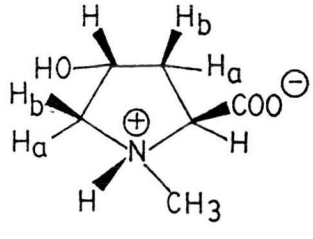
CHAPTER III

RESULTS AND DISCUSSION

From the extracts of A. odorata flowers, a novel compound was isolated from the methanol extract which was named "odoram". The odoram was colourless needle crystal and was recrystallised from methanol to m.p. 236-238°C (decompose) and it was insoluble in non-polar solvent. The chemical reaction of odoram with potassium permanganate solution gave the positive test but the reaction with bromine water, Benedict's solution, alkaloid reagent and ceric nitrate gave the negative results. Elemental analysis of this compound found C 49.93 %, H 7.62 % and N 9.79 % , molecular weight was 145 gram per mole. The formular structure was $C_6H_{11}NO_3$.

3.1 The Structure of Odoram

Odoram was shown to be a five-membered ring pyrrolidine derivative that was identified as "1-methyl-4-hydroxy-L-proline" of which the structure was shown below. [40-42]



The spectroscopic data could be assigned as follow :

The **IR** spectrum of odoram in Fig.1 gave important absorption peaks at 3260 and 2500 cm^{-1} of OH streching and 1710-1530 cm^{-1} of C=O streching of carboxylate group.

The **PMR** spectrum was shown in Fig.2. The chemical shift (δ scale(ppm)) and coupling constants (J (Hz)) were obtained with the aid of extensive double resonance experiment as shown in Fig.3 which was assigned to :

$\delta_{\text{H-3b}}$ 2.04 ppm (ddd, $J_{3b,3a}$=14.14, $J_{3b,2}$=11.01, $J_{3b,4}$=4.86 Hz,1H);

$\delta_{\text{H-3a}}$ 2.28 ppm (dddd, $J_{3a,3b}$=13.66, $J_{3a,2}$=7.51, $J_{3a,4}$=1.99 Hz,1H);

δ_{Me} 2.84 ppm (s, N-Me);

$\delta_{\text{H-5a}}$ 2.98 ppm (ddd, $J_{5a,5b}$=12.97, $J_{5a,4}$=2.03 Hz, 1H);

$\delta_{\text{H-5b}}$ 3.75 ppm (dd, $J_{5b,5a}$=13.00, $J_{5b,4}$=4.76 Hz, 1H);

$\delta_{\text{H-2}}$ 3.98 ppm (dd, $J_{2,3b}$=11.01, $J_{2,3a}$=7.50 Hz, 1H);

