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PII: S0040-4039(19)30697-5
DOI: <https://doi.org/10.1016/j.tetlet.2019.150949>
Article Number: 150949
Reference: TETL 150949

To appear in: *Tetrahedron Letters*

Received Date: 10 June 2019
Revised Date: 15 July 2019
Accepted Date: 16 July 2019

Please cite this article as: Shangguan, L., Shi, B., Chen, Q., Li, Y., Zhu, H., Liu, Y., Yao, H., Huang, F., Water-soluble pillar[5]arenes: a new class of plant growth regulators, *Tetrahedron Letters* (2019), doi: <https://doi.org/10.1016/j.tetlet.2019.150949>

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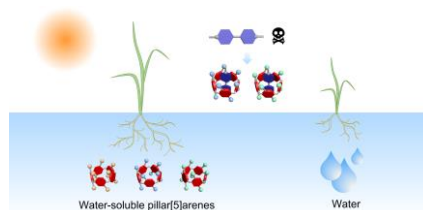


Graphical Abstract

Water-soluble pillar[5]arenes: a new class of plant growth regulators

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Water-soluble pillar[5]arenes: a new class of plant growth regulators

Liqing Shangguan,^a Bingbing Shi,^{a,*} Qi Chen,^a Yang Li,^a Huangtianzhi Zhu,^a Yuezhou Liu,^a Hong Yao,^{b,*} Feihe Huang^{a,*}

^aState Key Laboratory of Chemical Engineering, Center for Chemistry of High-Performance & Novel Materials, Department of Chemistry, Zhejiang University, Hangzhou 310027, P. R. China

^bKey Laboratory of Eco-Environment-Related Polymer Materials of Ministry of Education of China, College of Chemistry and Chemical Engineering, Northwest Normal University, Lanzhou 730070, P. R. China

*Corresponding author

Email address: bingbingshi@zju.edu.cn; yaohong@nwnu.edu.cn; fhuang@zju.edu.cn

ARTICLE INFO

Article history:

Received

Received in revised form

Accepted

Available online

Keywords:

plant growth regulators

pillararenes

Host-guest systems

Paraquat

ABSTRACT

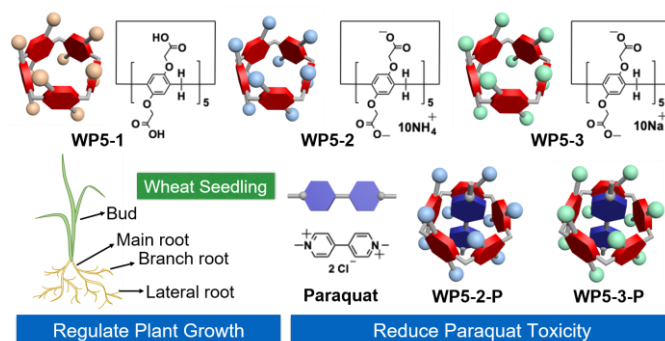
It is highly valuable to develop bifunctional chemical fertilizers that can both regulate plant growth and reduce the toxicity of pesticides. Here we report three water-soluble pillar[5]arenes **WP5-1**, **WP5-2** and **WP5-3** established as efficient plant growth regulators for wheat to efficiently improve the development of buds and roots in the seedling cultivation stage. Moreover, as macrocyclic hosts for the poisoning herbicide paraquat, **WP5-2** and **WP5-3** can also be employed to reduce its toxicity based on host-guest interactions, as confirmed by cell viability experiments towards HEK 293 and Raw 264.4 cell lines.

