

CHAPTER II

EXPERIMENT AND RESULTS

2.1 Instrumental Analyses

2.1.1 Infrared Spectroscopy (IR)

The IR spectrum was recorded on a Perkin-Elmer Model IR 1430 Infrared Spectrophotometer. Solid sample was generally examined as a potassium bromide pellet.

2.1.2 Mass Spectrometry (MS)

The mass spectrum were obtained by Jeol Mass Spectrometer Model JMS-DX-300/JMA 2000 at 70 eV.

2.1.3 Proton and Carbon-13 Nuclear Magnetic Resonance Spectroscopy (PMR and CMR)

The ^1H (PMR) and ^{13}C (CMR) spectra were recorded on Bruker Model ACF 200 Spectrometer operating at 200.13 MHz for proton and 50.32 MHz for carbon-13 nuclei. The chemical shifts (δ) were given in ppm. against residual proton peak from deuterated solvents.

2.1.4 Elemental Analysis

The elemental analysis was made by using a Perkin-Elmer CHNO Analyzer Model 240C.

2.1.5 Melting Point (m.p.)

The melting point was determined on a Fisher-John melting point apparatus and was uncorrected.

2.1.6 X-ray Crystallography

The X-ray crystallographic structure of odoran was obtained on a Enraf-Nonius CAD-4F automated diffractometer with a Cu anode tube.

2.2 The Spectroscopic Data of Odoran

The spectroscopic data of odoran were shown below :

IR ν_{\max}^{KBr} (cm⁻¹) : 3260, 3040, 2940, 2500(broad), 1710-1530, 1400, 1300, 1080, 710.

(Fig.1).

PMR (D₂O) δ (ppm) : 1.96-2.11 (ddd, J₁=14.14, J₂=11.01, J₃=4.86 Hz, 1H);

2.23-2.34 (dddd, J₁=13.66, J₂=7.51, J₃=J₄=1.99 Hz, 1H);

2.84 (s, 3H);

2.94-3.02 (ddd, J₁=12.97, J₂=J₃=2.03 Hz, 1H);

3.70-3.79 (dd, J₁=13.00, J₂=4.76 Hz, 1H);

3.94-4.03 (dd, J₁=11.01, J₂=7.50 Hz, 1H);

4.40-4.45 (m, 1H);

4.60 (s, D₂O 1H).

(Fig.2) as reported in lit.[41,46-47] and

(Fig.4) was the ¹H COSY correlated spectrum.

CMR (D_2O) δ (ppm) : 37.4(t), 42.0(q), 61.7(t), 68.5(d), 69.7
(d), 172.0(s).

(Fig.5) as reported in lit. [41,46-47] and
(Fig.6) was the $^{13}C/^1H$ two-dimensional
correlated spectrum.

MS m/e (% rel. int.): 145(M^+ ,3.0), 101(9.6), 100(100.0), 82(35.0),
72(3.3), 44(10.1), 42(23.6) .

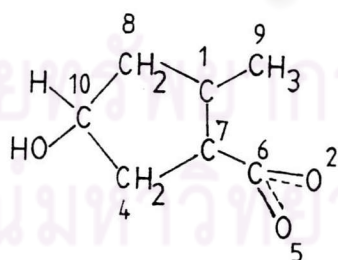
(Fig.7) as reported in lit. [41].

Elemental analysis found: C 49.93 %, H 7.62 %, N 9.79 %

$C_6H_{11}NO_3$ required : C 49.66 %, H 7.59 %, N 9.66 %, O 33.09 %.

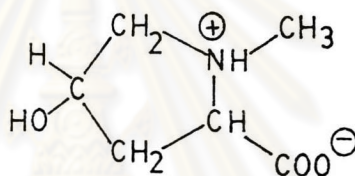
2.3 The X-ray Crystallographic Data of Odoram

The X-ray crystallographic data was reported as a
monoclinic colourless prisms. The space group was $P2_1$ with
two molecules per unit cell. Current discrepancy indices
(refinement) were $R_1=0.059$, $R_2=0.086$, $GOF=4.8$. This model
was shown below.



