

Handbook of Derivatives for Chromatography

Edited by

KARL BLAU and GRAHAM S. KING

Handbook of Derivatives for Chromatography

Edited by

KARL BLAU and GRAHAM S. KING

*Bernhard Baron Memorial Research Laboratories,
Queen Charlotte's Maternity Hospital, London, W6 0XG.*



London • Bellmawr, N.J. • Rheine

Heyden & Son Ltd., Spectrum House, Alderton Crescent, London NW4 3XX, U.K.

Heyden & Son Inc., Kor-Center East, Bellmawr, N.J. 08030, U.S.A.

Heyden & Son GmbH, Münsterstrasse 22, 4440 Rheine/Westf., Germany.

© Heyden & Son Ltd, 1977

All Rights Reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of Heyden & Son Ltd.

ISBN 0 85501 206 4

Printed in Great Britain by J. W. Arrowsmith Ltd, Bristol BS3 2NT

Foreword

Chromatography is a complicated business these days. When I first became the proud co-owner (with Evan Horning) of a gas chromatograph at the National Institutes of Health in the middle 1950s, however, there was not much a biochemist could do with the thing except fatty acid analyses. Historians of the field who inspect the literature from 1957 to 1960 will certainly be puzzled by the extraordinary amount of scientific manpower that went into that one subject of fatty acid analysis. Of course, it was not the perfection of the chromatographic separation alone that provided the driving force for this work. Much was done to delineate the theory of chromatography, many new liquid phases were invented, new detectors were described, support-coating procedures were developed, and several new firms were stocking their shelves with chromatographic supplies and announcing that they were ready to provide the chromatographer with everything he needed.

The explosive growth of gas-liquid chromatography since 1960 has depended in large measure on the development of lightly loaded columns and on the growing realization that 'almost anything' can be made volatile under the proper circumstances. One is impressed now if a compound (outside the macromolecular domain) is found that cannot be volatilized; I confess that I do not know of many such examples.

Unlike the efforts devoted to perfection of the chromatographic process with fatty acids, work in the amino acid field was involved with the problem of quantitative derivative formation with all of the amino acids, and with selection of derivative and partition phases that would maximize separation of the mixture in a reasonable period of time. Derivatives and the reactions by which they are formed thus became extremely important considerations in the chromatographic procedure. In this and many other examples, derivatization ranks equally with sample selection, preprocessing and chromatography as an important part of the overall analytical technique.

Derivatization in liquid chromatography has a different rationale and hence quite different rules regarding choice of reagents, etc. The derivative group(s) added to the molecule almost inevitably become the detectable residue in the derivative. Sensitivity is of primary concern, while the molecular weight of the product is of little or no consequence. Reactions are usually carried out *after* the chromatographic step, whereas derivatization precedes gas-liquid chromatography.

Here is a book about the chemistry of derivative reactions that will be consulted by experts and beginners alike. It contains detailed information about the chemical reactions of a wide variety of functional groups and compares strategies of derivatization of common classes of compounds such as amino acids, steroids and carbohydrates. The book should help overcome the initial problem in making choices among various derivatives and will certainly steer the reader to appropriate literature about yields, derivative purification, stability, etc. Processing of biological samples is an entirely separate subject and the editors have appropriately not considered this aspect of the overall chromatographic procedure. The chapter on ion-pair extraction and chromatography does address itself to this subject to some extent, but extraction and chromatography are treated together in a smooth and comprehensive review of a technique that should gain in popularity.

This is a thoroughly delightful book to me personally, as it contains so much of the information I and my students need to refer to when dealing with new compounds and different kinds of biological samples. The handbook will enjoy a wide and well-deserved popularity and should be a reference work in every laboratory where chromatography is a serious business.

