

Inositol Phosphates

LINKING AGRICULTURE AND THE ENVIRONMENT

This page intentionally left blank

Inositol Phosphates

LINKING AGRICULTURE AND THE ENVIRONMENT

Edited by

Benjamin L. Turner

*Smithsonian Tropical Research Institute
Balboa, Ancón, Republic of Panama*

Alan E. Richardson

*CSIRO Plant Industry
Canberra, Australia*

and

Edward J. Mullaney

*United States Department of Agriculture
New Orleans, USA*



CABI is a trading name of CAB International

CABI Head Office
Nosworthy Way
Wallingford
Oxfordshire OX10 8DE
UK
Tel: +44 (0)1491 832111
Fax: +44 (0)1491 833508
E-mail: cabi@cabi.org
Website: www.cabi.org

CABI North American Office
875 Massachusetts Avenue
7th Floor
Cambridge, MA 02139
USA
Tel: +1 617 395 4056
Fax: +1 617 354 6875
E-mail: cabi-nao@cabi.org

©CAB International 2007. All rights reserved. No part of this publication may be reproduced in any form or by any means, electronically, mechanically, by photocopying, recording or otherwise, without the prior permission of the copyright owners.

A catalogue record for this book is available from the British Library, London, UK.

A catalogue record for this book is available from the Library of Congress, Washington, DC.

ISBN-10: 1 84593 152 1
ISBN-13: 978 1 84593 152 1

Typeset by SPi, Pondicherry, India.
Printed and bound in the UK by Biddles Ltd, King's Lynn.

Contents

Contributors	vii
Preface	ix
Acknowledgements	xi
1. Nomenclature and Terminology of Inositol Phosphates: Clarification and a Glossary of Terms	1
<i>Stephen B. Shears and Benjamin L. Turner</i>	
2. Identification of Inositol Phosphates by Nuclear Magnetic Resonance Spectroscopy: Unravelling Structural Diversity	7
<i>Pushpalatha P.N. Murthy</i>	
3. High-performance Chromatographic Separations of Inositol Phosphates and Their Detection by Mass Spectrometry	23
<i>William T. Cooper, Matthew Heerboth and Vincent J.M. Salters</i>	
4. Origins and Biochemical Transformations of Inositol Stereoisomers and Their Phosphorylated Derivatives in Soil	41
<i>Michael F. L'Annunziata</i>	
5. Isolation and Assessment of Microorganisms That Utilize Phytate	61
<i>Jane E. Hill and Alan E. Richardson</i>	
6. Phytate-degrading Enzymes: Regulation of Synthesis in Microorganisms and Plants	78
<i>Ralf Greiner</i>	
7. Phytases: Attributes, Catalytic Mechanisms and Applications	97
<i>Edward J. Mullaney and Abul H.J. Ullah</i>	
8. Seed Phosphorus and the Development of Low-phytate Crops	111
<i>Victor Raboy</i>	
9. Phytase and Inositol Phosphates in Animal Nutrition: Dietary Manipulation and Phosphorus Excretion by Animals	133
<i>Xin Gen Lei and Jesus M. Porres</i>	

10. Environmental Implications of Inositol Phosphates in Animal Manures	150
<i>April B. Leytem and Rory O. Maguire</i>	
11. Ligand Effects on Inositol Phosphate Solubility and Bioavailability in Animal Manures	169
<i>Thanh H. Dao</i>	
12. Inositol Phosphates in Soil: Amounts, Forms and Significance of the Phosphorylated Inositol Stereoisomers	186
<i>Benjamin L. Turner</i>	
13. Abiotic Reactions of Inositol Phosphates in Soil	207
<i>Luisella Celi and Elisabetta Barberis</i>	
14. Interactions Between Phytases and Soil Constituents: Implications for the Hydrolysis of Inositol Phosphates	221
<i>Timothy S. George, Hervé Quiquampoix, Richard J. Simpson and Alan. E. Richardson</i>	
15. Plant Utilization of Inositol Phosphates	242
<i>Alan E. Richardson, Timothy S. George, Iver Jakobsen and Richard J. Simpson</i>	
16. Inositol Phosphates in Aquatic Systems	261
<i>Ian D. McKelvie</i>	
Index	279

