# APPENDIX

**A : Determination of 2AP in rice leaves samples**

In this study, the determination of 2AP in rice leaf samples was performed by using internal standardization method. The calibration curve was constructed by poltting peak area ratio of 2AP/2,6-DMP corresponding to concentration of standard of 2AP. The calculation of the concentration of the internal standard and quantity of 2AP in rice leaf samples are demonstrated as follows.

1) Stock internal standard, 2,6-DMP, 100, 250 and 500 ppm

Pipetted stock 2,6-DMP 2.7, 6.8 and 13.6 µl into volumetric flask 25 ml and adjusted volume to 25 ml with toluene. The concentrations of internal standard, 2,6-DMP, were 100, 250 and 500 ppm, respectively.

2,6-DMP were 0.922 g/ml of density and assay as 99%.

Thus, 0.922 g/mL = $0.922\times10^6$ mg/L (ppm)

The equation for calculated concentration of 2,6-DMP was

$$C_1\times V_1 = C_2\times V_2 \qquad (1)$$

When C= concentration and V = volume of solution

Preparation of 100 ppm of 2,6-DMP in toluene 25 mL is follow by equation 1.

$$0.922\times10^6 \text{ ppm} \times V_1 = 100 \text{ ppm} \times 25 \text{ mL}$$

$$V_1 = \frac{100 \text{ ppm} \times 25 \text{ mL}}{0.922\times10^6 \text{ ppm}}$$

$V_1 = 0.0027$ mL

$V_1 = 2.7$ µL

At concentration of 100 ppm 2,6-DMP, the stock of 2,6-DMP solution was pipetted of 2.7 µL and then dissolved in 25 mL of toluene.

The concentration of 2,6-DMP in each amount can be calculated same as the calculation in 100 ppm of 2,6-DMP.

2) Quantity of 2AP in rice leaf samples

The quantity of 2AP in rice leaf samples were determined by comparison with the calibration curve in Figure 3.19. The calibration curve obtained gave regression line with correlation coefficient, $R^2 = 0.9998$ and linear equation is $Y = 0.0413X - 0.0009$ (Y= peak area ratio of 2AP/2,6-DMP, X= concentration of 2AP). The data for calculation the quantities of 2AP are shown in Table A1.

**Table A1.** Data for calculation of quantities of 2AP in KDML 105 (organic) rice leaf sample

| Replication Number | Sample Weight (g) | Peak area ratio of 2AP/2,6-DMP (Y) | Concentration of 2AP, ppm (X) | Average of Concentration of 2AP, ppm |
|---|---|---|---|---|
| **1** | 0.2000 | 1.65 | 39.98 | |
| **2** | 0.2000 | 1.74 | 42.20 | **40.83** |
| **3** | 0.2000 | 1.66 | 40.33 | |

From the linear equation is Y= 0.0413X - 0.0009.

Thus, the concentration of 2AP (X) $= \frac{Y+0.0009}{0.0413}$

For example, the calculation of 2AP in KDML 105 (organic) rice leaf sample in replication number 1 is shown as follow.

The concentration of 2AP in replication number 1 $= \frac{1.65+0.0009}{0.0413}$

= 39.98 ppm

Therefore, the quantity of 2AP in KDML 105 (organic) rice leaf sample is 39.98 ppm (replicated number 1).

Therefore, the average of concentration of 2AP = $\frac{\sum X}{N}$

When X= Concentration of 2AP and N= Number of replication

The average of concentration of 2AP in replication number 1 $= \frac{39.98+42.20+40.33}{3}$

= 40.83 ppm

Therefore, the average of concentration of 2AP in KDML 105 (organic) rice leaf sample is 40.83 ppm.

The quantities of 2AP in rice leaf samples in each sample can be calculated same as the calculation in replicated number 1.

