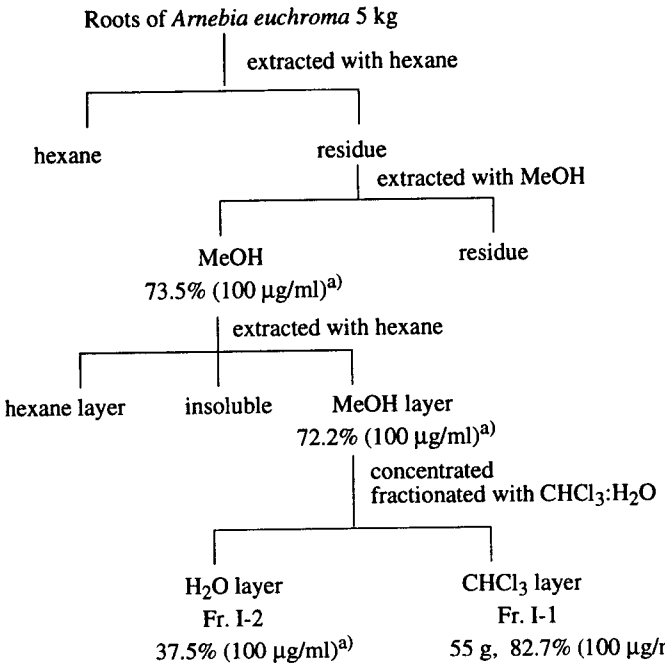
PL-2 Chemistry , Biology and Natural Products

Ushio SANKAWA
Faculty of Pharmaceutical Sciences
Toyama Medical & Pharmaceutical University

The medicinal plants have been used as drugs for thousands of years and many of them are used in traditional medicines. How long are these medicinal plants used as drugs? Ebers papyrus (BC 1550) is one of the oldest documents which described more than 700 kinds of herbal drugs. Some of herbal medicines described in the papyrus are familiar and 'Opium' is a good example. Some of the drugs described in the papyrus are hardly recognized as drugs by the standard of present day. We can trace back the history of medicinal plants from quite different approach. The investigations on group behavior of chimpanzees in Africa by 'The Primate Institute of Kyoto University' has revealed that chimpanzees suffered illness take specific plants which are not taken as daily food stuff. The chemical analysis clarified that the plants contain very bitter constituents and suggested that chimpanzees take medicinal plants to cure their illness. It is worth to note that young chimpanzees just learn what elder ones take when they suffer illness, but elders never teach to their children! It is no doubt that the chimpanzee's medicinal plants were found by random, and try and error screening. The medicinal plants found in this study have been used as herbal medicines by the peoples in the region.

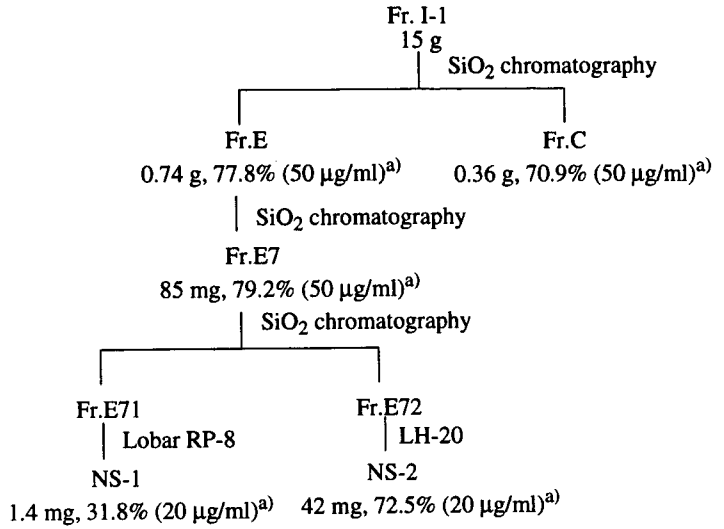
Systematic traditional medicines such as Ayurveda and Chinese medicines have been established thousands of years ago in various regions of the world. Numerous medicinal plant constituents were isolated and their structures were clarified by chemical and physical methods. In these two decades studies on medicinal plants have been oriented to isolate bio-active compounds by bioassay guided fractionation and isolation. The most of bioassay systems used in the isolation and identification of bio-active natural products were in vitro bioassays. Recent advent of 'Combinatorial Chemistry' (Combi Chem) brought a revolution in new drug development. Combi Chem consists of the creation of compound library by automated chemical synthesis and evaluation of biological activity with in vitro bioassay by using robot. If we compare the investigation on medicinal plant constituents and microbial metabolites with Combi Chem, source material can be regarded as the library of combinatorial chemistry. The structures in the last page are the compounds isolated and identified by bioassay guided investigation on medicinal plant constituents. Combi Chem and natural product isolation are not competitive, but complemental. The characteristic feature of natural products is the unpredictablility and unexpectedness in their structures. The processes of identification, isolation and structural determination will be discussed on several medicinal plans.

