CHAPTER IV

DISCUSSION

There have been only two compounds isolated from *Murraya siamensis* Craib recently; murrayanine (6) (Fiebig *et al.*, 1985) and girinimbine (37) (Kong *et al.*, 1986b). In the present work, the residue from chloroform extract of the dried chipped roots was separated into eight pure compounds by a combination of chromatographic techniques as described in the experimental section. The structure elucidation of these compounds will be described in the order in which they were eluted from the chromatographic columns.

The first and least polar compound was shown to be the carbazole, heptaphylline (26), MS-1, by comparison with the melting point and spectral data reported in the literature (Joshi *et al.*, 1972). As the aromatic protons in the previous ^{1}H NMR spectrum were not resolved (Joshi *et al.*, 1972), the 400 MHz ^{1}H NMR spectral data of MS-1 in three different solvents: chloroform-d, acetone-d_6 and benzene-d_6 have been reported. The acetone solvent was particularly effective in resolving the aromatic protons H-5 to H-8. In addition, the previously unreported ^{13}C NMR spectrum of MS-1 was presented.

After the structures of the second and third

components have been determined independently , these new carbazoles, MS-2, 7-methoxyheptaphylline (28) and MS-3, 2-hydroxy-3-formyl-7-methoxycarbazole (18) were reported to be present in *Clausena harmandiana*, another Rutaceae species (Chaichantipyuth *et al.*, 1988). ^{1}H and ^{13}C NMR data of these components were included for comparison with compounds to be described latter. In the previous report (Chaichantipyuth *et al.*, 1988), it is believed that the assignments for H-5 and H-8 in MS-2 should be reversed and the assignments for C-2 and C-7 in MS-3 should also be reversed.

The fourth component was a pale yellow solid which exhibited a parent ion in its electron impact mass spectrum (EIMS) at *m/z* 255 and an accurate mass consistent with the molecular formula $C_{15}H_{13}NO_3$. The UV spectrum of the compound indicated a 3-formylcarbazole chromophore (Buchi and Warnhoff, 1959; Chakraborty, 1977) and as addition of base did not alter the spectrum, no phenolic hydroxyl groups are present. The ^{1}H and ^{13}C NMR spectra indicated the presence of two methoxyl groups and a formyl substituent. The ^{1}H resonances for H-5, -6 and -8 were very similar to those in MS-2 and MS-3 and indicated that one of the methoxyl groups was attached to C-7. The other two aromatic resonances were singlets with no meta coupling and were assigned to H-1 and H-4. Thus the second methoxyl group must be located on C-2 and this component is 3-formyl-2,7-dimethoxycarbazole, a new alkaloid and the methylated derivative of MS-3. The related 3-formyl-2,6-

dimethoxycarbazole, glycozolidal(21) has been isolated from *Glycosmis pentaphylla* (Bhattacharyya and Chowdhury, 1985a) and prepared by oxidation of the corresponding 3-methyl derivative (Chowdhury *et al.*, 1987).

The fifth compound, MS-5, was shown to be the known coumarin, xanthoxyletin (134). It was obtained as white crystals, melting at 127-128°C, IR spectrum (figure 31) reveals the presence of carbonyl group. The ^{1}H NMR spectrum (figure 32) indicates the presence of five aromatic protons, three protons of methoxyl group (δ=3.86 ppm) and six protons of gem dimethyl (δ=1.46 ppm). This compound was also isolated from *Clausena harmandiana* (Wangboonskul, Pummangura, and Chaichantipyuth, 1984) along with MS-2 and MS-3 as mentioned above (Chaichantipyuth *et al.*, 1988).

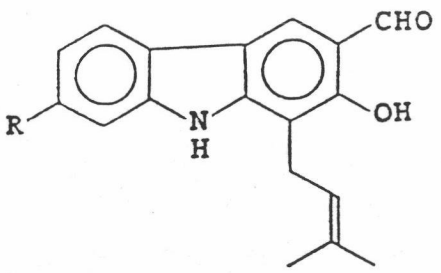
The next component was found to be mukonal (12), MS-6, a carbazole previously isolated from *Murraya koenigii* (Bhattacharyya and Chakraborty, 1984b). The ^{1}H NMR spectrum of MS-6 was reported in DMSO-d_6 but the resonances in the aromatic region were not resolved (Bhattacharyya and Chakraborty, 1984b) so its 400 MHz spectra in three other solvents were reported in this study. The ^{13}C NMR spectrum of MS-6 is given as a number of corrected assignments, particularly in the unsubstituted benzene ring, differ from those reported previously for mukonal (12) (Bhattacharyya and Chakraborty, 1984b). The assignments for this ring are similar to those reported for carbazole itself (Ahnod, Poupat, and Potier, 1978; Mester, Bergenthal, and Reisch,

1979).

