the transformation of testosterone by microorganisms [5, 15]. The results obtained in this work show that the effectiveness of *S. oligorrhiza* in the transformation of steroids is far smaller than that of microorganisms. However, it still has value in providing knowledge on the steroid metabolizing capabilities of this species.

EXPERIMENTAL

Cultivation of the clone of Spirodela oligorrhiza. The nutrient medium adapted for cultivation of cultures used directly for steroid transformations was prepared according to Bollard [7].



Scheme 1.

For cultivation of the parent strain, the nutrient was additionally enriched with L-asparagine (200 mg/l) and glucose (10 g/l).

Nutrient (100 ml) in 250 ml conical flasks was inoculated with the culture of *S. oligorrhiza* from the parent strain. The growth was carried out at 27° with continuous illumination (1000–1200 lx, fluorescent tube) for periods up to 14 days, until the whole surface area (38 cm^3) of the nutrient was covered with the plant. Substrate (10–20 mg) dissolved in *ca* 1 ml of Me_2CO or EtOH was added to the culture prepared in this way. The flask was then constantly shaken and the transformations carried out for 10–14 days. The experiments were repeated several times and were found to be satisfactorily reproducible. The rate and intensity of shaking were regulated to prevent the plant sinking. During the experiment the plants died and changed their colour from green to white. After the experiment, the plants did not reproduce when transferred into another nutrient.

The growth medium was extracted with $CHCl_3$ and the extract was analysed. No steroids were found to be present in the mass of the plant tissue after extraction.

TLC was carried out on silica gel (Merck, Darmstadt). C_6H_6–EtOAc (2:1) and petrol–EtOAc (3:2) mixtures were used as eluents.

GLC was carried out on an N 504-Elwro (Wrocław, Poland) apparatus equipped with 2 m columns of 3% OV-17 on Gas-Chrom Z 80–100 mesh or 3% OV-225 on Chromosorb W AW DMCS 60–80 mesh and a 1.3 m column of 2% OV-275 on Chromosorb W AW DMCS 80–100 mesh; temperatures: 270, 250 and 250°, respectively. Carrier gas: nitrogen (50 ml/min). A flame-ionization detector (sensitivity 4×10^{-10} AFS) was used.

The preparative separations were carried out on columns of silica gel 230–400 mesh, eluent: petrol–EtOAc (3:2) [9].

IR, UV, MS and ^{1}H NMR spectra were recorded on UR-10 (Carl-Zeiss, Jena), Specord UV-vis (Carl-Zeiss, Jena), LKB 9000 and Jeol JNM-4 (100 MHz) spectrometers, respectively.

Transformation of compound **1**. Acetate **1** was obtained by esterification of testosterone with Ac_2O. IR: 1750, 1690 cm^{-1}; mp 140–142°, $[\alpha]_{546}^{20} = +114°$. The transformation was carried out in the manner described by adding 110 mg of substrate to five flasks; 72 mg of the mixture of products was isolated. The results of identification and quantitative analysis made by GLC are shown in Table 2. The preparative chromatography of 36 mg of the transformation product provided pure compounds **1**, **3**, **5** and **6**. Product **3**: mp 150–152°, $[\alpha]_{546}^{20} = +134°$ ($c = 1.2$, $CHCl_3$); IR ν_{max}^{Nujol} cm^{-1}: 3640, 1690. Compound **5**: mp 168–172°, $[\alpha]_{546}^{20} = +199°$ ($c = 1.6$, $CHCl_3$); IR ν_{max}^{Nujol} cm^{-1}: 1750, 1690. Compound **6**: mp 129–133°, $[\alpha]_{546}^{20} = +113°$ ($c = 1.15$, $CHCl_3$); IR ν_{max}^{Nujol} cm^{-1}: 1738, 1725. Products **3**, **5** and **6** were identified by comparing their spectra with those of the authentic compounds.

Transformation of compound **2**. The starting material (**2**) had $[\alpha]_{546}^{20} = +6.0°$ ($c = 1.0$, $CHCl_3$), mp 169–171°; IR ν_{max}^{Nujol} cm^{-1}: 1750. The transformation of **2** was carried out with 126 mg of steroid distributed among six flasks; 93 mg of the product was isolated after the reaction. The results of GLC analysis are shown in Table 2.

Column chromatography of 77 mg of the product mixture yielded: 18 mg of **2**, 18 mg of **4** and 2 mg of **7**; all compounds being pure. The product **4** was identified by comparison with the authentic compound.

Transformation of compounds **3** *and* **5**. Both transformations were carried out as described above. The mixtures of products were analysed by GLC (Table 2).

Transformation of compound **4**. Substrate **4** (154 mg) (mp. 140–150°, $[\alpha]_{546}^{20} = +6.5°$, IR ν_{max}^{Nujol} cm^{-1}: 3630, 1750) was transformed as described above and 149 mg of product were isolated. GLC: Table 2. Column chromatography of 132 mg of the product yielded 81 mg of **4** and 38 mg of **7**. Compound **7**: GLC on 3% OV-17 on Gas-Chrom Z 80–100 mesh, 2 m, 270°, 50 ml/min of N_2: retention time: 515 sec (outside the range of retention times of the identified products and substrate). UV λ_{max}^{EtOH} nm: 220, $\varepsilon = 1.07$ ($c = 1.87$ μg/ml, $L = 5$ mm). IR ν_{max}^{Nujol} cm^{-1}: 2960, 2500–3400, 1680, 1710, 1430, 1450. ^{1}H NMR (100 MHz, $CDCl_3$): 0.61 (*s*), 0.90 (*m*), 1.13 (*s*), 2.5–3.0 (*m*). MS (70 eV) *m/z*: 149 (100%), highest mass ion at 482.

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