CHAPTER III

DISSCUSSION

In the investigation of *Cissus quadrangularis* Linn. (syn. *Vitis quadrangularis* Wall.), the ground fresh plant was exhaustively extracted with 95% ethanol by maceration method. The phytochemical screening suggested the presence of triterpenoids, flavonoids and sterols. The thin layer chromatography of the total crude petroleum ether extract showed triterpenoids and steroid-positive spots which indicated by a positive Liebermann-Burchard test. The concentrated alcoholic extract was partitioned with petroleum ether to remove triterpenoids, sterols, chlorophyll, and some other fat soluble materials from the aqueous alcoholic phase.

The separation process of individual substances was based on the use of adsorption chromatographic method. Most of the column chromatographic procedures followed the technique called "Short Column Chromatography". This technique was devised and pioneered by Hunt and Rigby (42) in 1967. The separative power of a short column chromatographic method was considerable. The separations are carried out more rapidly and with less solvent than conventional techniques. It is essential that the appropriate solvent system be employed, and that the column be packed uniformly. Examination of a mixture to be separated by analytical TLC in several mixed solvent systems enabled selection of an eluting mixture yielding the best separation of the components. Ideally, these components should appear as spots at about hRf values of 30-40 on the plates. Moving these spots around

with various solvent combinations of different polarities were carried out to ensure that each spot was a single compound. Having selected the best solvent system for TLC, the polarity for the column eluent can be chosen. Since a compound on a column runs somewhat faster than on a plate, the concentration of the more polar component in the column eluent was usually decreased to about 50% of that found to be suitable for analytical TLC. The best system for analytical TLC was found to be benzene : chloroform (1:1) ; the eluent mixture used for the column separation was benzene : chloroform (3:1). The main advantages of using this method of chromatography are that the columns are short resulting in rapid separations, efficient solvent utilization and excellent material recovery.

From this study, four compounds were isolated and characterized. Compound CQ-1 was identified as the known triterpene called lupenone which had been isolated from various plant sources as listed in Table III :

Table III

The Occurrence of Lupenone in Plants

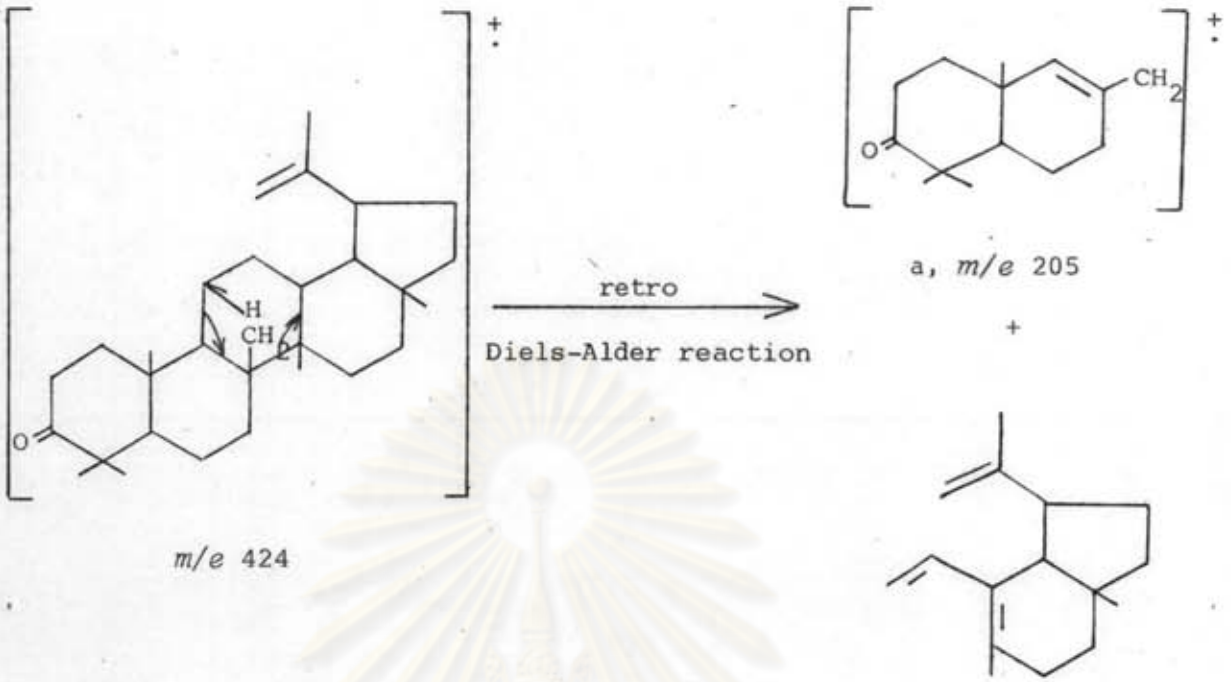
Plant	Family	Reference
Adenophora triphylla var. *japonica* Hara (rts)	Campanulaceae	43
Alnus rubra Linn. (lvs)	Betulaceae	44
A. hirsuta Linn.(lvs),. *A. fruticosa* Linn.(lvs)	"	45
Asteracantha longifolia Nees (rts)	Acanthaceae	46
Atylosia trivenia Gamble (pt)	Leguminosae (Papilionoideae)	47