Contributors

- Barberis, Elisabetta**, *University of Turin, DIVAPRA Chimica Agraria, via Leonardo da Vinci 44, Grugliasco, 10095 Torino, Italy*
- Celi, Luisella**, *University of Turin, DIVAPRA Chimica Agraria, via Leonardo da Vinci 44, Grugliasco, 10095 Torino, Italy*
- Cooper, William T.**, *Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL 32306, USA*
- Dao, Thanh H.**, *United States Department of Agriculture–Agricultural Research Service, Beltsville Agricultural Research Center, Room 121, 10300 Baltimore Avenue, Building 306 BARC-EAST, Beltsville, MD 20705, USA*
- George, Timothy S.**, *Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK*
- Greiner, Ralf**, *Federal Research Centre for Nutrition and Food, Centre for Molecular Biology, Haid-und-Neu-Straße 9, D 76131 Karlsruhe, Germany*
- Heerboth, Matthew**, *Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL 32306, USA*
- Hill, Jane E.**, *Environmental Engineering Program, Yale University, 9 Hillhouse Avenue, PO Box 8286, New Haven, CT 06520, USA*
- Jakobsen, Iver**, *Risø National Laboratory, Biosystems Department, Roskilde, DK 4000, Denmark*
- L'Annunziata, Michael F.**, *The Montague Group, PO Box 5033, Oceanside, CA 92052, USA*
- Lei, Xin Gen**, *Department of Animal Science, Morrison Hall 252, Cornell University, Ithaca, NY 14853, USA*
- Leytem, April B.**, *United States Department of Agriculture–Agricultural Research Service, Northwest Irrigation and Soils Research Laboratory, 3793 N. 3600 E., Kimberly, ID 83341, USA*
- Maguire, Rory O.**, *Crop and Soil Environmental Sciences, Virginia Tech, Box 0404, Blacksburg, VA 24061, USA*
- McKelvie, Ian D.**, *Water Studies Centre and Chemistry Department, School of Chemistry, Monash University, Clayton, Victoria 3800, Australia*

Mullaney, Edward J., *United States Department of Agriculture–Agricultural Research Service, Southern Regional Research Center, 1100 Robert E. Lee Blvd, New Orleans, LA 70124, USA*

Murthy, Pushpalatha P.N., *Department of Chemistry, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931, USA*

Porres, Jesus M., *Departamento de Fisiología, Universidad de Granada, Granada, Spain*

Quiquampoix, Hervé, *Unité de Science du Sol, INRA-ENSAM, 2 Place Pierre Viala, 34060 Montpellier Cedex 1, France*

Raboy, Victor, *United States Department of Agriculture–Agricultural Research Service, Small Grains and Potato Germplasm Research Unit, 1691 S. 2700 W., Aberdeen, ID 83210, USA*

Richardson, Alan E., *CSIRO Plant Industry, PO Box 1600, Canberra, ACT 2601, Australia*

Salters, Vincent J.M., *National High Magnetic Field Laboratory and Department of Geological Sciences, Florida State University, Tallahassee, FL 32306, USA*

Shears, Stephen B., *Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, NIH, DHSS, Research Triangle Park, PO Box 12233, NC 27709, USA*

Simpson, Richard J., *CSIRO Plant Industry, PO Box 1600, Canberra, ACT 2601, Australia*

Turner, Benjamin L., *Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancón, Republic of Panama*

Ullah, Abul H.J., *United States Department of Agriculture–Agricultural Research Service, Southern Regional Research Center, 1100 Robert E. Lee Blvd, New Orleans, LA 70124, USA*

Preface

Inositol phosphates are a group of organic phosphorus compounds found widely in the natural environment. They are common in eukaryotic organisms, especially plants, where they constitute most of the phosphorus in seeds. Soils and aquatic sediments also contain large amounts of inositol phosphates, some of which occur in forms that have not been detected anywhere else in nature.

The abundance of inositol phosphates in nature means that they are of widespread interest in the ecological and environmental sciences. However, it is in the science of animal nutrition that inositol phosphates have become a topic of considerable interest. This stems from the fact that monogastric animals cannot digest phytate (salts of *myo*-inositol hexakisphosphate), the most abundant inositol phosphate in cereal grains. Supplemental phosphate is therefore required in the diets of pigs and poultry to maintain productivity.

A consequence of phosphate supplementation is that animal manure can contain considerable concentrations of phosphorus. Not only does this represent a financial loss to the producer, but it also contributes to one of the most pervasive forms of environmental pollution from modern agriculture. Long-term application of manure to agricultural land leads to an accumulation of phosphorus in the soil and a gradual increase in phosphorus transport in runoff to water bodies. Such diffuse pollution is now widespread and there are numerous examples of regional-scale water quality deterioration in areas of intensive livestock operations. Two well-publicized examples are the Chesapeake Bay, USA, and the Gippsland Lakes, Australia. In both cases the problems have been severe and public – the high-profile detection of the neurotoxin-producing dinoflagellate *Pfiesteria piscicida* in the Chesapeake Bay being a particular cause for concern.