East Lansing, Michigan
March, 1977

Charles C. Sweeley

List of Contributors

- DUŠAN ANDERLE, Institute of Chemistry, Slovak Academy of Sciences, 809 33 Bratislava, Czechoslovakia (p. 201)
- KARL BLAU, Prenatal Biochemistry, Bernhard Baron Memorial Research Laboratories, Queen Charlotte's Maternity Hospital, London W6 0XG, United Kingdom (pp. 1, 104, 317)
- K. O. BORG, Department of Analytical Pharmaceutical Chemistry, Biomedical Centre, University of Uppsala, Box 574, S-751 23 Uppsala, Sweden (p. 500)
- HANS BRANDENBERGER, Gerichtlich-Medizinisches Institut der Universität Zürich, Chemistry Department, Zürichbergstrasse 8, Zürich, Switzerland (p. 234)
- ROBERTA H. BRANDENBERGER, Gerichtlich-Medizinisches Institut der Universität Zürich, Chemistry Department, Zürichbergstrasse 8, Zürich, Switzerland (p. 234)
- WILLIAM C. BUTTS, Clinical Chemistry Section Group Health Co-operative of Puget Sound, Group Health Hospital 200-15th Avenue East, Seattle, Washington 98112, U.S.A. (p. 411)
- ANDRÉ DARBRE, Department of Biochemistry, University of London King's College, Strand, London WC2R 2LS, United Kingdom (p. 262)
- LOTHAR DEMISCH, Max-Planck-Institut für Hirnforschung und Zentrum der Psychiatrie und Klinikum der Universität, Frankfurt am Main—Niederrad, West Germany (p. 346)
- DAVID J. EDWARDS, University of Pittsburgh School of Medicine, Department of Psychiatry, Western Psychiatric Institute and Clinic, 3811 O'Hara Street, Pittsburgh, Pa. 15261, U.S.A. (p. 391)
- BERT HALPERN, Department of Chemistry, University of Wollongong, P.O. Box 1144 Wollongong, N.S.W. 2500, Australia (p. 457)
- MAY N. INSCOE, Organic Chemicals Synthesis Laboratory, Agricultural Environmental Quality Institute, U.S. Department of Agriculture, Research Center East, Beltsville, Maryland 20705, U.S.A. (p. 317)
- GRAHAM S. KING, Mass Spectrometry Unit, Bernhard Baron Memorial Research Laboratories, Queen Charlotte's Maternity Hospital, London W6 0XG, United Kingdom (pp. i, 104, 317)
- PAVOL KOVÁČ, Institute of Chemistry, Slovak Academy of Sciences, 809 33 Bratislava, Czechoslovakia (p. 201)
- R. MODIN, Department of Analytical Pharmaceutical Chemistry, Biomedical Centre, University of Uppsala, Box 574, S-751 23 Uppsala, Sweden (p. 500)
- PAUL MUSHAK, Department of Pathology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, N.C. 27514, U.S.A. (p. 433)
- BENGT-ARNE PERSSON, Department of Analytical Pharmaceutical Chemistry, Biomedical Centre, University of Uppsala, Box 574, S-751 23 Uppsala, Sweden (p. 500)

COLIN F. POOLE, Department of Organic Chemistry, University of Ghent, Krijkslaan 271 (S.4), B-9000 Ghent, Belgium (p. 152)

GÖRAN SCHILL, Department of Analytical Pharmaceutical Chemistry, Biomedical Centre, University of Uppsala, Box 574, S-751 23 Uppsala, Sweden (p. 500)

NICKOLAUS SEILER, Max-Planck-Institut für Hirnforschung und Zentrum der Psychiatrie und Klinikum der Universität, Frankfurt am Main—Niederrad, West Germany (p. 346)

CHARLES C. SWEELEY, Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824, U.S.A. (p. xi)

List of Abbreviations

The following is a list of abbreviations in the *Handbook*. Other less common abbreviations are explained by the authors at their point of use. A number of generally accepted abbreviations such as those used for units and dimensions are not defined here

