CHAPTER IV

DISCUSSION

Discovery of pseudoginsenoside-RP_1 and RT_1

Above 12,000 feet, the timberline varnishes. Roaring winds, heavy snows and freezing temperatures make life hard for people and animals who live at this elevation. However, in the mountainous forest, Himalayan ginseng was found in the eastern Himalayas (74). It is said that the Himalayan ginseng is very similar to San-chi ginseng [*Panax pseudoginseng* Wall.var. *notoginseng* (Burkill). Hoo & Tseng] and Japanese ginseng [*P. pseudoginseng* Wall.subsp. *japonicus* (Mayer) Hara], and botanically it belongs to *Panax pseudoginseng* Wall. subsp. *himalaicus* Hara var. *angustifolius* (Burkill) Li. of Araliaceae. This newly discovered ginseng plant has been investigated by Professors H. Harra of Tokyo University, N. Kondo of Showa University and O. Tanaka of Hiroshima University (75).

Panax pseudoginseng Wall. (Jen-shen-san-chi) has also called Shan-chi (meaning a mountain varnish) of Chin-pu-huan (meaning a precious drug) in Chinese. Pharmacologically, Shan-chi is a very effective agent in arresting hemorrhage and bleeding in wounds, including snake and tiger bites. Internally, it has been prescribed in hematomesis, menorrhagia, etc. The leaves also have similar properties and are often combined with the root for medicinal preparations. It is said that the famous secret formula of Yun-nan-

-Pai-yao (a famous pharmaceutical antibleeding preperation made in Yun-nan province) contains the active principles of this particular plant as the effective constituent. Chemical studies of *Panax pseudoginseng* Wall. have been carried out in Japan recently. It was found that this plant contains saponin glycosides of Pseudoginsenoside-RP_1 and RT_1 as the principal active agent (71) along with minor components related to other ginseng saponins.

From *Panax* to *Randia*

In plant itself the natural variation tends to be morphological in the shape of leaves, the characters of flowers and fruits. The chemical variation in plants is not corresponded to the morphological one, but due to the period of growth at different seasons. The habitat is also playing an important role to the chemical contents of plants. The plant individuals of same species growing in different habitats often have the chemical variation.

The above mention is variation of chemicals in the same species. Interestingly, variation of either plant species or genus can produce the same compounds which possess biological effects. This can be back up by the cases of diosgenin, caffeine, β-sitosterol and so forth which widely distribute among several species in the plant kingdom. Thus in the same case of oleanolic saponins composed of glucuronic acid which are unique group of compounds found only in *Panax* and other Araliaceous species (76). On chemotaxonomic point of view, attention can be drawn for the second discovery of pseudoginsenoside-RP_1 and RT_1 in *Randia siamensis* Craib which is a

member of Rubiaceae. It can be said over interrelationship between *Panax* and *Randia* that ginseng saponins are not only restrict to Araliaceae (Polypetalae) but also occur in Rubiaceae (Sympetalae). It is of interest from a biogenetic standpoint that the key intermediate route of these two genera are illustrated their closed affinity.

Difficulties in purification and interpretation of isolated saponins

The presence of glucuronic acid in the three isolated saponins (RS-1, RS-2 and RS-3) which is extremely rare in nature has led to isolate in pure state arduously. In general, glucuronide saponins usually form complex with inorganic ions and in that case it shows broad signals on NMR in C_5H_5D. Therefore it is always treated with ion exchange resin (Amberlite MB-3) in the final purification. If the sample has not been deionized sufficiently, the signal due to -COOH (carboxylic) of glucuronic acid will appear at different positions from that should be.

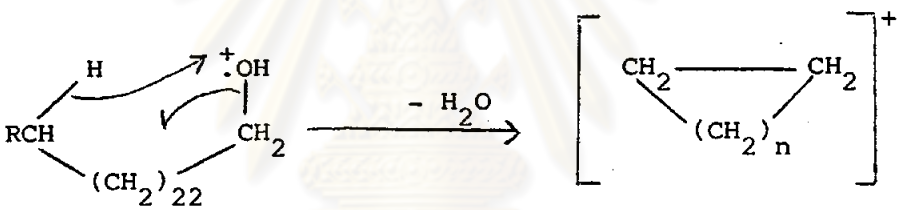
EIMS patterns of isolated compounds in this course of study

Mass spectral data of compounds which were isolated from two plants have shown patterns of fragmentation for four groups, e.g. long chain hydrocarbon, long chain alcohol, steroid and amyrins.

