Pure & Appl. Chem., Vol. 70, No. 3, pp. 547–554, 1998.
Printed in Great Britain.

Biologically active components from traditional Chinese medicines

Lian-Niang Li

Institute of Materia Medica, Chinese Academy of Medical Sciences, Peking Union Medical College, 1 Xian Nong Tan Street, Beijing 100050, China

Abstract. During the last two decades numerous natural compounds with novel structures and various biological activities have been isolated from Chinese traditional and herbal medicines. A new group of depsides with potent antioxidant activities and scavenging effects on free radicals, named salvianolic acids have been isolated from *Salvia miltiorrhiza* and related plants. Lignans and triterpenic acids with various biological activities from *Schisandra chinensis* and related plants not only shed light on the therapeutic effects of these medicinal plants , it also led to the discovery and development of the antihepatitis drug DDB.

INTRODUCTION

China is endowed with abundant resources of medicinal plants. Traditional Chinese medicine represents an accumulation of long periods practical experience in treating human diseases. During the past few decades research and development of these traditional medicines has attracted serious international scientific attention. A great number of these medicinal plants have been more or less investigated chemically in many countries. But to verify their clinical values, find the active principles and take them for new drug design, is not easy . Research and development of new drugs from traditional and herbal medicines is one of the main tasks in our Institute. Utilization of modern scientific techniques and methods as well as facilities for multidisciplinary research, such as phytochemistry combined with pharmacology, have led to the isolation of numerous natural compounds with novel structures and various biological activities. The discovery of these biologically active components provided new kinds of leading compounds for the research and development of new drugs. They also play an important role for the qualification control and quantitative analysis of new preparations from traditional and herbal medicines. The present paper deals with the studies on the biologically active components from the traditional Chinese medicines *Salvia miltiorrhiza, Schisandra chinensis* and related plants.

POLYPHENOLIC ACIDS FROM THE GENUS *SALVIA*

The genus *Salvia* has a variety of more than one hundred species distributed in several regions in China. Thirty of them are used as traditional and folk medicines (ref.1). The dried roots of *Salvia miltiorrhiza,* called Danshen, is the most well known traditional Chinese medicine among these species. It has the effect of "Promoting blood circulation and removing stasis", and is widely used for the treatment of coronary heart diseases, cerebrovascular diseases, hepatitis, hepatocirrhosis, chronic renal failure, dysmenorrhea and neurasthenic insomnia. The chemical constituents of *S. miltiorrhiza* have been studied for more than fifty years, but the studies have mainly been focused on the lipophilic diterpenoid quinones. According to traditional Chinese medicinal prescription it is used as a decoction. Since the seventies injections of

Danshen have been clinically used for the treatment of angina pectoris, myocardial infarction and various types of hepatitis. So there should still be other water soluble active components which are responsible for these biological activities. Studies on the aqueous extract of this medicinal plant yielded thirteen phenolic acids, seven of them were depsides constructed of a β-(3,4-dihydroxyphenyl) lactic acid and a caffeic acid derivative or a caffeic acid dimer forming several types of carbon skeletons. Except rosmarinic acid (**6**) and lithospermic acid (**7**), this type of depside have not been isolated from other plant materials before, so they were given the names salvianolic acid A(**1**), B (**2**), C (**3**), D (**4**) and E (**5**) (ref.2-4). Salvianolic acid F (**8**) and G (**9**) (ref.5,6) were two new polyphenolic acids, the former was a stilbene derivative, while the latter possessed an unusual tetracyclic dibenzooxepin skeleton. The other phenolic components were protocatechuic aldehyde, protocatechuic acid, isoferulic acid and *R*-(+)-β-(3,4-dihydroxyphenyl)lactic acid, named danshensu.

For a long time it was considered that protocatechuic aldehyde and danshensu were the major biologically active components of the aqueous extract of *S. miltiorrhiza.* Comparative studies of these depsides and phenolic components indicated that the antioxidant effects (ref.7) as well as antiplatelet aggregation and antithrombic activities of salvianolic acid A, B and rosmarinic acid were stronger than those of protocatechuic aldehyde and danshensu. Salvianolic acid A, B and rosmarinic acid showed significant scavenging effects on oxygen free radicals, but no scavenging effect was observed for danshensu. The scavenging effects on hydroxyl free radicals of these three depsides were stronger than that of mannitol, while the other phenolic components were inactive (ref.8). Further studies on salvianolic acid A and B showed protective effects on heart and brain injuries induced by ischemia reperfusion. The occurrence of ventricular fibrillation in isolated rat heart was reduced (ref.9). The impairment of learning and memory function induced by brain ischemia reperfusion in mice was improved (ref.10). These results provided a new understanding on the responsible biologically active components of *S. miltiorrhiza* used for the treatment of cardio and cerebrovascular diseases. It was obviously that the activities of these salvianolic acids may be ascribed to their free radical scavenging activities.

