CHAPTER IV

DISCUSSION

In the investigation of the leaves of *Dysoxylum grande* Hiern, compound As_1 and compound X were isolated from the chloroform extract. Addition amount of compound As_1 was isolated from the methanolic extract of the leaves. Compound As_1 gave positive test with modified Dragendorff's and Mayer's reagent suggested compound As_1 is an alkaloid.

Structure Elucidation of Alkaloid As_1

The alkaloids As_1 was obtained as pale yellow crystals, mp 218-219°C. The mass sprectrum of alkaloid As_1 (Figure 16) showed molecular ion peak at *m/z* 305, corresponding to the molecular formula $C_{16}H_{19}NO_5$.

The absorption bands in the UV spectrum recorded in Methanol (Figure 14) presented at λ max 220, 260 and 325 nm.

The IR spectrum (Figure 15) of the alkaloid As_1 revealed the presence of vibrations of hydroxyl group at (3400 cm^{-1},broad) and conjugated carbonyl (1660 cm^{-1}). The peaks at 1610 cm^{-1} and 1555 cm^{-1} suggested a

γpyrone moiety (Harmon, 1979). The N-H stretching absorption were absent.

The ^1H NMR spectrum (Figure 17) taken in pyridine-d_5, showed two one-proton singlet signals at δ6.12 ppm and δ6.73 ppm corresponding to the olefinic proton at C-3 and the aromatic proton at either C_6 or C_8, respectively. The low proton count in the aromatic/alkene region suggested a highly substituted aromatic ring. In ^1H-^1H COSY spectrum, the methyl proton singlet at δ2.18 ppm showed a correlation cross peak with the olefinic proton H-3, indicated their long range coupling relation. These evidence confirmed the presence of 2-methyl-chromone moiety. The assignments of the remaining aliphatic protons could be achieved by the analysis of ^1H-^1H COSY spectrum (Figure 18). In the upfield region of the spectrum, a three-proton singlet presented at δ2.23 ppm attributed to N-methyl group. A broad singlet integrating for one proton presented at δ4.39 ppm could be ascribed to a methine proton attached to a hydroxyl group. This proton showed connectivity with a methine proton at C-4' (δ3.60 ppm) as well as methylene proton at C-2' (δ3.12 ppm, δ3.34 ppm). The small coupling constant of H-3' suggested its equatorial orientation. H-4' showed correlation to the methylene proton at C-5' (δ2.96 ppm). The large coupling constant (J=13.4) suggested the axial-axial relation between H-4' and H-5'(δ2.96 ppm). The signal at δ1.53 ppm was assigned

as H-5' equatorial based on the "W" coupling with H-3' equatorial. The signal at δ 2.17 ppm and δ 2.96 ppm were assigned as the methylene proton at position 6'. The cross peak between H-6'(δ 2.96 ppm) and H-2' (δ 3.12 ppm) indicating the "w" coupling therefore these two protons were in equatorial orientation. Whit these cumulative data it was possible to identify the the compound as a dihydroxy chromone bearing a N-methyl-piperidinol group. Therefore the complete ^{1}H chemical shift assingments and their relative configurations assingment of N-methyl-piperidinol group were shown in Figure 3.

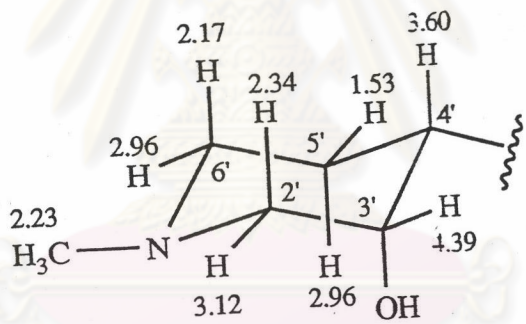

Figure 3 ^{1}H chemical shift assingments of N-methyl-piperidinol group.

In this experiment, the ^{13}C NMR assignment was based on interpretation of the spectra obtained from various ^{13}C NMR techniques which included the proton decoupling ^{13}C NMR spectrum (Figure 19).

