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STUDIES ON BIOACTIVE COMPONENTS FROM CHINESE MEDICINAL PLANTS

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Several novel bioactive components isolated from Chinese medicinal plants will be presented. These include novel maytansinoid tumor inhibitors, some new ent-kaurane and rosane diterpenoids from Mallotus anomalus *Meer et Chun (Euphorbiaceae), as well as novel insecticide,* stemona *alkaloids from* Stemona parviflora *C. H. Wright (Stemonaceae). Both are native plants of Hainan island, China. 2D NMR techniques such as mono and hetero-COSY, NOESY, COLOC as well as* ^{1}*H-NMR line broadening effect were utilized for structure elucidation. The separation techniques, structure elucidations and bioassay results will be reported.*

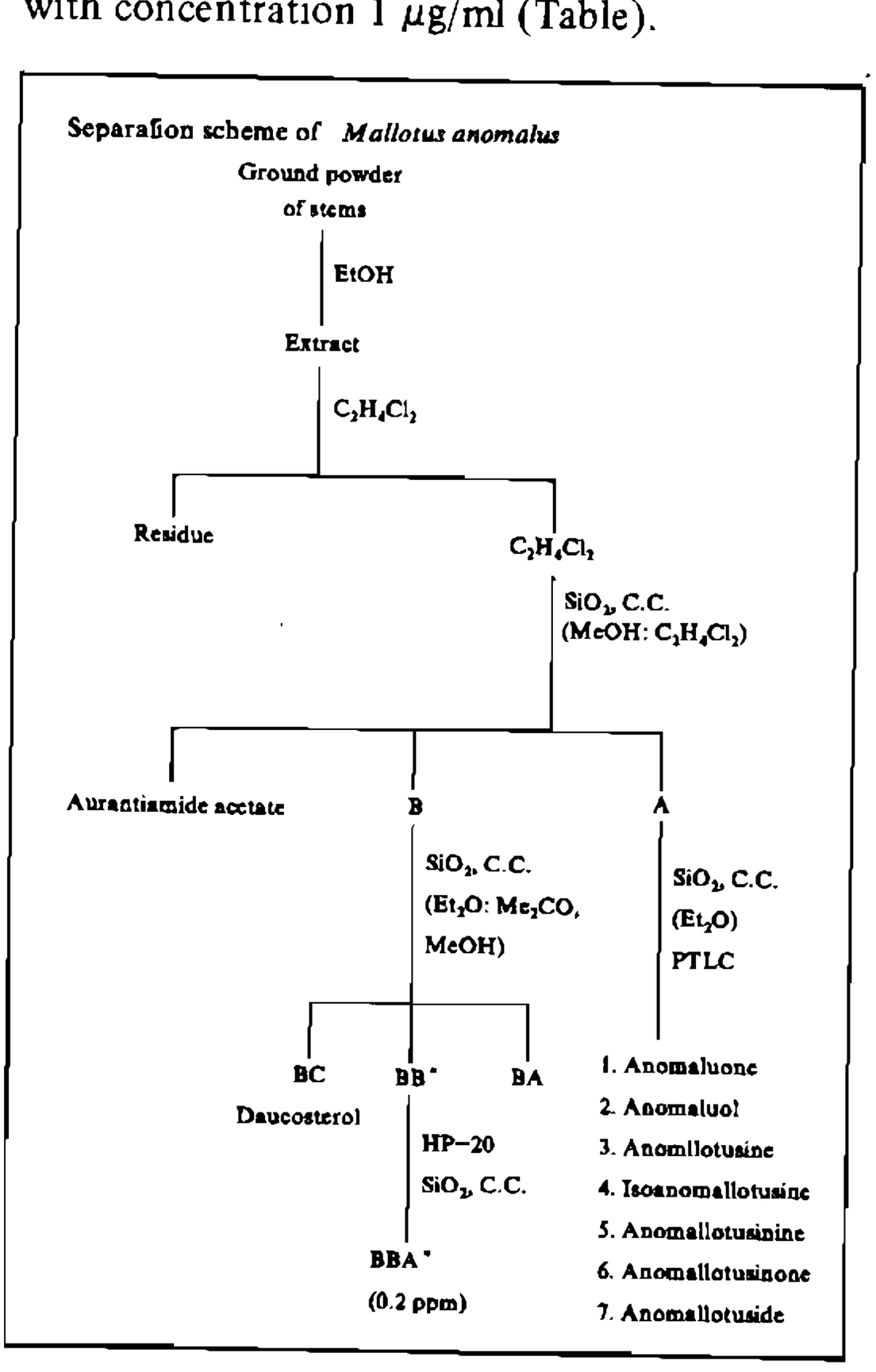
Key words: *Mallotus anomalus* – Euphorbiaceae – *Stemona parviflora* – Stemonoceae – ent-kourane – rosane – delabradane diterpenoids – maytansinoid – *stemona* alkaloids – 2D NMR techniques

