Preface

Steroids are terpenoid lipids with specific structure that occur widely in living systems. Over 250 sterols and related compounds have been reported in plants, insects, vertebrates, and lower eukaryotes such as yeasts. Steroid-based drugs have a wide range of therapeutic purposes, such as anti-inflammatory, immunosuppressive, progestational, diuretic, anabolic, and contraceptive, as well as other applications. As a result, about 300 approved steroid drugs exist to date, and the global market for steroid-containing products is in excess of $10 billion and more than one million tons annually.

Scientific research on steroid chemistry began in the early twentieth century and was encouraged in the 1950s, with the discovery of the pharmacological effects of cortisol and progesterone. Their production is being done by chemical synthesis pathways, but the replacement of some of the chemical steps by bioconversions is allowing, in many cases, fewer stages, higher yields, and new modifications leading to more competitive and robust processes.

One of the major raw materials for steroid industry is the natural steroid sapogenin called diosogenin. The conversion of diosgenin to valuable steroids is done by a well-established chemical route, despite presenting several shortcomings such as higher costs, multistep syntheses, waste of land resources, and exhaustion of wild plant resources. Alternative starting materials for the steroid industry are the natural sterols, e.g., phytosterols, a mixture of sterols industrially obtained as a by-product of the oil refining process or from the cellulose production process.

Microbial bioconversion of phytosterols into steroid intermediates remains a focus of research in the field of steroids. Growing numbers of microbial biotransformations of steroids have been reported, with an emphasis mainly on steroid hydroxylation, Δ1-dehydrogenation, and sterol side-chain cleavage. Many of these biotransformation reactions, in combination with chemical synthesis, enabled the production of large quantities of steroid compounds. Both phytosterol bioconversions and new steroid modifications are two areas of special interest for the industry.

This book is intended to provide practical experimental laboratory procedures for a wide range of steroid bioconversions. Although not an exhaustive treatise, it provides a detailed “step-by-step” description of the most recent developments in these biotechnological processes. The detailed protocols are cross-referenced in the Notes section, providing special details, minor problems, troubleshooting, and safety comments that may not normally appear in journal articles and can be particularly useful for those not familiar with specific techniques.

The two lead chapters of this volume are overviews on microbial bioconversions and chemical synthesis pathways, respectively. The following chapters show comprehensive experimental methods on strain characterization (genomics, transcriptomics, and proteomics) and improvement, bioconversion methods from sterols to androstenedione (AD) and androstadienedione (ADD), steroid hydroxylations, biocatalysis, downstream processes to purify steroid intermediates, and analysis.
This book has been written by outstanding experts in their field and provides a reference source for laboratory and industrial professionals, as well as for graduate students in a number of biological disciplines (biotechnology, microbiology, genetics, molecular biology, etc.).

We are indebted to the authors who, in spite of their professional activities, agreed to participate in this book, to Dr. J. Walker, Series Editor, for his encouragement and advice in reviewing the manuscripts, and to the staff of Springer for their assistance in assembling this volume and their efforts in keeping this project on schedule. Last but not least, we warmly acknowledge our families for their patience and support.

León, Spain
Bocillo, Valladolid, Spain

José-Luis Barredo
Ignacio Herráiz
# Contents

*Preface* .......................................................... v  
*Contributors* .......................................................... ix  

1 Steroid Bioconversions ........................................... 1  
   *Marina V. Donova*  

2 Chemical Pathways of Corticosteroids, Industrial Synthesis from Sapogenins ............................................... 15  
   *Ignacio Herráiz*  

3 Genome Sequencing of Steroid-Producing Bacteria with Illumina Technology ........................................ 29  
   *Victoria Y. Shtratnikova, Mikhail I. Schelkunov, and Marina V. Donova*  

4 Genome Sequencing of Steroid Producing Bacteria Using Ion Torrent Technology and a Reference Genome ................................ 45  
   *Alberto Sola-Landa, Antonio Rodríguez-García, Carlos Barreiro, and Rosario Pérez-Redondo*  

5 RNA-Seq-Based Comparative Transcriptomics: RNA Preparation and Bioinformatics ........................................ 59  
   *Antonio Rodríguez-García, Alberto Sola-Landa, and Carlos Barreiro*  