To address this issue, several strategies of dietary manipulation have been developed to improve the ability of monogastric animals to digest phytate. These include the use of ‘low-phytate’ grains – mutants selected for the low concentration of inositol phosphate in their seed – and the development of transgenic animals that produce phytase, an enzyme that degrades phytate but is not naturally present in the guts of monogastric animals.

By far the most successful strategy, however, has been the supplementation of animal diets with a microbial phytase. This is now standard practice in most large-scale animal feeding operations and is even mandated by law in some states of the USA. It has proved to be extremely effective in reducing phosphorus excretion in manure and has the added benefit of improving mineral nutrition by releasing metals from complexation with phytate.

Despite the wealth of information on inositol phosphates in animal nutrition, the environmental impacts of manure-derived inositol phosphates and associated dietary manipulations are not well understood. In particular, the fate of the large amount of inositol phosphates being cycled through

agriculture, especially in regions with high animal densities, is unknown. Importantly, only a handful of studies have assessed the impact of diet manipulation on phosphorus dynamics in the environment.

Inositol phosphates are rapidly and strongly stabilized in soil, which means that they are often considered to be biologically unavailable and unlikely to be transported in runoff to water bodies. Yet there is growing evidence that inositol phosphates are not as recalcitrant in the environment as once thought. It is now clear that many terrestrial microorganisms, including those associated with plants, have the capacity to use inositol phosphates. This trait appears widespread, although its ecological implications await investigation. Similarly, when inositol phosphates are transported in runoff to water bodies, they can degrade rapidly and contribute to the nutrition of cyanobacteria and other aquatic organisms linked to eutrophication. Inositol phosphates can therefore no longer be considered ecologically or environmentally benign.

Given the water quality problems associated with intensive livestock production and the widespread adoption of dietary modifications, there is an urgent need to improve our understanding of inositol phosphates in the environment. This was addressed at a conference held in August 2005 in Sun Valley, Idaho, USA, sponsored by the Soil Science Society of America. The meeting, entitled 'Inositol Phosphates in the Soil-plant-animal System: Linking Agriculture and Environment', was attended by scientists from a diverse range of disciplines with a common interest in inositol phosphates. This book is the output from that conference. Written by the invited speakers, it brings together critical reviews on the major topics in inositol phosphates in agriculture, ecology and the environment. The chapters cover three major themes:

1. State-of-the-art analytical methodology for assessing inositol phosphates in environmental samples, including nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry.
2. Inositol phosphates in animal nutrition, including the latest research on plant and microbial phytases, their interactions in soil and the manipulation of animal diets with phytase supplements and low-phytate grains.
3. Inositol phosphates in the environment, including the amounts, forms and behaviour in soils and aquatic systems, their biological availability and the fate of manure-derived inositol phosphates in the environment.

By covering all major aspects of inositol phosphates in agriculture and the environment, the book will serve as a unique reference source on this emerging topic. We hope that it will benefit those trying to unravel the complexity of inositol phosphates in the environment and reveal what is already known to a wider audience.

The inositol phosphate conference in 2005 was held a quarter of a century after the publication of Dennis Cosgrove's seminal text *Inositol Phosphates: Their Chemistry, Biochemistry, and Physiology* (Elsevier Scientific, Amsterdam). Formerly of CSIRO Plant Industry in Canberra, Australia, Cosgrove devoted his career to understanding inositol phosphates in the environment (an obituary can be found in *Soil Biology and Biochemistry*, vol. 14, pp. 77–78). His pioneering work in the two decades after he moved with his family from England to Australia in 1955 laid the foundations for many of the topics in this volume. His death in 1981 at the age of 56 marked the end of an era for studies on inositol phosphates in the environment, but his discoveries remain an inspiration to scientists in this field. We hope that this volume will go some way towards reinvestigating interest in these fascinating compounds.

Benjamin L. Turner

*Smithsonian Tropical Research Institute, Balboa, Ancón,
Republic of Panama*

Alan E. Richardson

CSIRO Plant Industry, Canberra, Australia

Edward J. Mullaney

United States Department of Agriculture, New Orleans, USA

Acknowledgements

The meeting would not have been possible without generous support from the Soil Science Society of America through the Bouyoucos Conference fund. Bouyoucos conferences were established to facilitate an intense, highly focused examination of a topic of critical importance to soil science. Scientists with a common interest are brought together in a forum that is not typically possible at large scientific meetings, with the aim of establishing personal relationships and promoting the free exchange of ideas. We hope this latest Bouyoucos Conference fulfilled these ideals.

Additional funding was provided by the Agricultural Research Service of the United States Department of Agriculture through a Professional Activities grant, and the Sun Valley Resort generously provided their conference facilities without charge. We thank those who gave their time to peer-review chapters for this volume – their expertise has contributed to the technical excellence of its contents. Finally, we thank all the delegates at the inositol phosphate conference for contributing to a vibrant and stimulating few days, and we look forward to the next meeting.

