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TRANSFORMATION OF STEROIDS BY ALGAE

YUSUF J. ABUL-HAJJ and XIADONG QIAN

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455

ABSTRACT.—Twenty-two algal cultures grown photoautotrophically were incubated with 4-androstene-3,17-dione and 17β-hydroxy-4-androstene-3-one. Most cultures were capable of effecting transformation. The metabolites were isolated and detected by tlc and hplc, and were identified by comparison of chromatographic and/or spectral properties with those of authentic compounds. Most cultures showed the presence of 17-oxidoreductase, some were capable of hydroxylating 4-androstenedione at the 6β- and 14α-positions, and a few were capable of utilizing substrates as an energy and/or a carbon source. Incubations with testosterone showed very little metabolism.

The use of microbial cells for transformation of complex chemicals resulting in single and specific transformations has been effectively exploited by the pharmaceutical industry, especially in the development of steroid hormone synthesis (1-3). Even though microbial transformations are used for large scale production of steroid hormones, only few enzymic reactions, namely 1,2-dehydrogention, 11α-, 11β-, and 16α-hydroxylation and sterol side-chain cleavage, are currently used (4). One of the major drawbacks of microbial transformations is the fact that microorganisms may result in the formation of multiple products due to reciprocal modes of binding that control multiple reactions (5). Thus, cell systems showing a more restrictive substrate fit may be of potential use. Studies by Stohs group (6,7) indicated that plant cell cultures do transform steroids. Cerniglia *et al.* (8,9) have shown that algae are capable of hydroxylating the alkyl side-chain of methyl naphthalene as well as catalyzing aromatic hydroxylation of naphthalene. In view of the fact that very little is known about the metabolic activity of algal cultures and because many pure cultures are available, we carried out an investigation of the ability of algal cultures to transform 4-androstene-3,17-dione.

MATERIALS AND METHODS

Melting points (uncorrected) were obtained on a Fisher-Johns apparatus. Ir spectra were recorded with a Perkin-Elmer 28 infrared spectrophotometer. Nmr spectra were obtained with a JEOL-90 Q spectrometer. 4-Androstenedione was purchased from Steraloids, Wilton, New Hampshire.

ORGANISMS.—Cultures were obtained from several sources: ATCC, American Type Culture Collection; UTEX, University of Texas at Austin, Collection, R.C. Starr, Department of Botany, Austin, 48712; CCAP, Culture Centre of Algae and Protozoa, 26 Storey's Way, Cambridge, England; and IVL, Institut for Vatten och Luftvards forskning, A. Nielson, Box 21060, S-10031 Stockholm, Sweden. All strains were maintained under photoautotrophic growth conditions and grown in Bg 11 (10) liquid medium and illuminated by "Sylvania Grolux" or "Easy grow" (Sears) light, 3,000-5,000 lux. Cultures were kept and maintained under constant illumination and at 27° without shaking in culture tubes.

FERMENTATION PROCEDURE.—Screening experiments were conducted in 125-ml erlenmeyer flasks and preparative scale incubations in 2-liter erlenmeyer flasks. Flasks contained culture medium one-fifth of their volume. A 10% inoculum from culture tubes was used to inoculate flasks and allowed to grow as described above for 10 days, after which 2.5 mg of substrate in DMF (50 μl) was added and incubated for 7 days using the above conditions. The solvent had no effect on algal culture growth as determined by turbidimetric methods.

ANALYTICAL PROCEDURES.—At the end of incubation for 7 days, the contents of the flasks from each incubation were extracted with three volumes of EtOAc. The extract was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue from each incubation was dissolved in MeOH and analyzed by tlc and hplc. Tlc was performed on Brinkman silica gel HF 254 plates developed in C_6H_6-

EtOAc (3:1). Starting material and metabolites were detected by uv light fluorescence at 254 nm and by spraying with a solution of 5% ceric sulfate in 3N H_2SO_4 and warmed using a heat gun to develop colors. Reverse-phase hplc was performed with a Beckman system consisting of a single 100A pump and a single wavelength absorbance detector at 254 nm. A 5μ C_{18} Ultrasphere ODS (Altex Scientific, Berkeley, CA) column (4.6 m×25 cm) was used, and the separation was achieved with a mixture of acetonitrile-H_2O (3:2) as the eluting solvent at a flow rate of 1 ml/min. The quantitation of metabolites was determined by comparison of peak areas to those observed for known amounts of authentic standards.