Bns	5-Di- <i>n</i> -butylamino-naphthalene-1-sulfonyl	HFB	Heptafluorobutyryl
BOC	Butyloxycarbonyl	HFBA	Heptafluorobutyric anhydride
b.p.	Boiling point	HMDS	Hexamethyldisilazane
BSA	<i>N,O</i> -Bistrimethylsilylacetamide	HPLC	High-pressure liquid chromatography
BSTFA	<i>N,O</i> -Bistrimethylsilyltri-fluoroacetamide	i.r.	infrared
Dis-Cl	2- <i>p</i> -Chlorosulfo-phenyl-3-phenylindene	MBTFA	Methyl-bis-trifluoroacetamide
DMAA	<i>N,N</i> -Dimethylacetamide	Mns	6-Methylanilinonaphthalene-2-sulfonyl
DMF	<i>N,N</i> -Dimethylformamide	MO-TMS	Combined methoxime-trimethylsilyl derivative
DMSO	Dimethylsulfoxide	m.p.	Melting point
DNP	Dinitrophenyl	MS	Mass spectrometry
2,4-DNP	2,4-Dinitrophenylhydrazine	MTH	Methylthiohydantoin
Dns	'Dansyl' i.e. 5-Dimethylaminonaphthalene-1-sulfonyl	n.m.r.	Nuclear magnetic resonance
EC	Electron capture	Nbd-Cl	4-Chloro-7-nitrobenzo-(c)-1,2,5-oxadiazole
ECD	Electron capture detector or detection	OD	Optical density
ECD-GC	Electron capture gas chromatography	OPT	<i>o</i> -Phthalaldehyde
EDTA	Ethylenediaminetetraacetic acid	PFB	Pentafluorobenzoyl— Chapter 3
FID	Flame ionization detector or detection		Pentafluorobenzyl— Chapter 2
GC	Gas chromatography	PFBCl	Pentafluorobenzimidyl— Chapter 6
GC-MS	Combined gas chromatography-mass spectrometry	PFP	Pentafluorobenzoyl chloride
GLC	Gas liquid chromatography	PFPA	Pentafluoropropionyl
GLC-EC	Electron capture gas chromatography	PTC	Pentafluoropropionic anhydride
GSC	Gas solid chromatography	PTFE	Phenylthiocarbamyl
		PTH	Polytetrafluoroethylene (e.g. Teflon®)
			Phenylthiohydantoin

RGC	Reaction gas chromatography	TFA	Trifluoracetyl
RLC	Reaction liquid chromatography	TFAA	Trifluoroacetic anhydride
RTLC	Reaction thin layer chromatography	TLC	Thin layer chromatography
SIM	Selected ion monitoring (also known as mass fragmentography, multiple ion detection, specific ion monitoring, etc.)	TMCS	Trimethylchlorosilane
		TMS	Trimethylsilyl
		TMSIM	Trimethylsilylimidazole
		TNP	Trinitrophenyl
		u.v.	Ultraviolet
		v/v	Volume for volume
		w/v	Weight for volume

Contents

Foreword	xi
List of Contributors	xiii
List of Abbreviations	xv

Chapter 1 **Introduction to the Handbook** G. S. KING and K. BLAU

1	Introduction	1
2	The Organization of the Book	4
3	Practical Considerations	27
4	Conclusion	37

Chapter 2 **Esterification** A. DARBRE

1	Theoretical Basis	39
2	Practical Methods	60
3	Determination and Reagents	90

Chapter 3 **Acylation** K. BLAU and G. S. KING

1	Introduction	104
2	Reaction Mechanisms	104
3	Types of Acylation Reagents	107
4	Detector-orientated Acylations	108
5	Practical Applications	110
6	Electron-capturing Ability and Chemical Structure	135
7	Mass Spectrometry of Perfluoroacyl Derivatives	143
8	Future Prospects of Acylation for Chromatography	145
9	Conclusion	145

