Studies in Fructus Evodiae

——A Multidisciplinary Exercise in Ethnomedical Research——

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Ethnomedicine refers to traditional health care systems using indigenous methods and materials for preventive and therapeutic purposes. Ethnomedicine of every ethnic group must be part of their cultural pattern and it is perpetuated because it is part of that cultural pattern.

There is an important linguistic element in every traditional health care system because the notions of normal physiology, pathology, materia medica (*i.e.* their selection) and diagnosis are all based on conceptual principles rather than concrete objects amenable to investigation by analytical tools.

In contrast, modern medicine requires an infrastructure that consists of diagnostic and therapeutic instruments, chemotherapeutic agents and the pharmaceutical industry, qualified personnel and medical education system. This infrastructure is only viable when it is supported sufficiently by financial capability and technical know-how. By the end of this cen tury, the economic situation in most countries would remain essentially the same(1). That is to say, more than half of the world population would still depend on indigenous health care systems. The World Health Organization recognized that the contribution from traditional and indigenous medical practices must not be ignored if we wish to provide everyone with efficient health care services by 2000. Indeed,traditional medicine is still appreciated where modern medicine is freely available, e.g. in Japan, Korea, Hong Kong, urban centres in China and other SE Asian countries.

Research with traditional drugs, or more precisely,the identification of active principles in currently used medicinal plants requires a multidisciplinary participation of the botanist, the chemist, the pharmacologist, and the clinician. It is important that each investigator must be able to relate the information and results in his own discipline to that of others so that the multidisciplinary information can be summed up and the whole group can be directed towards a specific objective.

There are several criteria for the selection of traditional drugs for laboratory studies.

a) Scientific interest: Is it necessary to develop this drug? Are there no similar modern drugs in the market? Is this drug representing a pharmacological profile differs from other modern drugs?

b) Operational constraint: Since most crude drugs cover a wide range of bioactivities, can we pick out one essential activity as the principal biological effect of this drug? Is it possible to establish a biological model to test this activity? If not testing in human subjects, can the result from animal experiments be extrapolated to clinical application?

c) Botanical support: Are we sure to get sufficient authentic supplies of this plant once we get serious with it? What is the possbility of domestication and cutivatlion in large quant-

ities?

d) Clinical experience: Is this drug effective and safe? How much is known about the chemistry of this plant? Since most traditional drugs are administered in small doses,what will be the toxicity of this drug when administered in large doses? What are the chances to produce carcinogenic, mutagenic and teratogenic effect?

e) Economic return: Is it cheaper to use this drug than to import a modern drug of similar therapeutic effect? Who will be profited from developing this drug other than scientific merit?

With these thoughts in mind, we perform some in-depth study of a few carefully selected traditional Chinese drugs. We try to capitalise on accumulated human experience rather than results of an extensive screening program. Screening a large number of test items to look for one active candidate does not apply to traditional Chinese drugs. For in Chinese medicine, the drug-disease pattern is quite clear and upheld by continuous human application. We can fairly that for every known disease, there is always an answer in Chinese medicine. Therefore our first concern is what kind of bioactivity we can investigate under laboratory conditions. Unfortunately, concepts in Chinese medicine are difficult to interpret in modern biomedical terms. All these notions of 'heat', 'cold' and 'phlegm' find no exact equivalent in modern medical language; they should not even be taken in the literary sense of the word in their own language. This situation is slightly better off with reproductive function.The cyclic events of menstruation and gestation, the different phasas of puberty, fertility and sterility due to senescence or otherwise can be described in rather plain language. Today with the advent of radioimmunoassay it is now possible to quantitate events of human reproduction in terms of the amount and sequence of appearance and disappearance of reproductive hormones. To bring this reasoning one step further, since the uterus is instrumental in menstruation, gestation and delivery, uterine contraction is one of the primary physiological parameter to describe uterine function. We try to look for oral active traditional drugs that can stimulate the contraction of the uterus in different functional states. This will allow us to gain more insight into such phenomenon as dysmenorrhea,amenorrhea, menostaxis etc. and all problems of fertility and infertility due to menstrual disortder in the light of treatment by traditional Chinese drugs. If we can bring the uterus under control we are more hopeful to bring human fertility under control. So the final objective is regulation of human fertility through the management of uterine function.

Of the dozens of traditional Chinese drugs used in obstetrics and gynecology, *Evodia rutaecarpa* (Juss) Benth. was chosen for this study.