Table III (Cont.)

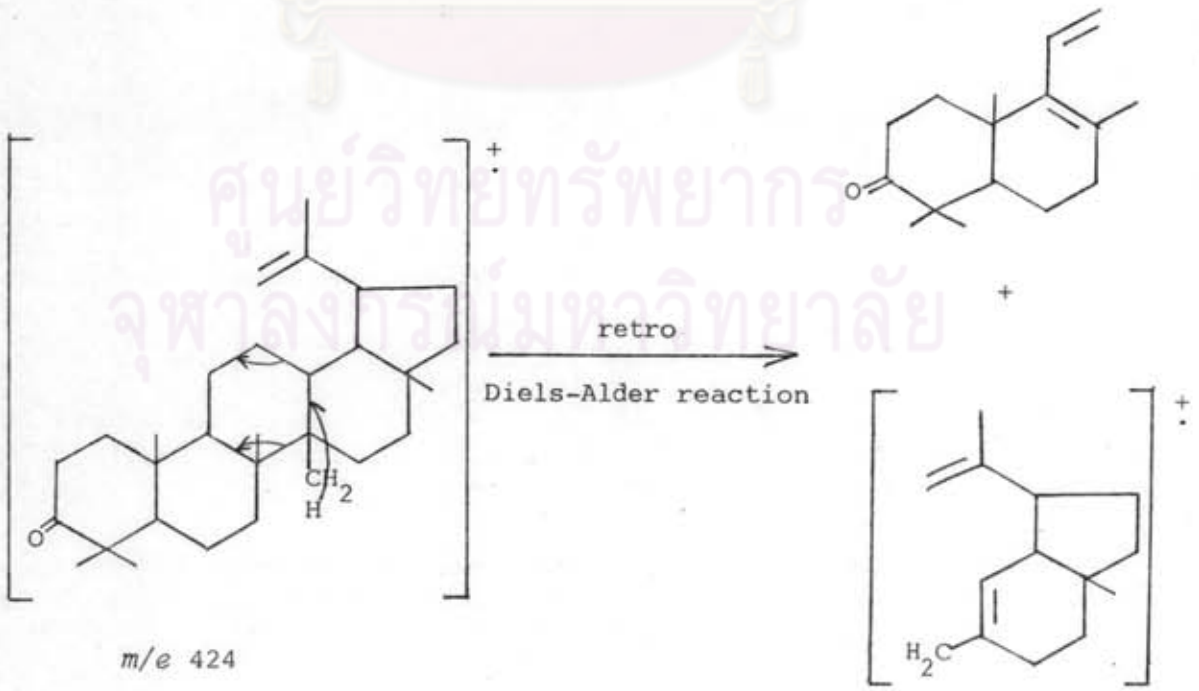
Plant	Family	Reference
Avicennia officinalis Linn. (rts)	Verbenaceae	48
Betula utilis D. Don. (bk)	Betulaceae	49
Caraipa grandifolia Mart. (wd)	Guttiferae	50
Carphephorus odoratissimus Hebert (lvs)	Compositae	51
Cassia siamea Britt. (bk)	Leguminosae (Caesalpinoideae)	52
Euphorbia balsamifera Ait. (lx)	Euphorbiaceae	53
Glochidion eriocarpum Champ. (lvs)	"	54
Lithocarpus polystachya Rhed. (st)	Fagaceae	55
L. harlandi Linn. (st)	"	56
Notonia grandiflora DC. (lvs)	Compositae	57
Phyllanthus emblica Linn. (st)	Euphorbiaceae	58
Pleurostylia opposita Rhed. (st)	Celastraceae	59
Pterocarpus santalinus Linn. (bk)	Leguminosae (Papilionoideae)	60
Salvia horminum Linn. (pt)	Labiatae	61
Sterculia foetida Linn. (bk)	Sterculiaceae	62
Voacanga papuana K. Schum. (bk)	Apocynaceae	63