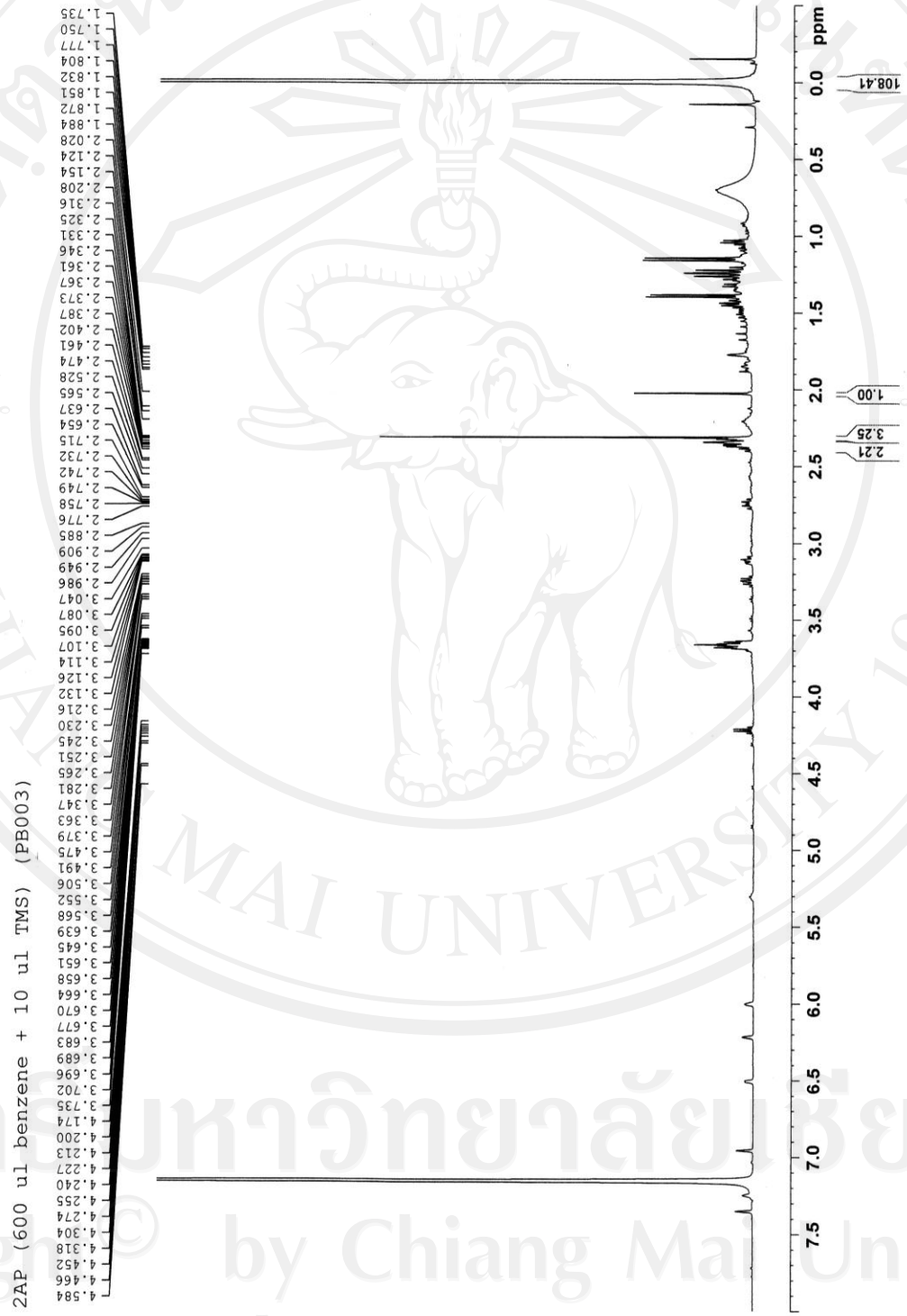
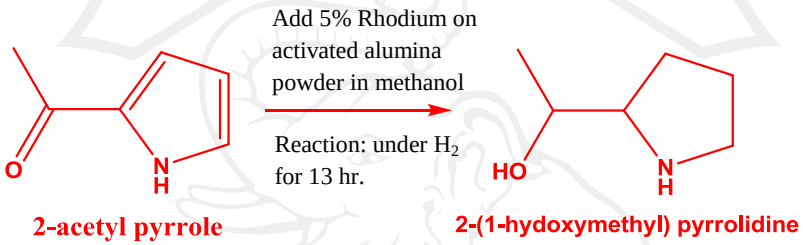



