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BIOTRANSFORMATIONS OF PROGESTERONE BY *CHLORELLA* SPP.

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Key Word Index—*Chlorella* spp.; biotransformation; progesterone; microalgae.

Abstract—Thirty-eight strains of *Chlorella* spp. were used as bioreactors on progesterone. Fourteen strains were ineffective whilst the others biotransformed the substrate. The observed bioreactions for progesterone were the hydroxylation, the reduction and the side-chain degradation. The kinds of biotransformation seem to fit the actual classification of the strains.

In connection with a study of the possible utilization of microalgae as bioreactors [1], we reported in a recent paper [2] that the green alga *Chlorella emersonii* C211/8h biotransformed progesterone (**1**). After 15 days, hydroxyprogesterones **2–7** were detected in the cultures and after 60 days these compounds, along with the dihydroxyl derivatives **8–10**, were isolated from the reaction mixture, 6β-hydroxyprogesterone (**3**) being the most abundant product.

In pursuing our screening on microalgae, we have investigated 38 strains belonging to different species of *Chlorella*. The algae were cultured in BBM [3], and **1** was added to the axenic cultures during the exponential phase of growth of the algae. The reactions were stopped after 15 days and the steroidal material was extracted with ethyl acetate and checked by GC for quantitative analysis. The bioproducts **2–11** were isolated as previously reported [2], while compounds **12–15** were isolated by preparative TLC. All the compounds were identified by comparison of their high-field ^{1}H MNR spectra with those of authentic samples [4].

All the experiments were carried out in triplicate and the yields of the bioproducts represent the average of the experiments. The strains causing hydroxylation of **1** are listed in Table 1, while those which reduce the substrate or degrade its side chain are listed in Table 2. In Tables 1 and 2, the strains are designated according to their classification in the algal Collections of Texas at Austin, U.S.A. (UTEX) [5] and of Cumbria, Ambleside, U.K. (CCAP) [6] and their yields are reported, along with those of recovered **1**. All the strains of *C. emersonii* induced hydroxylation of **1**; the strains C211/8c and C211/8b gave the same bioproducts of C211/8h, even if the amount of **1** biotransformed was lower. 6β-Hydroxyprogesterone (**3**) was the most

Table 1. Progesterone and bioproducts (%) from *Chlorella* spp. cultures

Species	Strain	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**11**
C. emersonii	C211/8h	67.4	3.1	7.6	5.2	4.0	2.1	4.8	1.1	—
C. emersonii	C211/8c	77.5	1.4	6.2	2.4	3.2	1.2	2.9	1.2	—
C. emersonii	C211/8b	75.6	4.0	7.1	1.4	1.4	4.2	1.6	1.4	—
C. emersonii	C211/8g	84.1	3.1	5.4	—	—	—	—	—	3.0
C. emersonii	C211/11m	90.0	2.7	2.8	—	—	—	—	—	1.9
C. emersonii	C211/15	85.8	3.2	5.1	—	—	—	—	—	1.9
C. emersonii	T1801	82.6	2.2	2.4	—	—	—	1.2	—	1.5
C. emersonii	C211/11n	91.1	2.1	2.1	—	—	—	—	—	—
C. pyrenoidosa	T395	UN								
C. pyrenoidosa	T343	74.5	5.6	4.5		1.1	1.6	1.2		
C. luteoviridis	C211/2a	UN								
C. luteoviridis	C211/5b	UN								
C. luteoviridis	C211/2b	84.2	1.5	6.4	2.9					1.6
C. glucotropha	T1802	95.1		2.4						

UN: unreacted.

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Table 2. Progesterone and bioproducts (%) from *Chlorella* spp.