The mass spectrum of pentacosane (TE-1) displays characteristic of saturated aliphatic hydrocarbon which groups a peak spaced 14 mass units apart (corresponding to a difference of CH_2) and each

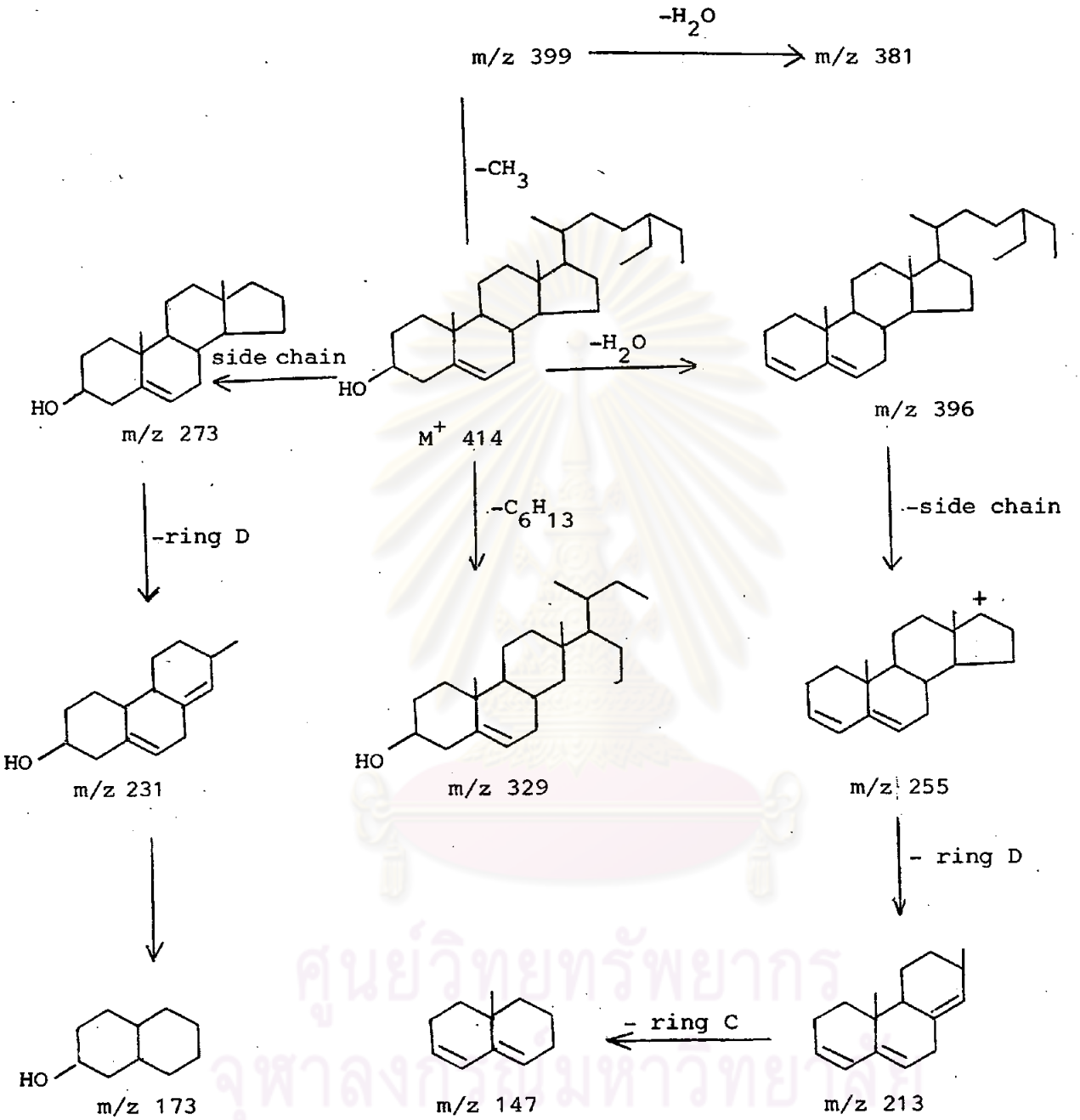
having as the most abundant species of C_nH_{2n+1} ion occurs with gradually decreasing abundance. Normal hydrocarbon shows a gradual decline in abundance of fragments with increasing fragment weight (77).

1-Triacontanol (TE-2), the second component is identified as aliphatic alcohol by confirmation with IR spectrum. It shows important peak of the elimination of water (M-18) by fragmentation of alcohol which McLafferty has suggested in the following general machanism (78).