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Currently the grain problem is one of the biggest challenges all over the world.¹ With the rapid development of agricultural science, plant growth regulators which can determine the directions of application and the potential of crops in agriculture to a large extent are being increasingly used for agricultural purposes.² Chemists and botanists become more and more interested in plant growth regulators. Phenoxyacetic acid derivatives and phenyl dioxyacetic acid derivatives are universally used in agriculture as plant growth regulators; trace quantities applied to crops efficiently promotes their maturity and increases their yields.³ On the other side, pesticides, including insecticides, fungicides and herbicides, which are usually used to resist the external enemies of crops and improve their living environments, are playing an important role in relieving the food crisis. Paraquat, one of the common pesticides, is used in an increasing number of agricultural applications as an herbicide.⁴ However, its high toxicity poses considerable risks to human health, animals, and the environment. Absorption of paraquat into the digestive tract, respiratory tract, and skin may result in various diseases or even death.⁵ In view of this, it is advantageous to develop novel materials that can both regulate plant growth and reduce the toxicity of pesticides, such as paraquat.

Pillar[*n*]arenes, in which methylene groups are linked at the 1- and 4-positions of 2,5-dialkoxybenzene rings, mainly include pillar[5]arenes and pillar[6]arenes.⁶ They are an emerging type of macrocyclic hosts after cyclodextrins,⁷ calixarenes,⁸ crown ethers,⁹ cucurbiturils,¹⁰ and cavitands.¹¹ Pillar[*n*]arenes have a pillar-like architecture, in sharp contrast to the basket-shaped architecture of calixarenes.⁶ Their symmetrical structures and easy functionalization

endowed them with excellent properties in host-guest chemistry. Based on great efforts made by chemists, numerous stimuli-responsive host-guest recognition motifs of pillararenes have been discovered and applied in the fabrication of various materials.¹² However, most of these studies have been focused on their responsiveness to external stimuli. Recently, Yang and coworkers applied carboxylated pillar[5]arenes as responsive controlling release valves in the application of promoting the growth of *Arabidopsis thaliana* and cabbage by hormone gibberellin acid.¹²ⁿ However, the role of pillar[5]arene itself played in plant growth regulation has been excluded from consideration. Here three water-soluble pillar[5]arenes, **WP5-1**, **WP5-2**, and **WP5-3**, were used as efficient plant growth regulators to improve the development of buds and roots in seedling cultivation (Scheme 1). Moreover, it was demonstrated that **WP5-2** and **WP5-3** reduce the toxicity of the herbicide paraquat efficiently (Scheme 1).



Scheme 1. Chemical structures and cartoon representations of **WP5-1**, **WP5-2**, **WP5-3**, paraquat, **WP5-2-P**, and **WP5-3-P** and applications of **WP5-2** and **WP5-3** in regulating plant growth and reducing paraquat toxicity.

Then, wheat seedling experiments were conducted to investigate the plant growth regulating performance of **WP5-1**, **WP5-2**, and **WP5-3**. Well-conditioned ungerminated wheat seeds were treated with a series of concentrations to observe the growing activity. First, the wheat seeds' germination rate was recorded after treatment with **WP5-1**, **WP5-2**, and **WP5-3**. The wheat seeds treated with distilled water and a commercialized plant growth regulator, 2,4-dichlorophenoxyacetic acid (2,4-DCP), were used as the control groups. As shown in Figure 1, all the seeds in the cultivation experiment exhibited good and stable germination behavior in the early three days of the growth period. We concluded that all three water-soluble pillar[5]arenes had no obvious influence in the germination rate of the tested wheat seeds.

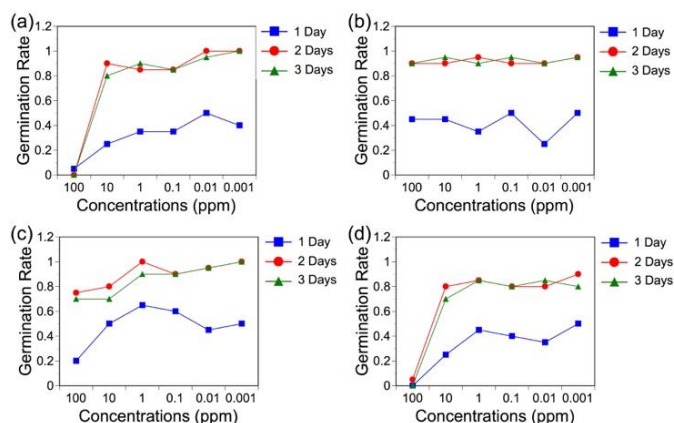


Fig. 1 Wheat seeds' germination rate in first three days of seedling treated with (a) **WP5-1**, (b) **WP5-2**, (c) **WP5-3**, and (d) 2,4-dichlorophenoxyacetic acid, respectively.