Studies on the aqueous extract of other herbal medicines of this genus yielded the same type of depsides. Besides the known salvianolic acids, four additional depsides, isosalvianolic acid C (**10**), salvianolic acid H (**11**), I (**12**) and J (**13**) were isolated from *Salvia chinensis* (ref.11), *Salvia cavaleriei simplifolia* (ref.12), *Salvia cavaleriei* (ref.13) and *Salvia flava* (ref.14). A glycoside of rosmarinic acid named salviaflaside (**14**) and its methyl ester were isolated from the polar fraction of *S. flava* (ref.15). The chemical structures of these depsides and phenolic acids were elucidated by chemical and spectral analysis. The mass spectrum of the methylated depsides showed characteristic fragmentation ions of a 3,4-dimethoxyphenyl lactic acid with *m/z* 222, 191, 181, 165 and 151. Application of 2D NMR such as C,H-COLOC led to the assignment of the dibenzooxepin skeleton of isosalvianolic acid C and the tetracyclic dibenzooxepin skeleton of salvianolic acid G. Salvianolic acid H and I were two *regio* isomeric depsides whose structures were defined by NOE analysis. The location of the aryl and carboxyl groups on the benzodioxane ring of salvianolic acid J was established by selective DEPT experiment. The position of the glucosyl linkage of salviaflaside was determined by HMBC analysis.

The fact that the carbon skeletons of salvianolic acid B, E, G, H, I and J were dimers of caffeic acid linked otherwise than $\beta-\beta'$ suggested that they might be recognized as neolignans (ref. 16). It is noteworthy that this is the first group of neolignans with free phenolic hydroxyls.

The biogenesis of these salvianolic acids may be explained by oxidative coupling of caffeic acid. It is known that caffeic acid possesses a mild antioxidant activity, so apparently the phenolic dihydroxyl group should be a necessary functional group for the antioxidant activities of these depsides. On the other hand the various structural skeletons showed different antioxidant activities. The fact that salvianolic acid A was the most potent depside, suggested that the presence of a highly conjugated double bond may play an important

role in the antioxidant activity.