Species	Strain	1	12	13	14	15
C. kessleri	C211/11g	UN				
C. nocturna	T1804	82.1		14.1		
C. protothecoides	C211/11a	UN				
C. saccharophila	C211/9aGö	UN				
	C211/9bGö	UN				
	C211/1a	90.1	7.3			
	C211/1b	UN				
	C211/1d	UN				
	C211/1f	UN				
C. sorokiniana	T1602	96.2	1.8			
	T1810	94.1	3.6			
C. vulgaris	C211/1e	81.1			8.8	5.2
	C211/11s	75.5	10.2	8.8		
	C211/21a	80.4	15.2			
	T396	82.3	16.3			
	C211/19	86.1	9.4			
	C211/11r	83.4	16.5			
	C211/8k	80.1	17.3			
	C211/11b	83.4	3.8			
	C211/11c	84.3	3.3			
	T398	UN				
	C211/9a	UN				
	T397	UN				
	C211/1c	UN				

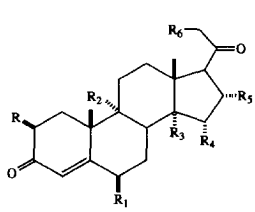
UN: unreacted.

abundant derivative and the yields of all the compounds were fairly comparable; the strains C211/8g, C211/11m, C211/15, T1801 and C211/11n produced the 2β (**2**) and 6β (**3**) hydroxyl derivatives and, except the latter, they gave also small amounts of 15α-hydroxyprogesterone (**11**).

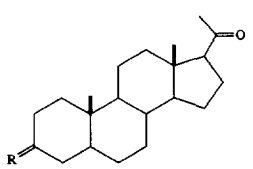
Chlorella pyrenoidosa T343 had the same effects of C211/8h and gave compounds **2**–**7** while the strain of the same species T395 did not induce biotransformations. Among the strains of the species *C. luteoviridis*, C211/2a and C211/5b were ineffective, whilst C211/2b afforded **2**, **3**, **4** and **11** and, finally, *C. glucotropha* T1802 gave small amounts of **3**.

A different kind of biotransformation was observed for the other *Chlorella* species. Among the six strains of *C. saccharophila*, C211/9aGö, C211/9bGö, C211/1b, C211/1d and C211/1f were ineffective, while C211/1a induced reduction of the substrate affording 5α-pregnan-3,20-dione (**12**). The same product was obtained by *C. vulgaris* C211/11s, C211/21a, C211/8k, C211/11r, C211/11b, C211/11c and T396, and by *C. sorokiniana* T1602 and T1810. The strains of *C. vulgaris* T398, C211/9a, T397 and C211/1c, of *C. kessleri* C211/11g and of *C. protothecoides* C211/11a did not modify the substrate. *C. vulgaris* C211/11s also gave 5α-pregnan-3β-ol-20-one (13) and the same product was obtained by *C. nocturna* T1804. Finally, *C. vulgaris* C211/1e caused the side chain degradation of **1** affording testosterone (**14**) and androst-4-en-3,17-dione (**15**).

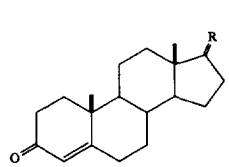
The taxonomy of *Chlorella* has been for a long time in a state of confusion, although this genus probably represents the most frequently studied green alga, for its importance both in basic and applied research [7]. One of the problems most frequently arising in the interpretation of physiological and biochemical data obtained from different species of *Chlorella* is that the strains from the algal Culture Collections are incorrectly named. For this reason, and to facilitate the cross-referencing of the strains, in Table 3 have been indicated, on one hand, the more recent assignments [8] suggested for each of the strains selected for our experiments and, on the other, the previous names of these strains, still retained in the algal Collections of Texas at Austin, U.S.A. (UTEX) [5] and of Cumbria, Ambleside, U.K. (CCAP) [6]. As can be seen, *C. emersonii* has been renamed as *C. fusca* var. *vacuolata*, according to the early morphological observations of Fott and Novakova [9]. It is noteworthy that Kalina and Punchocharova [10], on the basis of the biochemical



1 R = R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = H
2 R = OH R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = H
3 R_1 = OH R = R_2 = R_3 = R_4 = R_5 = R_6 = H
4 R_2 = OH R = R_1 = R_3 = R_4 = R_5 = R_6 = H
5 R_3 = OH R = R_1 = R_2 = R_4 = R_5 = R_6 = H
6 R_5 = OH R = R_1 = R_2 = R_3 = R_4 = R_6 = H
7 R_6 = OH R = R_1 = R_2 = R_3 = R_4 = R_5 = H
8 R_1 = R_2 = OH R = R_3 = R_4 = R_5 = R_6 = H
9 R_1 = R_6 = OH R = R_2 = R_3 = R_4 = R_5 = H
10 R_5 = R_6 = OH R = R_1 = R_2 = R_3 = R_4 = H
11 R_4 = OH R = R_1 = R_2 = R_3 = R_5 = R_6 = H