The fruit of *Evodia rutaecarpa*(*Fructus Evodiae*) is one of the 365 drugs described in *Sheng Nung Pents'ao Ching* (Ca. 200 A.D.) the prototype of Chinese Pênts'ao (herbal). It was classified as a medium category drug signifying that it must be used only to treat diseases as compared to superior category drugs whugs which are consumed continuously for the conservation of health. In an economy of 28 words, *Fructus Evodiae* is described as a 'warm' drug with a pungent flavour. It warms up the interior (body function): it removes tympanites; it is analgesic; it stops belching; it is good for 'hot' and 'cold' diseases; it removes wetness (antiedemic, diuretic), it improves circulation; it drives out the evil 'wind' element through the subcutaneous tissues; the root(*Radix Evodiae*) is anthelminthic.

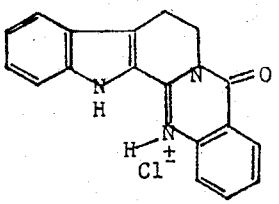
300 years later, in the first annotated version of *Sheng Nung Pênts'ao Ching* by the famous toaist herbalist *Tao Hung Ching* many new observations were added. *Fructus Evodiae* is further described to be a strongly 'hot' drug, sightly toxic, it removes 'cold' phlegm; stops intestinal colic and abdominal pain; removes indigestion and stops vomitting; the anthelminthic property of the root bark is oxyuricidal; improves laryngemphraxis; astringent; stops post-partum hemorrhage; cures dermatomycosis. The location and time of collection in the year are specified.

In *T'ang Pênts'ao* (659 A.D.), the first government edited version of *Pênts'ao*, all the above descriptions were confirmed. It was further noted that the young fruit was used and chalk was the contra-indicant.

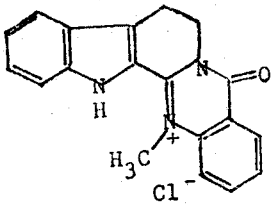
In *Ta Kuan Pênts'ao*(1108 A.D.), the government version of *Pents'ao* in Sung dynasty, the use of *Fructus Evodiae* to prevent epidemics in autumn (Chung Yeung Festival) was recorded. This preventive effect was further detailed in *Pênts'ao Kang Mu*(1598 A.D.) by *Li Shih Chên* who noted that if a *Evodia rutaecarpa* planted near a well into which the leaves fell, drinking water from this well could ward out diseases. Many prescriptions for gastrointestinal disorders such as diarrhea, cholera were presented in *Ta Kuan Pênts'ao*. The essentials of these information were faithfully recorded in *Tung I Pao Chiên* (1613 A.D.). It was further noted that *Fructus Evodiae* were used to treat 'yin' diseases, i.e. organ hypofunction.

In summary, *Fructus Evodiae* seem to act on the smooth muscle of the gastrointestinal tract, the cardio-vascular system and the uterus. In all cases, it can improve organ asthenia and thus increase the smooth muscle tone of these organs.

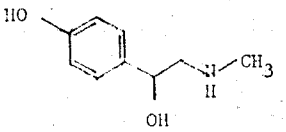
Chemical studies on the fruits of *Evodia rutaecarpa* were reported by-several investigators.(2-9) But none of them has been reported to be uterotonic or any other effects on female reproductive functions. Some of the pharmacological active compound were listed in Fig. 1.



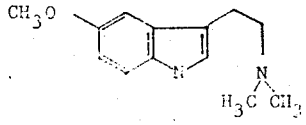
1. DHE
   a. Uterotonic effect b. Inotrophic effect



2. Rutaecarpine
   a. Uterotonic effect



3. Synephrine
   a. β-adrenergic agonist
      —Uterine relaxatory effect —Hypotensive effect



4. N,N-dimethyl-5-methoxytryptamine
   a. Serotoninergic agonist
      —Uterotonic effect

Fig. 1 Pharmacological effect of *Eructus Evodiae* compounds

Acocrding to ancient Chinese medical literat ure and modern international publications, the fruit of *Evodia rutaecarpa* may contain uterotonic compounds, probably alkaloid in nature. Therefore, special effort was put on the isolation of the alkaloid fraction. With the help of an appropriate bioassay method to guide the isola-

tion of the uterotonic compounds several were identified. Here are some significant findings.