Chapter 4 Recent Advances in the Silylation of Organic Compounds for Gas Chromatography

C. F. POOLE

1	Introduction	152
2	The Formation of Silyl Ethers	156
3	The Formation of TMS-Ethers of Some Biologically Important Compounds	169
4	The Mass Spectra of TMS-Ethers	182
5	Trialkylsilyl Ethers other than Trimethylsilyl	184
6	Conclusion	194

Chapter 5 Protective Alkylation

P. KOVÁČ and D. ANDERLE

1	Introduction	201
2	Alkylation Procedures	202
3	Appendix: Purification of Chemicals	227

Chapter 6 Derivatives by Ketone-Base Condensation

R. H. BRANDENBERGER and H. BRANDENBERGER

1	Analysis of Carbonyl Compounds as Schiff Bases	234
2	Analysis of Primary Amines as Schiff Bases	237
3	Analysis of Primary Amines as Mustard Oils	239
4	Selected Procedures	242
5	Tabular Summary of Ketone-Base Derivatives for Chromatography	246

Chapter 7 Cyclization

A. DARBRE

Section A—Theoretical Considerations

1	Introduction	262
2	Acetals and Metals	264
3	Siliconides	269
4	Boronates	270
5	Other Heterocyclic Derivatives	273
6	Cyclic Compounds in Protein Sequencing	279

Section B—Practical Methods

7	Acetals and Ketals	282
8	Siliconides	291

9	Boronates	293
10	Other Heterocyclic Derivatives	298
11	Cyclic Compounds in Protein Sequencing	308

Chapter 8 Microreactions

M. N. INSCOE, G. S. KING and K. BLAU

1	Introduction	317
2	Reaction Chromatography	319
3	Microchemical Reactions Prior to Chromatography	325
4	Conclusion	342

Chapter 9 Fluorescent Derivatives

N. SEILER and L. DEMISCH

1	Introduction	346
2	5-Dimethylaminonaphthalene-1-sulfonyl chloride (Dns-Cl)	349
3	5-Di- <i>n</i> -Butylaminonaphthalene-1-sulfonyl chloride (Bns-Cl)	363
4	6-Methylanilinonaphthalene-2-sulfonyl chloride (Mns-Cl)	365
5	2- <i>p</i> -Chlorosulfophenyl-3-phenylindone (Dis-Cl)	365
6	4-Chloro-7-nitrobenzo-(<i>c</i>)-1,2,5-oxadiazole (Nbd-Cl)	367
7	Isothiocyanates	370
8	9-Isothiocyanates	370
9	Fluorescein isothiocyanate	371
10	Fluorescamine	372
11	Pyridoxal and Pyridoxal-5-Phosphate	376
12	<i>o</i> -Phthaldialdehyde (OPT)	378
13	Condensation Reactions with Aldehydes	379
14	Fluorescent Reagents for Carbonyl Compounds	381
15	Sulfhydryl Reagents	383
16	Conclusion	383

Chapter 10 Dinitrophenyl and other Nitrophenyl Derivatives

D. J. EDWARDS

1	Properties of Nitrophenyl Derivatives	391
2	Reagents	393
3	Preparation of Nitrophenyl Derivatives	396

4	Separation of Nitrophenyl Derivatives	400
5	Detection	403
6	Applications	409

Chapter 11 Derivatives of Inorganic Anions for Gas Chromatography

W. C. BUTTS

1	Introduction	411
2	Halide Derivatives	411
3	Anion Esters	414
4	Phosphate	416
5	Substituted Phosphates	416
6	Mass Spectrometry	420
7	Specific Anion Derivatives	420
8	Appendix I: Reactions	423
9	Appendix II: Procedures	425

Chapter 12 The Gas-Liquid Chromatography of Metal Ions via Chelation and Non-chelation Techniques