As a typical example identification of inhibitors of prostaglandin (PG) biosynthesis from Arnebia euchroma is discussed. PGs have diverse physiological function in various organs and cells and non-steroidal anti-inflammatory drugs (NSAID) are the specific inhibitors of PG biosynthesis. NSAIDs are used as antipyretic, analgesic and anti-inflammatory drugs and spirin and idomethacin are representative NSAID. They inhibit first enzyme reaction of PG biosynthesis, cyclooxygenase (COX) which oxidized arachidonic acid (AA) to PGG. This reaction is typical of lipoxygenase and initiated with hydrogen radical abstraction from a methylene hydrogen of AA. PGG was converted into PGH which was further converted into PGE, PGF and PGD. Thromboxane and PGI are responsible in platelet coagulation and anti-platelet coagulation, respectively. The main constituent of Peony roots, panel, inhibited platelet aggregation by inhibiting COX reaction in platelet. This investigation was carried out in human and is rare example of study that the activity of medicinal plant constituent was proven in human. We prepared more than 200 medicinal plant extracts and ran screening for PG biosynthesis inhibitory activity. Extracts of Zingiberaceae plants showed higher activity in the screening and gingerol and diarylheptanoids were identified as active compounds. The structures of these pungent principles possess phenol and lipophylic side chain and it is reasonable to explain that these phenolic inhibitors act as radical quenchers in COX reaction. The extracts of *Arnebia euchroma* (Boraginaceae) showed significant inhibition against PG biosynthesis and the main constituent alkannin, an antipode of shikonin possessing naphthoquinone structure exhibit no inhibition against PG biosynthesis. Starting from 5 kg material, methanol extracts were partitioned between chloroform and methanol. The chloroform fraction showed more potent activity and then the fraction further fractionated with silica gel column chromatography. Further separation with LH20 and Lobar RP-8 finally gave seven compound, NS-1 to NS-7. NS-2 was identified as shikonofurans. NS-4 was a new compound with a unique ansa-type monoterpenyl hydroquinone structure and called arnebinol. Structure was finally confirmed by X-ray analysis. NS-3 was a red colour quinonic compound and its structure was determined by NMR spectra as well as biogenetic consideration. NS-6, arnebifuranone, was another furan containing compound. Biosynthesis of shikonin was firmly established in tissue cultures of *Lithospermum erythrorhizon*. Its biosynthetic intermediate is geranyl hydroquinone which derived from *p*-hydroxybenzoic acid and geranyl diphosphate. Biosynthesis of shikonin from geranylhydroquinone is explained by the formation of second ring from hydroxylated terpenyl moiety. The same intermediate is also serve as an intermediate to afford shikonofurane. Formation of arnebinone from geranyl-hydroquinone is also interpreted via spyran intermediate which is formed from hydroxylated geranylhydroquinone. The unique feature of arnebifuranone was that geranyl chain licked to quinone ring with its head methyl, but not tail methylene. Reverse linkage of geranyl moiety is accounted for by the biosynthetic pathway via another spyran intermediate. The double bond configuration of arnebifuranone was first proposed to be trans but not cis. However synthesis of trans isomer clarified that



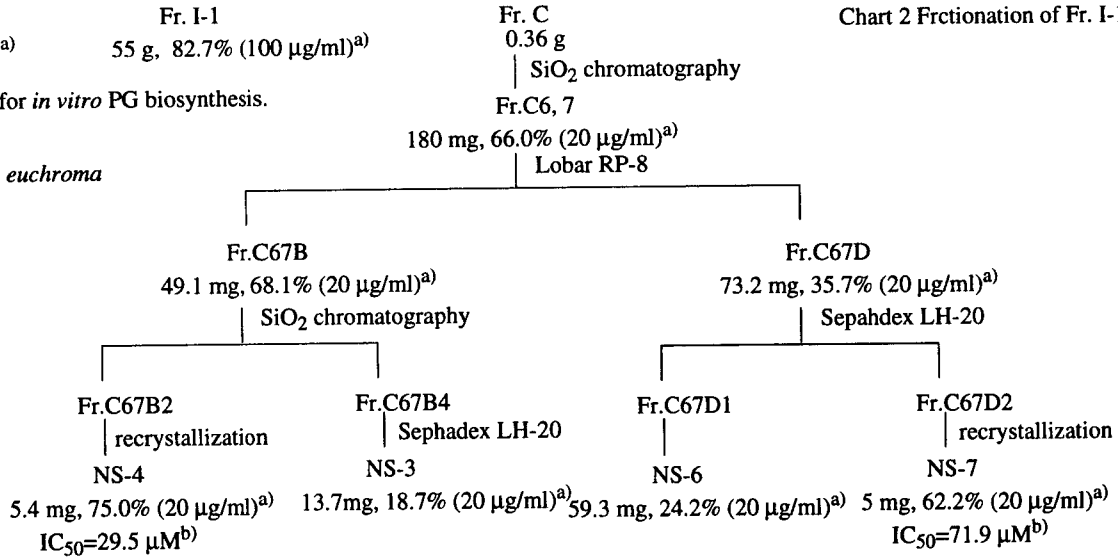
a) Inhibition % for *in vitro* PG biosynthesis.