The proton decoupling ^{13}C NMR spectrum (Figure 19) showed signals of all 16 carbons in the

molecule. The assignment was mainly based on the ^{1}H-^{13}C HETCOR spectrum (Figure 20). From this spectrum all signals of carbons possessing attached proton could be assigned in accordance with the ^{1}H NMR assignment. With the reference of complete proton chemical shift assignments and ^{1}H-^{13}C HETCOR data, the ^{13}C chemical shift assignment (Table 3) was established. Thus the assignment of C-3, C-2', C-3', C-4', C-5', C-6', N-CH_3 and 2-CH_3 were appeared at δ108.42, δ62.42, δ69.83, δ38.12, δ 25.29, δ 56.76, δ 19.90 and δ 46.09 ppm, respectively. The signal at δ 101.50 ppm was an aromatic carbon.

Those of 7 quaternary carbons in the molecule of As_1 were thus further investigated to assign all carbons they represented. Such carbon included C-2, C-4, C-4a, C-5, C-7, C-8 or (C-6) and C-8a. Among these 7 signals, one of the most downfield position appeared at δ183.14 was assigned to the carbonyl C-4. The other unassigned signals were related to 1 oxygenated olefinic (C_2) and 5 aromatic carbons. The aromatic carbons could be divided into two groups : three of which oxygenated type (C-5, C-7, C-8a) and two of non-oxygenated ones (C-4a, C-8 or C-6). Owing to the deshielding effect, signals of the former group were more downfield than those of the latter. Therefore four signals appearing in the comparative low field at δ166.80, δ 165.08, δ161.40 and δ156.23 ppm were

Table 3 : Proton and carbon assignments of alkaloids As_1

position	^1H (ppm)	^{13}C(ppm)
2	-	166.80
2-CH_3	2.18	19.90
3	6.12	108.42
4	-	183.14
4a	-	104.66
5	-	161.40
6	6.73	101.50
7	-	165.08
8	-	108.49
8a	-	156.23
N-CH_3	2.23	46.09
2'	2.34, 3.12	62.42
3'	4.39	69.83
4'	3.60	38.12
5'	1.53, 2.96	25.29
6'	2.17,2.96	56.76

attributed to the four oxygenated carbons. From the ^{1}H-^{13}C COLOC spectra (Figure 21,22) the signal of carbon at δ 166.80 ppm showing long range correlation with methyl proton at δ 2.18 ppm was assigned as C-2. The signal of carbon at δ 165.08 ppm, showed long range correlation with H-4' at δ 3.60 ppm and H-6 at δ 6.73 ppm, was assigned as C-7. The signal of carbon at δ 156.23 ppm showing long range correlation with H-4' at δ 3.60 ppm, was assigned as C-8a. Thus, the assignments of C-2, C-5, C-7 and C-8a were appeared at δ 166.80, δ 161.40, δ 165.08 and δ 156.23 ppm, respectively. The last signal of carbon at δ 161.40 ppm showed long range correlation with H-6 at δ 6.73 ppm was assigned as C-5.

The rest of unassigned signals appearing in the comparative high field at δ104.66 and δ 108.49 ppm attributed to the two non-oxygenated carbons. The first signal, showed long range correlation with both H-3' at δ 6.12 ppm and H-6 at δ 6.73 ppm, was assigned as C-4a. The last signal showing long range correlation with H-4' at δ 3.60 ppm and H-6 at δ 6.73 ppm and can be assigned as C-8. These evedence confirmed a dihydroxy chromone bearing a N-methyl-piperidinol group at C-8 (δ 108.49 ppm).

Therefore, the complete ^{13}C chemical shift assignments are summarized in Figure 4.