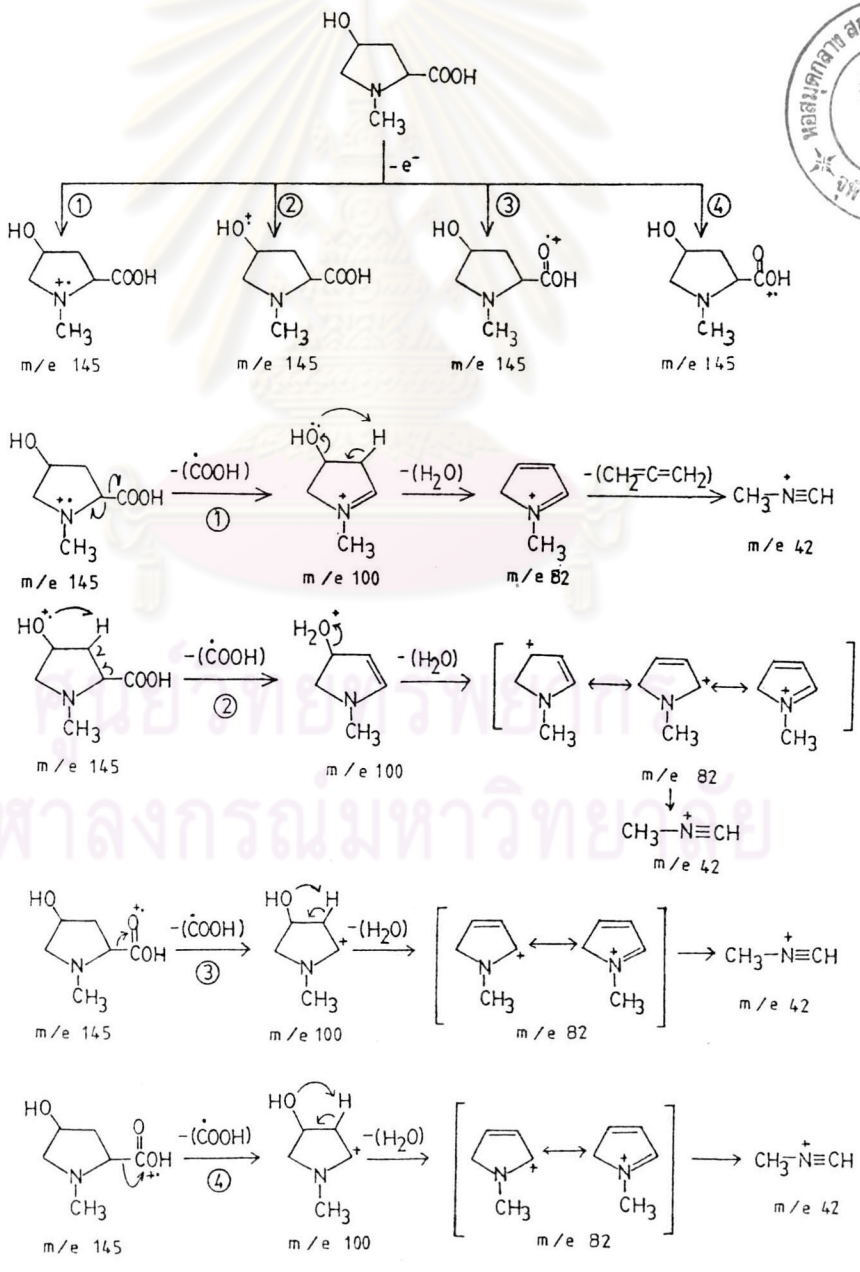
$\delta_{\text{H-4}}$ 4.43 ppm (m, 1H);

δ_{OH} 4.60 ppm (s, 1H exchanged with D_2O).

The **CMR** spectrum of odoram was shown in Fig.5 and the $^{13}C/^{1}H$ two-dimensional technique in Fig.6 was displayed to a singlet at δ 172.0 ppm (**COO^-**), two doublets at δ 68.5 ppm (**C-2**) and 69.7 ppm (**C-4**), two triplets at δ 61.7 ppm (**C-5**) and 37.4 ppm (**C-3**) and a quartet at δ 42.0 ppm (**N-Me**). According to the above assignment, the molecule of odoram adopted preferentially the conformation in which carboxylate and methyl groups were trans to each other (on the basic of steric factors). The conformation of carboxylate and hydroxy group were also trans to each other.

The mass spectrum (Fig.7) displayed the molecular ion peak at m/e (% relative intensity) 145(3.0, M^+) (cal. for $C_6H_{11}NO_3$: MW 145) and other fragmentation ion peaks at m/e 101 (9.6, M^+-CO_2), 100 (100.0, M^+-COOH), 82 (35.0, M^+-COOH-H_2O), 42 (23.6, (Me-$N^+\equiv$CH)). The possible mass fragmentation pattern of odoram or *trans*-4-hydroxy-N-methyl-L-proline is presented in scheme II.

Scheme II The possible mass fragmentation pattern of odoram.



3.2 Literature Surveys of Odoran

In 1919, 4-hydroxy-N-methylproline was isolated from the bark of *Croton gubouga,* S. Moore, for the first time [40]. *Croton gubouga,* S. Moore, was a small tree growing on the low veldt in the Eastern Transvaal near the Sabi and Selati rivers. The bark of the tree had a considerable local reputation among the natives as a remedy for malaria, and both the seeds and the bark had been used with opium for the treatment of malarial fever.