This page intentionally left blank

1 Nomenclature and Terminology of Inositol Phosphates: Clarification and a Glossary of Terms

Stephen B. Shears¹ and Benjamin L. Turner²

¹Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, NIH, DHSS, Research Triangle Park, PO Box 12233, NC 27709, USA;

²Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancón, Republic of Panama

In a book like this one, which brings together reviews from scientists working in such diverse areas as analytical chemistry, biochemistry, agronomy and environmental science, the consolidation of terminology is of considerable importance. This chapter reviews the nomenclature of inositol phosphates and provides a glossary of the terms that are used throughout this book.

An Overview of Inositol Phosphate Nomenclature

Much of what follows is based on the recommendations of the International Union of Pure and Applied Chemistry (IUPAC) (IUPAC–IUB Commission on Biochemical Nomenclature (CBN), 1973, 1977; Nomenclature Committee of the International Union of Biochemistry, 1989). Previous conferences, most notably the ‘Chilton Conference on Inositol and Phosphoinositides’, held in Dallas, Texas, USA, in 1984, have permitted the use of inositol phosphate nomenclature that is not IUPAC-approved (Agranoff *et al.*, 1985). The audience at the Chilton conference were largely animal biochemists and the number of known inositol-containing compounds was far smaller than today. The authors may therefore have underestimated the potential for confusion

that lay ahead. It is now arguable that the Chilton meeting was a missed opportunity to enforce a much-needed, unified nomenclature.

Inositol phosphate terminology continues to be misused even in the recent literature. For example, ‘phosphoinositide’ is a term that was intended to refer only to the inositol lipids (IUPAC–IUB Commission on Biochemical Nomenclature (CBN), 1977). Instead, conceptual difficulties arise when phosphoinositide is incorrectly used to describe inositol phosphates (e.g. De Camilli *et al.*, 1996; Luttrell and Lefkowitz, 2002; Liu *et al.*, 2004), especially as the physico-chemical properties and biological actions of these soluble inositol derivatives are markedly different from those of the membrane-bound inositol lipids.

Unfortunately, even such esteemed bodies as IUPAC are not immune from error; their intentions were confounded somewhat when the inositol lipid used to illustrate nomenclature was not the naturally occurring D-enantiomer, but the unnatural L-version (see Agranoff, 1978). The adoption of a consistent nomenclature clearly cannot eliminate mistakes, but it is an important first step towards limiting their frequency.

The inositol phosphate literature also contains a number of examples of the misuse of chemical nomenclature, so clarification is appropriate. For example, a newcomer to the field would be

forgiven for assuming that 'IP6' has only a single phosphate at the 6-position, when the abbreviation is incorrectly defined as 'inositol-6-phosphate' (Lee *et al.*, 2005). The structural relationship between the *scyllo*- and *myo*-forms of inositol hexakisphosphate can be misunderstood when they are erroneously described as 'conformers' (Fisher *et al.*, 2002). Of particular importance to this book is the term 'phytate'. This refers to any salt of *myo*-inositol hexakisphosphate. However, it has sometimes been considered that phytate is synonymous with phytin (Yoshida *et al.*, 1999), even though the latter term was introduced originally to describe calcium/magnesium phytate, which was thought to comprise much of the phytate in seeds (Ashton, 1976). Further examples of the continued confusion in this field caused by incorrect terminology are given in a recent review (Michell *et al.*, 2006).

A glossary of terms is provided below as a prelude to this volume. A determined effort has been made to ensure that these terms are consistently deployed throughout the various chapters.

Glossary

Conformer. This is one particular spatial arrangement of a molecule in space at any particular moment. For example, two conformers of ***myo*-inositol** are the so-called 'chair' and the 'boat' arrangements of the ring, the former being thermodynamically favourable. A switch between different conformers involves rotation around single bonds, but no chemical bonds are broken (if bonds were rearranged, and hence the configuration changed, the two molecules would be **stereoisomers**). For inositols, the end point of a conformational change can be a ring-flip, which involves the conversion between two alternate chair conformations. This occurs for ***myo*-inositol hexakisphosphate** when solution pH increases past a critical value, whereupon the phosphates switch from being in a 5-equatorial/1-axial arrangement to a 5-axial/1-equatorial grouping (see Murthy, Chapter 2, this volume).

Epimer. This is a special case of a pair of **stereoisomers** having two or more stereogenic centres, but differing at only one of these. For example, ***myo*-inositol** and ***scyllo*-inositol** are epimers, because of differences in the spatial

positioning of chemical bonds at one of their six stereogenic centres.