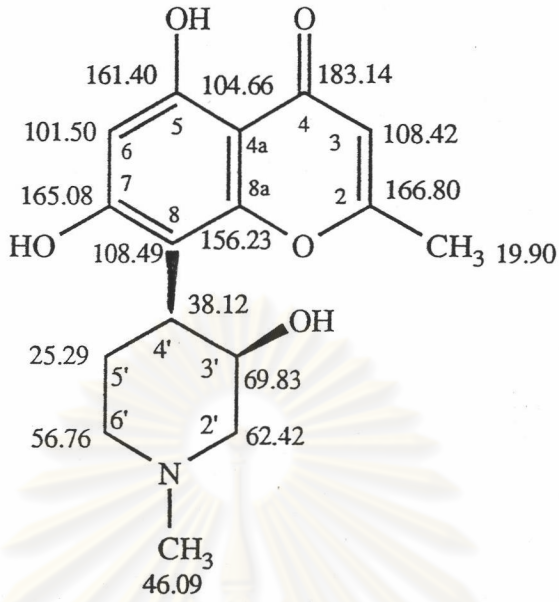

Figure 4 ^{13}C chemical shift assingments of alkaloid As_1.

The mass spectrum of alkaloids As_1 (Figure 16) showed the base peak at *m/z* 276 which resulted from the loss of carbon monoxide (CO) from the lactone carbonyl group subsequently loss of hydrogen radical ($H^{\bullet}$) to form more stable ion. The mass fragmentation pattern could be shown in Figure 5.

