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# Response to Comment on "Determination of *neo*- and D-*chiro*-Inositol Hexakisphosphate in Soils by Solution $^{31}$P NMR Spectroscopy"

We welcome the comment from Michael L'Annunziata on our article[1] and his clarification of the debate surrounding his previous identification of *muco*-inositol phosphate in leaf litter of velvet mesquite (*Prosopis velutina*).[2] We acknowledge our error in ascribing his report to *muco*-inositol hexakisphosphate, when in fact no specific ester form was mentioned in the original article.

We recently analyzed a sample of velvet mesquite litter collected by Dr. L'Annunziata from the same location in Arizona as the sample in his original study. Solution $^{31}$P NMR spectroscopy of an alkaline extract of the litter shows no evidence for the presence of inositol hexakisphosphate in any stereoisomeric form (Figure 1a). This includes *muco*-inositol hexakisphosphate, for which we show the spectrum of an authentic preparation of this compound[3] from Dennis Cosgrove's original collection (Figure 1b), kindly provided by Dr Alan Richardson of CSIRO Plant Industry, Canberra, Australia. The authentic *muco*-inositol hexakisphosphate yields four signals in a 1:2:2:1 ratio at $\delta$ = 4.19, 5.34, 6.13, and 7.16 ppm (Figure 1b). As well as being absent in the velvet mesquite litter, such signals have to our knowledge not been observed previously in solution $^{31}$P NMR spectra of any soil or sediment. This does not preclude the presence of trace amounts of *muco*-inositol hexakisphosphate in some samples, but does support the general assumption that this compound does not occur widely in the environment.

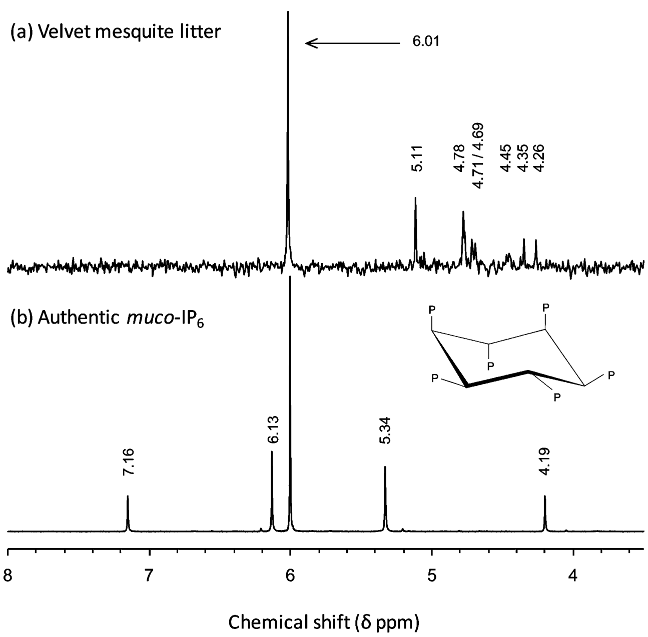


**Figure 1.** Solution $^{31}$P NMR spectra of (a) an NaOH−EDTA extract of velvet mesquite (*Prosopis velutina*) leaf litter from Arizona, and (b) an authentic *muco*-inositol hexakisphosphate (*muco*-IP$_6$) dissolved in NaOH−EDTA (pH > 13), showing four signals in a 1:2:2:1 ratio. The structure of *muco*-inositol hexakisphosphate is also presented, showing three axial and three equatorial phosphate groups (each group represented by "P"). Chemical shifts of individual signals are given in $\delta$ ppm. The strong signal at $\delta$ = 6.01 ppm in both spectra is inorganic orthophosphate. Samples were analyzed as described previously.[1]

The spectrum of velvet mesquite litter does, however, contain a series of phosphomonoester signals between $\delta$ = 4.26 and 5.11 ppm (Figure 1a). While most of these probably originated from the alkaline hydrolysis of RNA and phospholipids,[4] the presence of a number of simple phosphomonoesters in trace quantities supports Dr. L'Annunziata's suggestion that the *muco*-inositol phosphate detected in his original analysis was likely to have been a lower-order ester, presumably *muco*-inositol 1-phosphate.

Although more than four decades have passed since publication of Dr. L'Annunziata's original article,[2] it remains significant as the only report of a *muco*-inositol phosphate in the environment. Together with the apparent widespread occurrence of the *scyllo*, D-*chiro*, and *neo*-inositol hexakisphosphates in soils and sediments,[1] the presence of *muco*-inositol phosphate in leaf litter provides a further example of the remarkable deficiency in our understanding of the origins, behavior, and function of the inositol phosphate stereoisomers in nature.

**Benjamin L. Turner**[†,*]
**Alexander W. Cheesman**[†]
**H. Yasmin Godage**[‡]
**Andrew M. Riley**[‡]
**Barry V. L. Potter**[‡]
[†]Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Republic of Panama
[‡]Wolfson Laboratory of Medicinal Chemistry, Department of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY, United Kingdom

## AUTHOR INFORMATION

### Corresponding Author

*Phone: +507-212-8171; fax +507-212-8148; e-mail TurnerBL@si.edu.

### Notes

The authors declare no competing financial interest.

## REFERENCES

(1) Turner, B. L.; Cheesman, A. W.; Godage, H. Y.; Riley, A. M.; Potter, B. V. L Determination of *neo*- and D-*chiro*-inositol hexakisphosphate in soils by solution $^{31}$P NMR spectroscopy. *Environ. Sci. Technol.* **2012**, *46*, 4994−5002.

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(2) L'Annunziata, M. F.; Fuller, W. H. Soil and plant relationships of inositol phosphate stereoisomers: the identification of D-chiro- and muco-inositol phosphates in a desert soil and plant system. *Soil Sci. Soc. Am. Proc.* **1971**, *35*, 587−595.
(3) Cosgrove, D. J. The phosphorylation of *epi*-inositol and *muco*-inositol with polyphosphoric acid. *Carbohydr. Res.* **1975**, *40*, 380−384.
(4) Turner, B. L.; Mahieu, N.; Condron, L. M. Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH−EDTA extracts. *Soil Sci. Soc. Am. J.* **2003**, *67*, 497−510.