**Figure 1** $^{1}$H-NMR spectrum of 2AP

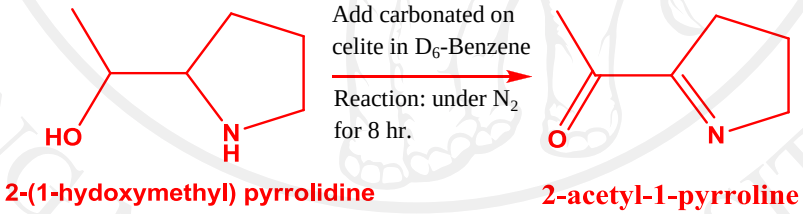
**B: Quantitative analysis of 2AP by nuclear magnetic resonance (NMR) spectroscopy**

In this study, 2AP was synthesized as a standard solution for create a standard calibration curve. The method for synthesis of 2AP has 2 steps including of hydrogenation and oxidation steps.

1. Hydrogenation reaction




2. Oxidation reaction




The calculation of the concentration of 2AP is demonstrated as follows.

1) The calculation of the concentration of tetramethylsilane (TMS)

$$M = \frac{10\%D}{MW} \quad (2)$$

When M= concentration (mol/L), % = assay of compound, D = density (g/L) and MW= molecular weight

TMS has 99.5% of assay, 88.23 of MW and density = 0.638 (g/L).

Thus, M of TMS followed by equation 1 = $\frac{10 \times 99.5\% \times 0.638}{88.23}$

= 7.1949 mol/L

In this study, TMS as a reference solution was used at 10 µl.

Thus, mole of TMS = $\frac{7.1949 \times 10^{-2}}{1000}$

= $7.1949 \times 10^{-5}$ mole

2) The calculation of the concentration of 2AP compared with TMS

In this experiment, the integrate value of $^{1}$H-NMR of TMS and $CH_3$ group of 2AP were 108.41 and 3.25, respectively.

The proton (H) of TMS 1 molecule contained 12.

The proton (H) of $CH_3$ group of 2AP at chemical shift 2.3 ppm was 3.

Thus, H of TMS was 4 times higher than 2AP at $CH_3$ group.

Thus, mole of 2AP = $\frac{\text{integrate value of 2AP} \times 4}{\text{integrate value of TMS}}$ × mole TMS

= $\frac{3.25 \times 4}{108.41} \times 7.1949 \times 10^{-5}$

= $0.8628 \times 10^{-5}$ mole

Therefore, the mole of 2AP in solution is $0.8628 \times 10^{-5}$ mole.

Then, the mole of 2AP was converted to be as concentration in ppm.

$$n = \frac{g}{MW} \quad (3)$$

$$g = n \times MW \quad (4)$$

The equation 3 and 4, when n = number of mole, g = gram of compound and MW = molecular weight

In this study, The MW of 2AP was 111.

g of 2AP $= n \times MW$

$= 0.8628 \times 10^{-5}$ mole $\times 111$

$= 95.7708 \times 10^{-5}$ g

$= 95.7708 \times 10^{-2}$ mg

The concentration of 2AP was calculated to ppm (mg/l).

In this experiment, 2AP dissolved in $D_6$-Benzene of 600 µl.

2AP in $D_6$-Benzene 600 µl was $95.7708 \times 10^{-2}$ mg

2AP in $D_6$-Benzene 1 L $= \frac{95.7708 \times 10^{-2} \text{ mg} \times 1 \text{ L}}{600 \times 10^{-6} \text{L}}$

$= 1596.18$ mg/L (ppm)

Therefore, the concentration of 2AP in in the product solution was 1596.18 ppm.

# VITA

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|---|---|
| **Name** | Miss Pittayaporn Boontakham |
| **Date of birth** | March 31, 1987 |
| **Academic status** | - High school certificate, Thoeng Wittayakom school, Chiangrai, 2000. |
| **Scholarship** | - B.S. (Chemistry), Maejo University, Chiangmai, 2004. Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC) |
| **National presentation** | -Application of an automated headspace-gas chromatographic (HS-GC) technique with a nitrogen-phosphorous detector (NPD) for the determination of an aroma compound in rice leaves, PERH-CIC congress VII, 4-7 May 2011, Jomtien Palm Beach Hotel&Resort.<br>- Poster presentation in the topic of Development of a Method Employing Headspace-Gas Chromatography for Determination of an Aroma Compound in Rice Leaves, The 1st National Rice Research Conference "Moving Rice Research Toward Innovation", 15-17 December 2010. |