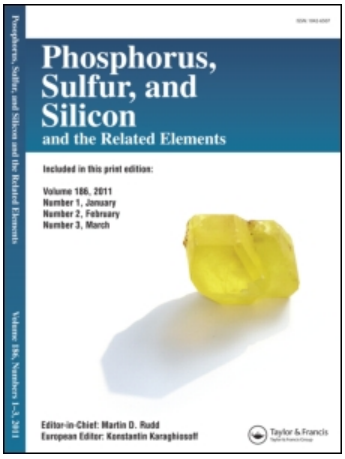


Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Synthesis, Potentiometric and ^{31}P-NMR Investigations of the Ionization State and Complexation Properties of Inositol-Phosphates : Biological Consequences

Laurent Schmitt[a]; Patrick Bortmann[a]; Bernard Spiess[a]; Gilbert Schlewer[a]
[a] Laboratoire de Pharmacochimie Moléculaire, STRASBOURG, FRANCE

To cite this Article Schmitt, Laurent , Bortmann, Patrick , Spiess, Bernard and Schlewer, Gilbert(1993) 'Synthesis, Potentiometric and ^{31}P-NMR Investigations of the Ionization State and Complexation Properties of Inositol-Phosphates : Biological Consequences', Phosphorus, Sulfur, and Silicon and the Related Elements, 76: 1, 147 — 150
To link to this Article: DOI: 10.1080/10426509308032380
URL: http://dx.doi.org/10.1080/10426509308032380

Phosphorus, Sulfur, and Silicon, 1993, Vol. 76, pp. 147–150
Reprints available directly from the publisher
Photocopying permitted by license only
Printed in the United States of America

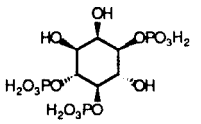
SYNTHESIS, POTENTIOMETRIC AND ^{31}P-NMR INVESTIGATIONS OF THE IONIZATION STATE AND COMPLEXATION PROPERTIES OF INOSITOL-PHOSPHATES : BIOLOGICAL CONSEQUENCES

LAURENT SCHMITT, PATRICK BORTMANN, BERNARD SPIESS, GILBERT SCHLEWER*

Laboratoire de Pharmacochimie Moléculaire, UPR 421 du Centre de Neurochimie du CNRS, 5, rue Blaise Pascal 67084 STRASBOURG, FRANCE.

Abstract The synthesis and the potentiometric studies of inositol-phosphates allow, particularly for Ins(1,4,5)P_3, the correlation between the ionization state of the the phosphate functions and the binding properties of the inositol-phosphates.

INTRODUCTION



1

Many recent biological studies have revealed that Inositol-Phosphates (IP's), especially D-*myo*-inositol-1,4,5-triphosphate (Ins(1,4,5)P_3) **1**, act as intracellular second messenger in different tissues[1-11].

