J. steroid Biochem. Vol. 22, No. 3, pp. 399–400, 1985
Printed in Great Britain. All rights reserved

0022-4731/85 \$3.00 + 0.00
Copyright © 1985 Pergamon Press Ltd

BIOTRANSFORMATION OF PROGESTERONE TO 14α-HYDROXYPREGNA-1,4-DIENE-3,20-DIONE, A NOVEL FUNGAL METABOLITE, BY *COLLETOTRICHUM ANTIRRHINI*

V. C. O. NJAR*, S. SHAPIRO†, T. ARUNACHALAM and E. CASPI‡
Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545, U.S.A.

(*Received* 18 *June* 1984)

Summary—The fermentation of progesterone by *Colletotrichum antirrhini* SC 2144 was examined. Instead of 15α-hydroxyprogesterone, the reported product, this fungus converted progesterone to androst-4-ene-3,17-dione, androsta-1,4-diene-3,17-dione, 14α-hydroxyandrosta-1,4-diene-3,17-dione, 11α-hydroxy-pregn-4-ene-3,20-dione, 14α-hydroxypregn-4-ene-3,20-dione, and a hitherto undescribed compound, 14α-hydroxypregna-1,4-diene-3,20-dione.

INTRODUCTION

Microorganisms have long been recognised as important means of achieving the regio- and stereospecific transformation of steroids [1, 2]. In connection with other studies, we explored the utility of *Colletotrichum antirrhini* for the production of 15α-hydroxyprogesterone [3, 4]. No 15α-hydroxy metabolites were obtained; instead, a mixture of six products was consistently recovered, one of which, 14α-hydroxypregna-1,4-diene-3,20-dione (**1**), has not previously been described. The results of our experiments with *C. antirrhini* strain SC 2144 are now reported.

EXPERIMENTAL

Chemicals and instrumentation

Chemicals and solvents were of analytical reagent grade. Progesterone, purchased from Sigma Chemical Co. (St Louis, MO), was judged to be >99% pure by TLC [pre-coated plastic sheets: silica gel 60 F_{254}, E. Merck A. G., Darmstadt, West Germany; ethyl acetate–benzene (4:1)] and by HPLC [silica gel column: 25 cm × 4.6 mm i.d., Alltech Associates, Deerfield, IL; mobile phase, isooctane–isopropanol (19:1)]. Authentic samples of 15α-hydroxypregn-4-ene-3,20-dione and 14α-hydroxypregn-4-ene-3,20-dione were gifts of Drs J. C. Babcock and P. W. O'Connell (The Upjohn Co., Kalamazoo, MI). 11α-Hydroxypregn-4-ene-3,20-dione was obtained from E. R. Squibb & Sons (Princeton, NJ); androsta-1,4-diene-3,17-dione, from Steraloids, Inc. (Wilton, NH); and androst-4-ene-3,17-dione, from Nutritional Biochemicals Corp. (Cleveland, OH).

Pmr spectra were recorded on a Varian EM-390 spectrometer at 90 MHz, in deuteriochloroform containing 1% TMS as internal standard. The spectrum of 14α-hydroxyandrosta-1,4-diene-3,17-dione was recorded in Unisol-*d* (Norell Chemical Co., Inc., Landisville, NJ). Mass spectra were recorded with a model 12-90G mass spectrometer equipped with a DA/CS 1.2 data analysis/control system (Nuclide Corp., State College, PA).

Organism

C. antirrhini strain SC 2144 was a gift of Ms J. Goodman and Dr F. L. Weisenborn, Squibb Institute of Medical Research, Princeton, NJ. An asporogenous culture was received on a medium containing, per l of distilled water: agar (20 g), D-glucose (10 g), yeast extract (2.5 g), and K_2HPO_4 (1 g). This mycelium was inoculated onto slants of nutrient agar and incubated at 23°C. The organism was maintained by biweekly transfers on nutrient agar slants.