(bk=bark, lvs=leaves, lx=latex, pt=plant, rts=roots, st=stem, wd=wood)

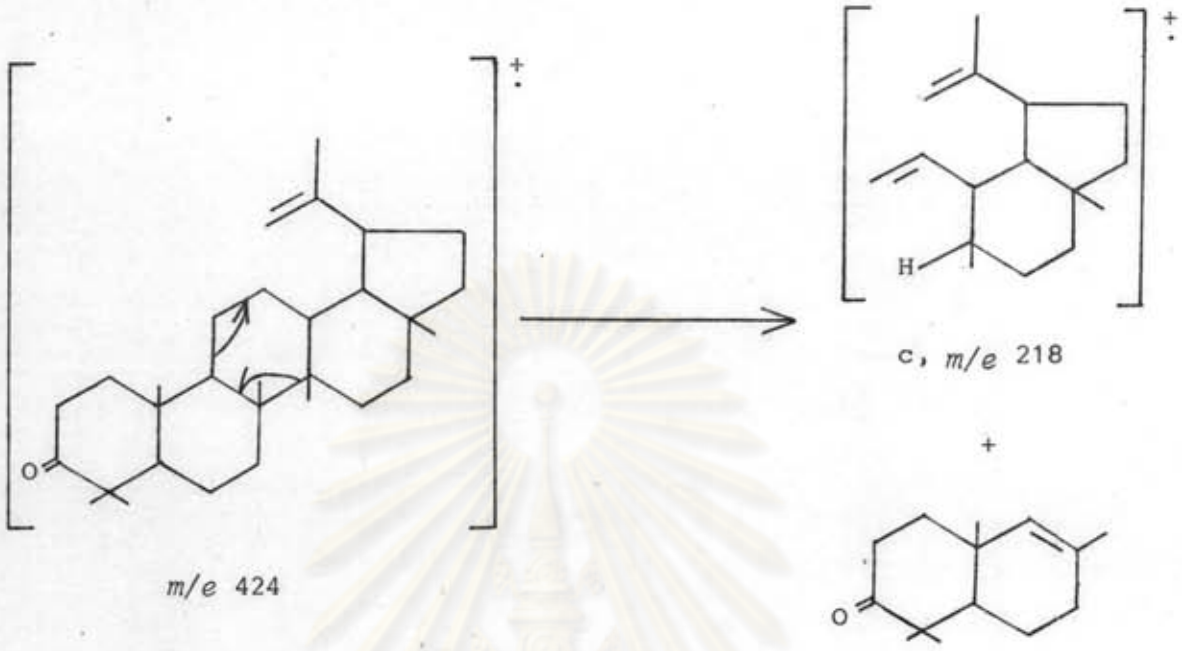
The mass spectral study of lupenone (see Figure 11) revealed the molecular ion at *m/e* 424 and the base peak at *m/e* 205. This characteristic peak exhibits fragment a *via* the retro-Diels-Alder reaction.



The peak at *m/e* 189 was also described by the retro-Diels-Alder reaction to form fragment b.



The peak at *m/e* 218 corresponded to the cleavage of ring C to form fragment c as shown below.



m/e 424

c, *m/e* 218

The other two characteristic peaks were at *m/e* 409 and *m/e* 381 which corresponded to the loss of CH_3 (M^+-15) and isopropenyl group (M^+-43) respectively.

The IR spectrum (see Figure 9) of lupenone showed a ketone functional group at 1720 cm^{-1} and a terminal alkene group at 1645 cm^{-1}. The ^1H nmr spectrum (see Figure 10) of lupenone revealed six unsplit methyl groups at δ 0.80, 0.94, 0.96, 1.03, and 1.07 ppm (two methyl groups) and one more methyl broaded by allylic coupling at δ 1.68 ppm. In the vinyl region, the two hydrogens of the terminal methylene showed broad doublet at δ 4.64 ppm.

The melting point and the spectral data of lupenone were in full agreement with the literature value (49). To confirm the structure of lupenone, the DNP derivative of lupenone was prepared. The melting point of the derivative was in full agreement with the

literature value (63).