China is a country rich in plant resources and long history of using medicinal plants. The tropic island, Hainan island, is the second largest island in China with area 33,000 km^2. There is a primeval forest and many plants are unexploited. Up-to-now we know at least 600 species of plants are special in the world due to its special natural enviroment and most of them have not been studied. These facts attract scientists to study them enthusiastically. Dozens of interesting compounds have been isolated from these plants, e.g. discovering of curine from *Cyclea barbata* and *C. hainanensis* (Xu et al., 1985), and many have new structures. Here several novel bioactive compounds from two such special plants will be reported.

Mallotus anomalus Meer et Chun

It belongs to the family Euphorbiaceae. It is a special shrub growing in some southern mountain area of the island. During our screening seaching for new anticancer compounds from the plants we found the ethylene chloride soluble fraction of the ethanol extract of the shrub showed significant activity *in vitro* and *in vivo* tests. Thus we start to study its chemical components systematically parallel with anticancer and other bioassays.

According to the following scheme an anticancer active substance BBA was separates from the overground part of the shrub. The BBA is white needles with m. p. 167-171 ^{o}C. Pharmacological test showed it exhibited obvious activity as high as maytansine. It prolonged the survival of Ehrlich ascites carsinoma mice 167.3% in a dose of 10 μg/kg and *in vitro* test it inhibited leukemia cell P_{388} 99% with concentration 1 μg/ml (Table).



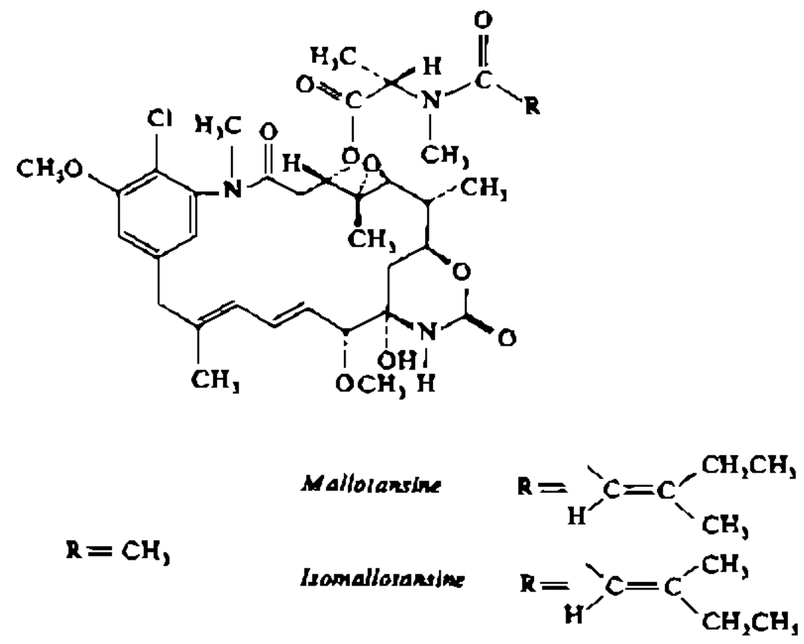
TABLE

Tumor inhibition of extract

Fraction	*in vivo* (EAC) dose	prolongation	*in vitro* (P388) dose	inhibition
B	15 mg/kg	180%	1 μg/kg	80%
BB	0.3mg/kg	267.7%	1 μg/kg	98%
BBA	10 μg/kg	167.3%	1 μg/kg	99.5%