6 Intra- and Extra-Cellular Proteome Analyses of Steroid-Producer Mycobacteria ........................................ 73  
   *Carlos Barreiro, Alejandro Morales, Inés Vázquez-Iglesias, and Alberto Sola-Landa*  

7 Genetic Techniques for Manipulation of the Phytosterol Biotransformation Strain *Mycobacterium neoaurum* NRRL B-3805 ............. 93  
   *Jessica K. Loraine and Margaret C.M. Smith*  

8 Identification and Characterization of the Genes and Enzymes Belonging to the Bile Acid Catabolic Pathway in *Pseudomonas* .................. 109  
   *José M. Luengo and Elías R. Olivera*  

9 Selection of Biodegrading Phytosterol Strains. .......................... 143  
   *María-Angélica Mondaca, Maricel Vidal, Soledad Chamorro, and Gladys Vidal*  

10 Stigmasterol Removal by an Aerobic Treatment System ................................. 151  
   *Soledad Chamorro, Claudia Xavier, Víctor Hernández, José Becerra, and Gladys Vidal*  

11 Production and Biotransformation of Phytosterol Microdispersions to Produce 4-Androstene-3,17-Dione ................................. 159  
   *Rodrigo A. Mancilla, Roberto Pavez-Díaz, and Alejandro Amoroso*  

12 β-Sitosterol Bioconversion to Androstenedione in Microtiter Plates ................................. 167  
   *Marco P.C. Marques and Pedro Fernandes*
13 Bioconversion of Phytosterols into Androstenedione by *Mycobacterium* ................................................. 177
*Kjell D. Jøsefesen, Anna Nordborg, and Håvard Sletta*

14 Scale-Up of Phytosterols Bioconversion into Androstenedione ......................................................... 199
*Sonia Martínez-Cámara, Esther Bahillo, José-Luis Barredo, and Marta Rodríguez-Sáiz*

15 Bioconversion of Phytosterols into Androstadienedione by *Mycobacterium smegmatis* CECT 8331 ........................................ 211
*Julia García-Fernández, Igor Martínez, Lorena Fernández-Cabezón, Carmen Felpe-Santero, José-Luis García, and Beatriz Galán*

16 Process for Biotransformation of Androsta-4-ene-3,17-Dione (4-AD) to Androsta-1,4-Diene-3,17-Dione (ADD) ........................................ 227
*Surya Prakash and Abhay Bajaj*

17 Laboratory-Scale Hydroxylation of Steroids by P450BM3 Variants ......................................................... 239
*Sabrina Hoebenreich, Martin Spinck, and Nathalie Nett*

18 Obtaining of 11α-Hydroxyandrost-4-ene-3,17-dione from Natural Sterols ............................................. 259
*Dmitry Dovbnya, Sergey Khomutov, Vyacheslav Kollerov, and Marina V. Donova*

19 Steroid 11-Alpha-Hydroxylation by the Fungi *Aspergillus nidulans* and *Aspergillus ochraceus* ............ 271
*Lidia Ortega-de los Ríos, José M. Luengo, and José M. Fernández-Cañón*

20 Biotransformation of DHEA into 7α,15α-diOH-DHEA ................................................................. 289
*Hui Li, Jin Sun, and Zhenghong Xu*

21 Stabilization of Enzymes by Using Thermophiles ................................................................. 297
*Ana Luisa Ribeiro, Mercedes Sánchez, Aurelio Hidalgo, and José Berenguer*

22 Biocatalysis of Steroids with *Mycobacterium* sp. in Aqueous and Organic Media .............................. 313
*Carla C.C.R. de Carvalho and Pedro Fernandes*

23 Downstream Process Synthesis for Microbial Steroids ................................................................. 321
*Fabian B. Thygs and Juliane Merz*

24 Analysis of Intermediates of Steroid Transformations in Resting Cells by Thin-Layer Chromatography (TLC) ........................................ 347
*Govinda Guevara, Julián Perera, and Juana-Maria Navarro-Llorens*

25 A Ligand-Binding Assay to Measure the Affinity and Specificity of Sterol-Binding Proteins In Vitro ........ 361
*Rabih Darwiche and Roger Schneiter*

Index ........................................................................................................................................... 369
Contributors

Esther Bahillo • Department of Biotechnology, Crystal Pharma, A Division of Albany Molecular Research Inc. (AMRI), Parque Técnológico de León, León, Spain