12 R = O
13 R = H,βOH



14 R = H,βOH
15 R = O

Table 3. Classification of *Chlorella* strains

Present assignment	UTEX*		CCAP*	
	Strain	Species	Strain	Species
C. fusca S. et K. var. *vacuolata* S. et K.			211/8b	*C emersonii* S. et K. var. *emersonii* S. et K.
C. fusca S. et K. var. *vacuolata* S. et K.			211/8c	*C emersonii* S. et K. var. *emersonii* S. et K.
C. fusca S. et K. var. *vacuolata* S. et K.			211/8g	*C emersonii* S. et K. var. *emersonii* S. et K.
C. fusca S. et K. var. *vacuolata* S. et K.			211/8h	*C emersonii* S. et K. var. *emersonii* S. et K.
C. fusca S. et K. var. *vacuolata* S. et K.			211/11m	*C emersonii* S. et K. var. *emersonii* S. et K.
C. fusca S. et K. var. *vacuolata* S. et K.			211/15	*C emersonii* S. et K. var. *emersonii* S. et K.
C. fusca S. et K. var. *vacuolata* S. et K.			211/11n	*C emersonii* S. et K. var. *emersonii* S. et K.
C. fusca S. et K. var. *vacuolata* S. et K.	1801	*C. emersonii* S. et K. var. *globosa* S. et K.		
C. fusca S. et K. var. *fusca* S. et K.	343	*C. pyrenoidosa* Chick		
C. vulgaris Bei	395	*C. pyrenoidosa* Chick		
C. luteoviridis Chod.			211/2a	*C. luteoviridis* Chod.
C. luteoviridis Chod.			211/2b	*C. luteoviridis* Chod.
C. luteoviridis Chod.			211/5b	*C. luteoviridis* Chod.
C. glucotropha S. et K.	1802	*C. glucotropha* S. et K.		
C. kessleri F. et N.			211/11g	*C. kessleri* F. et N.
C. nocturna S. et K.	1804	*C. nocturna* S. et K.		
C. protothecoides Krug.			211/11a	*C. protothecoides* Krug.
C. saccharophila (Krug.) Migula			211/9aGö	*C. saccharophila* (Krug.) Migula
C. saccharophila (Krug.) Migula			211/9bGö	*C. saccharophila* (Krug.) Migula
C. saccharophila (Krug.) Migula			211/1b	*C. saccharophila* (Krug.) Migula
C. saccharophila (Krug.) Migula			211/1d	*C. saccharophila* (Krug.) Migula
C. saccharophila (Krug.) Migula			211/1f	*C. saccharophila* (Krug.) Migula
C. saccharophila (Krug.) Migula var. *ellipsoidea* (Gern.) F. et N.			211/1a	*C. saccharophila* (Krug.) Migula var. *ellipsoidea* (Gern.) F. et N.
C. kessleri F. et N.	397	*C. vulgaris* Beij.		
C. kessleri F. et N.	398	*C. vulgaris* Beij.		
C. saccharophila (Krug.) Migula			211/1c	*C. vulgaris* Beij. var. *vulgaris*
C. sorokiniana S. et K.			211/11c	*C. vulgaris* Beij.
C. sorokiniana S. et K.			211/8k	*C. vulgaris* Beij. f. *tertia* F. et N.
C. vulgaris Beij.			211/1e	*C. vulgaris* Beij. var. *vulgaris*
C. vulgaris Beij.			211/11r	*C. vulgaris* Beij. var. *vulgaris*
C. protothecoides Krug.			211/9a	*C. vulgaris* Beij. var. *vulgaris*
C. vulgaris Beij.			211/11s	*C. vulgaris* Beij. var. *vulgaris*
C. vulgaris Beij.			211/11b	*C. vulgaris* Beij. var. *vulgaris*
C. vulgaris Beij.			211/19	*C. vulgaris* Beij. var. *vulgaris*
C. vulgaris Beij.			211/21a	*C. vulgaris* Beij. var. *vulgaris*
C. vulgaris Beij.	396	*C. vulgaris* Beij. var. *viridis* Chod.		
C. sorokiniana S. et K.	1602	*C. sorokiniana* S. et K.		
C. sorokiniana S. et K.	1810	*C. sorokiniana* var. *pacificensis* S. et K.		