Compound CQ-2 was identified as the known triterpene called epifriedelinol which had been isolated from various plant sources as listed in Table IV :

Table IV

The Occurrence of Epifriedelinol in Plants

Plant	Family	Reference
Antidesma bunius Spreng. (st)	Euphorbiaceae	64
Argyreia speciosa Sweet (lvs)	Convolvulaceae	65
Bridelia micrantha Baill. (bk)	Euphorbiaceae	66
Cannabis sativa Linn. (rts)	Cannabidaceae	67
Catha cassinoides G. Don. (lvs)	Celastraceae	68
Diospyros buxifolia Hiern. (lvs)	Ebenaceae	69
Euonymus europaea Linn. (lvs)	Celastraceae	70
Euphorbia antiquorum Linn. (st)	Euphorbiaceae	71
Haplopappus foliosus Linn. (st)	Compositae	72
Maytenus heterophylla Molina (pt)	Celastraceae	73
Mikania cordata Roxb. (rts)	Compositae	74
Notonia grandifolia DC. (lvs)	"	57
Piper aurantiacum Miq. (sds)	Piperaceae	75
Rhododendron niveum Hook. (pt)	Ericaceae	76
R. championae Linn. (lvs)	"	77
Scolopia schreberi Schreb. (bk)	Flacourtiaceae	78
Sphagnum sp. (peat moss) (pt)	Sphagnaceae	79
Syzygium cordatum Hochst. (bk)	Myrtaceae	80

(bk=bark, lvs=leaves, pt=plant, rts=roots, sds=seeds, st=stem)

The mass spectral study of epifriedelinol (see Figure 14) revealed the molecular ion at *m/e* 428 and the base peak at *m/e* 411 which corresponded to the loss of hydroxyl group (M^+-17). The peak at *m/e* 396 corresponded to the loss of MeOH (M^+-32).

Moreover, the computer search of mass spectrum collection at MIT (Massachusetts Institute of Technology) indicated that the spectrum of epifriedelinol is the most probable spectrum for CQ-2.

The IR spectral study of epifriedelinol (see Figure 12) showed hydroxyl functional group at 3480 cm^{-1}. The ^1H nmr spectrum of epifriedelinol (see Figure 13) revealed 8 methyl groups at the chemical shifts of δ 0.69, 0.88, 1.00, 1.14, 1.35, 1.42, 1.50, and 1.52 ppm respectively. The ^1H nmr spectrum did not indicate any double bond in the molecule but showed one hydrogen downfield at δ3.72 ppm which is in the right region for a CHOH of secondary alcohol.

Compound CQ-3 was identified as the known triterpene called isoarborinol which had been isolated from various plant sources as listed in Table V.

Table V

The Occurrence of Isoarborinol in Plants

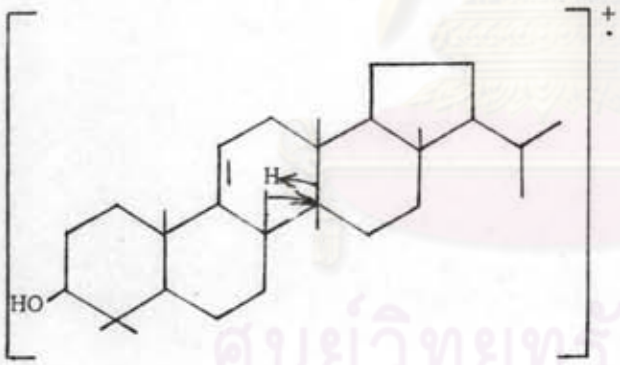
Plant	Family	Reference
Glycosmis arborea (Roxb.) DC.	Rutaceae	81-83
Hedyotis acutangula Champ. (st)	Rubiaceae	84
Madhuca neriifolia H.J. Lam. (bk)	Sapotaceae	85
Orixa japonica Thunb. (lvs)	Celastraceae	86

Table V (Cont.)

