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Research Article

Synthesis of the phytohormone [^{11}C]methyl jasmonate via methylation on a C_{18} Sep Pak^{TM} cartridge

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Summary

[^{11}C]labeled (±)-methyl jasmonate was synthesized using a C_{18} Sep Pak^{TM} at ~100°C to sustain a solid-supported ^{11}C-methylation reaction of sodium (±)-jasmonate using [^{11}C]methyl iodide. After reaction, the Sep Pak was rinsed with acetone to elute the labeled product, and the solvent evaporated rendering [^{11}C]-(±)-methyl jasmonate at 96% radiochemical purity. The substrate, (±)-jasmonic acid, was retained on the Sep Pak so further chromatography was unnecessary. Total synthesis time was 25 min from the end of bombardment (EOB) which included 15 min to generate [^{11}C]methyl iodide using the GE Medical Systems PET Trace MeI system, 5 min for reaction and extraction from the cartridge, and 5 min to reformulate the product for plant administration. An overall radiochemical yield (at EOB) of 17 ± 4.3% was obtained by this process, typically producing 10 mCi of purified radiotracer. A specific activity of 0.5 Ci/μmol was achieved using a short 3 min cyclotron beam to produce the starting ^{11}C.

Key Words: solid-supported ^{11}C-methylation; [^{11}C]methyl jasmonate; ^{11}C-labeled phytohormone

Introduction

Exposure of plants to jasmonates, a class of plant hormones, has been shown to increase growth rate,[1–5] partitioning of resources to stem and roots,[6,7] production of defensive secondary chemicals[8,9] and lignins.[10,11] Recently, we

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have shown that jasmonates will increase leaf ^{11}C-sugar intermediates,[12] with subsequent increase in phloem loading and ^{11}C-sucrose partitioning to roots.[13] Hence, we believe a role of jasmonates in resource partitioning and chemical fractionation may come from their regulation of sugar-transport proteins that mediate the long-distance transport of carbohydrates within the plant vasculature and partitioning between sink tissues.[14–17]

The purpose of the present work was to develop a rapid method for introducing carbon-11 ($t_{1/2}$ 20.4 min) into methyl jasmonate (MeJA). A key advantage for using this short-lived β^+-emitting radionuclide in plant biology is that tracer can be quantified *in vivo*, so that the same plant can be tested repeatedly over time. Additionally, the high specific activity achievable with ^{11}C allows us to administer non-physiological doses of tracer for observations of *in vivo* transporter binding. These measurements cannot be made with ^{14}C, ^{13}C or ^{3}H as tracers.

The approach described in this work makes use of a solid-supported ^{11}C-methylation reaction.[18] This approach holds significant appeal for ease of experimental setup, and minimal effort to purify the final product.

Results and discussion

Several experiments were conducted using unlabelled methyl iodide to optimize reaction conditions. The variable parameters included support-type, temperature, solvent-type, as well as the type and amount of base used. The amount of jasmonic acid (JA) substrate was fixed in these studies, as was the amount of carrier CH_3I administered, and its rate of introduction to the cartridge (25 ml min^{-1}).

Influence of support type on chemical yield and substrate retention

Several different cartridges were tested to determine whether the nature of the support influenced the extent of methylation. All conditions of reaction were maintained constant (i.e. temperature, 80°C; JA and methyl iodide, 13 μmol; NaOH, 6 μmol). C_{18} Sep Pak showed up to be the best cartridge; no product was detected with an Alumina B Sep Pak. To test the retention of JA on each different Sep Pak material, 0.4 ml of a standard solution (20 μl JA dissolved in 5 ml DMF) was loaded into each Sep Pak and later rinsed with 4 ml of acetone. Acetone was selected for its ability to readily solubilize both JA and MeJA. Aliquots of the rinse were analyzed via capillary gas chromatography to determine the total amount of bound JA. Results indicated that JA was irreversibly bound to the Silica and Alumina Sep Paks, and only 0.5% JA was eluted from the C_{18} Sep Pak. A similar measurement using MeJA showed full recovery of product from all of the cartridges.