A subculture of the organism produced spores on 2% (w/v) malt agar in a plastic Petri dish at 25°C under a regime of 12-h near ultraviolet ("black light") and 12-h darkness (D. Brewer, pers. commun.). These spores agreed in shape and size with those of *C. antirrhini* [5]. An agar slant of the fungus, returned to Squibb for taxonomic verification, was reconfirmed as *C. antirrhini* strain SC 2144 (Dr F. L. Weisenborn, pers. commun.).

Incubation conditions

Submerged seed cultures (1.2 l) were grown in a medium containing, per liter of demineralised water: glucose (10 g), $NH_4H_2PO_4$ (3 g), $CaCO_3$ (2.5 g), cornsteep liquor (A.E. Staley Mfg. Co., Decatur, IL) [3.0 g], and Proflo oil (Traders Oil Mill Co., Ft Worth, TX) (2.2 ml).

Biotransformations were performed in carboys containing 15 l of the above medium, from which

*Present address: Department of Chemistry, University of Ibadan, Ibadan, Nigeria.
†Present address: Department of Chemistry, Concordia University, Sir George Williams Campus, 1455 boulevard de Maisonneuve West, Montréal, Québec H3G 1M8 Canada.
‡To whom correspondence should be addressed.

glucose was omitted. Progesterone (5.5 g in 50 ml of 95% ethanol) was added to each carboy immediately after autoclaving, the medium was vigourously stirred until it cooled to room temperature, and then inoculated with a submerged seed culture of *C. antirrhini*. Fermentations were allowed to proceed for 5 days at 25°C with continuous stirring and aeration (8.5 l air min^{-1}).

Extraction and isolation of metabolites

Mycelium were separated from the medium by centrifugation, and both were extracted with chloroform. Combined extracts were dried (Na_2SO_4) and evaporated *in vacuo*. The crude residue (ca. 7.5 g per carboy) was fractionated on a column of acid-washed alumina (4 × 50 cm) packed in benzene. The column was eluted with ethyl acetate–benzene (4:1), and fractions (200 ml) were collected. Progesterone was eluted in fractions 2–5, and progesterone metabolites were eluted in fractions 6–25. Appropriate fractions were combined and purified by TLC (silica gel 60 $HF_{254+366}$, E. Merck A. G.; ethyl acetate–benzene (4:1)]. The various bands were eluted with methanol. Homogeneous compounds were brought to dryness by rotary evaporation at room temperature, weighed, and crystallised. Except for (**1**), the characterisation of which is described in the following section, recovered metabolites were identified by comparison with authentic samples (m.p., u.v., i.r., p.m.r.).

RESULTS AND DISCUSSION

About 27% of the incubated progesterone was transformed, and the metabolites were recovered. 11α- and 14α-Hydroxypregn-4-ene-3,20-dione were usually the predominant products, averaging 9 and 11% by weight, respectively, of the added progesterone. Other products, constituting ca. 8% by weight of added progesterone, were androsta-1,4-diene-3,17-dione, 14α-hydroxyandrosta-1,4-diene-3,17-dione, androst-4-ene-3,17-dione, and the hitherto unrecognised metabolite, 14α-hydroxypregna-1,4-diene-3,20-dione (**1**).

Compound (**1**) showed a m.p. 215–220°C (decomp.); i.r.: ν_{max} 3610 (free —OH), 3450 (hydrogen-bonded —OH), 1695 (satd C=O), 1655 (conj. C=O), 1610 (conj. double bond), 1095 (C—O stretch); u.v.: λ_{max}^{EtOH} 242 nm (log ε 4.18); p.m.r.: δ0.8 (13β—CH_3,s), 1.24 (10β—CH_3,s), 2.1 (20—CH_3,s), 3.2 (17α—H,t, J = 9 Hz), 6.1 (C—4,1H,s), 6.25 (C—2,1H,dd,J_1 = 3 Hz,J_2 = 9 Hz), 7.09 (C—1,1H,d,J = 10.5 Hz); ms; *m/e* 328 (M^+, 11.8%), 310 (M^+—H_2O, 3.9%).