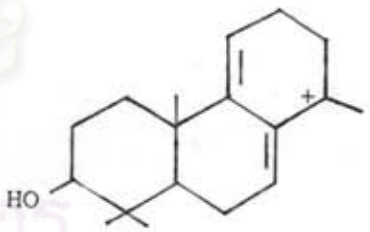
Plant	Family	Reference
Sorghum bicolor Linn. (sds)	Gramineae	87
Trema orientalis Lour. (st-bk)	Ulmaceae	88

(bk=bark, lvs=leaves, sds=seeds, st=stem, st-bk=stem-bark)

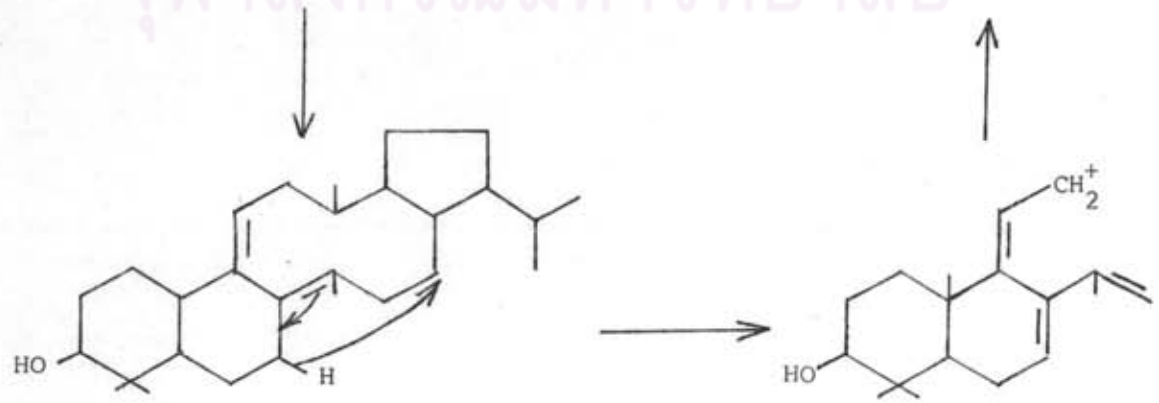
The mass spectral study of isoarborinol (see Figure 18) showed the molecular ion at *m/e* 426 and the base peak at *m/e* 411 which corresponded to the loss of methyl group. The peak at *m/e* 393 corresponded to the loss of element of CH_3-H_2O (M^+-33). The other peak at *m/e* 259 corresponded to the formation of fragment d by the mechanism suggested below.



m/e 426



d, *m/e* 259



The IR spectrum of isoarborinol (see Figure 16) showed the presence of hydroxyl group at 3475 cm^{-1} and alkene group at 1630 cm^{-1}. The ^{1}H nmr spectrum (see Figure 17) of isoarborinol revealed 8 methyl groups at δ 0.60-1.10 ppm, and saturated ethylene groups at δ 1.27-1.87 ppm. In the vinyl region, the hydrogen of the methylene showed broad singlet at δ 5.21 ppm.

The melting point and the spectral data of isoarborinol were in full agreement with the literature values (83, 85, 86). To confirm the structure of isoarborinol, the acetate derivative was prepared. The melting point of the derivative was in agreement with the literature value (83).

