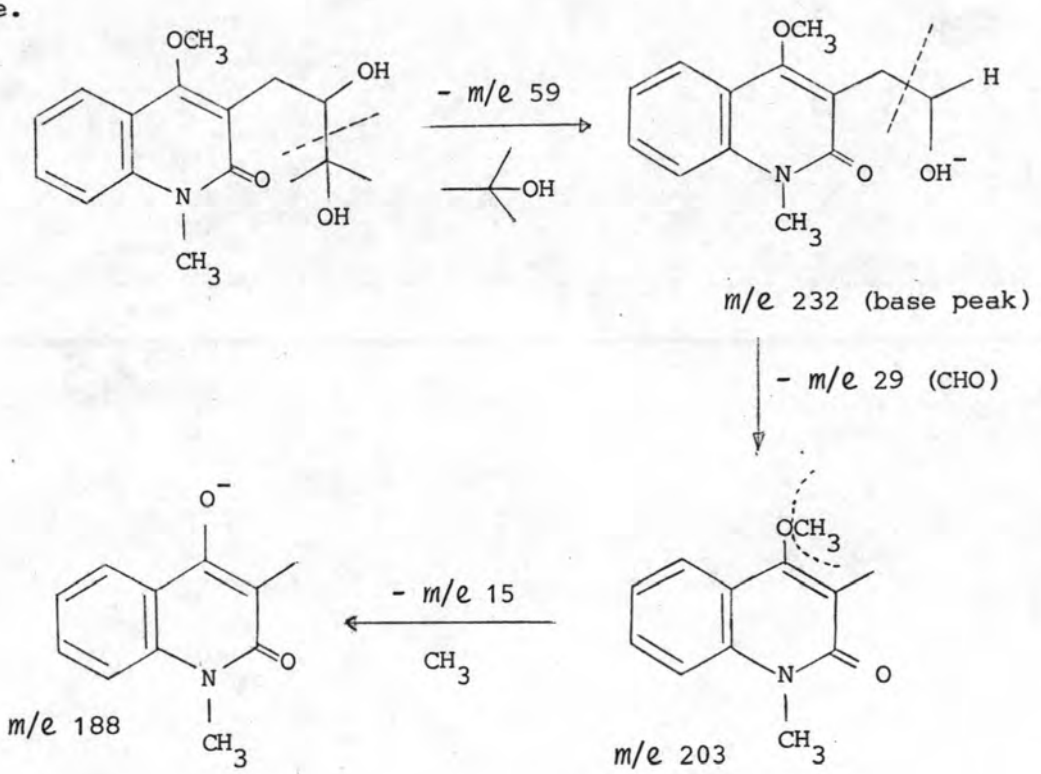
CHAPTER IV

DISCUSSION

The first component (EL-1) was isolated from the leaves of *Evodia lepta* Merr. and purified by silica gel column chromatography to yield a crystalline solid. The molecular formula was shown to be $C_{16}H_{21}O_4N$. It was further found to contain one OCH_3 group with corresponding to signal at δ 3.94 in the NMR spectrum is assigned to OCH_3 group. The UV spectrum is very similar to quinoline group (Cordell, 1981). The homocyclic ring is unsubstituted and its protons produce a first order ABCD system. The position of the C_5-proton resonance is indicative of a 4-OCH_3 group, the signal being shifted to lower field by about 0.3 ppm. in the absence of an oxygen function at position 8 (Toube *et al.*, 1967). The N-CH_3 group at δ 3.69 resonates at the same frequency as the corresponding group in N-methyl-2-quinolone.

The nature of side chain at C_3 may be deduced from NMR and the mass spectra. The geminal dimethyl group gives rise to a six-proton singlet at δ 1.29, a position characteristic of the dimethylcarbinol function. The proton on the carbon bearing the other OH group (δ 3.40) was shown by coupling to the benzylic protons at δ 2.69 and 3.13 only, with coupling constants of 10 and 2.3 Hz respectively, the benzylic protons exhibit a mutual geminal coupling of 14 Hz.