The observed downfield shift of the 17α-H signal from its normal position at *ca.* δ2.5 to δ3.2 is consistent with the deshielding effect of a 14α-hydroxyl group. Oxidation of (**1**) with Jones' reagent [6, 7] failed to give a *cyclo*pentyl ketone, instead yielding the 14,15-olefin. From these data, (**1**) was assigned the structure 14α-hydroxypregna-1,4-diene-3,20-dione.

Confirmation of this assignment was obtained by selective hydrogenation of the 1,2-double bond [8] to yield 14α-hydroxyprogesterone. A solution of (**1**) (20 mg) and Tris-(triphenylphosphine)chlororhodium (10 mg) in ethyl acetate–benzene (1:1) (5 ml) was stirred in an atmosphere of hydrogen (8 h). The solvent was removed, the residue taken up in diethyl ether, and filtered through a short column of silica gel. The eluate was concentrated *in vacuo*, fractionated by TLC [silica gel, hexane–ethyl acetate (1:1)], and the product recovered and crystallised (ethyl acetate). The TLC and p.m.r. spectrum of the product were identical with that of authentic 14α-hydroxyprogesterone.

The strain of *C. antirrhini* employed by us was direct, unselected descendant of the organism used by Fried *et al.*[3, 4] (Dr W. H. Trejo, pers. commun.); yet no 15α-hydroxymetabolites were recovered. The differences between the progesterone transformation products obtained in our fermentations and those reported by the earlier workers are ascribed to the genetic and metabolic changes that have occurred during some 25–30 years of maintenance of the fungus in the stock culture collection of the donor laboratory [cf. 9–12].

Acknowledgements—This work was supported by National Institutes of Health grant HD14906. The authors thank Dr D. Brewer (Halifax) for constructive comments on the manuscript.

REFERENCES

1. Akhrem A. A. and Titov Yu. A.: *Microbiological Transformation of Steroids*. Nauka, Moscow (1965).
2. Charney W. and Herzog H. L.: *Microbial Transformations of Steroids*. Academic Press, New York (1967).
3. Fried J., Thoma R. W., Perlman D. and Gerke J. R.: 7- and 15-hydroxyprogesterones. U.S. Patent 2,753,290 (1956).
4. Fried J., Thoma R. W., Perlman D. and Gerke J. R.: 15-Hydroxyprogesterones. U.S. Patent 2,879,280 (1959).
5. Von Arx J. A.: Die Arten der Gattung *Colletotrichum* Cda. *Phytopath. Z.* **29** (1957) 413–468.
6. Bowden K., Heilbron I. M., Jones E. R. H. and Weedon B. C. L.: Researches on acetylenic compounds. Part 1. The preparation of acetylenic ketones by oxidation of acetylenic carbinols and glycols. *J. chem. Soc.* (1946) 39–45.
7. Fieser L. F. and Fieser M.: *Reagents for Organic Syntheses*, Vol. 1. John Wiley, New York (1967) pp. 142–143.
8. Djerassi C. and Gutzwiller J.: Selective reduction of steroids by homogeneous catalytic hydrogenation. *J. Am. chem. Soc.* **88** (1966) 4537–4538.
9. Stakman E. C., Tyler L. J. and Hafstad G. E.: The constancy of cultural characteristics and pathogenicity in variant lines of *Ustilago zeae*. *Bull. Torrey Bot. Club* **60** (1933) 565–572.
10. Ullstrup A. J.: Studies on the variability of pathogenicity and cultural characteristics of *Gibberella saubinetti*. *J. Agr. Res.* **51** (1935) 145–162.
11. Lucas G. B.: Further studies on the deterioration of the red rot fungus in culture. *Proc. La. Acad. Sci.* **7** (1942) 35.
12. Miller J. J.: Cultural and taxonomic studies on certain Fusaria. I. Mutation in culture. *Can. J. Res.* **24c** (1946) 188–212.