Abhay Bajaj • Molecular Biology Laboratory, Department of Zoology, University of Delhi, Delhi, India

José-Luis Barredo • Department of Biotechnology, Crystal Pharma, A Division of Albany Molecular Research Inc. (AMRI), Parque Técnológico de León, León, Spain

Carlos Barreiro • Instituto de Biotecnología de León (INBIOTEC), Parque Científico de León, León, Spain; Área de Microbiología, Departamento de Biología Molecular, Campus de Ponferrada, Universidad de León, Ponferrada, Spain

José Becerra • Natural Products Chemistry Laboratory, Faculty of Natural and Oceanographic Sciences, University of Concepción, Concepción, Chile

José Berenguer • Centro de Biología Molecular Severo Ochoa (UAM-CSIC), Facultad de Ciencias, Universidad Autónoma de Madrid, Madrid, Spain

Soledad Chamorro • Engineering and Environmental Biotechnology Group, Environmental Science Faculty & Center EULA-Chile, University of Concepción, Concepción, Chile

Rabih Darwich • Division of Biochemistry, Department of Biology, University of Fribourg, Fribourg, Switzerland

Carla C.C.R. de Carvalho • IBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

Marina V. Donova • G.K. Skryabin Institute of Biochemistry & Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Russia; Pharmins LTD, Pushchino, Russia

Dmitry Dovbnya • G.K. Skryabin Institute of Biochemistry & Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Russia; Pharmins LTD, Pushchino, Russia

Carmen Felpeto-Santoro • Centro de Investigaciones Biológicas CSIC, Madrid, Spain

Pedro Fernandes • IBB – Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal; Faculty of Engineering, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal

Lorena Fernández-Cabezón • Centro de Investigaciones Biológicas CSIC, Madrid, Spain

José M. Fernández-Cañón • Instituto de Biología Molecular, Genómica y Proteómica (INBIOMIC), Dpto. Biología Molecular, Universidad de León, León, Spain

Beatriz Galán • Centro de Investigaciones Biológicas CSIC, Madrid, Spain

José-Luis García • Centro de Investigaciones Biológicas CSIC, Madrid, Spain

Julia García-Fernández • Centro de Investigaciones Biológicas CSIC, Madrid, Spain

Govinda Guevara • Department of Biochemistry and Molecular Biology I, Universidad Complutense de Madrid, Madrid, Spain

Víctor Hernández • Natural Products Chemistry Laboratory, Faculty of Natural and Oceanographic Sciences, University of Concepción, Concepción, Chile

Ignacio Herráiz • Research and Development Department, Gadea Pharmaceutical Group, A Division of Albany Molecular Research Inc. (AMRI), Parque Técnológico de Boecillo, Boecillo, Valladolid, Spain
Aurelio Hidalgo • Centro de Biología Molecular Severo Ochoa (UAM-CSIC), Facultad de Ciencias, Universidad Autónoma de Madrid, Madrid, Spain
Sabrina Hoebenreich • Fachbereich Chemie, Philipps-Universität Marburg, Marburg, Germany
Kjell D. Josefsen • SINTEF Materials and Chemistry, Department of Biotechnology and Nanomedicine, Trondheim, Norway
Sergey Khomutov • Institute of Biochemistry & Physiology of Microorganisms, Russian Academy of Sciences, Moscow, Russia
Vyacheslav Kollerov • Institute of Biochemistry & Physiology of Microorganisms, Russian Academy of Sciences, Moscow, Russia
Hui Li • School of Pharmaceutical Science, Jiangnan University, Wuxi, China
Jessica K. Lorraine • Department of Biology, University of York, Wentworth Way, York, UK
José M. Luengo • Departamento de Biología Molecular (Área de Bioquímica y Biología Molecular), Facultad de Veterinaria, Universidad de León, León, Spain
Rodrigo A. Mancilla • Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile
Marco P.C. Marques • Department of Biochemical Engineering, University College London, London, UK
Igor Martínez • Centro de Investigaciones Biológicas CSIC, Madrid, Spain
Sonia Martínez-Cámara • Department of Biotechnology, Crystal Pharma, A Division of Albany Molecular Research Inc. (AMRI), Parque Tecnológico de León, León, Spain
Juliane Merz • Department of Biochemical and Chemical Engineering, Laboratory of Plant and Process Design, TU Dortmund University, Dortmund, Germany
María-Angélica Mondaca • Microbiology Department, University of Concepción, Concepción, Chile
Alejandro Morales • INBIOTEC (Instituto de Biotecnología de León), Parque Científico de León, León, Spain
Alejandro Amoroso • Facultad de Ciencias, Universidad San Sebastián, Santiago, Chile
Juana-Maria Navarro-Llorens • Department of Biochemistry and Molecular Biology I, Universidad Complutense de Madrid, Madrid, Spain
Nathalie Nett • Fachbereich Chemie, Philipps-Universität Marburg, Marburg, Germany
Anna Nordborg • SINTEF Materials and Chemistry, Department of Biotechnology and Nanomedicine, Trondheim, Norway
Elías R. Olivera • Departamento de Biología Molecular (Área de Bioquímica y Biología Molecular), Facultad de Veterinaria, Universidad de León, León, Spain
Lidia Ortega-de los Ríos • Instituto de Biología Molecular, Genómica y Proteómica (INBIOMIC), Dpto. Biología Molecular, Universidad de León, León, Spain
Roberto Pavez-Díaz • Departamento de Ingeniería Química y Ambiental, Universidad Técnica Federico Santa María, Valparaíso, Chile
Julián Perera • Department of Biochemistry and Molecular Biology I, Universidad Complutense de Madrid, Madrid, Spain
Rosario Pérez-Redondo • Instituto de Biotecnología de León (INBIOTEC), Parque Científico de León, León, Spain
Surya Prakash • Hunan Yuxin Pharmaceutical Co. Ltd, Shaoyang, Hunan, China
Ana-Luisa Ribeiro • Centro de Biología Molecular Severo Ochoa (UAM-CSIC), Facultad de Ciencias, Universidad Autónoma de Madrid, Madrid, Spain
Contributors

