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Derivatization and Deuterium Labeling of Xanthoxin

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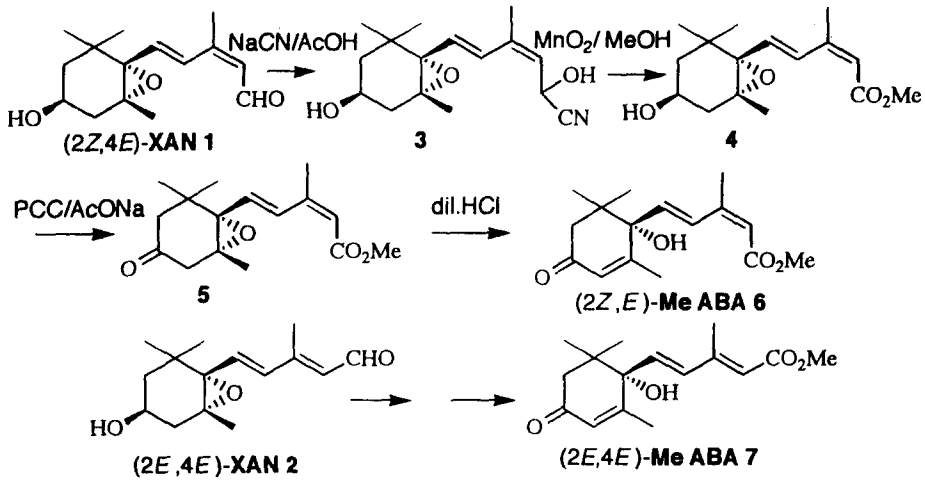
Abstract: Xanthoxin was derivatized to abscisic acid methyl ester via oxidative esterification. Deuterium labeling by $LiAlD_4$ and D_2O provided a useful internal standard for the quantificatin of natural xanthoxin level on GC-MS in combination with the new derivatization method.

Although xanthoxin (XAN) has frequently been proposed as a precursor of abscisic acid (ABA), a plant hormone, since the first isolation reported by Taylor et al,[1] the level and isomeric composition of XAN is still controversial.[2] The structure of XAN well explains the biosynthetic pathway via carotenoids, in which xanthophylls such as violaxanthin and neoxanthin suffer the oxidative cleavage induced by water stress. However, XAN in higher plants is mainly identified as a (2*E*,4*E*)-isomer contrary to the geometry of naturally occurring (2*Z*,4*E*)-ABA. Recently, authors found that (2*Z*,4*E*)-XAN **1** is completely isomerized to (2*E*,4*E*)-isomer **2** under the procedure reported by Parry et al.[2] and that the isomeric composition of natural XAN is exclusively shifted to **1**. Before reporting our biochemical results [3] in detail, we wish to describe the novel chemical approach to the measurement of natural XAN level on GC-MS.

GC-MS analysis of XAN itself has some shortcomings. The fragmentation pattern of XAN is rather complicated, and relatively large amount of XAN (1-5 μg) is necessary to measure a full EI-MS spectrum. The most fatal defect is the isomerization between **1** and **2** on GC even under mild conditions. Isomerization is also inevitable during the preparation of known XAN derivatives.[4] To overcomve these problems, the chemical conversion of XAN to ABA methyl ester (MeABA) was investigated (Scheme 1). The oxidative methyl esterification reported by Corey et al.[5] has been widely employed for the synthesis of ABA analogues, but in modest yields.[6] To achieve quntitative Corey reaction, the conventional procedure was modified into a stepwise method. Thus, **1** was preliminary converted to cyanohydrin **3** by a large excess amount of NaCN before subsequent addition of MnO_2. An exact ratio of NaCN and AcOH was found important as well as the stepwise addition of reagents to obtain **4** in more than 97% yields. Further oxidation of **4** by pyridinium chlorochromate (PCC) and acidic rearrangement of **5** by dil.HCl gave (2*Z*,4*E*)-MeABA (**6**) in excellent yields. Similarly, (2*E*,4*E*)-isomer **2** was converted to (2*E*,4*E*)-MeABA (**7**). General merits of new derivatization method are

as follows; no isomerization under the reaction condition and GC-MS analysis; high overall yield, high reproducibility and facile procedure; exceptionally high sensitivity of GC-MS for MeABA (empirically about 1000 times of XAN); and remarkably simple fragmentation pattern of MeABA on GC-MS [m/z (typical int.); 278 (M, 4), 190 (100), 165 (25), 135 (25) and 125 (40)]. It should be emphasized that this method is suitable to handle a small of XAN, as a large excess amount of reagents can be used and removed easily all through the procedure. A facile deuterium labeling of XAN was investigated.