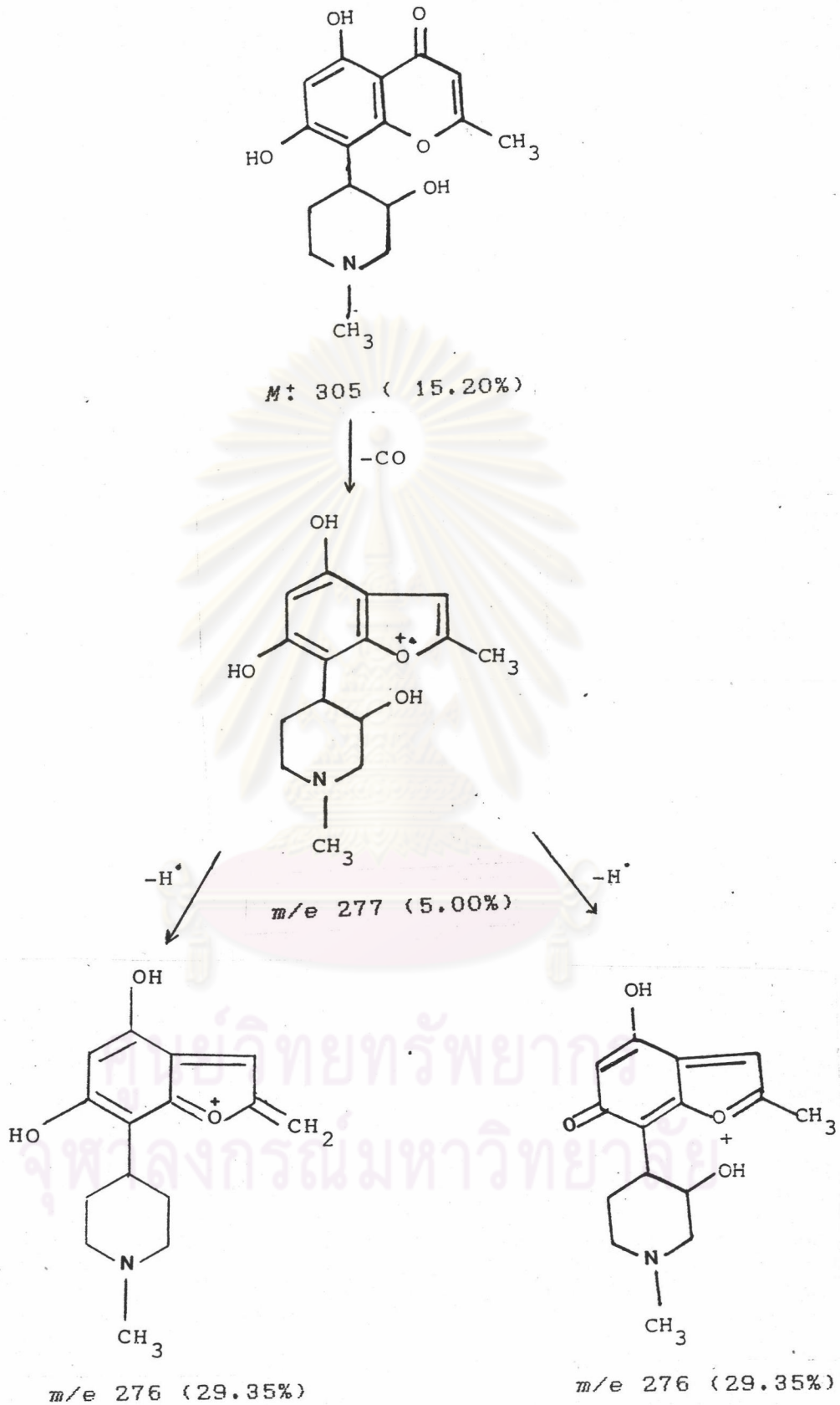

Figure 5 Mass fragmentation pattern in the EI mass spectrum of alkaloid As_1.

The melting point, IR, MS, ^{1}H and ^{13}C NMR data of As_1 are unambiguously identical with those of previously published of rohitukine (Harmon *et al*, 1979). Thus alkaloid As_1 can be identified as the known alkaloid rohitukine. From the sprectral evidence, it could be concluded that alkaloid As_1 is 5,7-dihydroxy-2-methyl-8(4-(3-hydroxy-1 methyl)-piperidinyl)-4H-1-benzopyran-4-one.

Rohitukine was previously found in dried leaves and stems of *Amoora rohituka* Wight & Arn (*Aphanamixis polystachya* (Wall) Parker), family Meliaceae (Harmon *et al.*,1979), in the root bark of *Schumanniophyton magnificum* Harms. family Rubiaceae (Houghton and Hairong., 1987) and in the stem bark of *Dysoxylum binectariferum* (Naik *et al.*, 1988). This compound was found to be analgesic, antiinflammatory and immunomodulatory principles (Naik, 1988),(Vasudev, 1985).

Structure Elucidation of Compound X

The compound X was obtained as colorless needles, mp 275-278° C (decomp). The mass spectrum (Figure 30) showed molecular ion peak at *m/z* 192, corresponding to the formula $C_{10}H_8O_4$.

The absorption bands in the UV spectrum recorded in Methanol (Figure 28) at λ max 227, 248, 255 and 294 nm

suggested the presence of chromone moiety (Fujita, 1967).

The IR spectrum (Figure 29) revealed the presence of hydroxy group (3400 cm^{-1}, broad) and α,β unsaturated carbonyl group (1650 and 1620 cm^{-1}) (Fujita,1967).

The ^1H NMR spectrum (Figure 31), taken in acetone showed a couple of doublets (J=2Hz) appeared at δ 6.21 ppm and δ 6.35 ppm which were assigned to two protons in meta relationship on a benzene ring. The hydroxyl proton signals were shown as singlets at C-7 (δ 9.57 ppm, s) and C-5 (δ 12.88 ppm, s). The C-5-OH was more deshielded than the C-7-OH, since it formed hydrogen bond with the C-4 carbonyl oxygen. Another proton signal on a double bond was observed as a singlet at δ 6.06 ppm. In addition, the methyl protons on a double bond appeared as a siglet at δ 2.36 ppm.

From these evidence Compound X was identical with noreugenin (Fugita, 1967). Therefore, the complete ^1H chemical shifts assingment was shown in Figure 6.

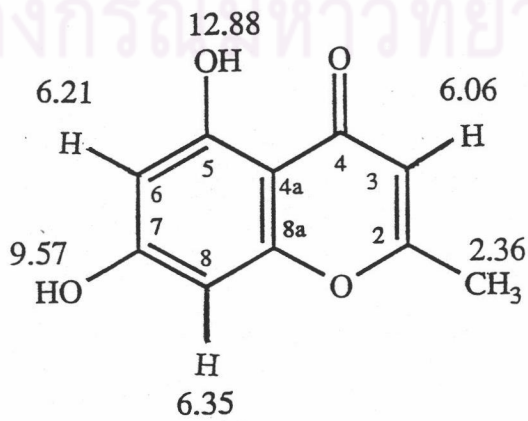

Figure 6 ^1H chemical shift assingments of compound X.

