Analysis of cuticular WAX composition

1. The cuticular waxes were extracted from 30-day old plants by immersing separated stems in hexane for 30 sec. (not homogenate).
2. Extracts were transferred to a new vial and evaporated to dryness.
3. Internal standard (*1) and BSTFA (*2) was added to the dried residue.
4. The derivatization was at 80℃ for 30min.
5. After the surplus BSTFA was evaporated, the samples were dissolved in hexane.
6. The quantitative composition of the samples was studied using capillary GC with FID following GC conditions.
7. Single compounds were quantified against the internal standard by automatically integrating the peak areas.

Gas chromatograph：SHIMAZU GC-14A

Condition： 170℃
↓2℃/min
265℃
↓4℃/min
310℃
↓
hold 10 min

AIR：0.5 kg/cm^2
Hydrogen：0.5 kg/cm^2
Carrier(P1)：1 kg/cm^2
Carrier(P2)：2 kg/cm^2
(Carrier : He)

Column：capillary column・DB-1 (J and W Scientific)
Length (meters) : 30
I.D. (mm) : 0.25
Film (um) : 0.25

(*1) Internal standard：10mM Nonadecanoic acid (Fuluka) / hexane
(*2) BSTFA : N,O-Bis (trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane (SIGMA (T6381-1AMP))

<Reference>
Plant Physiol. (1 995) 108: 369-377
M A. Jenk et, al.
Leaf E p i cu t icu lar Waxes of the Eceriferum Mutants in Arabidopsis