Phytochemistry, Vol. 26, No. 7, pp. 1959–1960, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00

TRANSFORMATION OF ISOPRENOIDS BY ORCHIDS IN TISSUE CULTURE*

AGNIESZKA MIRONOWICZ, KRYSTYNA KUKUŁCZANKA†, KRZYSZTOF KRASIŃSKI and ANTONI SIEWIŃSKI‡

Institute of Fundamental Chemistry, Agricultural University of Wrocław, ul. Norwida 25, 50-375 Wrocław, Poland; †Botanical Garden, The University of Wrocław, Poland

(*Revised received* 20 *October* 1986)

Key Word Index—*Epidendrum ochraceum*; *Cymbidium* 'Saint Pierre'; *Dendrobium phalaenopsis*; Orchidaceae; orchids; tissue culture; biotransformations; testosterone; androstenolone; menthyl acetate.

Abstract—Tissue cultures of *Epidendrum ochraceum*, *Cymbidium* 'Saint Pierre' and *Dendrobium phalaenopsis* were found to transform some isoprenoids. The transformation of the oxygen functions at C-17 in androstane derivatives and the hydrolysis of (±)-menthyl acetate were investigated in some detail. The degree of hydrolysis of the latter was about 75–85%.

INTRODUCTION

The development of tissue culture techniques for orchids has provided materials [2, 3] that can be used for transforming organic compounds which are either specific to the plant family or xenobiotic.

The knowledge that many orchid species produce secondary metabolites which are either isoprenoid compounds, including sterols [4–6], or derivatives of shikimic acid [4], prompted us to study the biotransformation of these types of compounds by tissue cultures of orchids.

RESULTS AND DISCUSSION

Tissue cultures of three orchid species, *Epidendrum ochraceum*, *Cymbidium* 'Saint Pierre' and *Dendrobium phalaenopsis* were used in these studies. The cultures had been developed earlier for both research and practical purposes [7].

The cultures were screened for their ability to modify steroids, monoterpenes and esters of aromatic-aliphatic alcohols that are analogues of shikimic acid or its derivatives. Of the steroids tested only the androstane derivatives were modified§, and then only very slowly. For the low-M_r compounds menthol, limonene, *trans*-pineol, cineol, 1,8-cineoldione, acetophenone and the acetates of menthol, *trans*-pineol, benzyl alcohol, 1-phenylethanol, 2-phenylethanol, 1-[2-naphthyl]-ethanol, and 1-[1-naphthyl]-ethanol the transformations were more rapid.

The androstane derivatives and (±)-menthyl acetate were selected for more detailed studies, the results of which are presented below. The transformations of the other monoterpenes and of the shikimic acid derivatives will be the subject of a separate report.

Androstenedione (1), testosterone (2) and androstenolone (3) were transformed by cultures of *Cymbidium* and *D. phalaenopsis*, but remained unaltered in the presence of tissue of *E. ochraceum*. The tissues of *Cymbidium* and *D. phalaenopsis* reduced the C-17 carbonyl group of 1 and 3 to a hydroxy group. In addition, the tissue of *D. phalaenopsis* oxidized testosterone (2) to androstenedione (1). The yield of transformation products never exceeded 10%.

The experiments did not reveal a reduction of the α,β-unsaturated ketone function in the A-ring of the C_{19}-steroids, a reaction frequently observed in microorganisms and in plant tissue cultures [8, 9], and which has been observed in the alga *Spirodela oligorrhiza* [10].

The transformation of racemic menthyl acetate (5) was investigated under the same conditions as for steroids. Menthol (6) and the unreacted substrate (5) were the only compounds present in the transformation mixture. Hence, the tissue cultures hydrolysed the ester group of 5. The hydrolysis was weakly enantiospecific with alcohol *R*-(−)-6 being formed at a slightly higher rate than alcohol (+)-6. The unreacted substrate had (+) configuration.

Since the transformation yielded just one product, the concentration of which was easy to monitor chromatographically, the system involving 5 was found particularly

Table 1. The degree of hydrolysis of 15 mg of menthyl acetate (5) in 100 ml of tissue culture of *Cymbidium* 'Saint Pierre' vs biomass of the culture

Time after addition of substrate (days)	Menthol formed (%)	Dry biomass (mg)	Dry biomass (mg) Mass of substrate (mg)
14	36	12	0.8
14	55	16	1.0
14	60	22	1.4
14	79	24	1.6
14	88	43	2.8
21	90	69	4.6
21	93	138	9.2

* Part 22 of the series "Biotransformations". For part 21 see ref. [1].

‡To whom correspondence should be addressed.

§Transformation did not proceed for cholesterol, β-sitosterol and some of their esters, diosgenin and pregnane derivatives.