According to the previous reports of noreugenin, only the ^{1}H NMR assignment had been provided. In this experiment the ^{13}C-NMR assignment was added. The reported data were based on the interpretation of ^{13}C-NMR spectrum obtained from the proton decoupling experiment, and comparison with those of rohitukine allowed us to ^{13}C assignment of noreugenin.

The proton decoupling ^{13}C NMR spectrum(Figure 32) showed signals of all 10 carbons in the molecule. The position of a signal correlated to the type of carbon represented (i.e. carbonyl, aromatic, aliphatic, etc.). The most high field and the most low field positions appeared at δ 19.72 and δ 182.58 ppm. The former was in the region typical for a methyl group and the latter was in the carbonyl range. The two signals were therefore assigned to the C-2 methyl and the C-4 carbonyl carbons, respectively.

The other unassigned signals were related to 6 aromatic and 2 olefinic carbons. These carbons could be divided into two groups : four of which oxygenated type (C-5, C-7, C-2, C-8a) and four of non-oxygenated ones (C-3, C-4a, C-6, C-8). Owing to the deshielding effect, signals of the former group were more downfield than those of the latter. Therefore, four signals appearing in the comparative low field at δ 162.74, δ 164.48, δ 167.81 and δ 158.66 ppm were attributed to the four oxygenated

carbons. The first two signals were assigned to C-5 and C-7, respectively, base on the comparison with resonated values of C-5 and C-7 of rohitukine which were δ161.40 and δ165.08 ppm (using pyridine-d_5 as a solvent). Signal of C-5 was more upfield than C-7, owing the shielding effect. The third signal was assigned to C-2 based on the comparison with resonated value of C-2 in the molecule of rohitukine which was δ166.80 ppm (using pyridine-d_5 as a solvent). The last signal of the group should be assigned to C-8a.

The rest of unassigned signals appearing in the comparative high field at δ 108.33, δ 104.33, δ 99.06 and δ93.94 ppm were attributed to the four non-oxygenated carbons. The first two signals were assigned to C-3 and C-4a based on the comparison with resonated value of carbons at positions C-3 and C-4a in the molecule of rohitukine which were δ 108.42 and δ 104.66 ppm (using pyridine-d_5 as a solvent). The last two signals in the range of non-oxygenated were assigned to C-6 and C-8 (or might be interchanged).

Thus the ^{13}C chemical shifts assignments were proposed as shown in Figure 7.

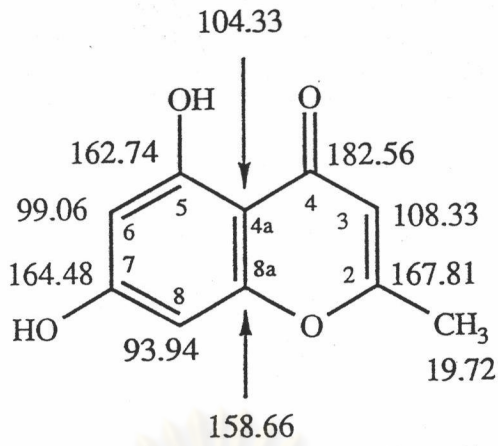

Figure 7 ^{13}C chemical shift assingments of compound X.

The mass spectrum (Figure 30) showed molecular ion peak at *m/z* 192 as well as a base peak and an intense peak at *m/z* 164 and *m/z* 136, respectively. Both peaks corresponded to loss of the a carbonyl groups (CO). The fragments ion at *m/z* 152 and *m/z* 124 corresponed to loss of MeC≡CH moiety from the molecular ion and subsequently loss of carbonyl function (CO), respectively. The mass fragmentation pattern could be shown in Figure 8 (Brown, *et al.*, 1975)