1. Purification and identification of dehydroevodiamine-HCl (DHE) and rutaecarpine.

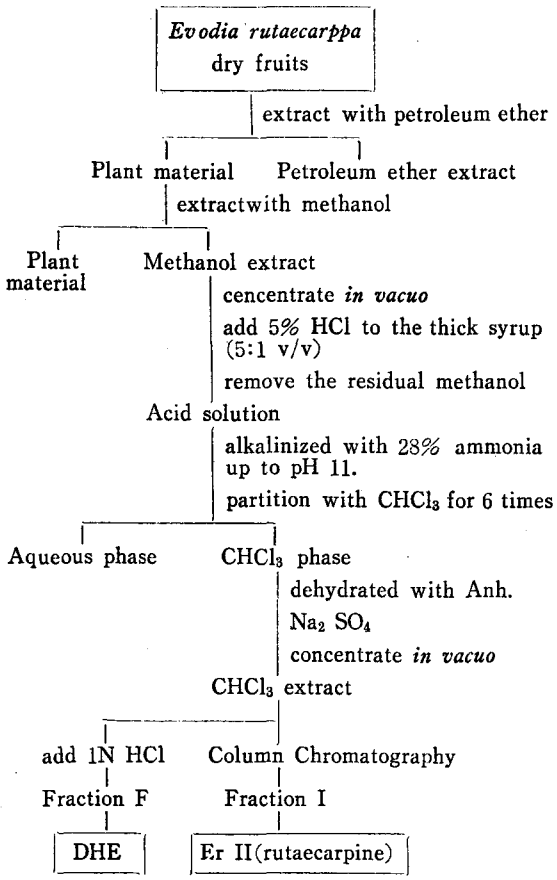


Fig. 2. Purification of DHE and rutaecarpine.

The procedure is summariezed in Fig. 2.

2. Another batch of *Fructus Evodiae* of Japanese origin was also processed in Japan. Two other compounds i.e. synephrine and N, N-dimethyl-5-methoxytryptamine were isolated. The procedure is summarized in Fig. 3.

3. Uterotonic effect of DHE, rutaecarpine-HCl and N, N-dimethyl-5-methoxytryptamine.

The uterotonic effect of these compounds are demonstrated *in vitro* by recording the tension produced by isometric contraction of the isolated rat uterine strip. The drc of DHE and N, N-

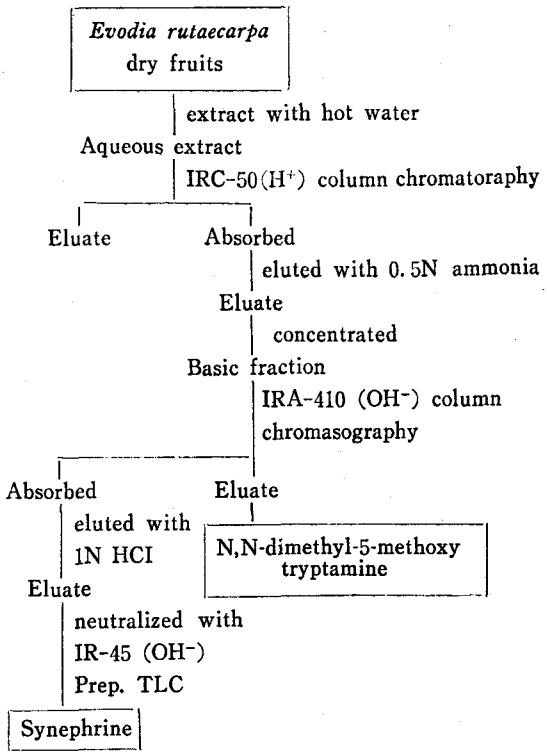


Fig. 3. Purification of synephrine and NN-dimethyl-5-methoxy tryptamine.

dimethyl-5-methoxytryptamine were shown in Fig. 4, 5, 6, 7.

The uterotonic effect of both DHE and N, N-dimethyl-5methoxytryptamine were inhibited by 3×10^{-9}M methysergise which was added 5 mins before the addition of the drug.