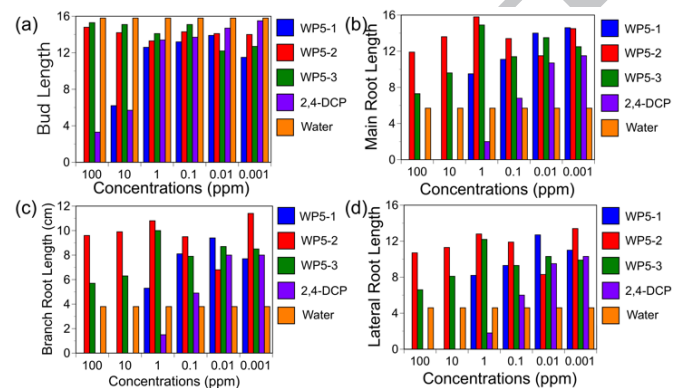


Fig. 2 Effect of **WP5-1**, **WP5-2**, **WP5-3**, and 2,4-DCP on (a) bud length, (b) main root length, (c) branch root length, and (d) lateral root length compared with the case of water.

In the following growing period, the lengths of buds and roots including the main root, branch root, and lateral root were recorded after cultivation to characterize the effectiveness of the water-soluble pillar[5]arenes in the growth process. As shown in Figure 2, **WP5-1** exhibited performance typical of plant growth regulators that only boost the growth in a suitable concentration range but inhibit it in a concentration that is too high or too low. **WP5-1** had a promoting effect on the wheat growth of both buds and roots at concentrations in the range of 0.1–0.01 ppm, which is obviously superior to water and comparable to the commercialized plant growth regulator 2,4-DCP. However, when the concentration of **WP5-1** was higher (100 ppm and 10 ppm) or lower (0.001 ppm) than that, the buds' and the roots' growing activities were inhibited. Interestingly, **WP5-2** and **WP5-3** showed excellent growth-promoting effects in all the measured concentrations unlike the pattern of **WP5-1**, which may be

attributed to the cations of **WP5-2** and **WP5-3**, Na^+ and NH_4^+ , respectively. The two cations were intrinsically beneficial to plant growth, so these results did not exhibit a typical plant growth regulator performance pattern.¹⁵ The collaborative effect of cations and macrocycles endowed **WP5-2** and **WP5-3** with better performance in growth promotion. These results clearly verified the potential of the three water-soluble pillar[5]arenes **WP5-1**, **WP5-2**, and **WP5-3** in plant growth regulating.

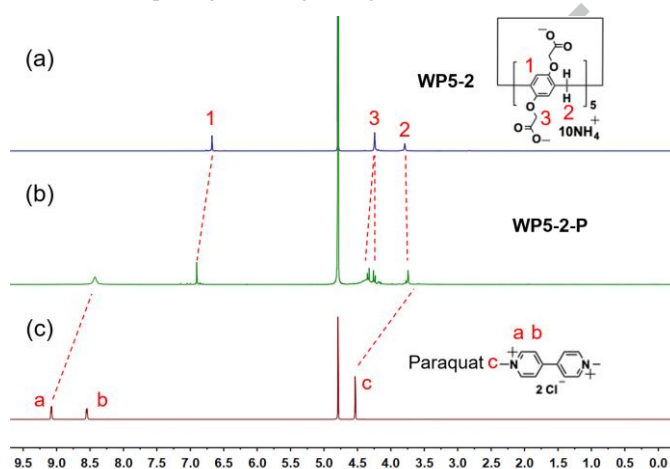


Fig. 3 Partial ^1H NMR (500 Hz, D_2O , 293 K) spectra: (a) **WP5-2** (5.0 mM); (b) **WP5-2** (5.0 mM) and paraquat (5.0 mM); (c) paraquat (5.0 mM).

