Methods in Enzymology

Volume XV

Steroids and Terpenoids

EDITED BY

Raymond B. Clayton

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Preface

This volume of "Methods in Enzymology" is a fission product of an undertaking originally planned jointly with Dr. J. M. Lowenstein in which lipids and steroids were to be covered in one book. Progress in these areas during the past few years has been so great that the need for separate treatment soon became apparent, for the bulk of material relating to steroids and terpenoids, on the one hand, and to lipids, on the other, clearly exceeded the limits that could be satisfactorily encompassed by any single volume.

This resulting volume, devoted to methodology in the steroid and terpenoid fields, is divided into four main sections that deal, respectively, with special analytical methods, methods of synthesis of labeled substrates, enzyme preparation methods, and steroid-binding protein methods as exemplified by corticosteroid binding globulin (C.B.G.). As in the other volumes of this publication, the aim has been to include details of new procedures or improvements in existing methods that have become available since the last contributions in the area of steroid biochemistry were made to the treatise. Thus, in the analytical section, thin-layer chromatography and a number of applications of gas–liquid chromatography receive particular attention, and, with regard to the latter, methods for the isolation of suitably purified extracts for analysis by the highly sensitive electron-capture technique are emphasized.

In the section devoted to methods of synthesis an extensive survey of chemical techniques for the labeling of steroids is given that represents the broad advances which have taken place since they were last summarized in this treatise (Volume IV, 1957). The outstanding progress in synthetic labeling methods for mevalonic acid and terpenoid pyrophosphate intermediates that has been achieved since 1960 is also represented in a separate chapter. This work, which underlies so many important recent advances in the understanding of biosynthetic mechanisms, will no doubt continue to be a keystone in the edifice of terpenoid and steroid biochemistry for many years.

In the coverage of enzyme preparative methods and assay techniques, the data presented in earlier volumes are now supplemented with more recent information in areas in which notable progress has been made, as, for example, in the analysis of steroid oxygenase systems and the characterization of several esterases and specific dehydrogenases. To some extent this volume departs from the general approach in other volumes in that several chapters deal extensively with enzyme systems that are obviously complex, such as, for example, a number of microsomal systems

of sterol and steroid metabolism. Since so much of steroid enzymology is still at this stage of development, it seemed appropriate to summarize the present status of work in these areas in the hope that the ready availability of the related methodology would help to promote further work toward their clarification.

The final chapter, devoted primarily to corticosteroid binding protein, summarizes methods that are useful in a number of areas of growing importance in hormone assay studies and in the characterization of specific binding proteins possibly involved in the mechanism of hormone action.

The understanding cooperation of the authors whose devotion to the consolidation and advancement of steroid and terpene biochemistry that has made this book possible has been most heartening and deserves the sincere thanks of all practitioners in the field. The expert attention of Dr. Charles H. Doering and his assistant Mrs. Joanne Richards to the indexing of the book and their help in proof reading are also most gratefully acknowledged. Indexing was greatly facilitated by the use of the Advanced Computer for Medical Research (ACME) at the Stanford University School of Medicine, supported by U.S.P.H.S. Grant FR-00311. We would like to thank Mr. Robert L. Bassett for his generous advice in computer programming. We sincerely appreciate the cooperation of Academic Press in the production of this work.

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June, 1969

METHODS IN ENZYMOLOGY

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II. Preparation and Assay of Enzymes
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Section I

Newer Analytical Techniques

[1] Thin-Layer Chromatography of Steroids, Sterols, and Related Compounds

By BELISÁRIO P. LISBOA

I. Introduction

As one of the most utilized analytical procedures for the investigation of steroids, thin-layer chromatography (TLC) is the subject of several recent reviews.[1-5] The simple apparatus and techniques required for the development of thin-layer chromatograms, the high-power of resolution of TLC, and the possibility of employing a large number of reactions of great specificity and high sensitivity for the visualization of the analyzed compounds are major reasons for the acceptance that TLC has found as a separation procedure.

[1] J. M. Bobbit, "Thin-Layer Chromatography." Reinhold, New York, 1963.
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[3] J. G. Kirchner, "Technique of Organic Chemistry; Thin Layer Chromatography" (E. S. Perry and A. Weissberger, eds.), Vol. XII. Wiley (Interscience), New York, 1966.
[4] E. Heftmann, *Chromatog. Rev.* 7, 179 (1965).
[5] A. A. Akhrem and A. I. Kuznetsova, "Thin-Layer Chromatography. A Practical Laboratory Handbook." Davey, New York, 1965.