P. MUSHAK

1	Introduction	433
2	Chelation Techniques in the Gas-Liquid Chromatographic Analysis of Metals	433
3	Non-chelation Techniques in Metal Ion Chromatography	449
4	Comparative Discussion of Metal Ion Analyses by Gas-Liquid Chromatography	452

Chapter 13 Derivatives for Chromatographic Resolution of Optically Active Compounds

B. HALPERN

1	Introduction	457
2	Application	459
3	The Mechanism of the Resolution Process	463
4	Resolution of Optical Isomers by Gas Chromatography of Diastereoisomers	464
5	Gas Chromatographic Resolution of Enantiomers on Optically Active Stationary Phases	492
6	Bibliography of Non-Gas Chromatographic Methods	496

Chapter 14	Ion-Pair Extraction and Ion-Pair Chromatography	
	G. SCHILL, R. MODIN, K. O. BORG and B.-A. PERSSON	
1	Introduction	500
2	Ion-Pair Extraction	501
3	Ion-Pair Partition Chromatography	509
4	Conclusion	525
5	Appendix: A Detailed Theoretical Treatment of Ion-Pair Extraction	525
Index	531

CHAPTER 1

Introduction to the Handbook

Graham S. King and Karl Blau

*Bernhard Baron Memorial Research Laboratories,
Queen Charlotte's Maternity Hospital, London W6 0XG*

1 INTRODUCTION

The enthusiastic adoption of chromatographic methods has had a great impact on chemical analysis, not only in chemistry and biochemistry but also in pharmacology, toxicology, clinical sciences, genetics, forensic science, environmental science and many other fields. Indeed chromatography has contributed greatly to recent advances in carbohydrate and lipid chemistry. The previously demanding discipline of amino acid, peptide and protein structural analysis has expanded and experienced unprecedented growth, transforming our understanding of large areas of biology. Unfortunately not all compounds are accessible to direct analysis by chromatography and much ingenuity has been expended into devising ways of making chemical derivatives that will render them easier to analyse. A whole new branch of chemistry has evolved around this problem. Much of the work is widely scattered, many derivatization schemes that were worked out for a limited objective are potentially much more widely applicable, and in spite of the extensive achievements that have already been described, a great deal of further development can still be anticipated.

For these reasons we feel that this is a good time to gather some of this experience in derivatization together into a more systematic arrangement. We hope that the underlying principles will, as a result, become more apparent, and that derivatization chemistry will become more readily appreciated, providing a solid foundation on which workers can base the development of new methods of analysis. There have already been a few useful reviews and monographs devoted to specific problems or techniques. The derivatization of amino acids for gas chromatography has received considerable attention¹⁻⁴ this is also true for steroids⁵⁻⁷ and drugs and pesticides.⁸ Review articles and a recent monograph have covered derivatization for liquid chromatography,^{9,10} gas chromatography^{11,12,21} and the analysis of pharmaceuticals by gas chromatography.¹⁴

The use of fluorescent derivatives^{10,15} and of chiral resolving agents¹⁶ has also received attention. The techniques of silylation have been thoroughly covered in Pierce's excellent *Silylation of Organic Compounds* which should be at the side of every practising gas chromatographer.¹⁷ Several books have been published on the subject of functional group analysis by gas chromatography. The book by Leathard and Shurlock¹⁸ deals briefly with some of the simpler methods of derivatization which were current in the late sixties and also describes many useful techniques of abstraction in GC and pyrolysis gas chromatography. Crippen¹⁹ has published his original and valuable approach to functional group identification, which necessarily relies upon derivatization to a large extent. Recently Ma and Ladas²⁰ have covered the area of functional group analysis in a slightly different way, briefly reviewing some areas of derivative formation, providing selected practical details and giving a very good coverage to the subject of reaction gas chromatography and abstraction techniques. The comprehensive *Handbook of Chromatography*, edited by Zweig and Sherma,²¹ contains a useful section on derivatization which is a first attempt, but necessarily a brief one, at a general coverage of this subject. Our plan is to cover derivatization chemistry along quite different lines, and to systematize the chemical reactions used on the basis of the chemical processes involved in derivative formation. We do not intend to duplicate or extend the publications on functional group analysis or reaction gas chromatography but we aim to provide a practical approach to derivative selection for the analyst. Our approach is two-fold: not only to present group reactions that have already been applied to specific problems and so reveal their underlying principles, common basis and applicability, but also to provide the information and ideas by which these principles may be extended to new applications and perhaps even to new derivatization methods.