4. Relaxation effect of synephrine on contracted rat uterus

The relaxation effect was demonstrated in both spontaneously contracting dioestrus rat uterus and acetylcholine-induced cotracting rat uterus. Synephrine tartrate was used in this experiment. The relaxation effect was reversed by a beta-adrenergic blocker, propanolol-HCl (0.6 ug/ml=2×10^{-6}M). Th drc was shown in Fig. 8. The comparative potency of the relaxation effect between epinephrine and synephrine was also tested and shown in Fig. 9. 20 ug/ml (4.13×10^{-5}M) of synephrine was used in this

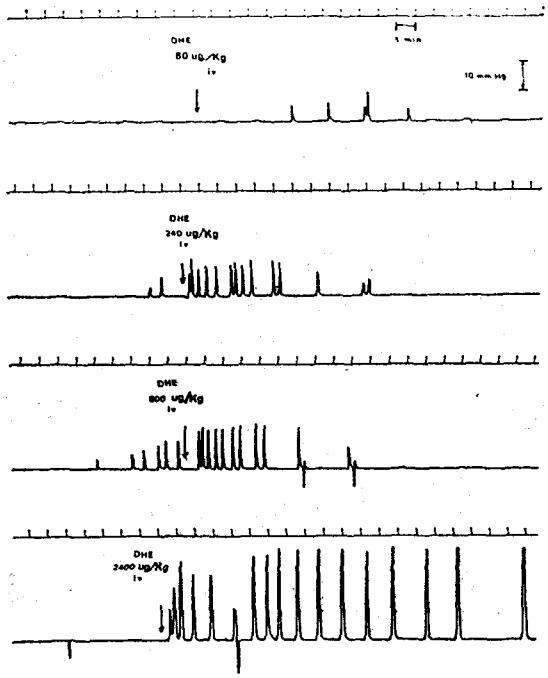


Fig. 4. Effect of DHH on rat uterus in intact rat. Arrow indicated the administration of DHE.

test.

The results present here can partly explain the properties of *Evodia rutaecarpa* in Pents'ao literature. Since both uterine stimulants and relaxant were found in the unripe fruits of *Evodia rutaecarpa* (*i.e.* DHE, rutaecarpine and N, N-dimethyl-5-methoxytryptamine are stimulants, while synephrine is a relaxant), it is interesting to know which predomiannt effect is referred to in ethnomedical information. There are two factors which can contribute to the uterotonic effect of *Fructus Evodiae*. First, the relative composition of the individual compounds, second, the relative potency of the compounds. It would be very difficult to assess the overall activity by just calculating the sum of the effects of the individual compounds. The best evidence is that the ethnomedical extract (aqueous decoction) has been demonstrated to be uterotonic *in vivo* in rat. It can be related to the therapeutic use of *Fructus Evodiae* as a treatment for post-partum hemorrhage.

In a prescription called *Tso Chin Wan* which is used for the treatment of dyspepsia, *Fructus Evodiae* itself is used as stomachic and deobstruent. Since DHE and N,N-dimethyl-5-metho-

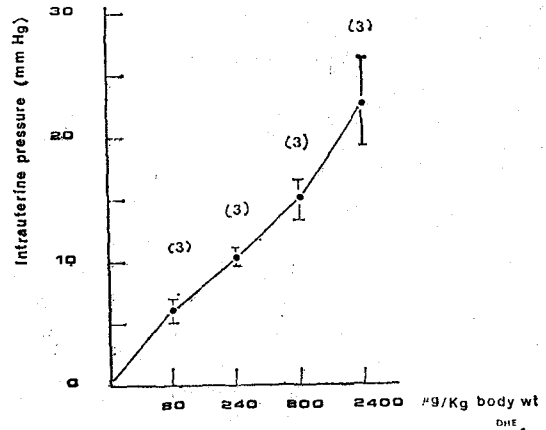


Fig. 5. Uterotonic effect of DHE. Maximum peak contractions in 10' were measured as response. Three rats used in this experiment.

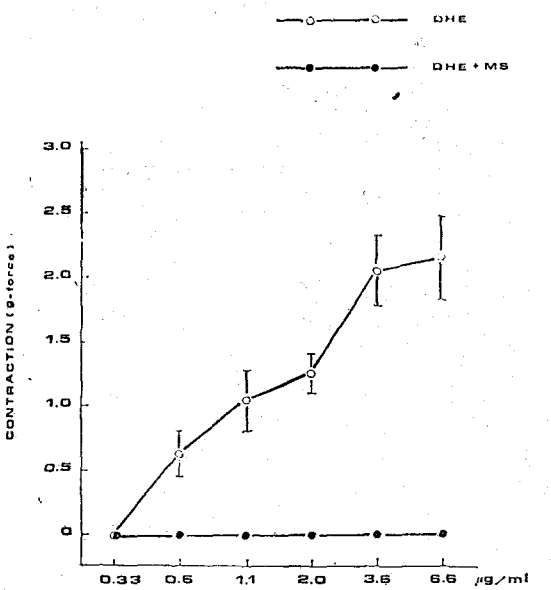


Fig. 6. Dose response curve of *in vitro* uterotonic effect of DHE. The maximum peak tension within 20 minutes after drug addition was measured as the response.Its effect effect was totally suppressed by 3×10^{-9}M methysergide (MS) which was added 5 minutes before the addition of DHE. The data were represented as mean ±S.E.M. with n=12 at every point.