RESULTS AND DISCUSSION

Twenty-two algal cultures were screened for their abilities to transform 4-androstene-3,17-dione, and the results are shown in Table 1. After incubation for 7 days,

TABLE 1. Percent of Metabolites from Algal Transformation of 4-Androstenedione (**1**)

No.	Strain (no.)	Metabolites[a] (%)					
		1	**2**	**3**	**4**	**5**	Unknown
1.	*Anabaena flos-aquae* (UTEX 1444)	65	30	—	—	—	5
2.	*Anabaena* sp. (IVL 7119)	—	—	—	—	—	15
3.	*Anabaena cylindrica* (IVL)	25	35	—	5	trace	25
4.	*Anacystis nidulans* (SVAL 1402-1)	63	12	trace	3	trace	25
5.	*Ankistrodesmus angustus* (ATCC 30448)	—	—	—	—	5	20
6.	*Aphanocapsa* sp. (A.J. Smith 6308)	10	—	—	—	—	60[b]
7.	*Characium californicum* (UTEX 2097)	55	25	—	—	—	20
8.	*Chlorococcum macrostigmatum* (UTEX 109)	55	45	—	—	—	—
9.	*Chlorogloea fritschii* (A.J. Smith)	100	—	—	—	—	—
10.	*Coccochloris peniocystis* (UTEX 1548)	77	21	—	—	—	—
11.	*Coelastrum proboscideum* var, *grucile* (ATCC 30410)	15	15	5	10	—	30
12.	*Fremyenadiplosiphon* sp. (UTEX 481)	100	—	—	—	—	—
13.	*Glococapsa alpicola* (CCAP 1430-1)	100	—	—	—	—	—
14.	*Lyngbya koet-zingii* (UTEX 1547)	100	—	—	—	—	—
15.	*Oocystis* sp. (ATCC 30417)	50	20	—	trace	—	15
16.	*Oscillatoria proliyeva* (UTEX 1270)	90	10	—	—	—	—
17.	*Phormidium autumnale* (UTEX 1580)	20	80	—	—	—	—
18.	*Plectonema boryanum* (UTEX 594)	100	—	—	—	—	—
19.	*Scenedesmus quadricauda* (ATCC 11460)	10	27	5	10	7	35
20.	*Selanastfum capricornutum* (ATCC 22662)	—	—	—	—	—	20
21.	*Synechococcus* sp. (R.Y. Stanier 6312)	—	—	—	—	—	10
22.	*Synechococcus* sp. (ATCC 27146)	100	—	—	—	—	—

[a](−)=Not detected.

[b]Of these 30% is possibly due to 1,4-androstadiene-3,17-dione.

each culture was analyzed for metabolites by tlc and hplc. Tlc analysis indicated that many of the algal cultures did transform 4-androstenedione primarily to 17β-hydroxy-4-androstene-3-one (testosterone) indicating the presence of 17-ketoreductase. Other enzymes were also detected, including 6β-hydroxylase and 14α-hydroxylase. Culture No. 6 showed the formation of possibly 1,4-androstadiene-3,17-dione (30%), suggesting the presence of Δ^1-dehydrogenase. Other unidentified products were detected by tlc. Some of these compounds showed no uv absorption, indicating either the saturation of the 4,5-double bond and/or reduction of the 3-keto group. The identity of these metabolites was not elucidated further because of the low yields obtained in these fermentations. Control experiments using sterilized cultures showed no transformation. (See Figure 1.)