The seventh component was a yellow crystalline solid which showed a weak parent ion in its EIMS at *m/z* 307 and an accurate mass consistent with the molecular formala $C_{19}H_{17}NO_3$. The UV spectrum suggested the presence of a 3-formylcarbazole partial structure (Buchi and Warnhoff, 1959; Chakraborty, 1977) and as addition of base did not shift the UV maxima, the other oxygens are not present as phenolic hydroxyl groups. The ^1H NMR spectra in the three solvents used in this study indicated the presence of a 7-methoxy substituent as the familiar pattern seen in the spectra of MS-2, MS-3 and MS-4 for H-5, -6 and -8 was observed here also. The only other aromatic proton, a significantly deshielded singlet, was assigned to H-4. Two vinyl protons, which were coupled to each other, and a 6-proton singlet were readily accounted for by a 2,2-dimethyl-Δ^3-pyran system fused to C-1 and C-2 of the carbazole nucleus and thus this component is a new alkaloid possessing the structure depicted in page 114. A compound without the methoxyl substituent, murrayacine (38), has been isolated previously from *Murraya koenigii* (Chakraborty *et al.*, 1971) and from *Clausena heptaphylla* (Ray and Chakraborty, 1976) and thus we suggest that MS-7 be called 7-methoxymurrayacine. A comparison of the ^1H NMR spectrum of murrayacine (38) (Chakraborty *et al.*, 1971) with that of MS-7 fully supports the structure proposed for the latter as does the ^{13}C NMR spectrum reported in page 193. The weaker parent ion in the

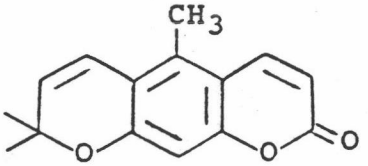
EIMS of MS-7 is explained by the facile loss of one of the methyl groups at C-11 to give a carbazolopyrilium ion (Mester, Bergenthal, and Reisch, 1979).

The final component isolated was a cream-colored solid which gave a parent peak in its EIMS (*m/z* 225) and an accurate mass corresponding to the molecular formula $C_{14}H_{11}NO_2$. The UV spectrum again suggested a 3-formylcarbazole skeleton and as it was unchanged upon addition of base, the remaining oxygen is not present as a phenolic hydroxyl group. The ^1H NMR spectrum was quite simple and other than the aromatic, amine and formyl protons, it showed only one methoxyl group. The aromatic protons H-5 to H-8 displayed a pattern similar to that found in MS-1 and MS-6. In addition, two aromatic singlets (no ortho coupling), with similar chemical shifts to the corresponding protons in MS-6, were assigned to H-1 and H-4. Thus, component MS-8 is 3-formyl-2-methoxycarbazole or O-methylmukonal. This compound has been synthesized (Chowdhury *et al.*, 1987) but at present knowledge, this is the first time it has been reported to occur in nature. The 3-formyl-1-methoxy isomer has been reported to be present in *Murraya koenigii* (Chakraborty, Barman, and Bose, 1965) and *Clausena heptaphylla* (Bhattacharyya and Chakraborty, 1973). ^1H or ^{13}C NMR spectra of MS-8 were not reported along with the synthesis (Chowdhury *et al.*, 1987) and so these are included in this report completely.