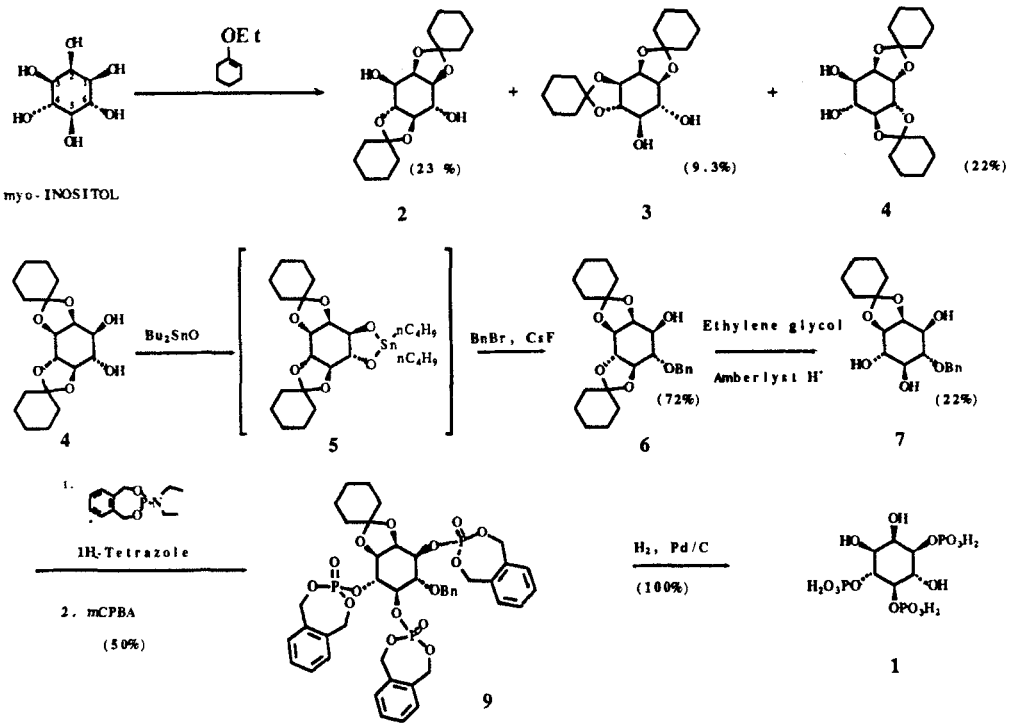
The structure-activity relationships of such molecules have to be analysed in terms of their supramolecular behaviour. This involves, in addition to the study of the complementarity between the molecules and the targets (receptors or enzymes), to take into account the effect of the variations of the ionic environment of the cellular medium (pH, ionic concentrations). Indeed, for the phosphate groups of these molecules, varying in number and position, the ionization state is closely related to the above-mentioned parameters. Depending on the medium, the possibility of setting more or less strong intramolecular hydrogen bonds between phosphates or hydroxyl groups is also of great importance. Moreover, metallic cations (alkali or alkali-earth cations) contained or liberated in the cellular medium during the IP's cascade compete with the second acidic

proton of the phosphates[12,13]. By considering the IP structures one can envisage that a complexe of the type : M_xH_yL (where : x is the number of metallic cations M, y is the number of protons, and L is the inositol phosphate,) can act as a biological species. The structure and conformation of such complexes should also be closely related to the dynamic variations of the cellular content.

The understanding of the behaviour of the IP's, as well as their analogs, requires an interdisciplinary approach involving synthesis, physico-chemical (potentiometry, ^{31}P-NMR) and pharmacological studies.

SYNTHESIS

The synthesis of the IP analogs needs a series of selective protection and deprotection steps as well as efficient and selective functionalisation procedures (such as phosphorylation). An illustration is given in scheme 1 with the synthesis of Ins(1,4,5)P_3 **1**.



Scheme 1.

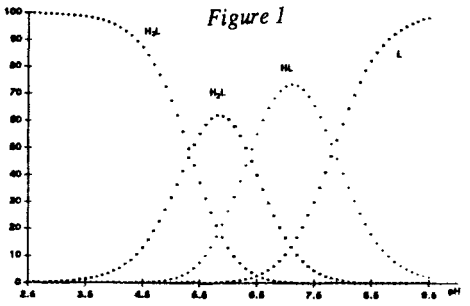
Myo-inositol treated with 1-ethoxy-cyclohexene gave a mixture of three dicetal **2-4** which were separated by chromatography. Treatment of dicyclohexyliden derivative **4** with dibutyltinoxide[14] gave the cyclic intermediate **5**. Opening this intermediate by reaction with benzylbromide yielded compound **6** selectively benzylated in position 4. The more strained *trans*-cetal in position 5 and 6 is then hydrolyzed to yield the triol **7**. Phosphorylation of this triol was achieved in a two step, but one pot, procedure. Thus the triol **7** was treated with diethylamino-1,5-dihydro-2,3,4-benzo-dioxaphosphepine **8**[15] to obtain an intermediate phosphite which was oxidized in situ to the phosphate **9** by means of *m*-CPBA[16]. Hydrogenolysis with palladium catalysis in ethanol at room temperature and at atmospheric pressure directly gave Ins(1,4,5)P_3 **1** which was kept as cyclohexylammonium salt to avoid phosphate migrations and other side reactions.

PHYSICO-CHEMICAL STUDIES.