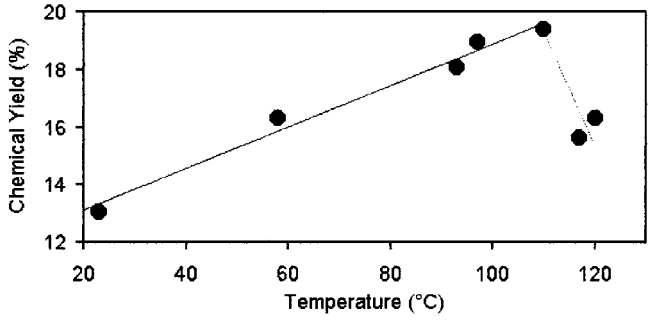

Figure 1. Influence of C_{18} Sep Pak temperature on chemical yield

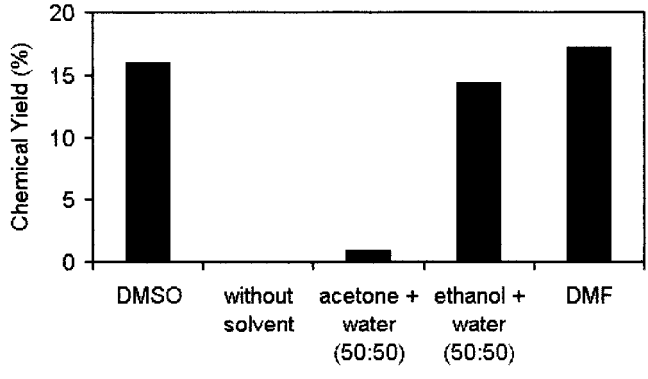

Figure 2. Influence of solvent on chemical yield

Influence of C_{18} Sep Pak temperature on chemical yield

Raising the temperature of the C_{18} Sep Pak cartridge led to a higher yield (Figure 1). A linear dependence was exhibited up to 110°C, but at higher temperatures the yield decreased. A temperature of 90°C was chosen to avoid melting of the frit within the cartridge that sometimes occurred at temperatures over 105°C.

Influence of solvent nature and polarity on chemical yield

Five solvents were tested with C_{18} Sep Paks (including no solvent: Figure 2) to determine whether there was an effect of solvent polarity: this would be expected to influence substrate anion formation and thus chemical yield. Interestingly, Figure 2 shows that some solvent was necessary to sustain a reaction, implying that chemistry was occurring within an organic film coating the support. DMF and DMSO are both moderately polar aprotic solvents and showed the highest chemical yields. Acetone on the other hand, which was only slightly less polar, resulted in a much lower yield than DMF and DMSO. Furthermore, ethanol, a much more polar solvent than DMF and DMSO,

gave a lower yield. However, ethanol could potentially hydrogen bond with the substrate which may have reduced overall chemical reactivity.

The exact role of the solvent is unknown. Our calculations reveal that for the amount of DMF added to the Sep Pak cartridge, a film thickness of roughly 0.5 μm occurs (approximately 1000 monolayers). It seems that the reaction is well sustained when substrate is in contact with a polar non-hydrogen-bonding solvent. However, we also found that the solid-support played a key role in activating reactants: different support materials had a different degree of influence on the MeJA chemical yield. Furthermore, an important aspect of the reaction efficiency was whether labeling substrate was in intimate contact with the solid support or uniformly mixed within the organic film. We tested this aspect in a single experiment by first depositing substrate dissolved in acetone onto the C_{18} Sep Pak and completely drying the support prior to coating it with DMF to the equivalent film thickness. The radiochemical yield of [^{11}C]MeJA nearly halved by this process suggesting that chemistry was optimized when substrate was uniformly mixed within the solvent film of DMF.

Influence of base on chemical yield

During these studies two different bases were tested (NaOH and triethylamine). Only NaOH resulted in a measurable yield of product. The amount of 2 N NaOH used in the reaction was then varied while all other parameters were fixed. A linear dependence between the moles of NaOH and chemical yield was observed for amounts of base less than the moles of labeling substrate. After this point, increasing the amount of base had no effect on the chemical yield (Figure 3).