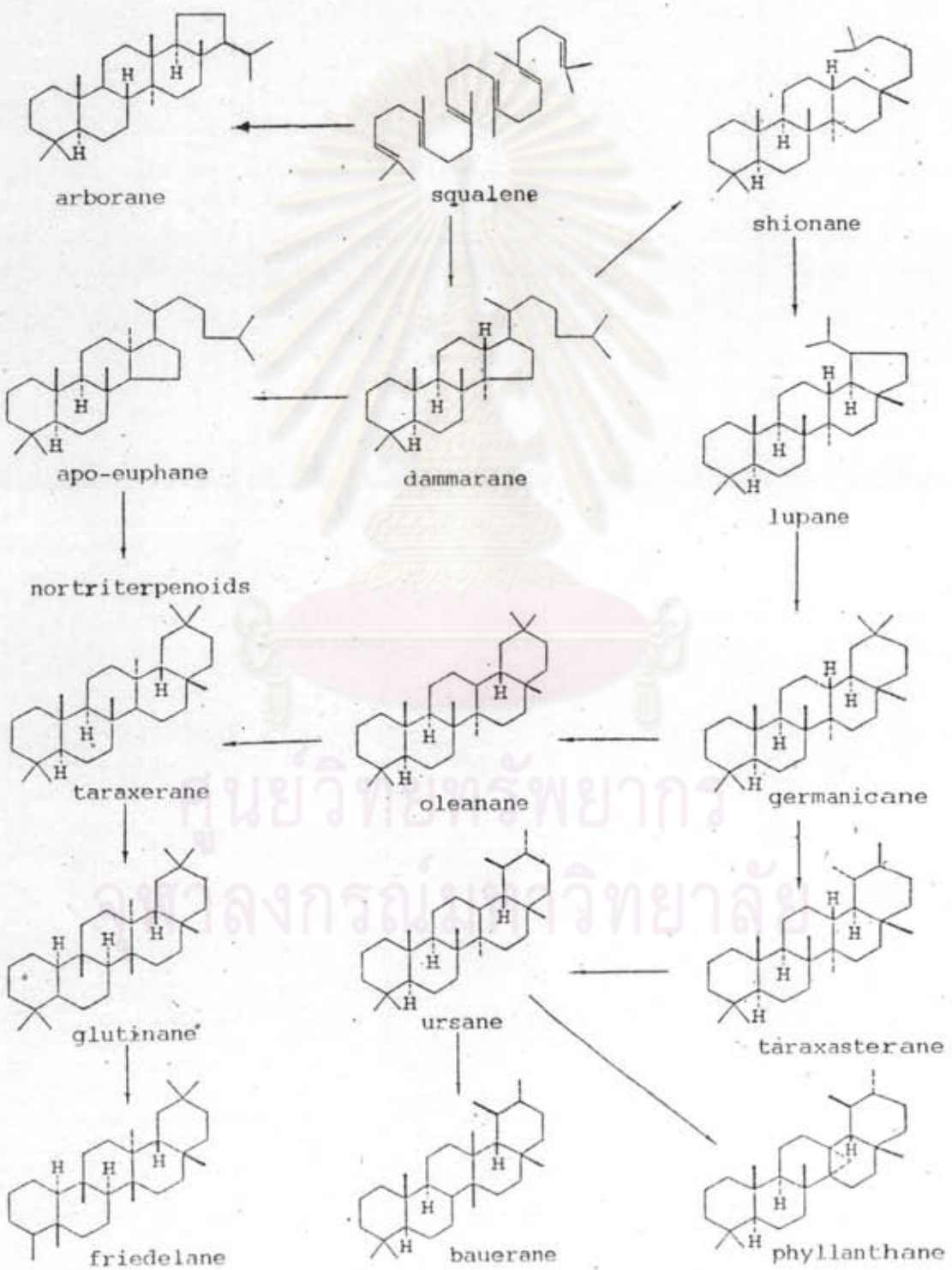
Compound CQ-4 which is apparently the main constituent, was identified to be the known sterol called β-sitosterol. This compound is distributed widely in various plant sources (42-73). The melting point of β-sitosterol was in full agreement with the literature value (81). Further proof of the identity of β-sitosterol was obtained by a peak by peak comparison of the IR and ^{1}H nmr spectra of our sample with those of the published spectra (89,90).

The triterpenoids form the largest group among the terpenoid classes, and are widely distributed in plant kingdom, either in free state or as esters or glycosides, although a few important members have been found in the animal kingdom, such as squalene, which is isolated from shark liver oil (91). All the triterpenoids originate biogentically from squalene, a tail-to-tail condensate of farnesol, which is a sesquiterpene alcohol (91). However, great structural variation has been found in nature. With the accumulation of structure data, Ruzicka (91) was able to rationalize the biogenesis of this

group of compounds and develop the basic concept of terpenoid biosynthesis. The following correlation charts shown below are based essentially on his biogenetic views.

Figure 2

The Correlation of Main Triterpene Skeleton (92)