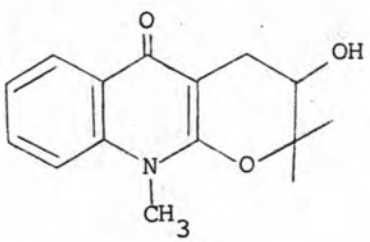
The mass spectrum contains only a trace of molecular ion (*m/e* 291). Small peaks indicate the loss of water (*m/e* 273), and of a CH_3 radical (*m/e* 276) followed by water. By far, the most intense peak in the spectrum is *m/e* 232, which corresponds to the loss of the terminal dimethylcarbinol radical (59 mass units) of the side chain. The side chain also fragments at the benzylic position (*m/e* 203) and 4-OCH_3 (*m/e* 188) and -C=O (*m/e* 188 - *m/e* 28) respectively. The major fragmentation is shown in the following scheme.



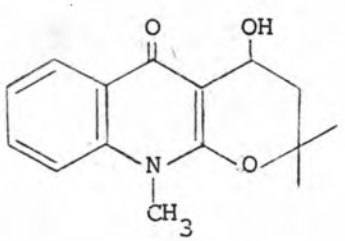
The structure of EL-1 is interesting in that it lies between those of the furoquinoline alkaloids and pyroquinoline alkaloids. In the basis of nucleophilic attack of 2'-OH on 2-CO to form pyroquinoline while nucleophilic attack of 3'-OH on 2-CO would lead to furoquinoline, therefore the biogenetic route of such a compound required appropriate enzyme for cyclisation.

The interesting point to note is that finding EL-1 or related alkaloids which have 3-prenyl quinolone in *E. lepta* Merr. has led to a key intermediate which gives rise to both of pyro and furoquinoline alkaloids.

The second component was isolated as colourless plates with λ_{max} 213, 238, 316 and 328 nm were made up. Proton counting by NMR and the molecular ion at *m/e* 257 in the mass spectrum establish the molecular composition as $C_{15}H_{17}NO_3$. Functional group analysis (NMR) of EL-2 reveal one N-CH_3 and no O-CH_3 group. It also contains a secondary alcoholic function as indicated by IR absorption band at 3200 cm^{-1}. Further structural aspects are also revealed by the NMR spectrum and electronic integration of areas. EL-2 shows a N-CH_3 signal at δ 3.44 and two unsplitted CH_3 peaks at δ 1.31 and 1.52 respectively, these data indicate a gem-dimethyl supported by a carbon atom without hydrogen and bonded to an oxygen. The doublet at δ 2.93 is ascribed to a methylene group adjacent to the secondary alcoholic function (triplet of the α-hydrogen at δ 3.91) so that double resonance measurements is strongly confirmed these assignments (J = 4.2 Hz). The foregoing results and the fact that the molecular formula of EL-2 requires another ring in addition to the 4-quinolone portion, lead to the structure (17) or of the alternative with alcoholic function located at position 4' (28).



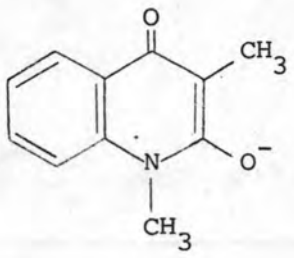
(17)



(28)

Structure (17) is firmly supported by the chemical shift of the methylene group (δ 2.93) in comparison with values found in similar compounds. In lunasia-II or isobalfourodine (8-methoxy derivative of 17) the 4'-methylene signal appears as a doublet centered at δ 2.88 (Corral and Orazi, 1967).

The mass spectrum of EL-2 also merits some additional comment. The particular interest in the mass spectrum is the ion at *m/e* 188 (base peak, $C_{11}H_{10}O_2N$) which exhibits N-methyl 4-quinolone nucleus (29).

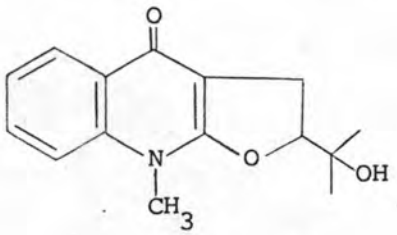


m/e 188

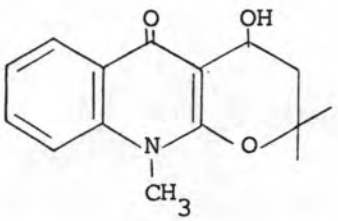
(29)

Small peak at *m/e* 229 indicated the loss of gem dimethyl (-*m/e* 30) and *m/e* 242 indicated the loss of 3'-OH (-*m/e* 17)

The third component was isolated as colourless prisms. The spectral data obtained for this alkaloid shown great similarities to that obtained for EL-2, notably identical empirical formulae, that both are 4-quinolones (UV and NMR) and that both possess cyclised hydroxy-dimethylallyl side chain (IR, NMR and Mass spect.). The ready loss of *m/e* 71 mass unit (C_4H_7O) indicated a hydroxy dimethylallyl side chain that had to be incorporated into a third ring system. There were a number of alternative structures could be deduced to two (20 or 28).