In view of the fact that many cultures are capable of reducing the 17-keto group to 17β-hydroxyl group, we investigated the reversibility of this reaction as shown in Fig-

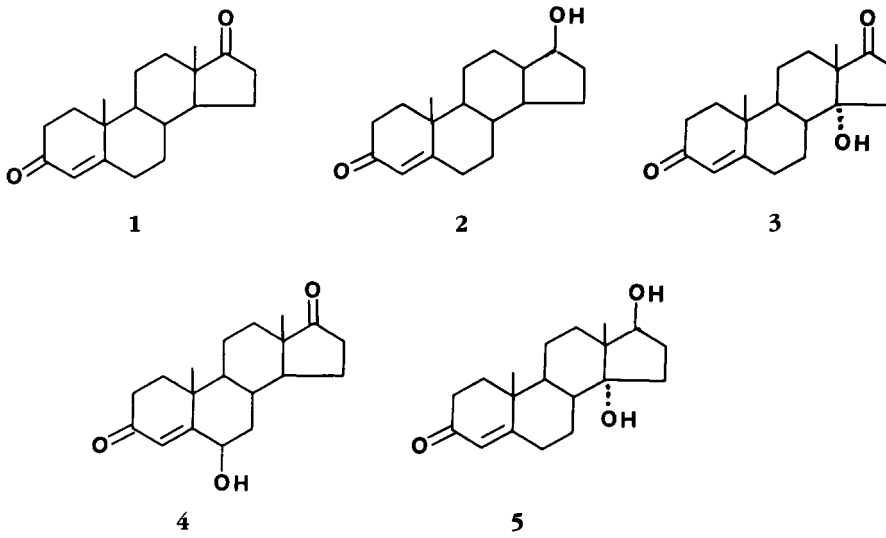

FIGURE 1. Structures of 4-androstene-3,17-dione (**1**); testosterone (**2**); 14α-hydroxy-4-androstenedione (**3**); 6β-hydroxy-4-androstenedione (**4**); and 14α-hydroxytestosterone (**5**).

ure 2. Incubations with testosterone as substrate (Table 2) showed very little oxidation of the 17β-hydroxy group, indicating the lack of 17β-hydroxy steroid dehydrogenase in these cultures. The oxidation-reduction reaction presented in Figure 2 has been

TABLE 2. Percent of Metabolites from Algal Transformation of Testosterone (**2**)

No.	Strain (no.)	Metabolites[a] (%)					
		2	**1**	**3**	**4**	**5**	Unknown
3.	*Anabaena cylindrica* (IVL)	100	—	—	—	—	—
5.	*Aukisrodesmus augustus* (ATCC 30448)	80	—	—	—	trace	10
6.	*Aphanocapsa* sp. (A.J. Smith 6308)	50	35	—	—	—	—
7.	*Characium californicum* (UTEX 2097)	85	5	—	—	—	—
11.	*Coelastrum proboscideum* var. *grucile* (ATCC 30410)	40	—	—	—	20	20
17.	*Phormidium autumnale* (UTEX 1580)	100	—	—	—	—	—
19.	*Scenedesmus quadricauda* (ATCC 11460)	70	—	—	—	5	20
20.	*Selanastfum capricornutum* (ATCC 22662)	15	—	—	—	—	25

[a](−)=Not detected.

shown to occur in many cell systems, and the enzyme catalyzing both the oxidation and reduction is a single enzyme (11,12). Inasmuch as oxidation of the 17β-hydroxy group was not carried out by the cultures investigated, it suggests that lack of oxidation of the

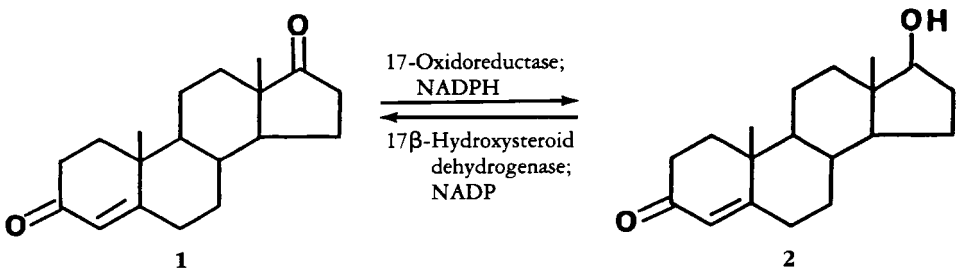

FIGURE 2. Oxidation of testosterone by 17β-hydroxysteroid dehydrogenase and NADP, and reduction of 4-androstenedione by 17-oxidoreductase and NADPH.