Table 2. The degree of hydrolysis of 15 and 30 mg of menthyl acetate (5) vs time of transformation in 100 ml of tissue culture of *E. ochraceum* and *Cymbidium* 'Saint Pierre'

Wt of substrate added (mg)	Transformation time (days)	*E. ochraceum*		*Cymbidium* 'Saint Pierre'	
		Menthol formed (%)	Biomass / Substrate (mg/mg)	Menthol formed (%)	Biomass / Substrate (mg/mg)
15	2	8	2.8	7	2.4
15	5	9	2.8	10	2.9
15	7	36	2.5	41	2.9
15	14	67	2.8	88	2.8
15	21	74	2.7	86	3.0
30	6	18	2.3	27	4.3
30	21	60	2.5	83	2.6

convenient for the study of the transformation capabilities of orchid tissues.

Table 1 presents the degree of hydrolysis of 15 mg of **5** in 100 ml of culture medium vs dry mass of the tissue cultures. The degree of hydrolysis increased with the amount of biomass in the culture. A biomass/substrate ratio equal to 2.8 seemed to be optimal for the transformation.

Next, the effect of transformation time upon the degree of hydrolysis was analysed. Tissue cultures of *E. ochraceum* and *Cymbidium* were used to transform 15 or 30 mg of **5**. The experiments lasted for up to 21 days. Due to the difficulties in controlling the biomass of the cultures, the experiments were repeated several times and the results presented in Table 2 are those for which the biomass/substrate ratio was close to that assessed to be optimal, i.e. 2.8.

In both systems, the degree of hydrolysis of **5** increased with time. A characteristic four-fold increase was observed between the fifth and the seventh day of a run. Later, the reaction slowed down. A two-fold increase in either the substrate concentration or the biomass of the culture did not change significantly the transformation process.

The results presented above indicate that tissue cultures of orchids transform C_{19}-steroids to a small extent. The only reaction observed being the ketone to alcohol transformation at C-17. By contrast menthyl acetate (**5**) is readily hydrolysed with a yield comparable to that observed with microorganisms [11].

This suggests that among tissue cultures of Orchidaceae there are species capable of transforming isoprenoids.

EXPERIMENTAL

The tissues of *Cymbidium* 'Saint Pierre', *Dendrobium phalaenopsis* (clone 303/15) and *Epidendrum ochraceum* (clone 83/7) had been maintained for several years at the Botanical Garden of the University of Wroclaw [7, 12].

The tissue of *Cymbidium* was passaged to PB liquid medium [11], while the tissues of *D. phalaenopsis* and *E. ochraceum* were cultivated using RM liquid medium [13]. After 4 or 7 weeks (*E. ochraceum*) the protocorm agglomerates were placed in 200 ml conical flasks containing 100 or 50 ml of fresh medium. The tissue with liquid medium was referred to as the tissue culture. The cultures were constantly shaken at 20–24° and given an 18 hr photoperiod (2000 lx).

After 2 weeks of cultivation 10 mg of steroidal substrates or 15–30 mg of menthyl acetate (**5**) were added to each flask and the incubations continued for 14 days (steroids) or for the times shown in Tables 1 and 2. The tissue cultures died within 1–4 weeks.

The transformation products were extracted with $CHCl_3$ and purified by chromatography. The spectral data of substrates and products were identical with those of authentic standards.

REFERENCES

1. Dmochowska-Gładysz, J., Kołek, T., Siewiński, A., Derdziński, K., Zabża, A. and Nespiak, A. (1986) *J. Basic Microb.* (in press).
2. Kukulczanka, K. (1980) *Orchideen* **15**, 38.
3. Kukulczanka, K. (1982) *Orchideen* **16**, 9.
4. Hills, H., Williams, N. and Dodson, C. (1968) *Am. Orchid Soc. Bull.* **37**, 967.
5. Wan, A. S. C., Aexel, R. T. and Nicholas, H. J. (1971) *Phytochemistry* **10**, 2267.
6. Luning, B. (1974) *The Orchids* (Withner, C. L., ed.) p. 349.
7. Kukulczanka, K. and Wojciechowska, U. (1983) *Acta Horticult.* **131**, 105.
8. Dmochowska, J., Siewiński, A. and Nespiak, A. (1968) *Bull. Acad. Polon. Sci. Ser. Sci. Biol.* **17**, 133.
9. Hirotani, M. and Furuya, T. (1974) *Phytochemistry* **13**, 2153.
10. Tłomak, E., Pawłowicz, P., Czerwiński, W. and Siewiński, A. (1986) *Phytochemistry* **25**, 61.
11. Yamagucki, Y., Oritani, T., Tajima, N., Komatsu, A. and Moroe, T. (1979) *J. Agric. Chem. Soc. Jap.* **50**, 475.
12. Kukulczanka, K. (1976) *Proc. 8th World Orchid Conference, Frankfurt a/M.* p. 384.
13. Reinert, R. A. and Mohr, H. C., (1967) *Proc. Am. Soc. Hort.* **91**, 664.