The distance between atoms were $C_1-C_8 = 1.51 \text{ \AA}$,
 $C_1-C_7 = 1.49 \text{ \AA}$ and $C_1-C_9 = 1.48 \text{ \AA}$. The highest peak on the
difference electron density map was 0.71 \AA to C_1 and made
angles of 106.7, 108.9 and 106.8, respectively to C_7 , C_8 and C_9 .
Furthermore, the thermal parameter on C_1 was smaller than C_7 , C_8
and C_9 which would be a nitrogen atom. And the C_1 was the

highest peak obtained from the solution by direct method. Therefore, the N atom would appear at C_1 that might be a quaternary N^+ . The geometry of C_6 was supported that two oxygens appeared completely equivalent because the distance was $C_6-O_2=1.245$, $C_6-O_5 = 1.242$ and the angles were $C_7-C_6-O_2 = 116.9^\circ$ and $C_7-C_6-O_5 = 116.0^\circ$. Moreover, the highest peak on the difference electron density map was 0.71 \AA from C_1 which was reasonable to its Sp^3 angles. So this structure of odoram was shown below.



And the conformation of odoram was determined by X-ray diffraction studies as shown in the figure 7.

2.4 The Spectroscopic Data of *trans*-4-Hydroxy-L-proline

The spectroscopic data of *trans*-4-hydroxy-L-proline were shown below :

PMR (D_2O) δ (ppm) : 1.84-1.98 (m, 2H);

2.14-2.25 (m, 2H);

3.14-3.23 (q, 1H);

4.06-4.11 (t, 1H);

NH, OH, COOH (exch. with D_2O). (Fig.9)

CMR (D_2O) δ (ppm) : 36.34(t) , 51.87(t) , 58.77(d) , 68.98(d) ,

173.17(s). (Fig.10)

2.5 Syntheses

To confirm the structure of odoram, the methylation of odoram and *trans*-4-hydroxy-L-proline were carried out in order to compare the structure of the methylated products.

2.5.1 Methylation of *trans*-4-hydroxy-L-proline

A mixture of *trans*-4-hydroxy-L-proline (0.30 g., 2.29 mmol) and sodium methoxide which was *in situ* generated, in abs. methanol (20 mL), was stirred overnight (~ 10-12 hrs.) at room temperature. Then a small piece of Na-metal was added into the reaction mixture while the excess amount of methyl iodide (1 mL, 2.28 g., 16.06 mmol) was added and stirring was continued overnight. The methanol solution was evaporated on a rotary evaporator to give a residue, pale viscous liquid with white precipitate (0.25 g., 69.44 % yield).

PMR (D_2O) δ (ppm) : 2.23-2.36 (m, broad, 1H);

2.54-2.67 (m, 1H);

3.06 (s, 3H);

3.30 (d, 1H);

3.35 (s, 3H);

3.45-3.53 (dd, 1H);

3.97-4.07 (dd, 1H);

4.32-4.61 (dd, 1H). (Fig. 11)

CMR (D_2O) δ (ppm) : 35.05(t), 47.96(q), 53.67(d), 65.49(d),
73.14(t), 75.32(d), 169.73(s). (Fig. 12)

2.5.2 Methylation of Odoram

A mixture of odoram (0.25 g., 1.72 mmol) and sodium methoxide in abs. methanol (20 mL) was stirred at room temperature overnight. The excess amount of methyl iodide (1 mL, 16.06 mmol) was added and stirring was continuous overnight. Then the methanol was evaporated under reduced pressure to give a pale viscous liquid with white precipitate (0.16 g., 59.26 % yield).

The spectroscopic data of this product was in agreement to those of the product obtained from the methylation of *trans*-4-hydroxy-L-proline. These spectra were shown in figure 13-14.

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