Radiosynthesis of (±)-[^{11}C]methyl jasmonate

The synthesis of (±)-[^{11}C]methyl jasmonate was carried out through a solid-supported [^{11}C]methylation at 90°C on a C_{18} Sep Pak cartridge. This approach

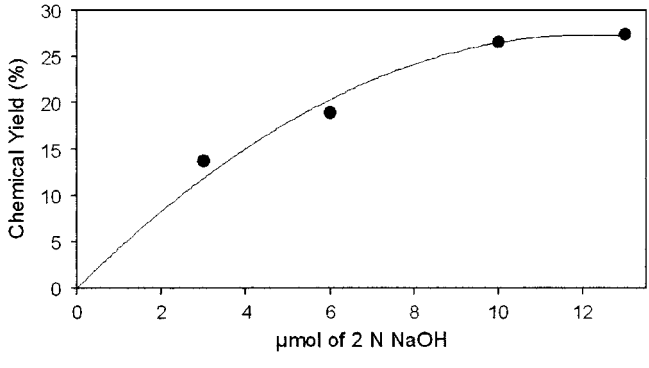

Figure 3. Influence of base on chemical yield

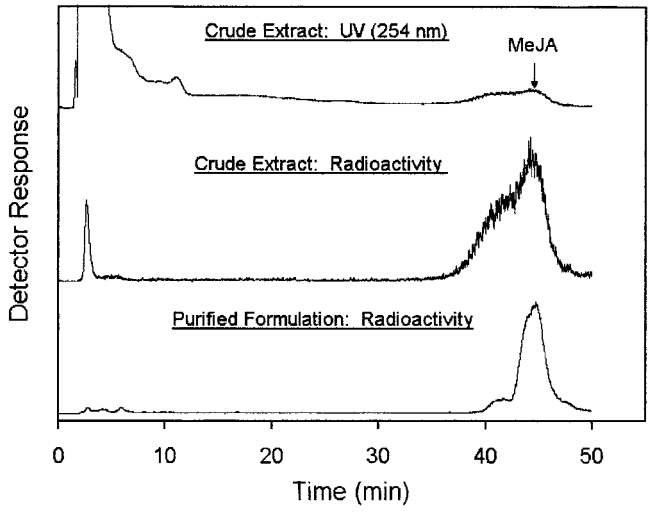

Figure 4. Radio-HPLC traces

has simplified the methylation and its possible automation. Quick changes could be made to the apparatus to vary the method, e.g. loading the crude mixture into a HPLC for separation. The reaction time from EOB (including 15 min to make and deliver [^{11}C]methyl iodide using the GE MeI box) is very short, $\sim$25 min. Because substrate was retained on the cartridge, extraction using acetone gave a crude product which, besides the [^{11}C]MeJA, included volatiles that could be removed via evaporation. Radio-HPLC analysis was used to quantify radiochemical yield and purity, as well as product specific activity (Figure 4). The elution time for MeJA on the HPLC system was verified by trapping cold product and reanalyzing it using capillary GC. The radiochemical yield of $17 \pm 4.3\%$ ($n = 3$), based on [^{11}C]methyl iodide delivered to the Sep Pak, was sufficient for plant studies without further development of the chemical process. Radioactive purity was 96%, and a specific activity of 0.5 Ci μmol^{-1} could be achieved for 40 μA min of beam.

Unless the substrate-loaded Sep Pak was pre-rinsed prior to reaction, specific activity was 7 times lower than that cited above because the commercial substrate, JA, contained as much as 3% unlabelled MeJA. Further increase in specific activity is possible by using a larger activity of methyl iodide, from longer beam times.

Experimental

Materials

Reagents and solvents were used as purchased, unless specified. ($\pm$)-Jasmonic acid (93%), acetonitrile (99.9%) and ($\pm$)-methyl jasmonate (95%) were obtained from Sigma-Aldrich (St. Louis, USA). The solvent DMF (99.8%)

and NaOH were purchased from Aldrich (St. Louis, USA), as well as CH_3I (99%) and triethylamine (99.5%). Acetone (99.7%) and HC1 (37%) were obtained from Mallinckrodt (Phillipsburg, USA), DMSO (99.5%) from Fluka (St. Louis, USA) and ethanol (95%) from Aaper (Shelbyville, USA). Acetic acid (99.9%) was purchased from Baker (Chicago, USA). All solvents were used dry unless mentioned. All cartridges were obtained from Waters (Milford, USA). [^{11}C]methyl iodide was produced from [^{11}C]CO_2 that was produced on the BNL Ebco TR-19 cyclotron. The radioactive gas was immediately released from the target and automatically converted to [^{11}C]methyl iodide using the GE Medical Systems (Upsala, Sweden) PET Trace Mel Synthesis Module.