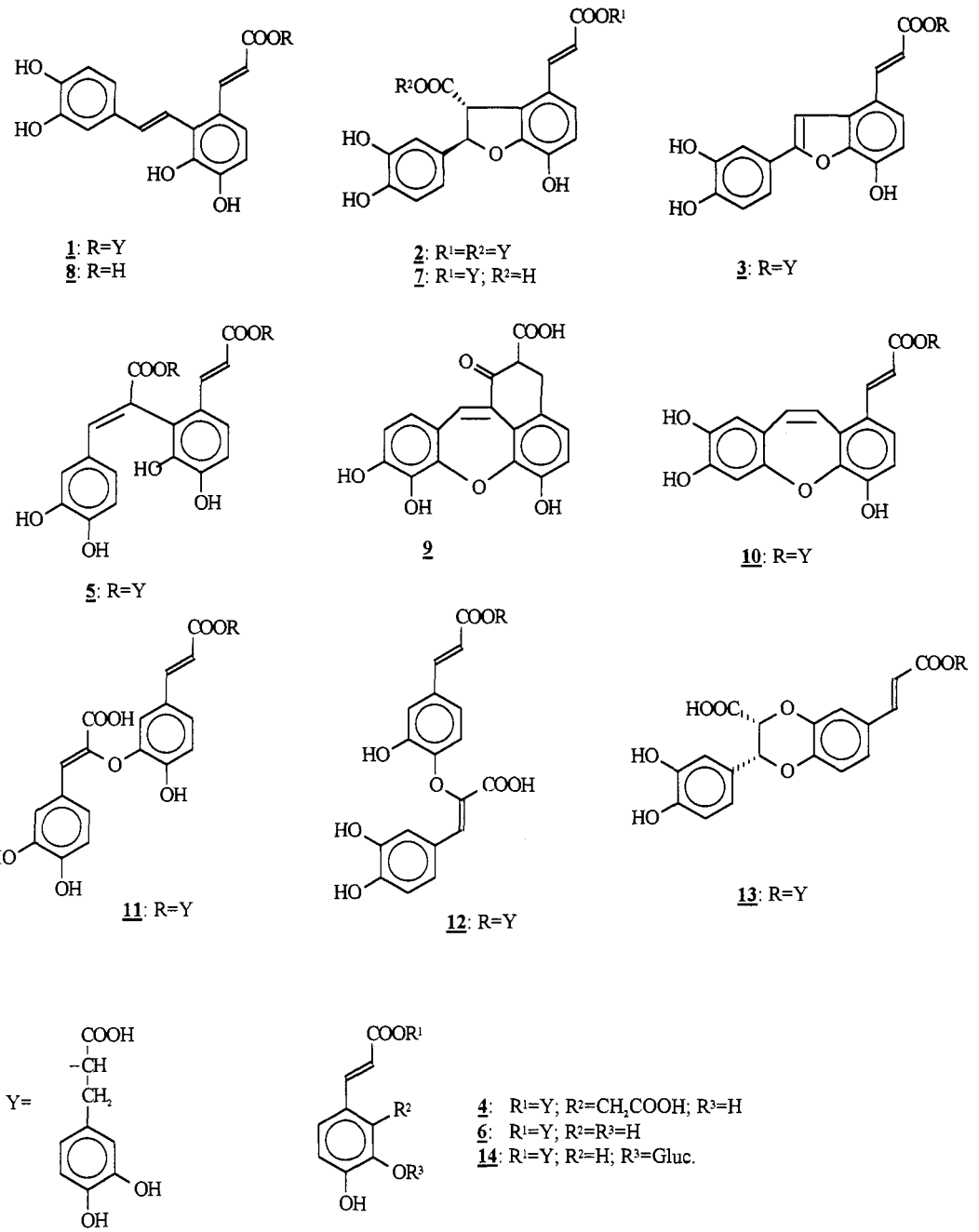


Fig. 1. Depsides and polyphenolic acids from *Salvia* species

LIGNANS FROM SCHISANDRACEAE

The dried fruit of *Schisandra chinensis* (Schisandraceae) is a widely used traditional Chinese medicine, which was listed in classical compendium of Materia Medica as one of the "superior medicines". It is used

as a common ingredient in prescriptions and can also be used alone as a tonic and astringent. During the 1970s. honey pills of *S. chinensis* was used for the treatment of hepatitis with satisfactory results in lowering elevated SGPT levels. Investigation on the biologically active components led to the isolation of seven dibenzocyclooctadiene lignans (ref.17). Pharmacological studies of these lignans showed protective effects against CCL_4 - induced hepatotoxicity in mice and inhibition of lipid peroxidation (ref.18).

The family Schisandraceae consists of the genus *Schisandra* and *Kadsura*, there are more than fifty species of these two genera in China and nineteen species are used as traditional and folk medicines (ref.19). Further studies on the biologically active components of six *Schisandra* species and three *Kadsura* species yielded forty nine lignans. Most of these lignans possess a dibenzocyclooctadiene skeleton in *R* biphenyl configuration twist boat chair (TBC) cyclooctadiene conformation (**I**), *S* configuration TBC conformation (**II**) or *S* configuration twist boat (TB) conformation (**III**). Generally the TBC conformation is more stable than the TB conformation, however the presence of an axial β-hydroxyl group at C-9 and an axial α-methyl group at C-8 may cause a severe crowding with the corresponding benzene ring, therefore a TB conformation for this type of lignans prevails over the TBC conformation (ref.20). Neokadsuranin (**15**) a unique member of this group, possesses an oxygen bridge across the cyclooctadiene ring forming a tetrahydrofuran ring with an envelope conformation (ref.21). From the genus *Kadsura* a group of lignans possessing an α - dienone - spirobenzofuranoid skeleton (**IV**) have been isolated (ref.22). Treatment of this type of lignans with Zn/HOAc under reflux yielded the corresponding dibenzocyclooctadiene lignans of type **II** (ref.23).