*UTEX and CCAP defined in text.

and ultrastructural characters of the cell wall, separated this species from the genus *Chlorella* and included it in the subfamily Scotiellocystoideae as *Graesiella vacuolata*, genus et species novae.

It must be stressed that the strain T343 *C. pyrenoidosa*, which showed the same bioconversion pattern of *C. fusca* var. *vacuolata*, has been reclassified by Kessler and Huss [8] as *C. fusca* var. *fusca* and is, therefore, very similar to the above mentioned strains while the strain T395 has been reclassified as *C. vulgaris* Beij.

The strains of *C. luteoviridis* C211/2a, C211/2b and C211/5b are included in Table 1 because the strain C211/2b gave the same bioproducts of *C. fusca* var. *vacuolata* although this species is morphologically closest to the *Chlorella* species listed in Table 2 [9].

Among the *C. saccharophila* strains, only C211/1a gave bioconversion and it is noteworthy that Huss *et al.* have reported that this strain is characterized by a DNA base composition which is different from the other strains of *C. saccharophila* [11]. The early works, carried out by Kessler and coworkers [12, 13], evidenced that this strain had biochemical and ecophysiological characters intermediate between *C. saccharophila* and *C. vulgaris* and, more recently, on the basis of two peculiar characters (no growth on mannitol and extreme cadmium sensitivity), it has been proposed the assignment of C211/1a to *C. saccharophila* var. *ellipsoidea* [14].

Among the strains belonging to *C. vulgaris* only C211/1e, C211/11s, C211/21a, T396, C211/11r, C211/8k, C211/11b and C211/11c biotransformed progesterone, while T397, T398, C211/9a and C/211/1c were ineffective. However, the latest assignment includes T397 and T398 in *C. kessleri*, C211/9a in *C. protothecoides* and C211/1c in *C. saccharophila*, and the strains of these species tested do not bioconvert the substrate.

In conclusion, the results obtained on *Chlorella* spp. seem to fit enough of the more recent taxonomic classification. The strains of *C. fusca* S. et K. var. *vacuolata* S. et K. and *C. glucotropha* give hydroxyl derivatives of progesterone while the strains of *C. vulgaris* Beij., *C. nocturna* and *C. sorokiniana* reduce the substrate.

These latter strains seem to have the more promising biotechnological applications since they gave only one bioproduct (except strain C211/11s, which afforded two bioproducts), instead of a mixture of numerous derivatives, as observed within the *C. emersonii* group. The side-chain degradation of progesterone afforded by C211/1e is probably the most interesting transformation carried out by a strain of *Chlorella* and further work is required to understand if different side chains such as those of phytosterols and cholesterol may be degraded.

EXPERIMENTAL

The strains were supplied by The Culture Collection of Algae and Protozoa, Ambleside, Cumbria, U.K., and The Algal Collection of Texas at Austin, U.S.A. In the standard procedure **1** (100 mg sterilized at 100° for 1 hr), dissolved in dioxane (0.7 ml), was added to the axenic culture of the *Chlorella* strain in BBM (50 ml) [3] during the exponential phase of growth of the strain. The inoculum was *ca.* 1.7×10^5 cells ml^{-1} and after 5 days the algal concn was 6×10^5 cells ml^{-1}. The suspensions were stirred at room temp. with a photoperiod of 16 hr light–8 hr dark. After 15 days the cultures were extracted with EtOAc (2×50 ml) and the organic layers after evapn of solvent were monitored by GC on a Fractovap 4160 chromatograph (Carlo Erba) equipped with a 30 m glass capillary column coated with OV-101 at 280°. Standard solns of authentic samples of the hydroxyprogesterones **2–11**, **12**, **13**, **14** and **15** were used for quantitative analysis. For identification, **2–11** were isolated as previously reported [2]. Compounds **12–15** were isolated by prep. silica gel TLC (hexane–EtOAc, 4:1). ^1H NMR spectra of all the compounds were recorded in $CDCl_3$ soln on a Bruker AM 400 spectrometer. Each experiment was carried out in triplicate. The yields represent the average of the 3 experiments and the differences among values were not higher than 7%.

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