Chart 1 Fractionation of *A. euchroma*



a) Inhibition % for *in vitro* PG biosynthesis.

Chart 2 Frctionation of Fr. I-1



a) Inhibition % for *in vitro* PG biosynthesis. b) IC_{50} values for *in vitro* PG biosynthesis.

Chart 3 Fractionation of Fr. C

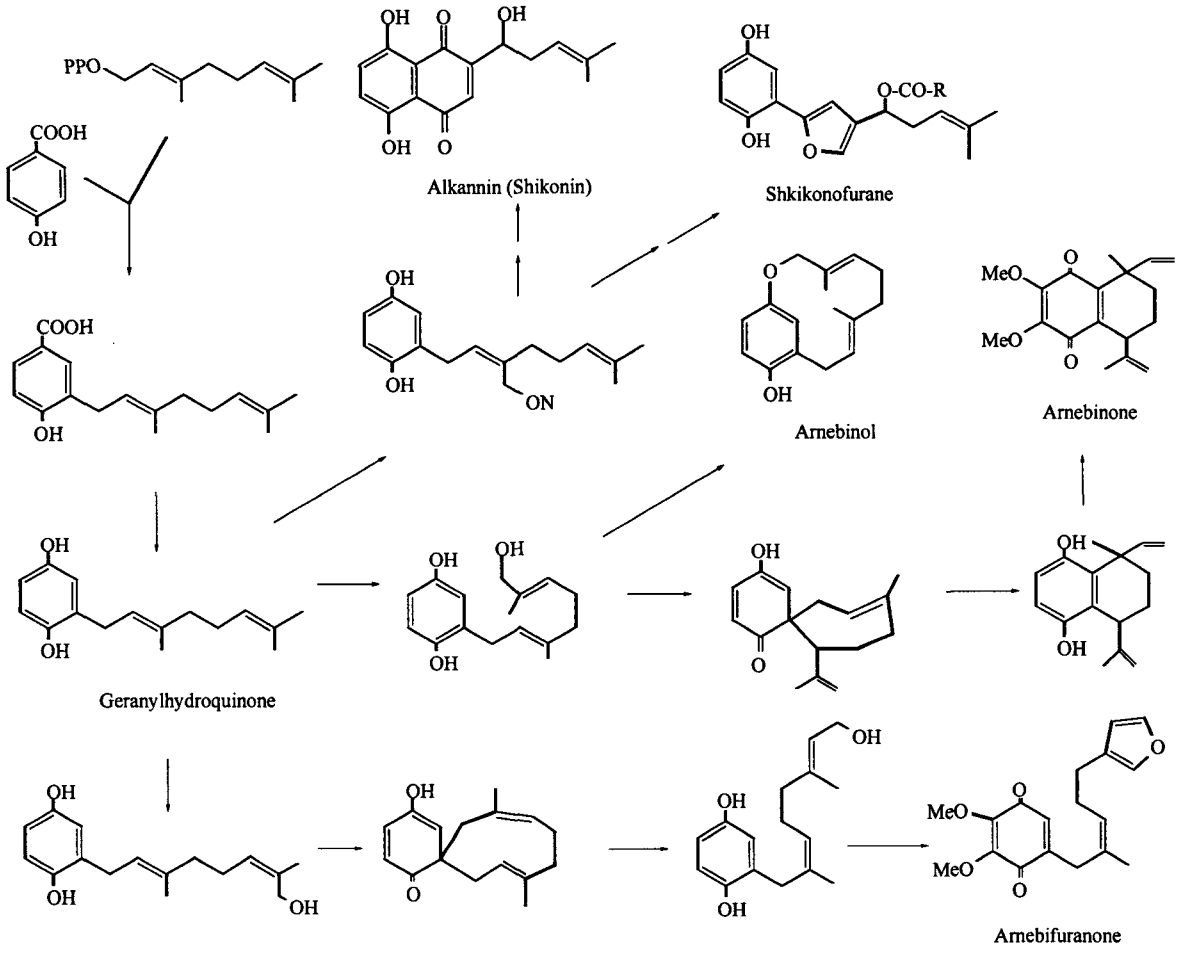
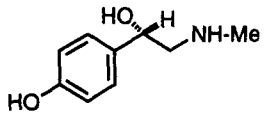