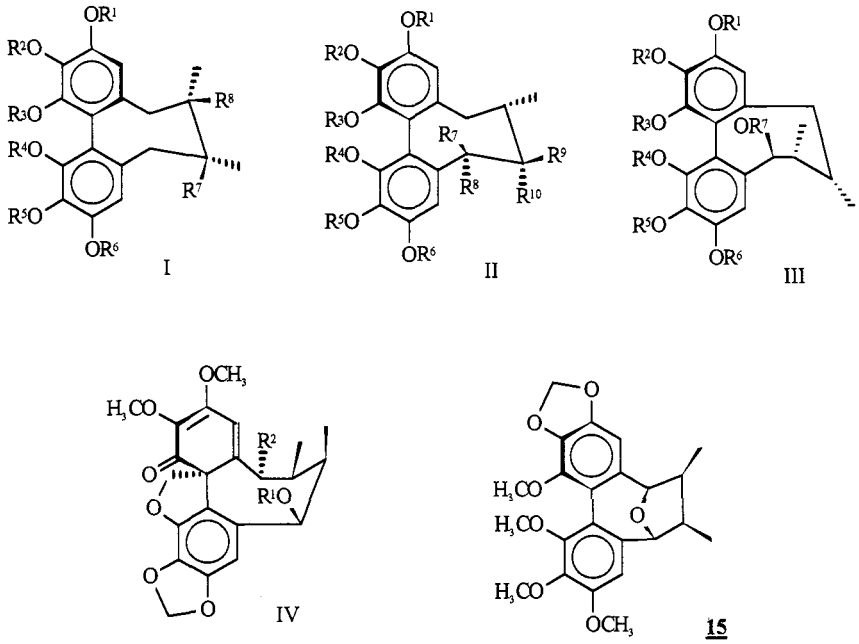
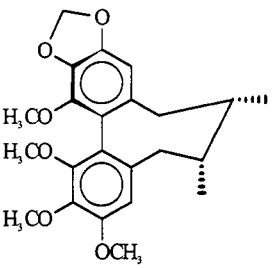


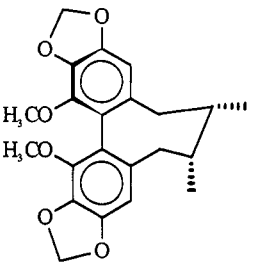
Fig. 2 Stereostructures of dibenzocyclooctadiene lignans

The protective actions of eighteen lignans against CCl_4 induced hepatotoxicity in mice were examined, thirteen of them significantly decreased the elevation of SGPT levels induced by CCl_4. Liver lesions such as inflammation and necrosis were also ameliorated. The relationship between the functional groups as well as the stereostructures of these lignans and their activities were examined. The activities of *S* -wuweizisu B (**16**) and *S* -wuweizisu C (**17**) were stronger than those of their *R* enantiomers. Angeloylgomisin R (**18**) possesses an *S* configuration and the same functional groups on the benzene ring as wuweizisu C, the presence of an additional angeloxy group at C-9 caused a conversion of the TBC conformation to a TB

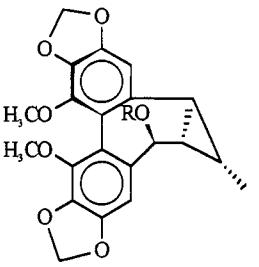
conformation. This compound was less active than wuweizisu C. Wuweizisu C which has two methylene-dioxyl groups at the benzene rings was more active than wuweizisu B which has only one methylene-dioxyl group, while deoxyschisandrin (**19**) which has no methylene-dioxyl group showed only weak activity. The effects of these lignans on lipid peroxidation induced by CCl_4 were measured in vitro. The results indicated that the lignans with hepatoprotective activities considerably inhibited MDA formation induced by CCl_4. However, schisandrol B (**20**) and schisantherin B (**21**) which possess a hydroxyl group at the cyclooctadiene ring showed only weak inhibition of MDA formation. On the other hand the antioxidation effect of schisanhenol(**22**) which has an *R* configuration and a hydroxyl group instead of a methylene-dioxyl at the benzene group, was the strongest among these lignans, but it only showed weak activity in lowering elevated SGPT level (ref.24).