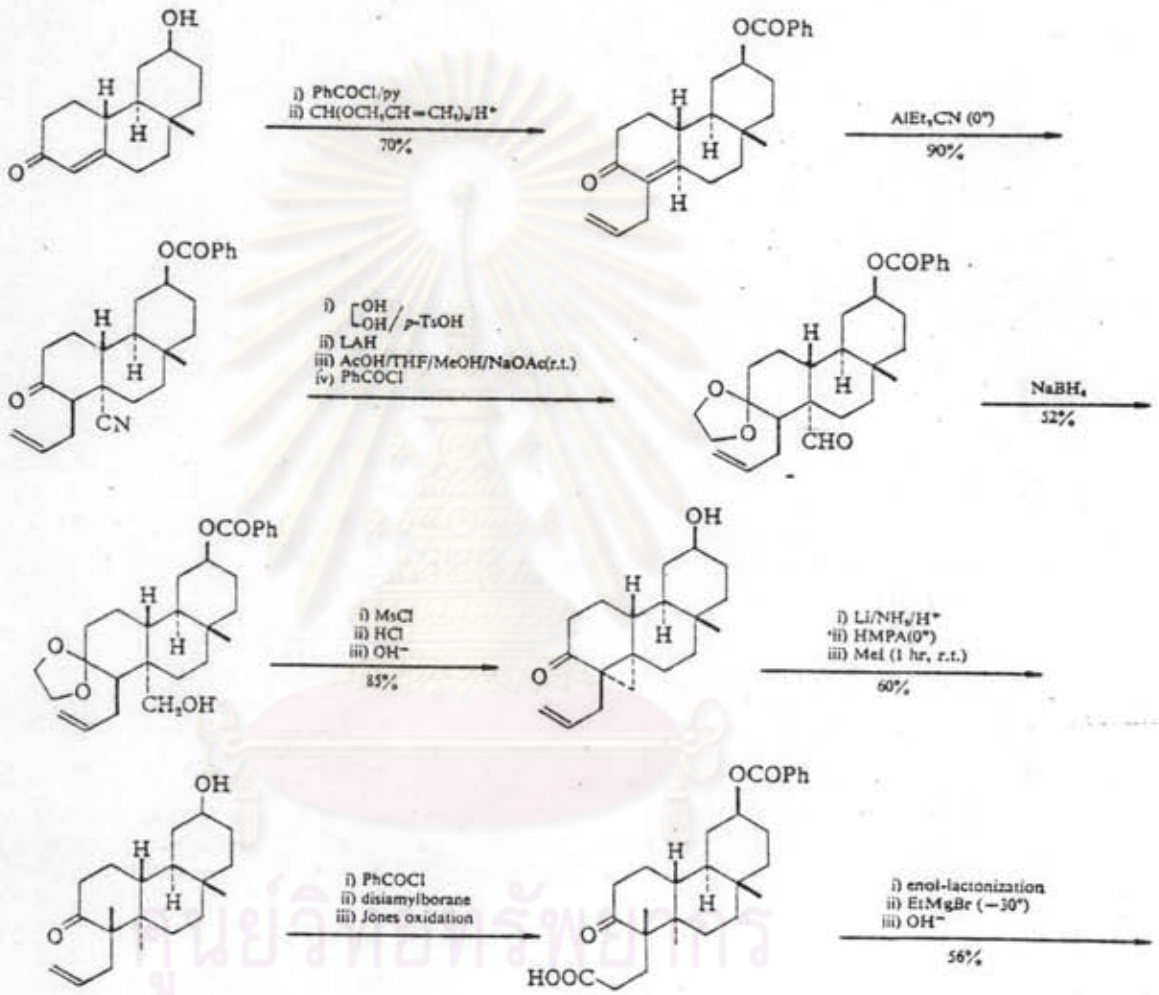


The triterpenoids exist as acyclic, tricyclic, tetracyclic, and pentacyclic structures. No triterpenoid so far has been found to have monocyclic or dicyclic structures. Tricyclic ones are rare such as ambrein, $C_{30}H_{52}O$ (93). Several tetracyclic triterpenoids are known. The most important and widely distributed triterpenoids are the pentacyclic compounds. They have been found in plants as primitive as *Sphagnum* (79) but are most common among the seed plants. All known members of this group are oxygenated at C-3 usually as alcohols but some as ketone. They are distinguished from eąch other by unsaturations, additional hydroxyl groups and frequently carboxyl groups.