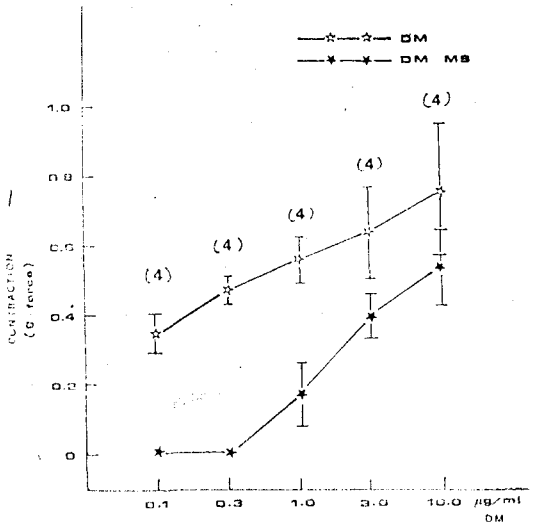


Fig. 7. Effect of N,N-dimethyl-5-methoxytryptamine (DM) on isolated rat uterus. The inhibitory effect of methysergide (MS) on DM is also presented. The maximum peak contraction was measured as response.

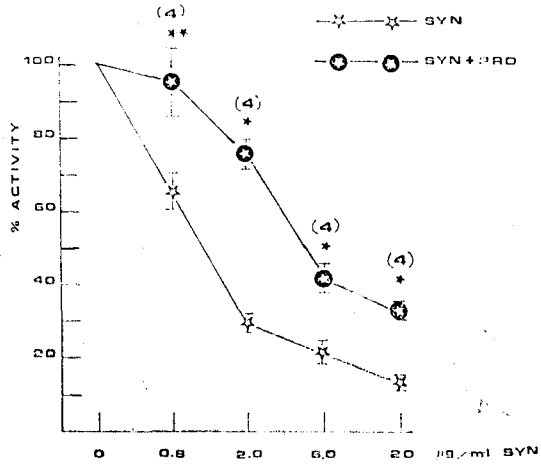


Fig. 8. Relaxation effect of synephrine tartrate(SYN) on isolated rat uterus. The inhibitory effect of propranolol (PRO) on SYN is also shown. The maximum peak contraction was recorded as response. The spontaneous contraction was regarded as 100% activity. ★P<0.001 ★★P <0.05

xytryptamine are serotoninergic agonist, they may be able to contract the intestinal tract thus causing the above effects. On the other hand, synephrine can mitigate conditions of excessive bowel movement by inhibiting peristalsis and belching. Synephrine is a catecholamine analogue which was first synthesised before it was discovered in several kinds of citrus peels. Tod ay, *Fructus Evodiae* and citrus peels(like *Citrus aurantium*) are used to treat indigestion, meteorism, anorexia and dyspepsia. They may all contain synephrine as their active ingredient.

Our recent investigation showed that DHE-causes a positive inotrophic effect as shown in figure 10. Those effects cannot be prevented by the administration of the posthanglionic blocker pentolinium and by bilateral vagotomy. The inotropic effect of DHE can surely improve circulation and hence anti-edemic and diuretic. The vasopressor effect of synephrine can synergise with DHE in improving peripheral circultion.

The effect of N, N-dimethyl-5-methoxytryptamine can act as a partial agonist of serotonin to minimize the psychotogenic effect of serotonin, hence resulted in analgesia. By the same token, one of the antagonists of serotonin, methysergide, is used for the treatment of migraine. DHE and N, N-dimethyl-5-methoxytryptamine may be responsible for the therapeutic effect *Fructus Evodiae* for headache.