Scheme 1. Conversion of XAN to Me ABA

Thus, **1** was treated with LiAlD4 in dry THF, and the reaction mixture was quenched by D_2O in a usual manner. The produced allylic alcohol was again oxidized to XAN by MnO_2 oxidation. This procedure was usually repeated twice to achieve the typical isotopic composition of $[^2H_0]$: $[^2H_1]$: $[^2H_2]$: $[^2H_3]$: $[^2H_4]$: $[^2H_5]$ = 3 : 14 : 28 : 32 : 17 : 6. Though the isotopic composition was slightly improved after the repeated treatment, repetitions more than three times were not effective. The overall yield of $[^2H_{0\text{-}5}]$-**1** and $[^2H_{0\text{-}5}]$-**2** from nonlabeled **1** were 12% and 48% , respectively. The isomeric mixture was easily separated by preparative HPLC in the same isotopic composition.

To examine the mode of deuterium scrambling during the derivatization to MeABA, $[^2H_{0\text{-}5}]$-**1** (Fig. 1) was converted to $[^2H_{0\text{-}4}]$-**6** (Fig. 2). EI-MS analysis of $[^2H_{0\text{-}4}]$-**6** showed the typical isomeric composition of $[^2H_0]$: $[^2H_1]$: $[^2H_2]$: $[^2H_3]$: $[^2H_4]$ = 17 : 28 : 32 : 17 : 6. This deuterium scrambling pattern indicates the complete elimination of aldehyde proton of **1** which was almost exclusively labeled by deuterium, while the at other positions were secured. When $[^2H_{0\text{-}5}]$-**1** was used as an internal standard [4] and converted to **6** together with natural XAN, the ratio of m/z 192 : 193: 194 on GC-MS was kept unchanged while the intensity at m/z 190 and 191 increased according to the levle of natural **1**.[7]

In summary, facile and novel methods for the derivatization and deuterium labeling of XAN were developed. The combination of these two techniques on GC-MS analysis promised the precise measurement of natural XAN.