The DC1-MS of the molecular ion m/z 745 and ^{13}C-NMR DEPT analyses indicated BBA has the molecular formula $C_{38}H_{52}NClO_3$. Further careful examination of its MS and NMR spectral data and comparison with that of maytansine showed both have the same basic ansamacroring and similar side chain, but BBA has one more isoprene group instead of the end metyl group of the side chain in maytansine. The high-resolution EIMS showed BBA had similar fragments as maytansine: m/z 684 (M^+-61) due to loss of HCNO and H_2O from the cyclic carbamate and m/z 485 due to further loss of the whole side chain. Moreover the NMR spectra predicted BBA is a mixture of two isomers, namely, mallotusine and isomallotusine, with ratio of equal quantity. It was further confirmed by HPLC and could be separated by lichrosobar CN column with fluent solvent CH_2Cl_2 : Et_2O : n-C_6H_{14} : H_2O 70 : 25 : 5 : 0.5. Both isomers are stereoisomer on the double bond of the end isoprene group. Their proton NMR data showed in the Fig. 1.

1



Besides the above mentioned active substances, 7 new diterpenoids were also isolated. The structures of two new ent-kaurane type, i.e. anomaluone ($C_{20}H_{30}O_3$, M. P. 193-194 °C, $[\alpha]_D$ −76.9 ° (MeOH)) and anomaluol ($C_{20}H_{34}O_4$, M. P. 241-244 °C, $[\alpha]_D$ 309.86 ° (Py)) were deduced by ^{1}H-NMR and ^{13}C-NMR spectral analyses including the noesy experiments and the structure of compound anomaluone was confirmed by X-ray diffraction method (Fig. 2).

The structures of other 5 compounds (Fig. 3) are new rare occured rosane type and dolabradone type diterpenoids.

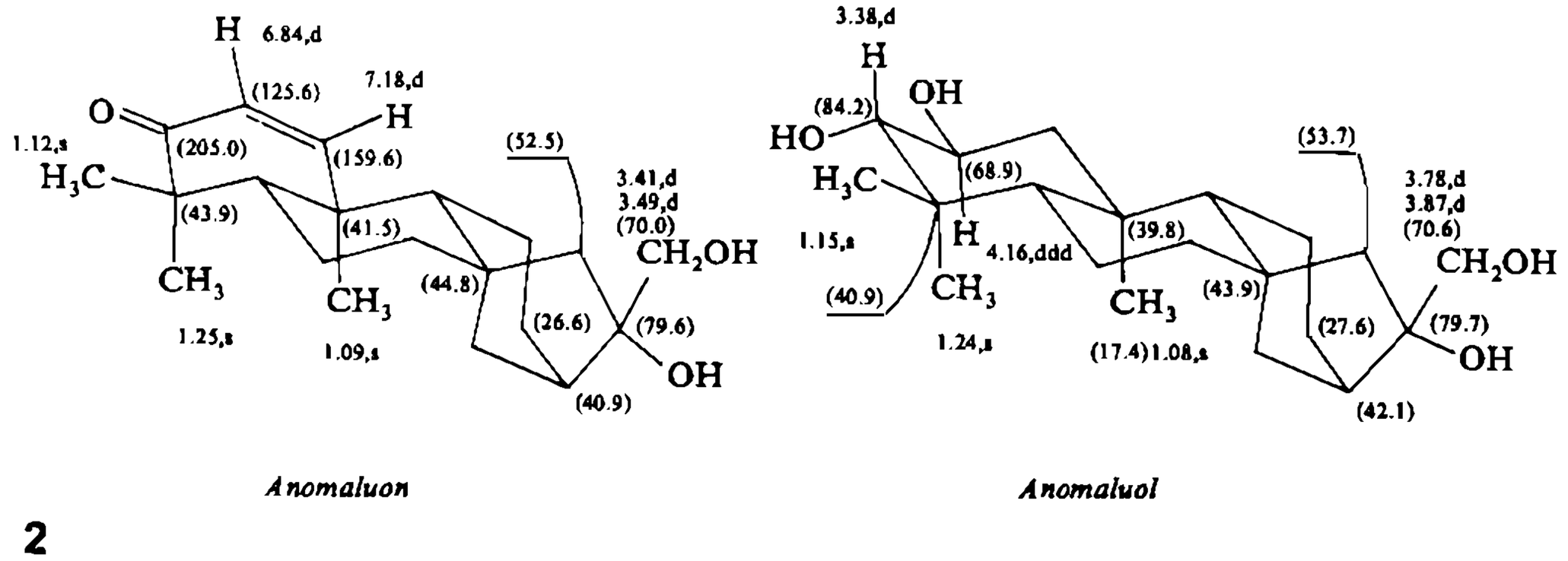
anomallotusin; $C_{20}H_{32}O_3$; m.p. 155-156 °C; $[\alpha]_D$ 52.6 °C (MeOH)