Epimerization. The process by which two **epimers** are interconverted.

Inositol. A cyclitol (cyclohexanehexol) with a hydroxyl group associated with each of the six carbon atoms on the ring. See also ***myo*-inositol** and ***scyllo*-inositol**.

Inositol phosphate. The addition to the inositol ring of an ascending number of phosphate groups gives rise to a series of phosphorylated compounds (Table 1.1). The multiplicative prefixes (no part of which should be italicized) highlight the fact that each carbon atom has only one phosphate attached to it. Thus, 'bis', which is Latin in origin, means twice; 'tris' is Greek, meaning thrice or three times; and 'kis' is a general prefix from Greek that means times (Sarma, 2004). This distinguishes 'n' from 'n-times'. Thus, if there were an inositol derivative with a chain of three phosphates attached to a single carbon atom, it would be a triphosphate, not a trisphosphate. The reader who is new to this field may be relieved to know that inositol triphosphates have not been detected (yet). However, diphosphate groups can be attached to the inositol ring (Table 1.1). These 'inositol pyrophosphates' occur naturally inside cells from a wide range of organisms (Shears, 2005). Throughout this book the term **inositol phosphate** is used in a general sense for all phosphorylated inositols present in environmental samples.

***myo*-Inositol.** This is one of the nine possible stereoisomers of cyclohexanehexol (Fig. 1.1). In the literature, when the exact nature of the **stereoisomer** is not defined, it can typically be presumed to be ***myo*-inositol**. In fact 'Ins' is an IUPAC-approved term for ***myo*-inositol** (Nomenclature Committee of the International Union of Biochemistry, 1989). The 'Ins' abbreviation is not used in this volume, so as not to undervalue the significance of the other **stereoisomers** that figure prominently in the environment.

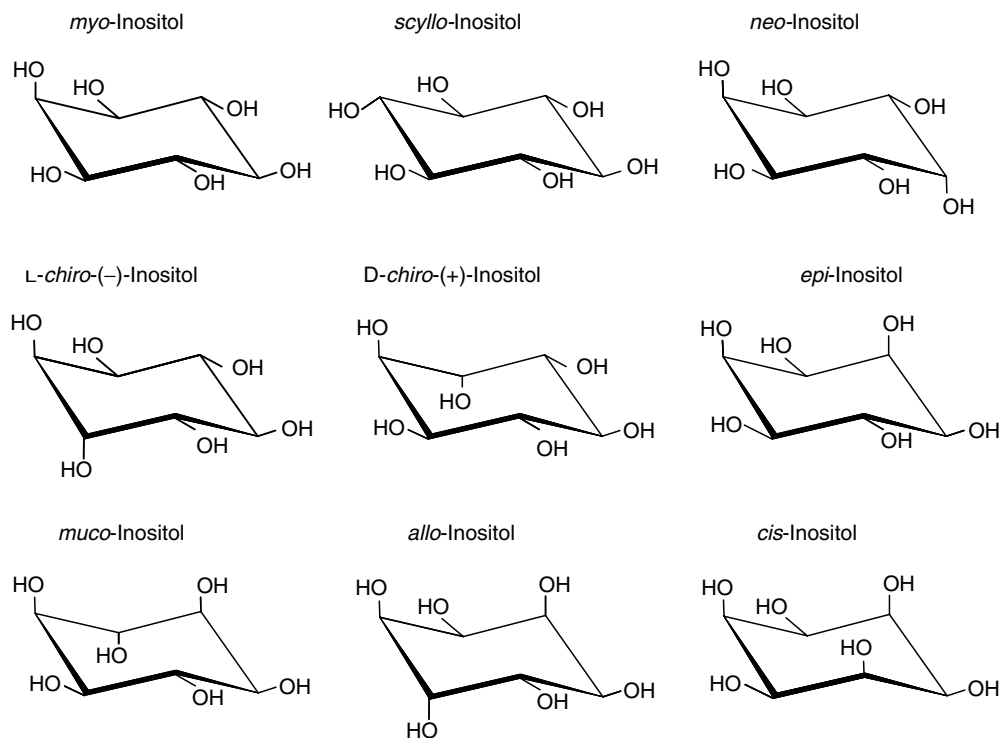
***myo*-Inositol hexakisphosphate.** A compound in which all six hydroxyl groups of ***myo*-inositol** are esterified as phosphates. ***myo*-Inositol hexakisphosphate** is a systematic name and is also popular in the cell-signalling literature (Irvine and Schell, 2001). Outside that field, this compound is more usually known as **phytic acid**. This is strictly defined as *myo*-inositol hexakis(dihydrogen phosphate), but the commonly used *myo*-inositol hexakisphosphate is used in this book.

