3-Indole[^{11}C]acetonitrile synthesis and biological application to study 3-Indole[^{11}C]acetic acid biosynthesis in Zea mays

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Introduction: In recent years, application of PET radiotracers to plants advanced to be a field of great interest. Understanding plant growth and development, and the mechanisms regulating these functions are key to optimizing crop productivity for agriculture and for improving biomass quality for better conversion to biofuels. 3-Indoleacetic acid (IAA) is the most important and abundant plant growth hormone. It is known to impact root growth affecting the extent of lateral root branching [1]. Still the mechanism of IAA transport and signaling for cell division and elongation is not fully understood. 3-Indoleacetonitrile (IAN) and 3-indoleacetamide (IAM) are natural biosynthetic precursors for this important hormone. Therefore, the *in-vivo* biodistribution of [^{11}C]IAN (**2**) and its conversion to [^{11}C]IAA (**3**) was investigated in intact plants. Recently, the BNL group has been studying the effects of western corn rootworm (*wcr*) infestation on belowground plant defense responses using PET radioisotopes. One interesting phenomenon is a root re-growth response which is hypothesized to link with IAA signaling.

Experimental: ^{11}C was generated as [^{11}C]CO_2 using 17.4 MeV proton irradiation of a N_2 gas target containing 100 ppm O_2 to induce the ^{14}N(p,α)^{11}C nuclear reaction. [^{11}C]CO_2 was catalytically reduced to [^{11}C]CH_4 over reduced Ni at 365 °C. [^{11}C]CH_4 was converted to [^{11}C]HCN over Pt at 1000 °C using gaseous NH_3. The synthesis of **[**^{11}C**]**IAN was conducted as described by Reid *et al.* [2] with slight changes. 1 mg of Gramine (**1**) was added to 0.3 ml of DMSO in a reaction vial. [^{11}C]HCN was trapped and the mixture was heated to 140 °C for 5 minutes.

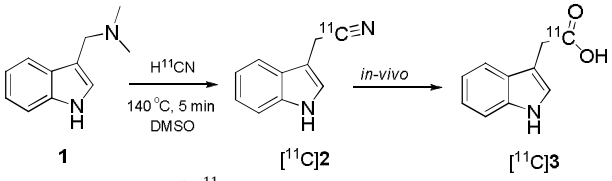

Fig.1. Synthesis of [^{11}C]IAN (**2**) and *in-vivo* conversion to [^{11}C]IAA (**3**)

The compound was purified by semi-prep HPLC. [^{11}C]IAN eluted at 14-17 min using 0.1% formic acid/acetonitrile (60:40). The product fraction (10-15 ml) was diluted with 50-60 ml water and the solution was passed through a C18 Sep-Pak (Waters, Cartridges). The Sep-Pak was washed with 15 ml water and eluted with 1.5 ml diethyl ether. The diethyl ether was evaporated using argon and, if needed, a part of the residual water was evaporated (T= 85-90 °C) prior to plant administration. The total radiochemical yield at EOB was ca. 50.6±15.6%. The specific activity was 9.6 GBq/µmol. The tracer was injected into targeted second generation crown roots using a gas chromatography syringe (20-30 µl). Root extracts were analyzed with two TLC methods at different time points (30, 60, 120 min.). One TLC plate was treated with a mixture of 1.5:1 hexane/ethyl acetate (0.1% formic acid) and the other was pretreated with ammonia vapor and developed using ethyl acetate.

Results: [^{11}C]IAN was successfully administered to the root system of intact maize plants. The *in-vivo* conversion of [^{11}C]IAN to [^{11}C]IAA and other metabolites was demonstrated and quantified (see Fig. 2). The free [^{11}C]IAA can be stored and transported as ester or amid conjugates [3], which were not further identified. Conversion rate of [^{11}C]IAN slightly increased from about 4% to 9% for healthy plants in a range of 2 h. *Wcr* infested plants showed a higher conversion rate of [^{11}C]IAN to [^{11}C]IAA (12-16% over 2 h). This suggests the local tissue biosynthesis may account, in part, for the higher levels of endogenous IAA seen in damage root sections (see Fig. 3).

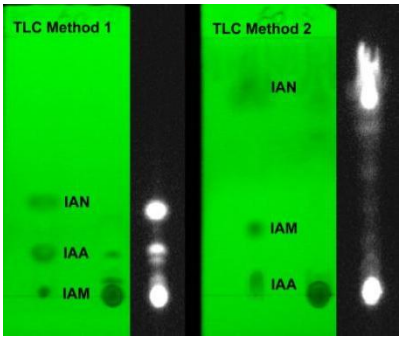

Fig. 2. TLC of root sample (control plant). Right image: autoradiography, left image: UV profile.

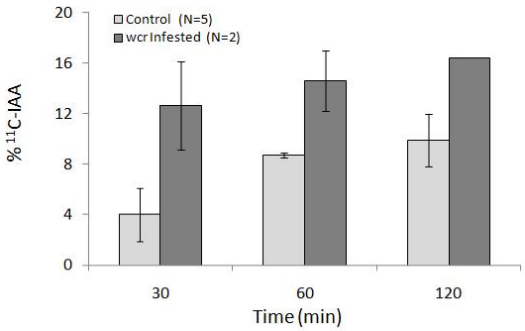

Fig. 3. Effect of *wcr* herbivory on metabolic turnover of [^{11}C]IAN to [^{11}C]IAA.

Conclusions: The synthesis route for **[**^{11}C**]**IAN was optimized. A protocol for administration of **[**^{11}C**]**IAN and analysis of plant tissue was developed. The *in-vivo* conversion of [^{11}C]IAN to [^{11}C]IAA was demonstrated by TLC analysis. *wcr* infested plants showed a higher conversion rate of [^{11}C]IAN to [^{11}C]IAA.

Literature

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[2] Reid, Kim, Fowler J. Label Compd. Radiopharm. 2011.
[3] Zazimalova, Napier, Plant Cell Rep.21 2003 625-634.