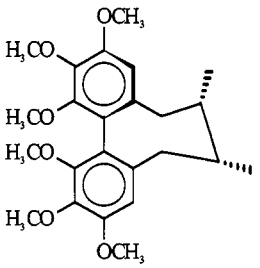
16



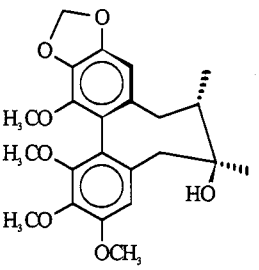
17



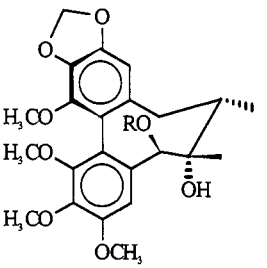
18 R=Ang



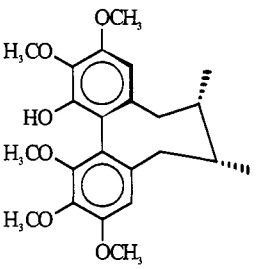
19



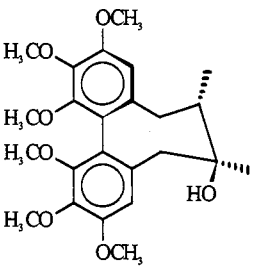
20



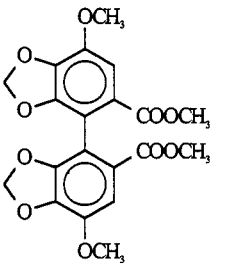
21 R=Ang



22



23



DDB

Further pharmacological studies on wuweizisu B which is the major active component of *S. chinensis* showed strong protective effects on lipid peroxidation damage of cultured rat hepatocytes surface treated by Fe^{2+}/cystein. It also scavenged free radicals. Oral administration of wuweizisu B significantly increased the activities of antioxidant enzymes. The ethanolic extract of *S. chinensis* as well as wuweizisu B showed stimulation of liver glycogenesis and serum lipid protein biosynthesis. All these results are of importance for

the protection and repairing of liver injuries (ref.25).

In addition, schisandrin (**23**) which had no effect on the liver function and only weak antioxidant activity, possessed extensive inhibiting effects on the CNS, which is characteristic of neuroleptics (ref.26).

The different activities of these lignans not only provided an understanding on the pharmacological bases of *S.chinensis* as an antihepatitis drug and sedative, they also shed light on the clinical effects of this traditional Chinese medicine used as a tonic and antiaging remedy.

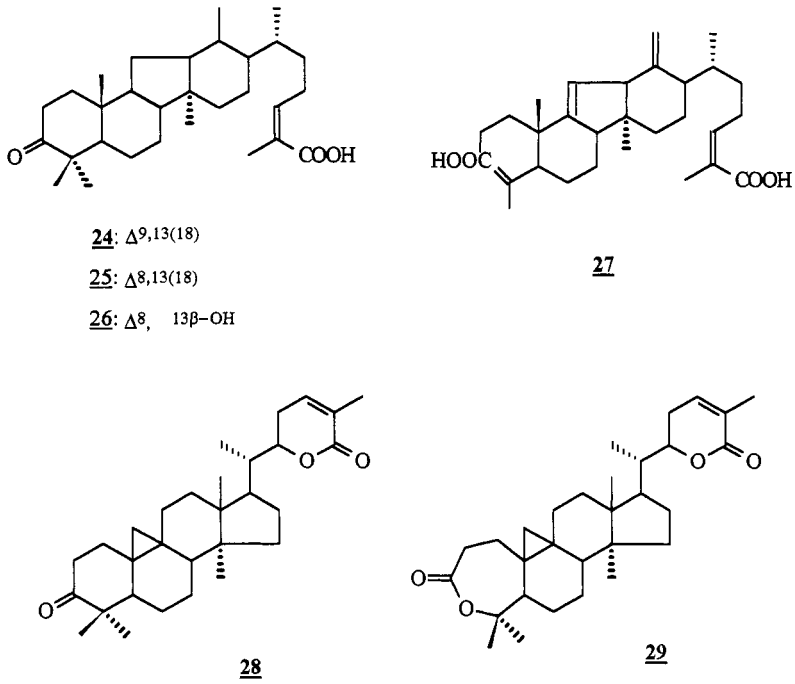
During the total synthesis of wuweizisu C and its analogues, one of the intermediates, the substituted biphenyl ester (DDB) also showed significant hepatoprotective activities, inhibition of lipid peroxidation and induction of liver microsomal cytochrome P-450. Its toxicity was very low. Clinical trials on a large number of patients with chronic hepatitis showed that about 85% of these patients showed reduction of SGPT level and relief of other symptoms. DDB was more active than silymarin (Legalon) and glycyrrhizin (Stronger Neo Minophagen C.SNMC) in parallel clinical trials. It is also highly effective for the treatment of drug induced hepatitis. Pilulles of DDB are now manufactured in China and exported to several countries. The discovery of DDB from the studies of *S. chinensis* is a successful example of traditional Chinese medicine as a lead for new drug research (ref.27).