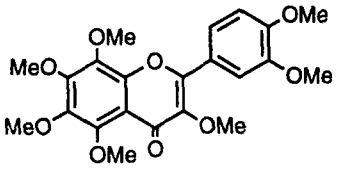
Chart 4 Biosynthesis of Consttituents of *Arnegia euchroma*

the configuration of double bond of arnebifuranone is cis. NS-7 is macrocyclic lactone and it is evident that the lactone is not derived from geranylhydroquinone, but it should be formed by plyketide biosynthesis.

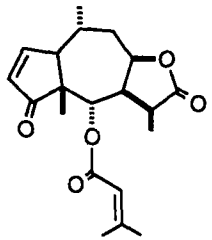
In the last page the compounds obtained by our investigation on medicinal plant constituents obtained during the course of bio-assay guided isolation and identification. It is worth to note here that the isolated compounds cannot explain the activity in the extracts as it is clear from the yields of compounds. The activity of the extracts should be the sum of compounds contained in the extracts including unisolated compounds.



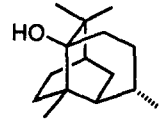
(-)-synephrine
(β–agonist)
Citrus unshu



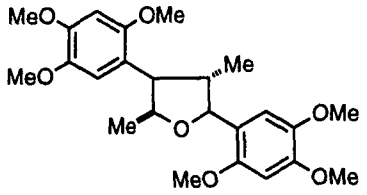
nobiletin
(anti-allergy)
Citrus unshu



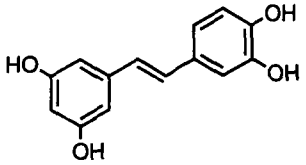
senecioylplenolin
(anti-allergy, PAF-antagonist)
Centipeda minima



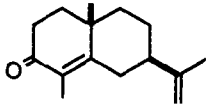
pachouli alcohol
(Ca antagonist)
Pogostemon cablin



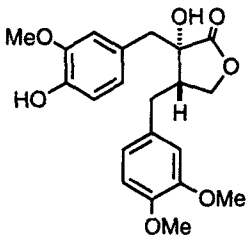
magnosalicin
(anti-allergy)
Magnolia salicifolia



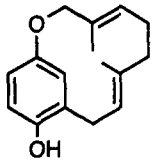
piceatanol
(anti-allergy, inhibitor of PG, LT biosynthesis)
Meraleuka leukadendron



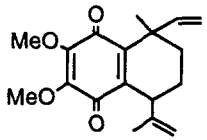
α-cyperone
(inhibitor of PG biosynthesis)
Cyperus rotundus



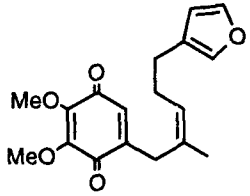
trachelogenin
(anti-allergy, Ca antagonist)
Arcticum lappa



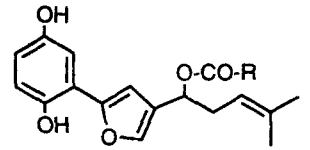
arnebinol
(inhibitor of PG biosynthesis)
Arnebia euchroma



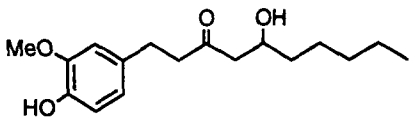
arnebinone
(inhibitor of PG biosynthesis)
Arnebia euchroma



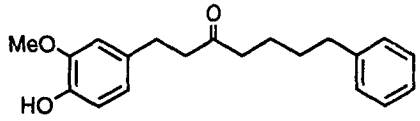
arnebifuranone
(inhibitor of PG biosynthesis)
Arnebia euchroma



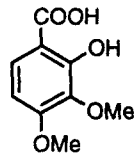
Shikonofurans B, C
(inhibitor of PG biosynthesis)
Arnebia euchroma



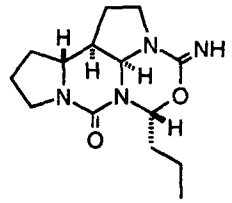
[6]-gingerol
(inhibitor of PG, LT biosynthsis)
Zingiber officinale



yakuchinone A
(inhibitor of PG, LT biosynthesis)
Alpinea oxyphylla



benzoate derivative
(anti-platelet aggregation)
Dalbergia odorifera



fissoldimine
(anti-platelet aggregation)
Fissistigma oldhamii

Bioactive Compounds Contained in Medicinal Plants