Potentiometric methods allowed us to measure the protonation constants of the phosphates as well as the nature and the stability of the calcium complexes expressed according to the general equilibria :

$$xM^{2+} + yH^{+} + L^{6-} \rightleftharpoons M_xH_yL^{(6-2x-y)-}$$

Figure 1



As reported on the distribution curves of the various protonated species (figure 1), Ins(1,4,5)P_3 mainly exist, in a biominitic medium (KCl 0.2M, 37°C) and near the physiological pH, under a HL and a L form[12].

In addition, it has been shown that Ca++ form with Ins(1,4,5)P_3 more or less protonated mononuclear and homodinuclear complexes. By such reactions, a specific geometry or a particular conformation of the ligand may be induced, influencing so its binding ability[13].

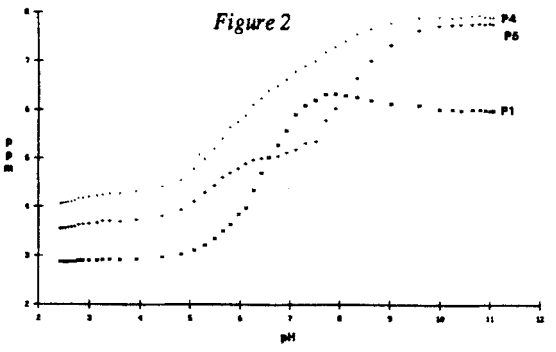


Figure 2

The attribution of the potentiometrically determined macroconstants was done by ^{31}P-NMR which enables the resonance of each phosphorus nucleus to be related to the degree of protonation of a given phosphate group.

The ^{31}P-NMR titrations also lead to unusual and complexe chemical shift curves which indicate large interactions between the various phosphates as can be seen in figure 2 for Ins(1,4,5)P_3.

CORRELATION WITH BINDING STUDIES.

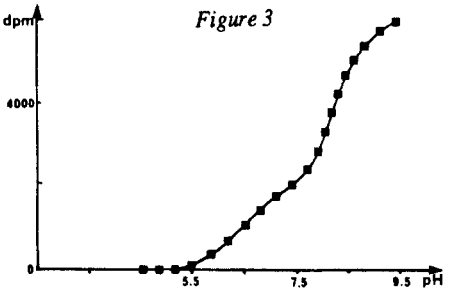


Figure 3

The comparison of these curves with that of the binding taken from the litterature[17] seems to show that the affinity of Ins(1,4,5)P_3 for the central receptors could be due mainly to the ionization of the phosphate in position 5.(figure 3)

REFERENCES

1. M.J. Berridge, R.F. Irvine, Nature, 306, 67, (1984).
2. M.J. Berridge, R.F. Irvine, Nature, 312, 315, (1984).
3. M.J. Berridge, Biochem. J. 220, 345, (1984).
4. M.J. Berridge, R.F. Irvine, Nature, 341, 197, (1989).
5. M. J. Berridge, JAMA, 262(13), 227, (1986).
6. A.A. Abdellatif, Pharmacological Reviews, 38(3), 227, (1986).
7. R. Parthasarathy, F. Eisenberg, Biochem. J., 235, 313, (1986).
8. J.P. Heslop, R.F. Irvine, A.H. Tashjian, M.J. Berridge, J. Exp. Biol., 119, 395, (1985).
9. R.F. Irvine, A.J. Letcher, J.P. Heslop, M.J. Berridge, Nature, 320, 631, (1986).
10. Hahorski, I. Batty, Trends Pharmacol. Sc. 7, 83, (1986).
11. M.J. Vallejo, T. Jackson, S.Lightman, M.P. Hanleyt, Nature, 330, 656, (19897).
12. L. Schmitt, G. Schlewer, B. Spiess, Biochem. Biophys. Acta, 1075, 139, (1991).
13. L. Schmitt, G. Schlewer, B. Spiess, J. Inorg., Biochem., 45, 13, (1992).
14. N. Nagashima, Chem. Lett., 141, (1987).
15. J.W. Perich, R.B. Johns, Tetrahedron Lett., 28, 101, (1987).
16. J.W. Perich, R.B. Johns, Tetrahedron Lett., 29, 2369, (1988).
17. P.F. Worley, J.M. Baraban, S. Supattapone, V.S. Wilson, S.H. Snyder, J.Biol. Chem., 262, 12132, (1987).