CHAPTER IV

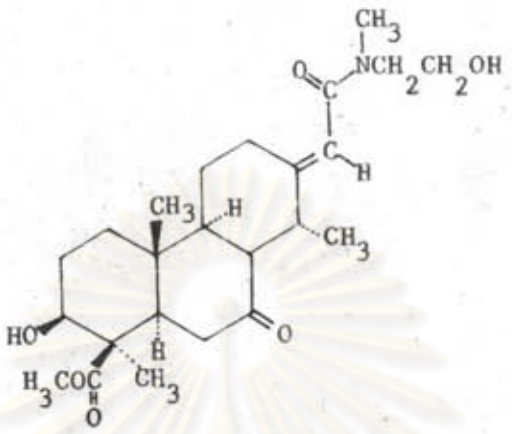
DISCUSSION

The genus *Erythrophleum* has attracted the attention of the workers in the fields of phytochemistry and plant pharmacology since 1875. The genus consists of 20 species which are distributed in Africa, Asia and Australia. These plants have been commonly known to be extremely poisonous and to contain alkaloids (at least) in their barks, leaves and seeds. In general, the alkaloids isolated from species of *Erythrophleum* are proved to be esters or amides of N-methylated ethanolamines with diterpenic or cinnamic acids which possess a digitalis-like action on the heart.

There have been no previous informations from the phytochemical point of view on *Erythrophleum* species growing in Thailand whereas the African and Australian *Erythrophleum* species have been studied in more details. Chromatographic studies of bark extract of *Erythrophleum teysmannii* Craib *var. puberulum* Craib showed that the crude extract contained at least ten alkaloids. These alkaloids are present in mixture, each of small quantity and are difficult to separate from one another.

The present work has led to the isolation of a crystalline alkaloid KS_1. The alkaloid KS_1 was identified as norerythrophlamide (62) by comparison with the ultraviolet, infrared, nuclear magnetic resonance and mass spectra with the published data of norerythrophlamide isolated from *E. ivorense* A. Chev. and *E. chlorostachys* Baill. (Cronlund and Sandberg, 1971 and Loder *et al.*, 1974). Norerythrophlamide is

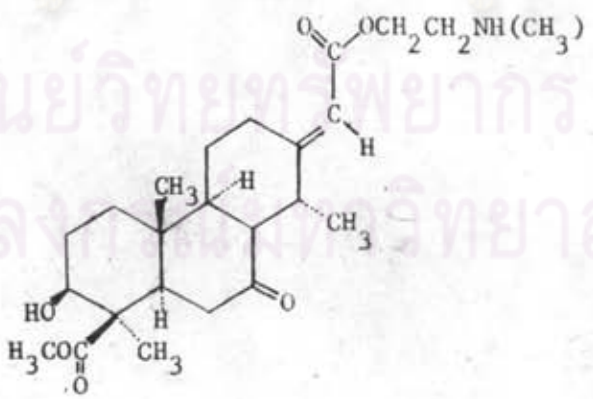
classified in the group of *Erythrophleum* alkaloid containing a β-carbomethoxy group at C-4 position.



(62) Norerythrophlamide

The alkaloid KS_1 exhibits an ultraviolet maximum absorption at 214 nm and infrared bands at 1645 and 1610 cm^{-1}. These data indicate the presence of an α,β unsaturated amide grouping of the type also characteristic of the amide type of *Erythrophleum* alkaloids. In the ir spectrum of KS_1, the bands at 1735 and 1720 cm^{-1} indicate the presence of β-carbomethoxy group at C-4. The nmr spectrum of amide of N-methyl ethanolamine exhibits a broad four-proton multiplet at δ 3.52-3.62 ppm and shows the amide N-methyl signal at δ 3.08 ppm. The signal at δ 5.90 ppm is assigned to the olefinic proton of the α,β unsaturated amide. The characteristic of the mass spectrum of the amide KS_1 is having the M^+ ion, *m/e* 435, as base peak because the amide bond prevents ready fission of the side chain. However, cleavage of the side chain still occurs and so the amide KS_1 has the M-74 ion, *m/e* 361, and the M-75 ion, *m/e* 360, which represents loss with hydrogen transfer. KS_1 has an M-59 peak, *m/e* 376, attributed to the loss of its C-4 carbomethoxy group.

Loder *et al.* (1974) reported the isolation of norerythrophlamide from the bark of *Erythrophleum chlorostachys* Baill. and proposed that most of the amides might probably derive from base-catalyzed rearrangement of the alkaloidal esters of N-methylethanolamine since the characteristic nmr signals of the amides were not detectable in the spectra of freshly isolated alkaloids, when the pH of the extract did not exceed 10, or in neutral fraction. They summarized that the rearrangement occurs when isolated by standard procedures with alkaline solutions of high pH, when chromatographed on alumina, or simply on standing. In the following year, Falkiner *et al.* (1975) reported the occurrence of the isomeric ester, norerythrophlamine from the same species and also converted the latter ester alkaloid into the amide, norerythrophlamide by using 5 N sodium hydroxide solution. According to this information, norerythrophlamide from this work might possibly be derived from naturally occurring isomeric amine, norerythrophlamine (63).



(63) Norerythrophlamine

The occurrence of norerythrophlamide which might probably be derived from norerythrophlamine indicates that the other alkaloids which have not yet been isolated might be the monoethylaminoethanol esters of diterpenic acids. The possibilities also include the dimethylaminoethanol esters. Both types of esters have been found commonly in other *Erythrophleum* species.

The poison in *Erythrophleum* acts as a local anesthetic and as a cardiac poison which are proportional to their alkaloid contents (Irvine, 1961). The pharmacological test of the ester alkaloids, on the isolated papillary muscle system showed that they increased the contractility of the heart muscle while the amide alkaloids decreased this activity (Clarke *et al.*, 1967). In general, monoethylamino esters are more potent than the dimethyl analogs (Cronlund, 1971).

The cytotoxic activity of norerythrophlamide was reported by Loder *et al.* (1974). Norerythrophlamide was not significantly active against the KB cell culture and had the ED_{50} value more than 100 µg/ml. However, accurate figures for the cytotoxicity of the pure amine are not yet available. Loder and Nearn (1975) found that 3β-acetates of diterpenoid alkaloids have more cytotoxic effect against KB cell culture than the corresponding 3β-hydroxy alkaloids.