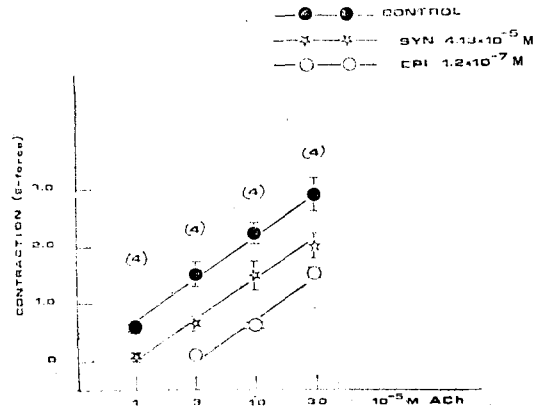


Fig. 9. Relaxation effect of synephrine tartrate(SYN) and epinephrine (EPI) on the stimulatory effect of acetylcholine on isolated rat uterus. The maximum peak contraction was measured as response.

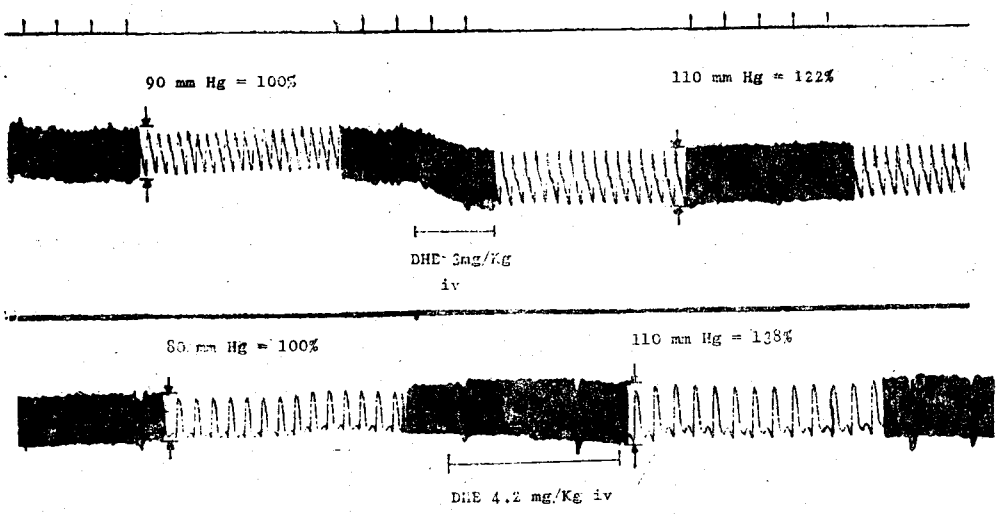


Fig. 10. Inotrophic effect of DHE in intact rat

As we are all aware that the therapeutic value of herbal medicines lies in its chemical constituents. Studies in Chinese medicinal herbs will eventually give an explanation to the therapeutic use of these plants. By quantitative analysia, one can justify whether the plants were used appropriately and give grounds for improvement.

Lastly, it is important to point out that in Chinese medicine, multi-item prescriptions are often used. The therapeutic effect is based on the synergistic and antagonistic actions among individual drugs or compounds. As in the case of *Fructus Evodiae* where many bioactive compounds were found, it is not easy to correlate every therapeutic properties to the known individual compound. Although many pharmaceutical scientists are interested in finding out the active ingredients, the concept that the therapeutic effects are caused by the integrated actions among many compounds will still hold until the principle of medication with Chinese drugs are better known.

Acknowledgements:

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References

1. World Bank Annual Report, Press of 17th August, 1979
2. Chen, A.L. and K.K. Chen: *J. Amer. Pharm. Ass.*, **22**, 716 (1933)
3. Tscheche, E. and W. Werner: *Tetrahedron*, **23**, 1973 (1967)
4. Li, M.T. and H.I. Huang: *Yao Hsueh Hesueh Pao*, **13**, 265 (1966)
5. Kamikado,T., S. Murakoshi and S. Tamura: *Agr. Biol. Chem.* **42**, 1515 (1978)
6. Nakasto, T., S. Asada and K. Marui: *Yakugaku Zasshi*, **82**, 619 (1962)
7. Hirose, Y., K. Kondo, H. Arita and A. Fujita: *Shoyakugaku Zasshi*, **21**, 126 (1967)
8. Kurono, G., S. Aburano and K. Yamada: *Yakugaku Zasshi*, **93**, 691 (1973)
9. Chu, J.H.: *Sci. Rec.*, **4**, 279 (1951)