Table 1.1. The *myo*-inositol phosphates and their accepted abbreviations.

Full name	Number of phosphate groups	IUPAC abbreviation ^a	Common abbreviation
<i>myo</i> -Inositol	0	Ins	Ins
<i>myo</i> -Inositol monophosphate	1	InsP ₁ ^b	IP ₁
<i>myo</i> -Inositol bisphosphate	2	InsP ₂	IP ₂
<i>myo</i> -Inositol trisphosphate	3	InsP ₃	IP ₃
<i>myo</i> -Inositol tetrakisphosphate	4	InsP ₄	IP ₄
<i>myo</i> -Inositol pentakisphosphate	5	InsP ₅	IP ₅
<i>myo</i> -Inositol hexakisphosphate	6	InsP ₆	IP ₆
Diphospho- <i>myo</i> -inositol tetrakisphosphate	6	PP-InsP ₄	PP-IP ₄
Diphospho- <i>myo</i> -inositol pentakisphosphate	7	PP-InsP ₅	IP ₇
Bis-diphospho- <i>myo</i> -inositol tetrakisphosphate	8	[PP] ₂ -InsP ₄	IP ₈

^aThe italicization of the *P* denotes its use as an abbreviation for phosphate, rather than the chemical symbol for phosphorus.

^bAlthough it is not explicitly stated, we infer that InsP (without a numeric subscript) is actually the IUPAC-preferred abbreviation for *myo*-inositol monophosphate. However, we recommend InsP₁, to avoid confusion with 'InsP', which is sometimes incorrectly used as a collective abbreviation for inositol phosphates (e.g. Tavares *et al.*, 2002; Woodcock *et al.*, 2003).

**Fig. 1.1.** The nine stereoisomeric forms of inositol.

Phytase. An enzyme (*myo*-inositol hexakisphosphate phosphohydrolase) that initiates the cleavage of one or more phosphate groups from ***myo*-inositol hexakisphosphate**. Several phytases are now known to exist and are described in detail in this volume (see Mullaney and Ullah, Chapter 7). Some authors prefer the term **phytate-degrading enzyme**.

Phytate. This refers to any salt of **phytic acid**. Phytate can be soluble or insoluble and can occur in both dissolved and precipitated forms. Insoluble phytate involves polyvalent cations (e.g. iron phytate), whereas soluble phytate usually involves monovalent cations (e.g. sodium phytate). However, a recent study concluded that the neutral pentamagnesium salt is the predominant soluble form in animal cells (Torres *et al.*, 2005). Nevertheless, phytate will precipitate out of solution, in a pH-dependent manner, once a critical concentration of divalent cations is exceeded. In most cases, ***myo*-inositol hexakisphosphate** exists as a salt, in both precipitated and dissolved forms, and can thus be termed phytate. However, to avoid confusion, the term phytate is used in this volume only when additional information about the cation or solubility is known.

Phytate-degrading enzyme. This is an alternative term for phytase that is preferred by some authors when the *in vivo* function of the enzyme has not been unambiguously demonstrated (see Greiner, Chapter 6, this volume).

Phytic acid. This is a non-systematic but widely used alternate name for the free-acid form of ***myo*-inositol hexakisphosphate**. As the salt-free form is unlikely to occur widely in nature, the term ***myo*-inositol hexakisphosphate** is preferred over phytic acid in this volume, although the term phytate is used when the salt is known. Phytic acid should not be used to describe other phosphorylated **stereoisomers** such as *scyllo*-inositol hexakisphosphate.

Phytin. This term was originally introduced to describe 'insoluble' calcium/magnesium **phytate** deposits in the globoids of plant seed (e.g. Ashton, 1976). The term is largely obsolete, because **phytate** in the seeds of many species is now known to consist predominantly of magnesium/potassium salts (Ockenden *et al.*, 2004). The use of 'insoluble' as an absolute description of this material also seems unwarranted, as the deposits are mobilized during seed germination.

Positional isomers. This is a form of structural isomerism in which side chain groups (in this

case phosphates) are found attached to different carbons of the inositol ring. That is, atoms are bonded together in a different order, as opposed to **stereoisomers**, in which the connectivity is the same. *myo*-Inositol 1,3,4,5-tetrakisphosphate and *myo*-inositol 3,4,5,6-tetrakisphosphate are examples of positional isomers. The numbering of the carbon atoms follows rules developed by IUPAC (IUPAC–IUB Commission on Biochemical

