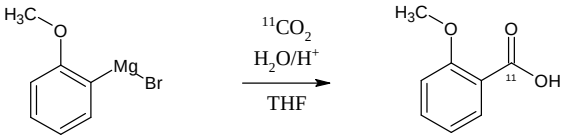
Radiosynthesis of the plant hormone [^{11}C]Salicylic Acid and Autoradiography Imaging of [^{11}C]Salicylic Acid in *Nicotiana tabacum*

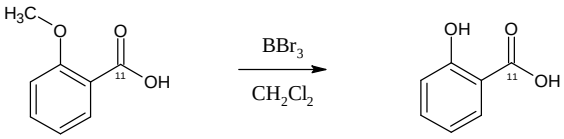
Tatjana Judt[1], Sung Won Kim[2], Joanna S. Fowler[2]
[1]Fachbereich Chemie, Johannes Gutenberg University, 55099 Mainz, Germany
[2]Medical Department, Brookhaven National Laboratory, 11973 Upton, NY, USA

Introduction: Plants possess multiple mechanisms to protect themselves against pathogens. An attack by fungi, bacteria or viruses often causes a formation of necrotic lesions. These dead tissues can prevent the development and growth of additional pathogens. Also, a signaling molecule is produced which activates the plant's defense mechanisms, similar to the human immune system. This phenomenon is called systemic acquired resistance (SAR)[1]. The plant hormone salicylic acid plays a key role in the disease resistance to pathogens by inducing the production of pathogenesis-related proteins (PRs) [2].

Experimental: Radioactive salicylic acid was obtained in a two-step reaction, starting from a Grignard compound and radioactive $^{11}CO_2$ and continuing with demethylation with the aid of BBr_3.



Scheme 1: Grignard Reaction (Synthesis of Anisic Acid)



Scheme 2: Demethylation (Synthesis of Salicylic Acid)

0.15 ml (1.5 mmol) of the precursor 2-methoxyphenylmagnesium bromide (1.0 M solution in THF) was diluted with 0.15 ml THF. After trapping $^{11}CO_2$, a solution of 0.1 ml (1M) hydrochloric acid in water was added. A few drops were taken for analytical HPLC (Gemini 5u C18 110A, 250 × 4.60 mm, 5 micron, phenomenex) and TLC analysis. The product of the first step is anisic acid. The reaction solution was dried by blowing argon and heating at 130°C to evaporate the reaction solvent and water. For synthesis of the final product, salicylic acid, a solution of 1.0 ml (1.0 mmol) boron tribromide in dichloromethane (1 M) was added [3]. After five minutes the product was dried again with argon and was heated at 130°C to speed up the drying. Salicylic acid was dissolved in 1 ml acetonitrile and purified in a preparative HPLC (Gemini 5u C18 110A, 250 × 10.00 mm, 5 micron, phenomenex; solvent: 70% of 0.1% formic acid in water, 30% of acetonitrile) and analyzed by an analytical HPLC.

Results: Figure 1 is the analytical HPLC spectra of salicylic acid. The upper spectrum shows the radio peak of synthesized radioactive [^{11}C]salicylic acid with a retention time of 17.216 min. The lower spectrum contains the peak of the commercial salicylic acid with a retention time of 16.816 min. The co-injection was made to prove that the radioactive product is [^{11}C]salicylic acid. The reaction was successful since both peaks come out with similar retention times. The plant hormone [^{11}C] salicylic acid was synthesized with a radiochemical yield of 19%.

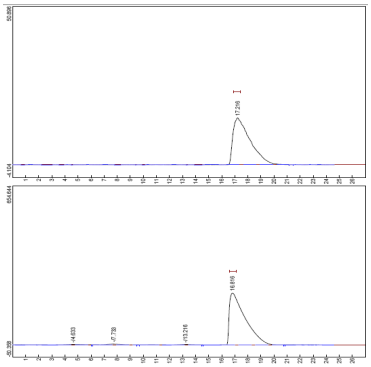


Figure 1: Analytical HPLC of Salicylic Acid

A treatment of *Nicotiana tabacum* with the radioactive plant hormone is shown in Figure 2, which is an autoradiography image. [^{11}C]Salicylic acid was added to the tip of the tobacco leaf. After 1 h the plant transported the hormone from the tip to the stem through its vascular tissue.

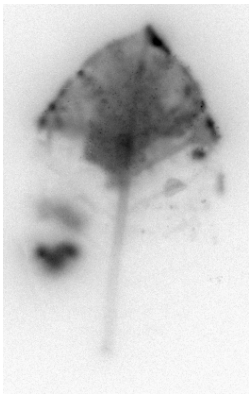


Figure 2: [^{11}C]Salicylic Acid in Nicotiana tabacum

References
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