Direct chromatographic analysis of certain classes of compounds is difficult. For example, the application of paper chromatography to lipophilic substances was hampered for years by their hydrophobic nature, and the expedient of 'reversed-phase' chromatography was used until the development of thin-layer chromatography on silica gel.²² Difficulties were often experienced with the gas chromatography of very polar compounds such as free acids and amines, with thermolabile substances and with more complex polar compounds such as the amino sugars and carbohydrates. It was soon found that chemical modification of polar functional groups improved the compound's accessibility to gas chromatographic analysis. In amino acid analysis it was found that the coloured dinitrophenyl derivatives, and later the fluorescent 'dansyl' derivatives had distinct advantages for paper, thin-layer and liquid chromatography. During these early stages in the development of derivatization techniques, the chemical reactions used were mostly those currently available from preparative organic chemistry and the groups introduced were those commonly used in organic synthesis for 'blocking' or protecting groups. These were usually designed to be easily removed at the end of a synthetic sequence. It was soon

obvious that for chromatographic analysis, the susceptibility of the protecting group to easy removal was of no importance and might even be a disadvantage. This in turn led to the expansion of a new area of derivatization chemistry: the preparation of chemical derivatives selected or specifically designed to improve chromatograph analysis. This might involve incorporating a chemical function which gives a high detector sensitivity, or a particularly volatile derivative, or good chromatographic separation or even a simple and convenient preparation method. One of the most important contributions was the development of silylation methods; Pierce's handbook on silylation techniques¹⁷ was an important publication and helped to transform a relatively unfamiliar chemical reaction into one of the most widely used derivatization methods. Silylation effectively blossomed and scattered seeds in all directions; recent advances are covered in this volume and considerable progress has been made since the publication of Pierce's book. To some extent Pierce's book has prompted the presentation of our *Handbook* and we gladly acknowledge its influence.

There is now a very wide choice of reactions for the preparation of derivatives to assist in chromatographic analysis. The selection of suitable methods depends on many factors. We hope to convey an appreciation of these factors which, apart from those already mentioned, may also involve considerations such as chemical stability of the substrate and the analytical sensitivity required. It may not be very easy at first to choose the best derivative for a given application from the many methods presented here. It may not even be apparent that the compound might chromatograph quite well without any derivative formation. However, we hope to make it easier to reach a rational decision by presenting the information in a convenient and fairly unified form in a single volume. Much of the book is basic chemistry that has stood the test of time and describes methods that have become standard and so we do not anticipate that it will be out of date as soon as publications in some other areas of research.

The procedures and discussion given by the different authors are inevitably varied, both in presentation and in scope. We have encouraged the contributors to standardize only to the extent that each chapter includes enough details to make the book a truly practical handbook; we have not attempted to cover isolation methods (apart from the chapter on ion-pair extraction), because they are so diverse that a whole separate volume would hardly cover the field. It must be stressed that the isolation of a compound from the sample matrix in good yield and ready for derivatization and chromatography is of paramount importance, and it is this that is very often the most difficult part of an assay. Nevertheless, both isolation and analysis are essential links of the chain. Solutions to the problems of extraction, derivatization and separation are more easily tackled once the analytical procedure has been worked out with standard samples.

We have attempted to obtain some degree of uniformity between chapters and also a degree of balance, especially between different types of