ANTONIO RODRÍGUEZ-GARCÍA • Instituto de Biotecnología de León (INBIOTEC), Parque Científico de León, León, Spain; Área de Microbiología, Departamento de Biología Molecular, Campus de Ponsferrada, Universidad de León, León, Spain
MARTA RODRÍGUEZ-SÁIZ • Department of Biotechnology, Crystal Pharma, A Division of Albany Molecular Research Inc. (AMRI), León, Spain
MERCEDES SÁNCHEZ • Centro de Biología Molecular Severo Ochoa (UAM-CSIC), Facultad de Ciencias, Universidad Autónoma de Madrid, Madrid, Spain
MIKHAIL I. SCHELKUNOV • Institute for Information Transmission Problems, Russian Academy of Sciences, Moscow, Russia
ROGER SCHNITTER • Division of Biochemistry, Department of Biology, University of Fribourg, Fribourg, Switzerland
VICTORIA Y. SHTRATNIKOVA • A.N. Belozersky Research Institute of Physico-Chemical Biology, M.V. Lomonosov Moscow State University, Moscow, Russia
HÅVARD SLETTA • SINTEF Materials and Chemistry, Department of Biotechnology and Nanomedicine, Trondheim, Norway
MARGARET C.M. SMITH • Department of Biology, University of York, Wentworth Way, York, UK
ALBERTO SOLA-LANDA • Instituto de Biotecnología de León (INBIOTEC), Parque Científico de León, León, Spain
MARTIN SPINCK • Fachbereich Chemie, Philipps-Universität Marburg, Marburg, Germany
JIN SUN • School of Pharmaceutical Science, Jiangnan University, Wuxi, China
FABIAN B. THYS • Department of Biochemical and Chemical Engineering, Laboratory of Plant and Process Design, TU Dortmund University, Dortmund, Germany
INÉS VÁZQUEZ-IGLESIAS • Instituto de Biotecnología de León (INBIOTEC), Parque Científico de León, León, Spain
GLADYS VIDAL • Engineering and Environmental Biotechnology Group, Environmental Science Faculty & Center EULA-Chile, University of Concepción, Concepción, Chile
MARCELO VIDAL • Microbiology Department, University of Concepción, Concepción, Chile
CLAUDIA XAVIER • Engineering and Environmental Biotechnology Group, Environmental Science Faculty & Center EULA-Chile, University of Concepción, Concepción, Chile; Federal University of Technology – Paraná – UTFPR, Curitiba, PR, Brazil
ZHENGHONG XU • School of Pharmaceutical Science, Jiangnan University, Wuxi, China