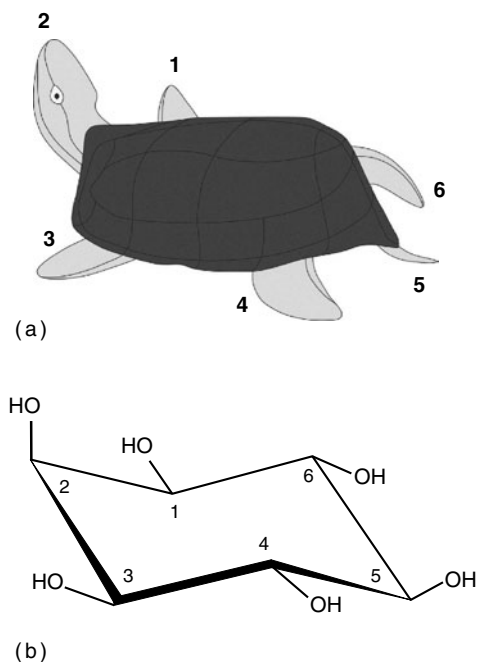


Fig. 1.2. (a) Agranoff's turtle and (b) *myo*-inositol. (From Shears, 2004.) In its most stable chair conformation with 1-axial and 5-equatorial hydroxyl groups, *myo*-inositol has been said to resemble a turtle (Agranoff, 1978). International Union of Pure and Applied Chemistry (IUPAC) rules state that the 1-*D*-numbering of each carbon begins with the turtle's front right flipper and proceeds in an anticlockwise direction around the ring (viewed from above). The axial hydroxyl is therefore represented by the turtle's head (position number 2) and the equatorial hydroxyls by the limbs and tail. For further details of the numbering system and stereochemistry of the inositol phosphates the reader is referred to the IUPAC recommendations (IUPAC–IUB Commission on Biochemical Nomenclature (CBN), 1977; Nomenclature Committee of the International Union of Biochemistry, 1989) and comprehensive reviews published elsewhere (Parthasarathy and Eisenberg, 1991; Murthy, 2006).

Nomenclature (CBN), 1973; Nomenclature Committee of the International Union of Biochemistry, 1989). Agranoff's **turtle** (see Agranoff, 1978; Shears, 2004) provides a timeless, visual mnemonic to the numbering of **myo-inositol** (Fig. 1.2). Unfortunately, there are no such aids for the other **stereoisomers** of **inositol**.

scyllo-Inositol. This is one of the nine possible **stereoisomers** of cyclohexanehexol (inositol). It differs from **myo-inositol** at only one stereogenic centre – i.e. it is an **epimer** – and is unique in that it has a stable chair conformation in which all six hydroxyl groups are equatorial to the plane of the ring (Fig. 1.1).

Stereoisomer. This refers to compounds that have the same chemical formula, the same atoms and the same connectivity, but differ in the fixed spatial positioning of bonds at a particular stereogenic carbon (for the inositols, a stereogenic carbon is one lacking a plane of symmetry). Hydroxyl groups on the **inositol** ring can be oriented in either an axial or equatorial manner, which gives nine possible

stereoisomers (Fig. 1.1). These stereoisomers are distinguished by a configurational prefix, which must be italicized (IUPAC–IUB Commission on Biochemical Nomenclature (CBN), 1973). The most abundant stereoisomer in nature is **myo-inositol**, but several others occur in plants and animals. Only four inositol stereoisomers (*myo*-, *neo*-, *scyllo*- and *D-chiro*-) occur naturally in phosphorylated forms, predominantly in soils (see L'Annunziata, Chapter 4, and Turner, Chapter 12, this volume).

Turtle. A marine reptile that provides an *aide-mémoire* for easy recall of the nomenclature for numbering the carbon atoms that comprise the **myo-inositol** ring (Fig. 1.2; Agranoff, 1978; Shears, 2004).

Acknowledgements

We thank Dr Andrew Riley, University of Bath, UK, and Victor Raboy, USDA–ARS Aberdeen, USA, for their valuable contributions.