Methods

0.4 ml JA (20.0 μmol of JA) of a standard solution (30 μl of (±)-JA, 0.249 mmol, dissolved in 5 ml DMF) was mixed with 9 μl of 2 N NaOH (18 μmol) and loaded on a preheated (~90°C) C_{18} cartridge prior to the start of the synthesis. The precursor was allowed to contact the Sep Pak for 5 min prior to introduction of [^{11}C]methyl iodide. The temperature of the cartridge was regulated using an Omega temperature controller to drive a resistance element with temperature feedback provided by a thermocouple (Omega, Inc., Stamford, CT). [^{11}C]Methyl iodide was administered to the substrate loaded cartridge in a flow of argon (25 ml min^{-1}). Trapping efficiency of [^{11}C]methyl iodide on the treated cartridge was approximately 70%. After the reaction the cartridge was rinsed with 4 ml of acetone and this crude mixture was evaporated under reduced pressure to remove the radioactive volatiles and excess solvent. Afterwards the product was dissolved in a solution of 1% aqueous acetone for topical administration to intact leaf tissue. Figure 5 shows a schematic diagram of the system.

The radiochemical purity was determined by radioanalytical HPLC using a Phenomenex Spherosorb 5 ODS 2 (250 × 4.6 mm; PP/1307 D). The mobile phase consisted of acetonitrile, water and acetic acid (30:70:0.1 by vol.) at a flow rate was 1.5 ml min^{-1}. The eluate was monitored using a Biodex radiation detector connected in series with a Knauer UV absorption detector (254 nm) for mass measurements. Additional mass analyses were carried out on a Hewlett-Packard capillary gas chromatograph (model 5890 series II; Hewlett-Packard, Stamford, CT) coupled with a flame ionization detector (350°C). The analyses employed a Hewlett-Packard Ultra 1 capillary column (25 m × 0.32 μm i.d.) with the column oven temperature increased from 70°C at the time of injection to 200°C at 5°C min^{-1}. Split injections were made at a 1:50 ratio using a column flow 2 ml min^{-1} and the total injector flow of 100 ml min^{-1}. The injector was maintained at a temperature of 225°C.

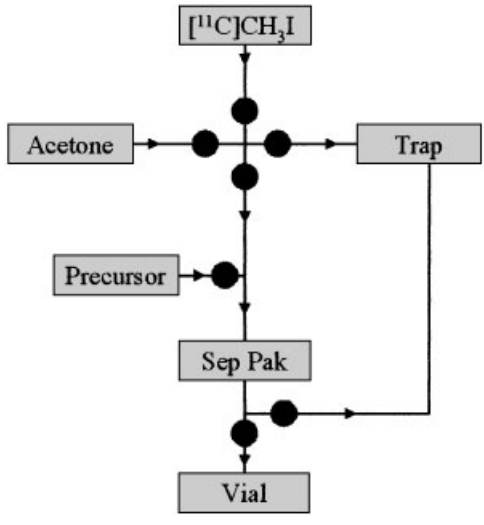

Figure 5. Schematic diagram of the reaction system

Conclusions

(±)-[^{11}C]Methyl jasmonate was successfully produced in a short time, with high radiochemical purity of 96%, and in sufficient quantities that will allow for topical administration of tracer to intact leaves of plants. Although tracer specific activity was low, using larger starting amounts of ^{11}C might be expected to improve on this.

The method described makes use of a disposable C_{18} Sep Pak which has great appeal due to the ease of setup. Additionally, the approach is amenable to automation, or at least remote operation which can minimize personnel exposure to radiation hazards.

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