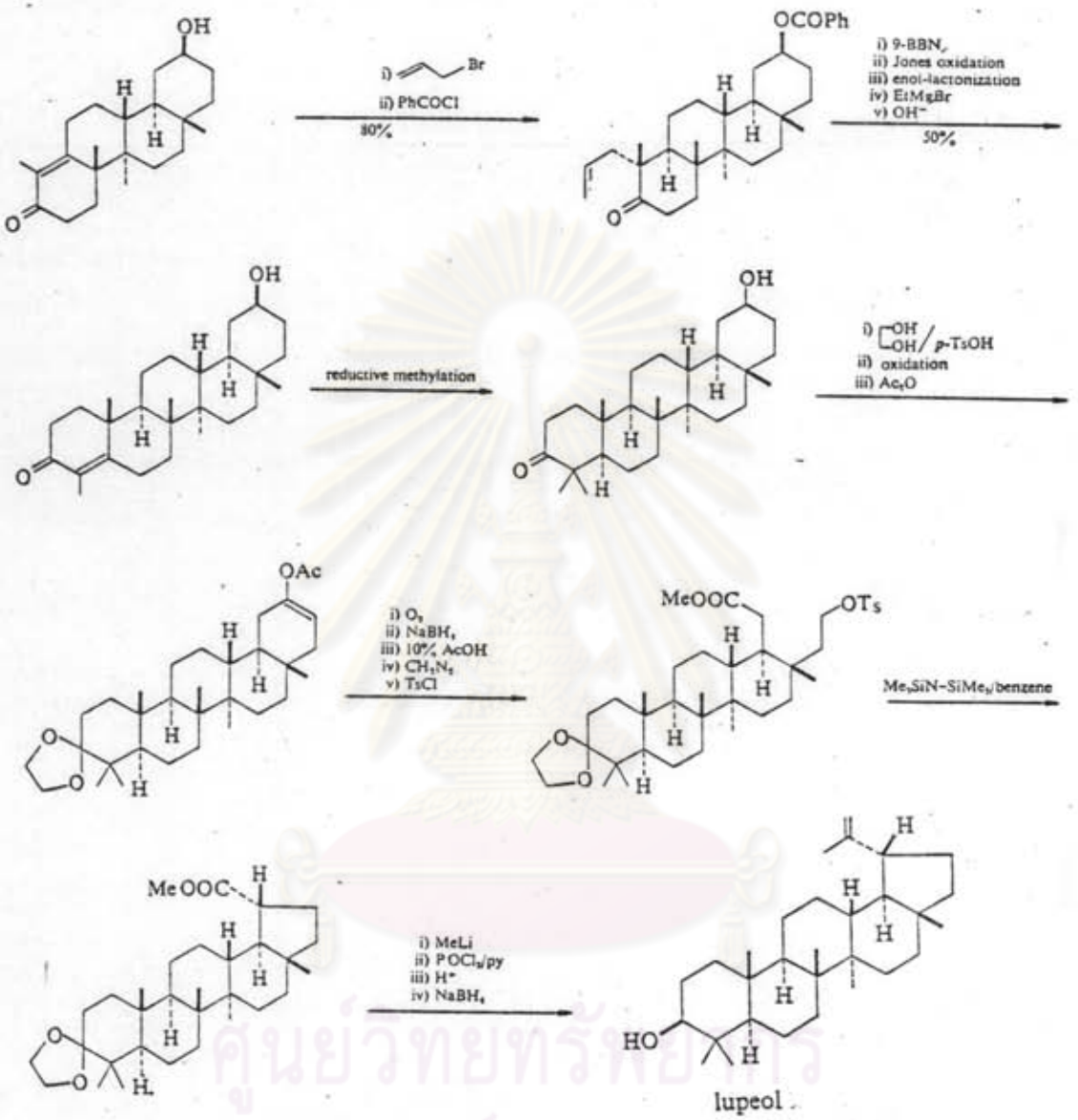
Lupenone was found widely distributed in various plant sources (43-63). It could also be produced synthetically by oxidation of lupeol with Jone's oxidation (94). The total synthesis of lupeol was described by Stork *et al.* (95) in 1971 by applying the enolate trapping method (95) of which the schematic reactions were shown in Figure 3 :

Figure 3

Synthesis of Lupeol



(Figure 3 Cont.)



Epifriedelinol was isolated from various plants both of higher plants (64-78,80) and lower plant (79). The synthetic pathways of this compound has not been reported, however, the semisynthetic process of epifriedelinol was described by Corey and Urspreng in 1956 (95), by the reduction of friedelin with lithium aluminium hydride.

Isoarborinol was first isolated with arborinol from the leaves of *Glycosmis arborea* (Roxb) DC. family Rutaceae by Roy and Pakrassi (81) in 1961. Four years later its structure was proved to be epimeric triterpene alcohol by Vorbrüggen and Djerassi (83). Moreover, isoarborinol could be obtained semisynthetically from arborinone by sodium borohydride reduction (83).

So far no pharmacological study of compounds isolated in this investigation are reported. All isolated compounds are simple triterpenes and sterol, which are found to be widely distributed in various plants. The data obtained from this investigation are not sufficient to prove Sen's proposal (23-25) about the presence of bone healing principles, ketosteroids until more exhaustive studies of nonpolar fraction of *C. quadrangularis* Linn. are done.