isoanomallotusin; $C_{20}H_{32}O_3$; m.p. 213-215 °C; $[\alpha]_D$ 102.9 ° (MeOH)

anomallotusinin; $C_{20}H_{32}O_3$; m.p. 175-176 °C; $[\alpha]_D$ 117.6 ° ($CHCl_3$)

anomallotuside; $C_{20}H_{32}O_2$; m.p. 186-188 °C; $[\alpha]_D$ −57.1 ° ($CHCl_3$)

anomallotusinon; $C_{20}H_{28}O_2$; m.p. 152-154 °C; $[\alpha]_D$ 43.4 ° ($CHCl_3$).



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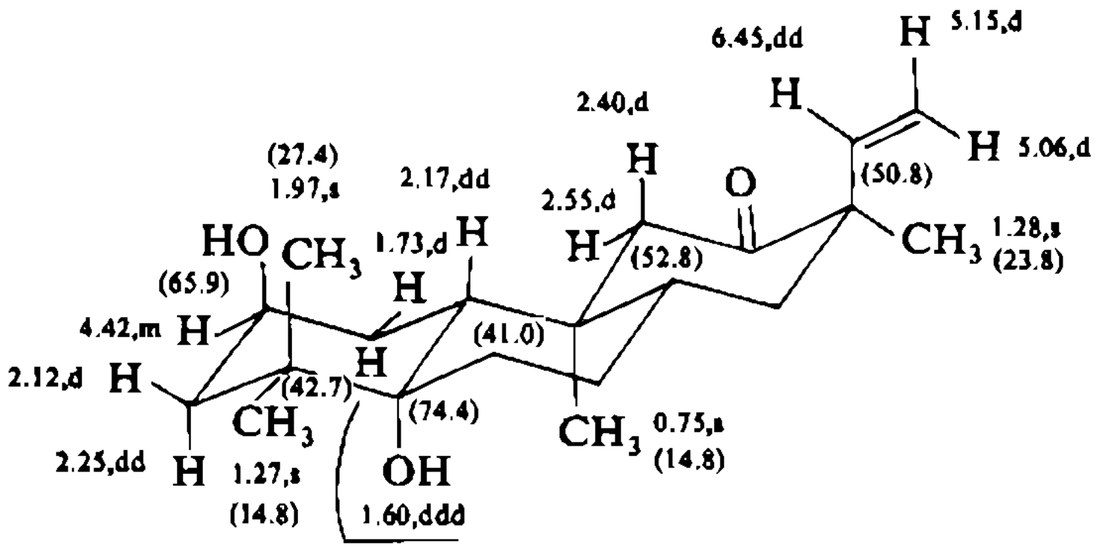
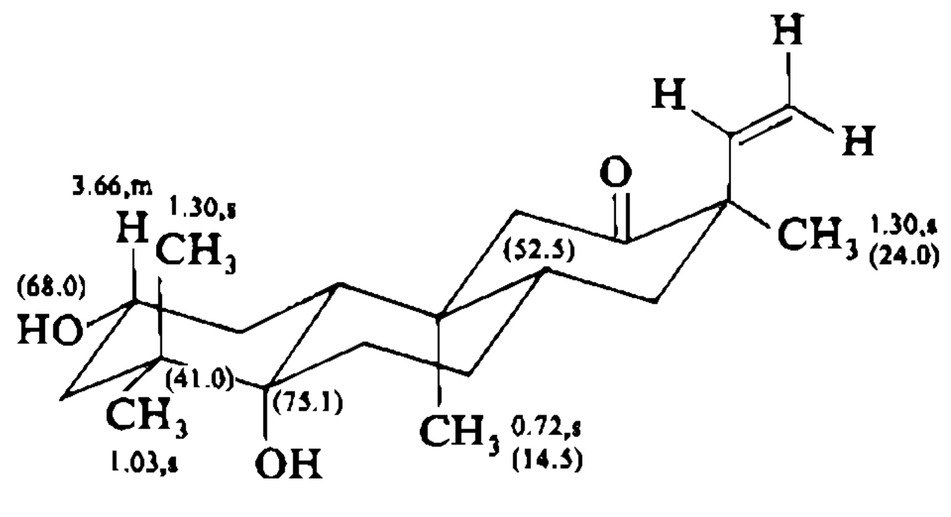
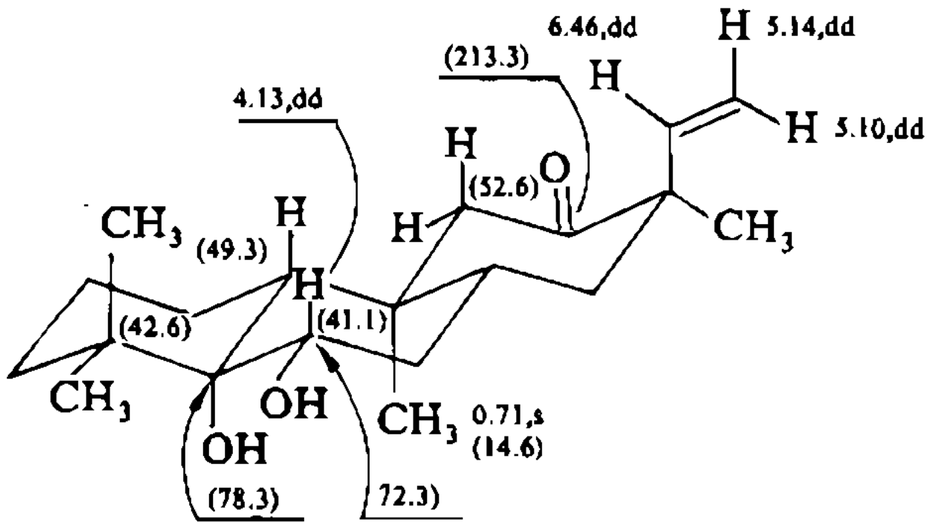