Paraquat is used as an effective universal herbicide since its introduction to agriculture with high toxicity that can cause irreversible damage to human lung, liver and kidney tissues by interfering with the oxidative process in cells and after such poisoning has no antidote.⁴ Water-soluble macrocycles that can wrap up the paraquat molecules were designed for removal of paraquat.^{13,16} In our research, the high binding affinity ($K_a = (8.2 \pm 1.7) \times 10^4 \text{ M}^{-1}$ for **WP5-2** and paraquat in water¹⁴) between water-soluble pillar[5]arenes and paraquat was employed to efficiently reduce the toxicity of paraquat. First of all, the three host–guest complexations between the chosen water-soluble pillar[5]arene hosts **WP5-1**, **WP5-2** and **WP5-3** and guest paraquat were investigated by ^1H NMR (Figure 3 and Figure 4). For both the cases of **WP5-2** and **WP5-3**, the proton signals on guest paraquat (H_a and H_c for the complex with **WP5-2**, H_a' and H_c' for the complex with **WP5-3**) shifted upfield while those on hosts (H_1 , H_2 and H_3 on **WP5-2**, H_1' , H_2' and H_3' on **WP5-3**) shifted downfield. The signal related to H_b or H_b' on paraquat disappeared after mixing with **WP5-2** or **WP5-3** with a molar ratio of 1:1. This was attributed to the shielding by the pillar[5]arene electron-rich cavity. These evidences confirmed that paraquat was threaded into the cavity of the host **WP5-2** or **WP5-3** and thus formed stable host–guest complexes (**WP5-2-P** or **WP5-3-P**). However, in the ^1H NMR spectra of **WP5-1**, paraquat, and a 1:1 molar ratio mixture of **WP5-1** and paraquat (Figure S2), no obvious changes in proton signals were observed after mixing **WP5-1** and paraquat, which indicated that the host–guest interactions between **WP5-1** and paraquat were very weak and it was difficult to form a valid host–guest complex between **WP5-1** and paraquat. It was possibly due to the poorer water-solubility of **WP5-1** comparing to **WP5-2** and **WP5-3** and the lack of electrostatic interactions between the **WP5-1** host and the paraquat guest in water.

The cytotoxicity of the chosen three water-soluble pillar[5]arenes, paraquat and the host–guest complexes **WP5-2-P** and **WP5-3-P** were evaluated at different concentrations through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. As shown in Figure 5, all of the three types of water-soluble pillar[5]arenes exhibited relatively low toxicity at low concentrations towards both the two cell lines (HEK 293 and Raw 264.7) treated in the experiments, while at high concentrations ($\geq 400 \text{ mM}$) the Raw

264.7 cell viability went down below 50% (Figure 3a and 3b). These data indicate that all the three water-soluble pillar[5]arenes are relatively low in toxicity and promisingly applicable in pharmaceutical or agricultural additives. Furthermore, cell viability after treatment with the host-guest complexes (**WP5-2-P** and **WP5-3-P**) and paraquat was also measured to evaluate the detoxifying effectiveness of **WP5-2** and **WP5-3**. Decrease in relative cell viability was detected with the increase of the concentration of paraquat. In contrast, the relative cell viability of paraquat was lower than that of the host-guest complexes **WP5-2-P** and **WP5-3-P** at the same concentrations, which indicated that the formation of the host-guest complexes reduced the toxicity of paraquat. Hence we believe **WP5-1** has no detoxification effect towards paraquat. However, it's still hopeful that the water-soluble pillar[5]arenes **WP5-2** and **WP5-3** can be applied as agricultural plant growth regulators and reduce the toxicity of poisoning herbicide paraquat at the same time.

