Development of a C-11 labeling method for the plant hormone azelaic acid to investigate the molecular specifity of the systemic acquired resistance (SAR) in Nicotianatabacum

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Introduction: Azelaic acid is a nine-carbon dicarboxylic acid (1,9-nonane diacid), which has been known for years as an antibacterial agent in dermal creams. A recent study showed that azelaic acid has a direct function in the SAR (systemic acquired resistance) of plants [1]. It has been determined that azelaic acid can move systemically, most likely through the vasculature. The SAR is one of the most important parts of the plant immune system. It has been shown that azelaic triggers the elevated accumulation of salicylic acid in the leaves after local infection. Azelaic acid induces the expression of azelaic acid induced gene 1 (AZI1), which has a very important role for the downstream systemic resistance of the plant. It has been discovered that azelaic acid induced effects are very specific. The related molecules, suberic acid (1,8-octane diacid) and sebacic acid (1,10-dekane diacid), do not cause any resistance. The result of an induced SAR is the excited state of a plant after a primary infection, which leads to a stronger defence against opportunistic, secondary infections. In this work, we labelled azelaic acid with the isotopes ^{11}C and ^{14}C. In addition all other dicarboxylic acids mentioned above were labelled with ^{11}C with the goal to get a better understanding of their transport.

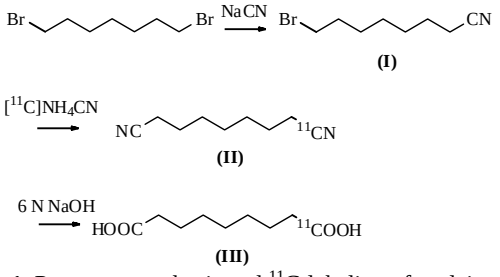

Figure 1: Precursor synthesis and ^{11}C-labeling of azelaic acid.

Methods and Materials: The 8-Bromooctanenitrile (**I**) was produced via a Kolbe nitrile synthesis in isopropanol starting from 1,6-dibromohexane (Figure 1). [^{11}C]Hydrogen cyanide was produced with a cyanide production system at the Brookhaven National Laboratory by following the same procedure as described in the literature [2]. Trapping and labeling was conducted in a basic DMSO solution. The labeled [^{11}C]azelanitrile (**II**) was purified with a Sep-Pack® (C-18 plus) and hydrolyzed with 6N NaOH to obtain the labeled [^{11}C]azelaic acid (Figure 1). Purification was conducted with a KNAUER HPLC system by using a Gemini C-18 column. The final product was dissolved in water and applied to the tip of the leave of either corn or tobacco plants. Chemistry was verified with ^{13}C-NMR by adding K^{13}CN as carrier. An Agilent LC-MS system yielded supporting results. This method was carried out for all three diacids. ^{14}C-Labeling of azelaic acid was performed with the same procedure. The tracer was applied to the tip of the leaf of *Arabidopsis*.

Results and Discussion: The synthesis of the precursors (**I**) was performed with moderate yields. $^{11/14}$C-labeling to obtain the [$^{11/14}$C]dinitriles (**II**) could be accomplished in very high yields. The hydrolysis with 6N NaOH solution was almost quantitative. A 50 minute application of the ^{11}C-labeled diacids to the leaves showed a clear difference between azelaic acid and the other two acids: sebacic und subericacid were both lacking transport (Figure 2).

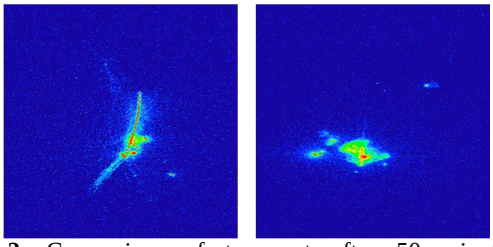

Figure 2: Comparison of transport after 50 minutes of incubation (tobacco leaves); left image: [^{11}C]azelaic acid; right image: [^{11}C]suberic acid.

The co-application of ^{11}C-labeled azelaic acid with the ^{12}C-reference showed that transport can be blocked. This indicates that there is a specific enzyme that loads azelaic acid into the midrib of the leaf for transport. The reference might utilize its activity. ^{14}C-Studies over a 24 hour application indicated that azelaic acid is transported in the phloem to phloem sink tissues; namely the roots, flower stalk, flower buds and immature leaves:

Figure 3: Plant image of *Arabidopsis* after 2-4 hours of application of ^{14}C-labeled azelaic acid.

Outlook: Further studies with $^{11/14}$C-labeled diacids to proof the hypothesis that a very specific carrier for azelaic acid is responsible for the fact that suberic acid and sebacic acid do not induce SAR are needed. ^{14}C-Studies might clearly show that the transport into the phloem has an active mechanism.

References

[1] HW Jung *et al. Science* **2009**, 324:89-91
[2] DR Christman *et al. Int J Appl Radiat Isot* **1985**, 26:435-442