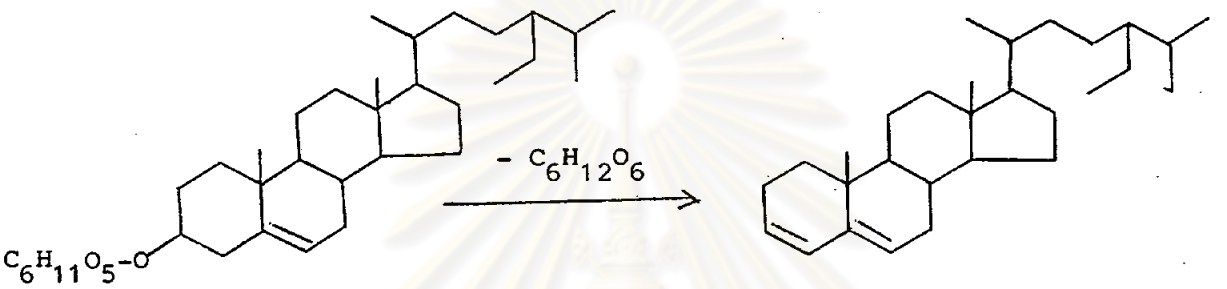


In general, the mass spectra of long-chain alcohols resemble greatly to those of the corresponding hydrocarbon with peak groups of fourteen mass unit apart and increasing in intensity with decreasing molecular weight (78).

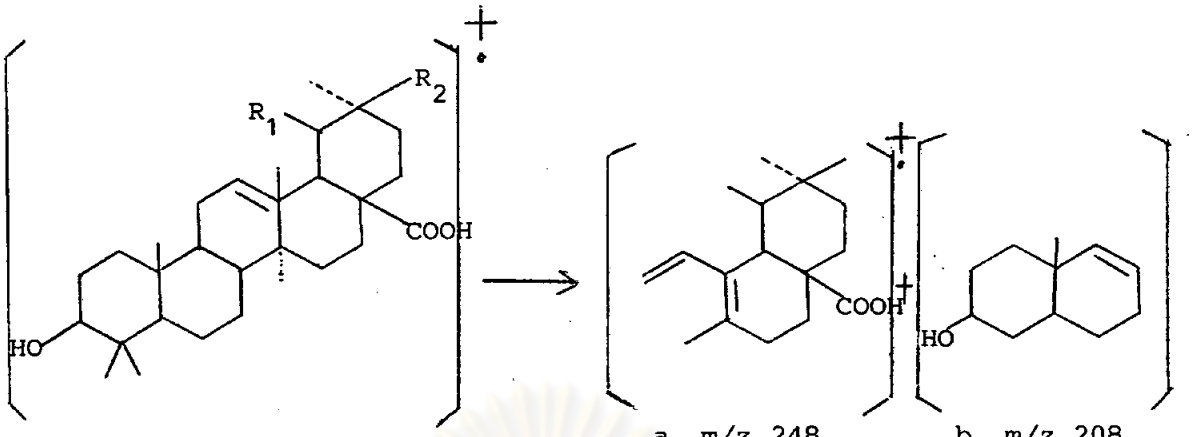
β-Sitosterol, as representative of steroid pattern in mass spectral data shows peak at m/z 414 (M^+) 399, 396, 381, 303, 273 255, 231, 213, 147 which agreed well with the proposed fragmentation pattern (79,80) as shown on page 107.



The mass spectrum of β-sitosteryl 3-O-β-D glucopyranoside in high resolution mass spectrum showed $M^{+}-C_6H_{12}O_6$ or m/z 396 which is the first step of fragmentation. The latter steps are similar to β-sitosterol as shown below.



The most common naturally occuring pentacyclic triterpenes are member of the α-amyrin or ursene type and β-amyrin or oleanene type, all of them being characterized by the presence of a $C_{12}-C_{13}$ double bond. This feature has proved to be readily recognizable by mass spectrometry, since the molecular ion undergoes the equivalent of a retro-Diels Alder fragmentation to furnish a very characteristic peak due to an ion of type a. In the absence of substituents which constitute an addition to the basic skeleton, type a will occur at m/z 248 and m/z 208 (79).



R_1 = CH_3, R_2 = H; ursolic acid

R_1 = H, R_2 = CH_3; oleanolic acid

a m/z 248 b m/z 208

- COOH

m/z 203

- COOH

m/z 411

Mass spectrum of oleanolic acid (β-amyrin type) and ursolic acid (α-amyrin type) have a very similar fragmentation. Difficulties in determining or distinguishing of their structures that the identification of them are performed by using high field ^{1}H-NMR and ^{13}C-NMR. In ^{1}H-NMR spectrum, multiplicities of ursolic acid at C-29 and C-30 are doublet (CH_3-CH) whilst those of oleanolic acid are singlet (CH_3-C-). Proton or carbon signals at C-29 of ursolic acid in nmr spectrum is shifted to lower field than of oleanolic acid.