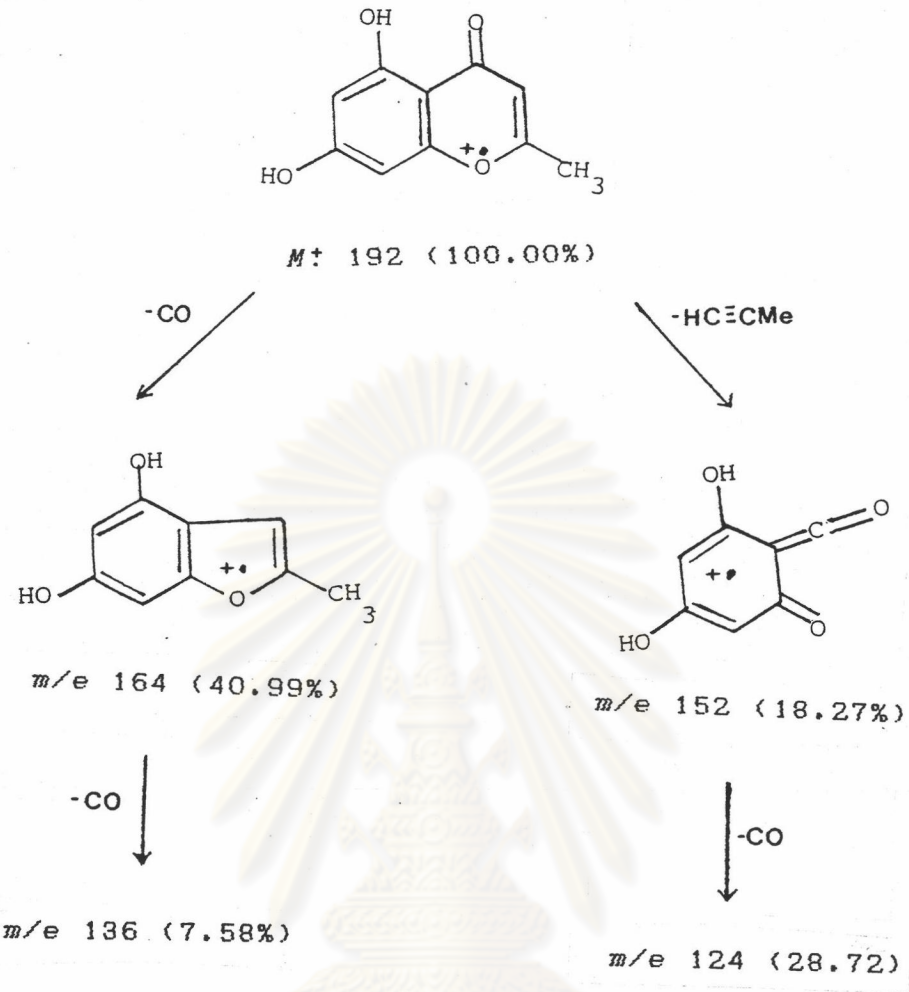

Figure 8 Mass fragmentation pattern in the EI mass spectrum of compound X.

The melting point, IR, MS, ^{1}H and ^{13}C NMR data of compound X are unambiguously identical with those previously published values of noreugenin (Harmon *et al.*,1979), (Brown *et al.*,1975). Thus compound X can be identified as the known chromone noreugenin, 2-methyl-5,-7-dihydroxychromone.

Noreugenin has been isolated several times from higher plants, for example, *Nauclea orientalis* L., family Rubiaceae (Fujita *et al.*,1967), *Rhododendron collettianum* Hitch and Hensel, family Ericaceae (Ahmad *et al.*, 1973), *Adina rubescens*, family Rubiaceae (Brown *et al.*, 1975), *Schumanniophyton problematicum*, family Rubiaceae, (Schlittler *et al.*, 1978), *Schumanniophyton magnificum* ,family Rubiaceae (Okogum *et al.*,1983). In Meliaceous species, noreugenin has been isolated from *Amoora rohituka* (*Aphanamixis polystachya*) (Harmon *et al.*,1979)

From the phytochemical studies of the leaves of *Dysoxylum grande* Hiern, the alkaloid, Rohitukine and the hydroxychromone, noreugenin were isolated. The result of this present investigation exhibited the homogeneity in term of chemical constituents in the genus *Dysoxylum* (Naik *et al.*, 1988). However, the data obtained are not sufficient to conclude chemotaxonomy of the genus *Dysoxylum* until more studies of the plants in the genus *Dysoxylum* are done.