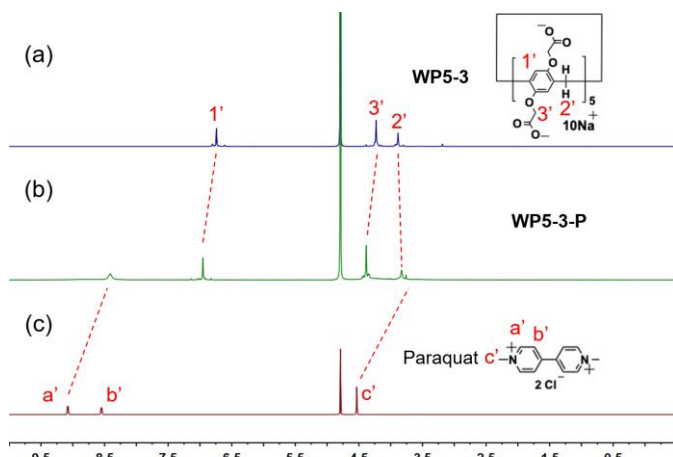


Fig. 4 Partial ^1H NMR (500 Hz, D_2O , 293 K) spectra: (a) **WP5-3** (5.0 mM); (b) **WP5-3** (5.0 mM) and paraquat (5.0 mM); (c) paraquat (5.0 mM).

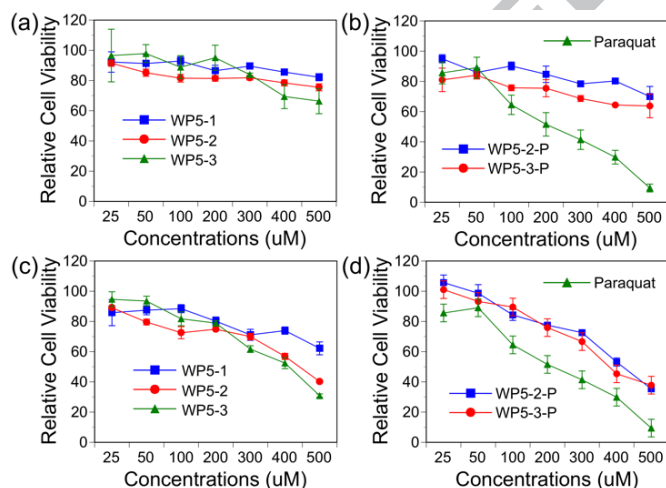


Fig. 5 Relative cell viability of (a) HEK 293 and (c) Raw 264.3 treated with different concentrations of **WP5-1**, **WP5-2**, **WP5-3**. Relative cell viability of (b) HEK 293 and (d) Raw 264.3 treated with different concentrations of paraquat and host-guest complexes **WP5-2-P** and **WP5-3-P**.

In conclusion, we demonstrated that three water-soluble pillar[5]arenes, **WP5-1**, **WP5-2**, and **WP5-3**, are efficient plant growth regulators to improve the development of buds and roots in the seedling cultivation stage of wheat. The treatment with these pillar[5]arenes expedites the growth rate of buds and roots. Meanwhile, the seeds' germination rates were the same as those treated with water. Moreover, **WP5-2** and **WP5-3** performed well not only in promoting the plant's growth but also in reducing the toxicity of poisoning herbicide paraquat by host-guest interactions, which was confirmed by cell viability experiments towards HEK 293

and Raw 264.3 cell lines. Therefore, these two water-soluble pillar[5]arenes are good candidates as novel bifunctional chemical fertilizers. We believe that our work provides a new understanding and applicable strategy for utilizing macrocycles as novel plant growth regulators with higher efficiency and multifunctionality in agricultural research.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (21434005, 91527301) and the fundamental research funds for the central universities.

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Supplementary Material

Synthetic procedures and characterizations and other materials. Supplementary data related to this article can be found at <http://xx>.

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2. Water-soluble pillar[5]arenes are performed well in reducing the toxicity of paraquat.
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