TRITERPENOIDS FROM SCHISANDRACEAE

Triterpenic acids with a C-24(25) double bond and a vinylic carboxyl in the side chain have been isolated from *Schisandra* as well *Kadsura* species. Besides the classical lanostane type triterpenoids, a group of triterpenic acids possessing an unusual 14(13→ 12)*abeo* lanostane skeleton , named neokadsuranic acid A (**24**), B (**25**), C (**26**) and seco-neokadsuranic acid A (**27**) were isolated from *K. longipedunculata* (ref.28) and *K. heteroclita* (ref.29). The occurrence of a 12-hydroxylanostane triterpenic acid together with this type of triterpenic acids in the same plant suggested the possibility that the biogenesis of neokadsurane triterpenoids might conceivable arise via a rearrangement pathway similar to Wagner-Meerwein rearrangement.

Two triterpenic lactones, kadsulactone (**28**) and kadsudilactone (**29**) were isolated from the roots of *K longipedunculata* and *K. coccinea* (ref.30). They all possessed an α , β unsaturated six membered lactone ring in the side chain. The CD spectrum of kadsulactone showed a negative Cotton effect at 300 nm and a positive Cotton effect at 258 nm. The former was similar to that of schisandronic acid, which possessed a 3-oxo;9,19-cyclolanostane skeleton, while the latter indicated a 22*R* configuration for the six membered lactone ring. Kadsudilactone showed only a positive Cotton effect at 260 nm, due to the replacement of a seven membered lactone ring for the cyclohexanone.

Pharmacological screening of twelve triterpenic acids showed inhibition of cholesterol biosynthesis. (24Z)-3-Oxo-lanosta-8,24-dien-26-oic acid (3-oxo-LA) was the most potent in depressing cholesterol biosynthesis from [2-^{14}C]mevalonic acid in the supernatant fraction of rat liver homogenate, it showed an 82% inhibition at 25μg/ml. The neokadsurane triterpenic acids were only moderately active. It was suggested from TLC analysis of radio-labeled products that these compounds inhibited the demethylation of lanosterol, 3-oxo-LA also inhibited cholesterol biosynthesis from [1-^{14}C]acetate in primary cultures of rat liver cells, while no cytotoxicity was observed. This result suggested that 3-oxo-LA has the possibility to be an antihyperlipidemic agent with low toxicity.



24: $\Delta^{9,13(18)}$

25: $\Delta^{8,13(18)}$

26: Δ^{8}, 13β–OH

27

28

29

Fig. 3 Triterpenoids from*Kadsura* species

The total triterpenic acid (containing kadsuric acid and nigranoic acid) from *K. longipedunculata*, showed prevention of gastric mucosal lesions induced by indomethacin and absolute alcohol, as well as inhibition of stress-induced gastric ulceration (ref.32). The roots of *K. longipedunculata* is used for the treatment of rheumatoid arthritis, gastric and duodenal ulcer (ref.33). The anti-ulcer activities of these triterpenic acids are in agreement with the clinical indications of their source plant.

CONCLUSION

Traditional Chinese medicines are based on long periods of practical experiences in treating human diseases. They provide medicinal chemist a vast, fertile field to be developed. However to verify the clinical values of these medicinal plants is a complicated task which requires collaboration of scientists in various fields and utilization of modern scientific techniques as well as knowledge of Chinese traditionally medical theory. The studies on the biologically active components of *Salvia miltiorrhiza*, *Schisandra chinensis* and related plants illustrated the potential value of leads for drug development, concealed in traditional Chinese medicines.