In 1964, 4-hydroxyhygric acid was obtained in the water-soluble portion of the methanol extract from the heartwood of *Afrormosia elata* in 0.2-0.3 % yield and was recrystallised from methanol to m.p. 238-240°C (decomp.). [42]

In 1983, 4-hydroxy-N-methylproline, an unusual amino acid was studied in the aqueous extract of the red alga *Chondria coerulescens* (Rhodomelaceae, Ceramiales). This compound, which was ninhydrin-negative but reacted with Dragendorff´s and iodoplatinate reagents, was isolated by ion-exchange chromatography (in a yield of 0.23 % dry wt.). [41]

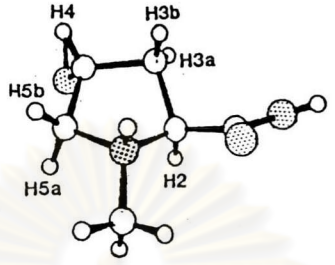
In 1984, *trans*-4-hydroxy-N-methyl-L-proline was found in aerial parts of *E. argentinum* and its spectra data (^{1}H-NMR, ^{13}C-NMR and mass spectrum) were reported for the first time in the genus *Erythroxylon*. [44]

In 1986, N-methyl-L-proline and *cis*- and *trans*-N-methyl-4-hydroxy-L-proline were drugs for the treatment of cancer, virus diseases , blood vessel diseases , and disorders of nervous system. [45]

In 1987, the unusual imino acid, N-methyl-*trans*-4-hydroxy-L-proline was isolated from leaves of five species of the deguminous tropical tree *Copaifera,* and, for the first time, characterized by ^{1}H and ^{13}C NMR and mass spectrometry. This nonprotein imino acid constituted ca. 3 % of the dry weight of mature leaves from two of *C. mutijuga* trees and one of *C. langsdorfii* tree. It also constituted 1-2 % of the dry weight of mature leaves of greenhouse-grown sampling of *C. pubiflora, C. officinalis* and *C. venezuelana* var. *laxa,* as well as 2-3 % of the dry weight of the seeds of the cited species. [46]

In the same year, 4-hydroxy-N-methylproline and 4-hydroxy-N,N´-dimethylproline were isolated from the aqueous extracts of *Melaleuca* spp. by ion-exchange chromatography. These compounds were found in *M. lanceolata* at level > 4 % dry weight and found in leaves of *M. uncinata* at level > 1.4 % dry weight. [43,47-48]

In 1988, the crystal structure of N-methyl-4-hydroxy-L-proline and its derivative [49] were determined as their hydrochloride. A trans-disposition of the carboxyl and hydroxy groups was found in the structure below.

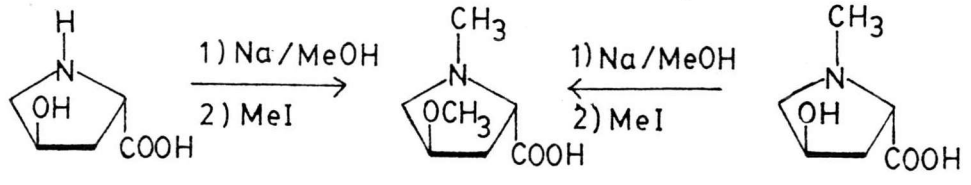


In 1990, the possible metabolic relationship between methylsulfonium and N-methylated compounds were investigated in red alga *C. coerulescens* by radiotracer techniques. Following administration, the [$^{14}CH_3$]4-hydroxy-N-methylproline was isolated from the aqueous fraction, indicating that sulfonium K-salts might function as Me donor in the biosynthesis of N-methylated compounds. [50]

3.3 Methylation of *trans*-4-Hydroxy-L-proline and Odoram

Trans-4-hydroxy-L-proline was methylated in order to prove the structure of odoram. The methylation was carried out by using, sodium methoxide in abs. methanol and an excess amount of methyl iodide. The physical properties of the methylated product were a pale viscous liquid with white precipitate which differed from the starting material. The white precipitate was presumably sodium iodide. This product was named "*trans*-4-methoxy-*N*-methyl-L-proline".

The PMR spectrum of *trans*-4-methoxy-*N*-methyl-L-proline (Fig.11) showed the singlet signal of N-methyl proton at δ 3.06 ppm and the singlet signal of methoxy proton at δ 3.35 ppm. The PMR spectrum of this product was different from odoram while the PMR spectrum odoram which showed only a singlet signal of N-methyl proton at δ 2.84 ppm. Therefore, the methylation of odoram was carried out under the same condition to compare the product with those obtained from the structure of the methylation of *trans*-4-hydroxy-L-proline. The physical properties of the methylated odoram agreed very well to *trans*-4-methoxy-*N*-methyl-L-proline. The reaction occured as shown in the equation below.



trans-4-hydroxy-L-proline

trans-4-methoxy-*N*-methyl-L-proline

trans-4-hydroxy-*N*-methyl-L-proline