References

- Agranoff, B.W. (1978) Textbook errors: cyclitol confusion. *Trends in Biochemical Sciences* 3, N283–N285.
- Agranoff, B.W., Eisenberg, F. Jr, Hauser, G., Hawthorn, J.N. and Michell, R.H. (1985) Comment on abbreviations. In: Bleasdale, J.E., Eichberg, J. and Hauser, G. (eds) *Inositol and Phosphoinositides: Metabolism and Regulation*. Humana Press, Totowa, New Jersey, pp. xxi–xxii.
- Ashton, F. (1976) Mobilization of storage proteins of seeds. *Annual Reviews in Plant Physiology* 27, 95–117.
- De Camilli, P., Emr, S.D., McPherson, P.S. and Novick, P. (1996) Phosphoinositides as regulators in membrane traffic. *Science* 271, 1533–1539.
- Fisher, S.K., Novak, J.E. and Agranoff, B.W. (2002) Inositol and higher inositol phosphates in neural tissues: homeostasis, metabolism and functional significance. *Journal of Neurochemistry* 82, 736–754.
- Irvine, R.F. and Schell, M. (2001) Back in the water: the return of the inositol phosphates. *Nature Reviews Molecular Cell Biology* 2, 327–338.
- IUPAC–IUB Commission on Biochemical Nomenclature (CBN) (1973) Nomenclature of cyclitols. Recommendations 1973. *Biochemical Journal* 153, 23–31.
- IUPAC–IUB Commission on Biochemical Nomenclature (CBN) (1977) Nomenclature of phosphorus-containing compounds of biochemical importance. Recommendations 1976. *Proceedings of the National Academy of Sciences of the United States of America* 74, 2222–2230.
- Lee, H.J., Lee, S.A. and Choi, H. (2005) Dietary administration of inositol and/or inositol-6-phosphate prevents chemically induced rat hepatocarcinogenesis. *Asian Pacific Journal of Cancer Prevention* 6, 41–47.
- Liu, J.W., Anderson, S.N., Meulbroek, J.A., Hwang, S.M., Mukerji, P. and Huang, Y.S. (2004) Polyphosphoinositides suppress the adhesion of *Haemophilus influenzae* to pharyngeal cells. *Lipids in Health and Disease* 3 (online-only journal: doi:10.1186/1476-511X-3-20).
- Luttrell, L.M. and Lefkowitz, R.J. (2002) The role of beta-arrestins in the termination and transduction of G-protein-coupled receptor signals. *Journal of Cell Science* 115, 455–465.
- Michell, R.H., Heath, V.L., Lemmon, M.A. and Dove, S.K. (2006) Phosphatidylinositol 3,5-bisphosphate: metabolism and cellular functions. *Trends in Biochemical Sciences* 31, 52–63.
- Murthy, P.P.N. (2006) Structure and nomenclature of inositol phosphates, phosphoinositides, and glycosylphosphatidylinositols. In: Lahiri Majumder, A. and Biswas, B.B. (eds) *Biology of Inositols and Phosphoinositides*. Springer-Verlag, Berlin, pp. 1–20.

- Nomenclature Committee of the International Union of Biochemistry (1989) Numbering of atoms in *myo*-inositol. Recommendations 1988. *Biochemical Journal* 258, 1–2.
- Ockenden, I., Dorsch, J.A., Reid, M.M., Lin, L., Grant, L.K., Raboy, V. and Lott, J.N.A. (2004) Characterization of the storage of phosphorus, inositol phosphate and cations in grain tissues of four barley (*Hordeum vulgare* L.) low phytic acid genotypes. *Plant Science* 167, 1131–1142.
- Parthasarathy, R. and Eisenberg, F. Jr (1991) Biochemistry, stereochemistry, and nomenclature of the inositol phosphates. In: Reitz, A.B. (ed.) *Inositol Phosphates and Derivatives*. American Chemical Society, Washington, DC, pp. 1–19.
- Sarma, N.S. (2004) Etymology as an aid to understanding chemistry concepts. *Journal of Chemical Education* 81, 1437–1439.
- Shears, S.B. (2004) How versatile are inositol phosphate kinases? *Biochemical Journal* 377, 265–280.
- Shears, S.B. (2005) Telomere maintenance by intracellular signals: new kid on the block? *Proceedings of the National Academy of Sciences of the United States of America* 102, 1811–1812.
- Tavares, P., Martinez-Salgado, C., Ribeiro, C.A., Elono, N., Lopez-Novoa, J.M. and Teixeira, F. (2002) Cyclosporin effect on rat aorta α_1 -adrenoceptors and their transduction mechanisms. *Journal of Cardiovascular Pharmacology* 40, 181–188.
- Torres, J., Domínguez, S., Cerdá, F.M., Obal, G., Mederos, A., Irvine, R.F., Diaz, A. and Kremer, C. (2005) Solution behaviour of *myo*-inositol hexakisphosphate in the presence of multivalent cations. Prediction of a neutral pentamagnesium species under cytosolic/nuclear conditions. *Journal of Inorganic Biochemistry* 99, 828–840.
- Woodcock, E.A., Mitchell, C.J. and Biden, T.J. (2003) Phospholipase C δ_1 does not mediate Ca²⁺ responses in neonatal rat cardiomyocytes. *FEBS Letters* 546, 325–328.
- Yoshida, K.T., Wada, T., Koyama, H., Mizobuchi-Fukuoka, R. and Naito, S. (1999) Temporal and spatial patterns of accumulation of the transcript of *myo*-inositol-1-phosphate synthase and phytin-containing particles during seed development in rice. *Plant Physiology* 119, 65–72.