17β-hydroxy group is most likely due to a deficiency in the oxidized form of the pyridine nucleotide (NADP) in algal cultures. However, it may be possible that in these algal cultures, the oxidation and reduction reactions may be catalyzed by two separate enzyme systems. Studies on cell-free systems are currently investigating this possibility.

Figure 3 is an hplc elution profile of compounds produced by *Scendesmus guadricanda* grown in the presence of 4-androstene-3,17-dione. Examinations of the hplc traces revealed the formation of compounds **2,3,4,** and **5** (Figure 1) by different cultures. The overall yields of testosterone ranged between 0 to 80%, and it was found to be the major metabolite in most of the cultures investigated (Table 1). The identity of this metabolite was compared with authentic testosterone by ir and nmr spectral analyses and by tlc and hplc. The ir spectrum (KBr) showed absorptions at 3550 (17β-OH) and 1690 (3-ketone) cm^{-1}, and all other peaks were identical to those obtained from an authentic sample. The ^{1}H-nmr spectrum ($CDCl_3$) gave signals at ppm values of 0.83 (s, 3H, 18-CH_3), 1.23 (s, 3H, 19-CH_3), 5.72 (s, 1H, 4-H). The other metabolites were identified using color reactions obtained on tlc as well as hplc. It is interesting to note that several algal cultures, including cultures 2, 5, 20, and 21, showed very little steroid left after the incubation, suggesting the utilization of the steroid skeleton by these cultures as a carbon and/or energy source. Studies using a ^{14}C-ring labeled steroid will be used to determine whether these cultures use the steroid skeleton as an energy source.

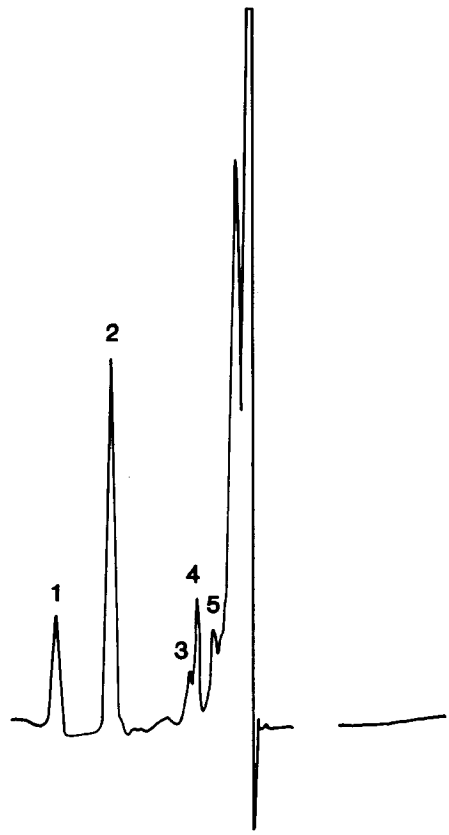

FIGURE 3. High-pressure liquid chromatogram of 4-androstenedione by *Scenedesmus quadricauda*. Numbers refer to compounds listed in Figure 1.

The results obtained from this study clearly demonstrate that algal cultures have the potential for metabolizing steroids. Even though most cultures showed only reduction of the 17-keto group, others did possess some hydroxylating activity. Furthermore, the fact that some algal cultures resulted in complete utilization of substrate as well as the lack of formation of the common metabolites suggests that algae could utilize steroid molecules as a carbon and/or energy source. This observation may be of significant importance if indeed these cultures are also capable of degrading and utilizing aromatic compounds as an energy source.

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