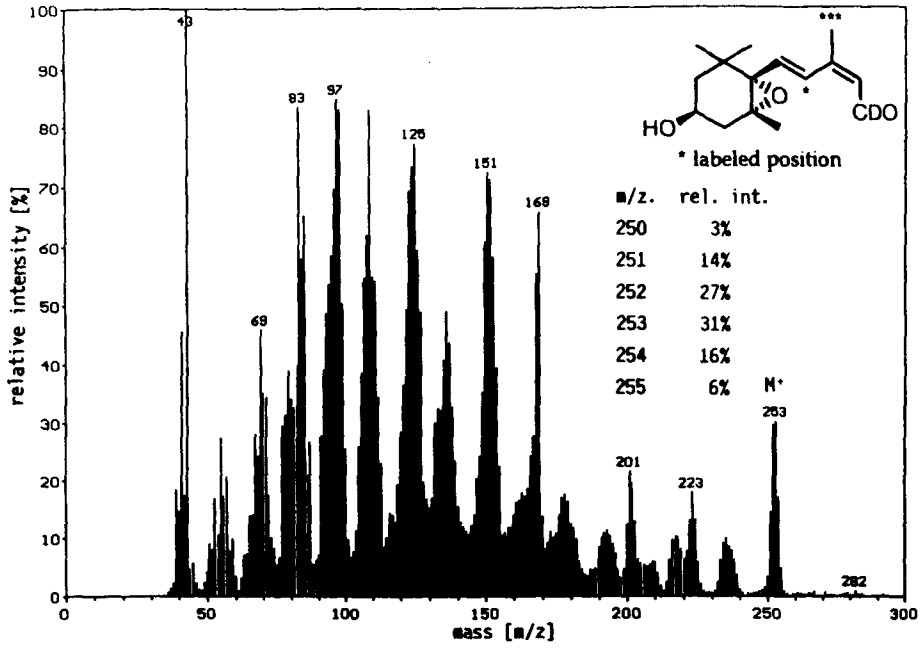


Fig.1. MS Spectrum of $[^2H_{0-5}]$-1

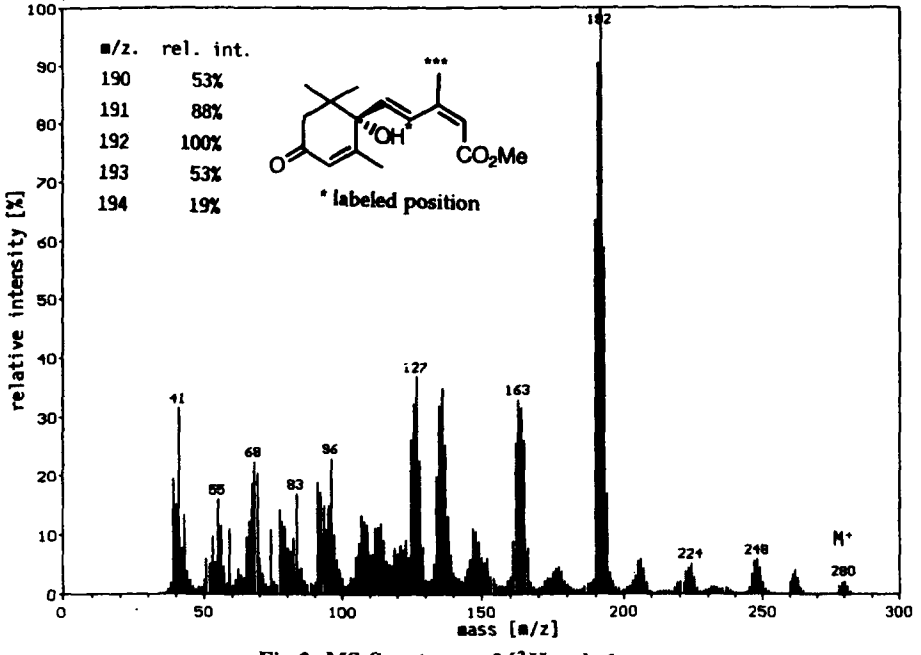


Fig.2. MS Spectrum of $[^2H_{0-4}]$-6

Typical procedure: Authentic **1** (or HPLC purified natural XAN, 5-5000 μg) in dry MeOH (5 .0 ml) was added to a mixture, prepared from powdered NaCN (200 mg) and absolute AcOH (200 mg) in dry MeOH (10 ml) . After stirring for 60 min. at room temp, the mixture was further stirred with additional MnO_2 (activated powder 2.0 g) overnight. The usual workup gave crude **4**, which was oxidized with PCC (500 mg) and NaOAc (50 mg) in dry CH_2Cl_2 (10.0 ml) under stirring for 2 hr to give crude **5**. Crude **5** in EtOAc (10 ml) was treated with 18% HCl (5.0 ml) with vigorous stirring for 2 hr , followed by the routine workup, to give **6**.[8]

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7. Quantification of natural XAN was carried out by the following equation.

$$1 - \left\{ \frac{\text{(int, of m/z 192)/0.32}}{\text{(total int. of m/z 190-194)}} \right\} \times \quad \text{(XAN added as an internal standard)}$$

8. The overall yield of **6** from **1** was estimated at 90-95% by HPLC (ODS Sphersorb 250 x 4.5 mm eluted with $CHCl_3$: EtOH = 100:1, 5.0 ml/min, Rt = 11.3 min). No peak which corresponds to **7** (Rt = 10.0 min) was recognized. The isomerization was not obserbed during GC-MS analysis (Mega-2 OV-bonded phase column 15 ml x 0.32 mm, Carlo Erba,150-210°C at 8°C/min, 70eV, **6**; Rt = 11.2 min, **7**; Rt = 9.5 min.

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