REFERENCE

1. S.L.Huang. In *Danshen-Biology and Its Application* (R.S.Xu,ed.), pp. 23-25, Science Press, Beijing (1991).
2. L.N.Li, R.Tan and W.M.Chen. *Planta Med.* **50**, 227-228 (1984).
3. C.B.Ai and L.N.Li. *J. Nat. Prod.* **51**, 145-149 (1988).
4. C.B.Ai and L.N.Li. *Planta Med.* **58**, 197-199 (1992).
5. C.B.Ai and L.N.Li. *Chinese Chem. Lett.* 7, 427-430 (1996).

6. C.B.Ai and L.N.Li. *Chinese Chem. Lett.* **2**, 17-18 (1991).
7. G.T.Liu, T.M.Zhang, B.E.Wang and Y.M.Wang. *Biochem. Pharmacology* **43**, 147-152 (1992).
8. L.N.Xu, D.Y.Li and J.Y.Tian. *Proceedings of the 3th National Symposium of Activating Blood Circulation and Removing Stasis Research,* pp. 195-201, Xue Yuan Press, Beijing 1990.
9. G.H.Du, Y.Qiu and J.T.Zhang. *Acta Pharm. Sin.* **30**, 731-735 (1995).
10. G.H.Du and J.T.Zhang. *Acta Pharm. Sin.* **30**, 184-190 (1995).
11. T.X.Qian and L.N.Li. *Phytochemistry* **31**, 1068-1070 (1992).
12. H.J.Zhang and L.N.Li. *Chinese Chem. Lett.* **4**, 501-504 (1993).
13. H.J.Zhang and L.N.Li. *Planta Med.* **60**, 70-72 (1994).
14. C.B.Ai, Q.H.Deng, W.Z.Song and LN.Li. *Phytochemistry* **37**, 907-908 (1994).
15. L.M.Zhao, W.Y.He, X.T.Liang and L.N.Li. *Chinese Chem. Lett.* 7, 449-452 (1996).
16. O.R.Gotttlieb. *Phytochemistry* **11**, 1537-1570 (1972).
17. Y.Y.Chen, Z.B.Shu and L.N.Li. *Scientia Sin.* **19**, 276-290 (1976).
18. T.T.Bao, G.T.Liu, Z.Y.Song, G.F.Xu and R.H.Sun. *Chinese Med. J.* **93**, 41-47 (1980).
19. W.Z.Song and P.G.Xiao. *Chinese Traditional and Herbal Drugs* **13**, 40-48 (1982).
20. L.N.Li. *Abstract of Chinese Medicines* **3**, 414-428 (1989).
21. L.N.Li, X.J.Qi, D.L.Ge and M.Kung. *Planta Med.* **54**, 45-46 (1988).
22. L.N.Li and H.Xue. *Phytochemistry* **29**, 2730-2732 (1990).
23. L.N.Li, H.Xue and X.Li. *Planta Med.* **57**, 169-171 (1991).
24. G.T.Liu. *Proceedings of TMMP*, pp.78-86, The People's Medical Publishing House, Beijing (1987).
25. G.T.Liu. *Recent Advances in Chinese Herbal Drugs* (J.H.Zhou, G.Z.Liu, eds.), pp. 100-111, Science Press, Beijing (1991).
26. X.Y.Niu, W.J.Wang, Z.J.Bian and Z.H.Ren. *Acta Pharm. Sin.* **18**, 416-421 (1983).
27. G.T.Liu. *Recent Advances in Chinese Herbal Drugs* (J.H.Zhou, G.Z.Liu, eds.), pp112-125, Science Press, Beijing (1991).
28. L.N.Li, H.Xue, K.Kangouri, A.Ikeda and S.Omura. *Planta Med.* **55**, 294-296 (1989).
29. L.N.Li, H.Xue, D.L.Ge, K.Kangouri, T.Miyoshi and S.Omura. *Planta Med.* **55**,300-302 (1989).
30. R.Tan, H.Xue and L.N.Li. *Planta Med.* **57**,87-88 (1991).
31. K.Kangouri, T.Miyoshi, A.Ikeda, S.Omura, L.N.Li and H.Xue. *Agric. Biol. Chem.* **54**,993-997 (1990).
32. S.R.Zhang. *Chinese Traditional and Herbal Drugs* **21**, 27-28 (1990).
33. *Compilation of Chinese Herb Medicine,* Vol. **1**, p. 581, People's Publishing House, Beijing (1988).