Pharmacological Activities of Isolated Compounds

β-Sitosterol which is widely distributed in plant kingdom has been used in therapy as cholesterol suppressing drug. It acts

by physiological competition with cholesterol. Atal *et al.* found that fixed oil of Isabgul has cholesterol reducing property which is attributed to unsaturated acid and β-sitosterol present in the oil (81). From Extrapharmacopoeia (82), sitosterol or cystellin® (Lilly, USA) is a mixture of 80-90 percent β-sitosterol and β-dehydrositosterol. It appears to reduce absorption and to facilitate fecal elimination of cholesterol. It is proposed for treatment of hypercholesterolemia and clinical reports (82,83) on the use of sitosterol in coronary atherosclerosis.

A study of 29 patients with gall-stones indicated that β-sitosterol might enhance the effect of chenodeoxycholic acid against cholesterol gall-stones (82).

Recently, β-sitosterol is used in the treatment of non-infective prostalitis and prostata-adenoma. In Germany, several formulations containing β-sitosterol are used in the above diseases. It has been found further that African plants belonging to Hypoxidiaceae, containing β-sitosterol, have given good results in rheumatism. Probably they act by inhibition of prostaglandin synthesis in the system similar to phenylbutazone in inflammatory rheumatism. Drug containing β-sitosterol is marketed in Germany by 'Intermuti', 3440-Eschwege (81). β-Sitosterol is known definitely to inhibit growth of the WA neoplasm. It is proposed for anti-neoplastic activity (84). Further work showed it to have some activity against WM, LL, Murphy-Sturm Lymphosarcoma and marginal activity against CA (85). It is of interest for *Typha elephantina* Roxb. that contains β-sitosterol as a major component. Therefore

it might use for the diseases that claimed above and might use as natural source in turning to medicinal one.

Dorner and Huschke (86) found that Crataegus acid, a mixture of ursolic acid, oleanolic acid and crataegolic acid have no coronary dilating action. Seel (87) studied in Clinical-Pharmacology with approximate 220 patients showed that crataegus acid improved coronory flow and thereby regulates the general circulation and are superior to digitalis in that they favor assimilation of sugar from the blood, thereby improving the glycogen metabolism of the heart. There are numerous indications in cardiac disease. The most toxic effect of crataegus acid causes lung damage because the formation of solid particles occur in the blood. Schimert (88) claimed that pure oleanolic acid, ursolic acid do not cause lung damage.

The acetates of ursolic acid and oleanolic acid were administered subcutaneously to adrenalectomized rats and the effect on Na and K excretion was measured. Found that acetate of ursolic acid induced a significant retention of Na but no reports of this drug has been used (89).

Li, Na and K ursolates were used in varying in preperation of Cod-liver oil emulsion N.F.IX, Liquid petrolatum emulsion U.S.P. XIV, Turpentine oil emulsion N.F. VIII and Benzyl benzoate lotion U.S.P. XIV. The results indicate that the stability of the emulsion is influenced by the identity and quantity of the emulsifier and the inherent properties of the oleagineous phase. The formation of a water-in-oil emulsion, which can be attributed to the lipophilic character of ursolate ion, is contrary to the oil-in-water emulsion

usually formed by a univalent salt. Extremely low concentration of the univalent ursolates generally produce stable emulsions. Nowaday methyl ursolate is used as an emulsifier for pharmaceutical preperation and food. It has higher water binding activity than cholesterol and its cost is cheap. Methyl ursolate ointment has high stability and is a pharmaceutical inert additive for internal drug (90).

In this course of studies, it is found that crude ethanolic extract of *Randia siamensis* Craib fruits exhibited acute ichthyotoxic activity in low dose by using method of Sprague (91,67). The various concentration and time of entirely lethal dose are shown in Table 7.

Table 7 Time (hour) of entirely lethal concentration

concentration of alcoholic extract / Fish used	Control	0.03125%	0.0675%	0.09375%	0.125%
Puntius gonionotus Smith (ปลาตะเพียนขาว)	36	12	5.51	3.25	1.08
Poecilia reticulata Peters (ปลาหางนกยูง)	72	36	12	8	3.4
Cyprinus carpio Linn. (ปลาไน)	18	6	5.5	3.25	1.33
Tilapia nilotica Linn. (ปลานิล)	20	7	5.66	3.3	1.25

Moreover, crude ethanolic extract also exhibits spermicidal activity in human and rat semina (a). RS-2 shows no activity on isolated uterus of albino rat (b). However, animal experiments in different systems of isolated compounds from *Randia siamensis* Craib are currently being studied.

a) This experiment is performed at Faculty of Science, Mahidol University.

b) This experiment is performed at Faculty of Pharmaceutical Sciences, Chulalongkorn University.