(20) Isoplatydesmine



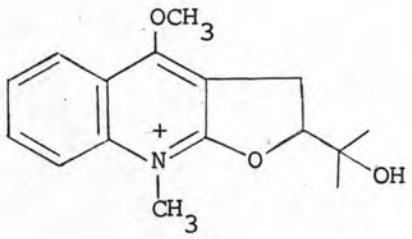
(28)

The only significant different noted was that the resonance of the methine proton of the dimethylallyl side chain was at δ 4.82. This resonance suggested that the hydroxyl group was attached directly to the methine carbon. In this case thereby indecating that this alkaloid was of the rare 4'-hydroxy pyroquinolone type. The signal of 4'-OH being shifted to higher field by δ 2.76 and replaceable by D_2O, while -OH of isoplatydesmine resonates at δ 4.85 (Fish *et al.*, 1976).

This alkaloid presumably occurring as the alcohol having an unusual 4'-OH pyroquinolone. The assigned skeleton has never been found elsewhere either naturally or synthetically therefore it can be deduced to the tentative structure (28) and named isoribalinine. In addition, it was noted that pyroquinoline alkaloids was first isolated from the genus *Evodia* and structure was elucidated on the basis of spectral data.

Recently, Boyd and Grundon have proved the structure of edulinine by synthesis of platydesminium metho-salt (18), which they had isolated from *Skimmia japonica* Thunb. Furthermore, they transformed the metho-salt (18) into edulinine with aqueous ammonia at 20℃ and suggested that edulinine might be an artifact

and that the metho-salt is the plant constituent (Boyd, 1970).



(18)

Higa *et al.* could also show that edulinine could only be isolated from *Pelea barbigera* Hillebr. (Rutaceae) when an aqueous alkaline solution was allowed to stand at room temperature for several hours or by continuous chloroform extraction of an alkaline solution. Conversely, chloroform extracts of acidic or neutral aqueous solutions contained no edulinine (Higa and Scheuer, 1974)

The fourth and fifth components (EG-1 and EG-2) were obtained from the leaves of *E. gracilis* Kurz. as isomeric form from the mass and NMR spectral data. Ultraviolet spectra of both alkaloids suggested furoquinoline nucleus (Cordell, 1981). Three $-OCH_3$ groups and two doublets of α, β-H furan ring of each alkaloid can be observed by NMR signals. The absolute configurations and rearrangement of substitution of both alkaloids are in accordance with previous publications (Higa and Scheuer, 1974; Robertson, 1963; Silva *et al.*, 1979; Mitscher *et al.*, 1975; Steck *et al.*, 1971). Therefore, it can be inferred that EG-1 and EG-2 are kokusaginine and skimmianine respectively.

Morphologically, *E. lepta* Merr. and *E. gracilis* Kurz. have been considered to be a homogeneous species by Smitinand. In

reappraising the character of these two species, it becomes apparent that there is a possibility that the *E. lepta* Merr. and *E. gracilis* Kurz. are also still a heterogenous assemblage of taxa. The investigation of this problem is still in the initial stage and at the moment little can be said over the interrelations of the remainders of the *E. lepta* Merr. and *E. gracilis* Kurz.. However, the exclusion of *E. lepta* Merr. from the *E. gracilis* Kurz. which having a strong affinity cannot be considered of significance. The distinctive of relationships have been considered only by vernacular names and usages by Thai tribes. Moreover, external appearance can be observed by scanty differentiation of shape, size, texture or so forth of leaves, flowers and fruits (see Fig. 27-28 pages 120-121).

Phytochemically, evidences have shown that alkaloids isolated from those two species as mentioned above are in different groups which might be illuminated the difficulties in distinguishing between the species. It was then thought that a knowledge of the alkaloids present might help to ditinguish these species and also to confirm identifications made on the basis of morphological and anatomical characters. In view of finding different groups of alkaloids in each plant, it is considered that alkaloidal content would be a useful character to be included in the parameters to be selected for a taxonomic study of an alkaloid-containing genus. It is known that compounds isolated from plants of the same species and environment should be identical. Therefore, it is proposed that these two plants might belong to separate species and not in homogeneous species as previously considered by Smitinand (Smitinand, 1980).