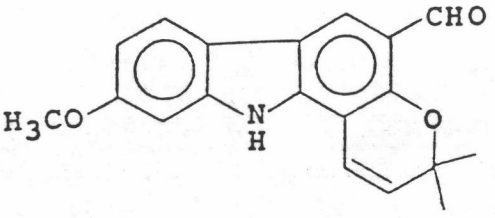


Heptaphylline (MS-1)
(R = H)

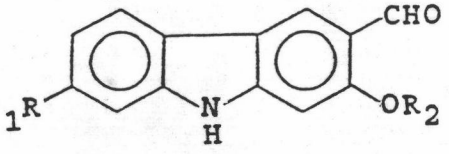
7-Methoxyheptaphylline (MS-2)
($R = OCH_3$)



Xanthoxyletin (MS-5)



* 7-Methoxymurrayacine (MS-7)



2-Hydroxy-3-formyl-7-methoxycarbazole (MS-3)
($R_1 = OCH_3$, $R_2 = H$)

* 3-Formyl-2,7-dimethoxycarbazole (MS-4)
($R_1 = OCH_3$, $R_2 = CH_3$)

Mukonal (MS-6)
($R_1 = H$, $R_2 = H$)

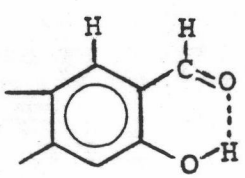
* O-Methylmukonal (MS-8)
($R_1 = H$, $R_2 = CH_3$)

The isolation of seven carbazole components from the species under investigation provides an unusual opportunity for a detailed comparison of the ^{1}H and ^{13}C NMR spectra of these compounds. It should be noted that a compilation of the ^{1}H NMR spectra of known carbazole alkaloids has been reported (Chakraborty, 1977). In this study, ^{1}H NMR spectra

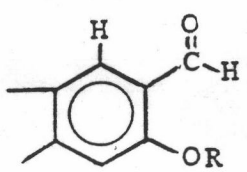
of the seven carbazoles in three solvents: the polar solvent acetone-d_6, the common NMR solvent $CDCl_3$ and the aromatic medium benzene-d_6 were presented. For all compounds, the amine proton is shielded in benzene while in acetone it is deshielded relative to its position in chloroform. The aromatic solvent-induced shift (ASIS) is explained by assuming that benzene is aligned perpendicular to the polar N-H bond at the positive end of the dipole, resulting in shielding of the amine proton by the π-cloud (Nikki and Nakagawa, 1978). It should be noted that with an alkyl group at the 1-position (e.g. in MS-1 and MS-2) this shielding effect is not as pronounced. Conversely, in acetone solvent, this proton is deshielded because of the interaction between the amine as proton-donor and the acetone oxygen as electron pair-donor (Reichardt, 1988). Interestingly, in compounds MS-1-3 and MS-6, the 2-OH, which is H-bonded to the 3-formyl oxygen, is deshielded in benzene compared with the other two solvents. Unfortunately, owing to the large amount of spectra obtained in the present investigation, the illustration of ^1H NMR of benzene-d_6 and $CDCl_3$ of seven carbazoles were omitted in this thesis.

A comparison of the chemical shift of H-4 in compounds containing a 2-OH substituent (MS-1-MS-3, MS-6) with those containing a 2-OR substituent (MS-4, MS-7, MS-8) is instructive. In the latter compounds in benzene solvent, H-4 resonates considerably further downfield than in the former compounds. It is suggested that in compounds MS-1-

MS-3 and MS-6 the 3-formyl group exists in conformation A because of intramolecular H-bonding with the 2-OH substituent while with compounds MS-4, MS-7 and MS-8 conformation B predominates. Application of the so-called carbonyl plane rule (Connolly and McCrindle, 1965; Reichardt, 1988) would predict that in benzene solvent H-4 in conformation B would be deshielded because it is in front of the carbonyl plane whereas in conformation A it would be shielded relative to its position in $CDCl_3$. It should also be noted that in the A-type compounds (MS-1-MS-3, MS-6) the formyl proton is more



Conformation A



Conformation B

shielded in all solvents compared with the B-type compounds (MS-4, MS-7, MS-8). On the other hand, the formyl carbon is deshielded in the A-type group (~ 196 ppm) because of intramolecular H-bonding, as compared with the other group (~ 188.5 ppm). A comparison of the chemical shifts of the two methoxyl groups in MS-4 with the single methoxyl groups in MS-2, MS-3, MS-7 and MS-8 establishes unambiguously that in MS-4 the downfield resonance in acetone and chloroform solvents (3.99 ppm), but the upfield resonance in benzene (3.32), is the 2-methoxyl group. It is hoped that knowdledge of these solvent effects may find application in the structural elucidation of new carbazole natural products.