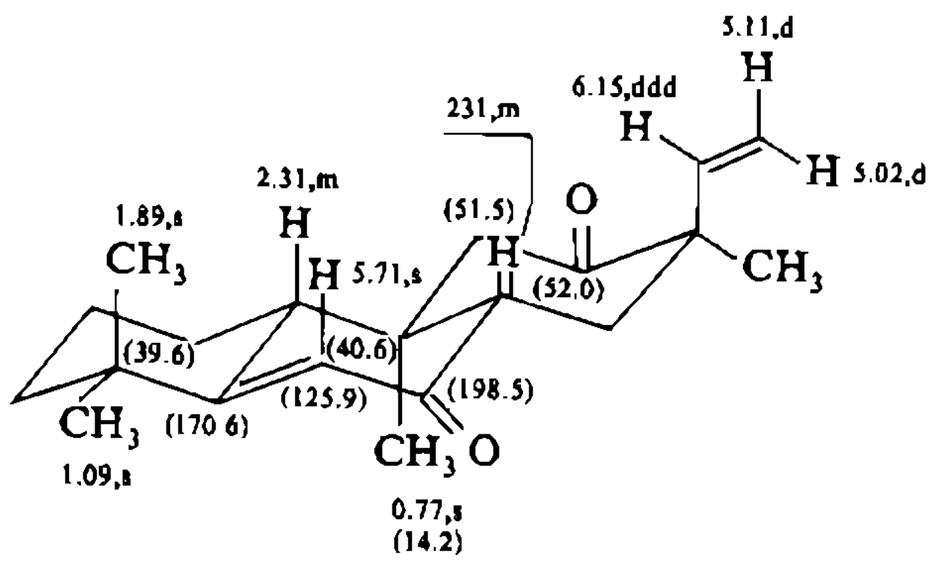
Anomallotusin



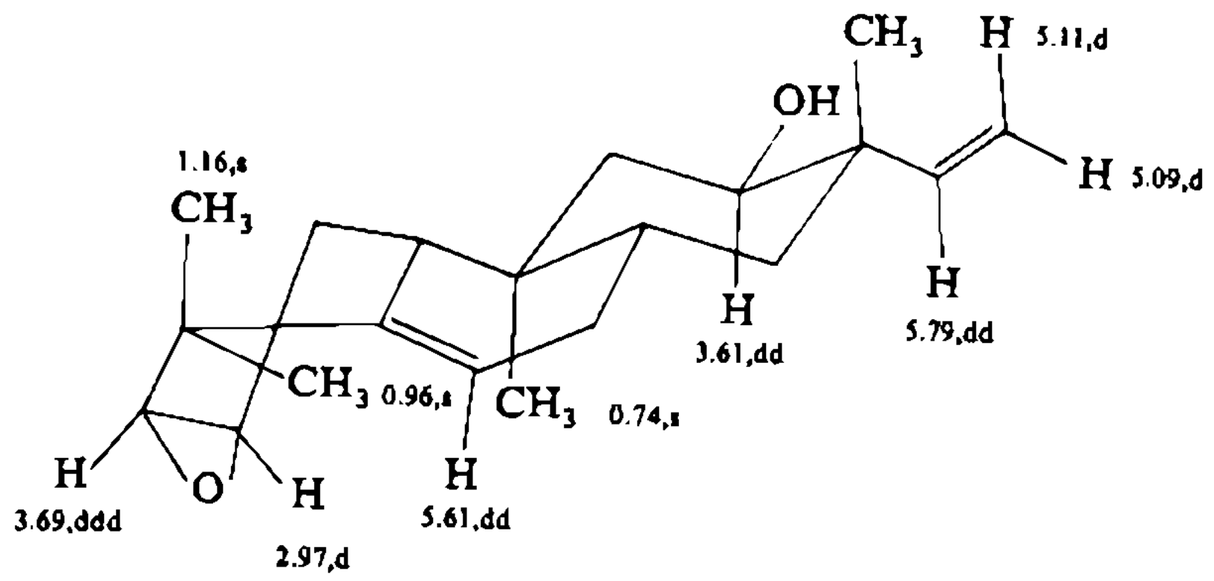
Isoanomallotusin



Anomallotusinin



Anomallotusinon



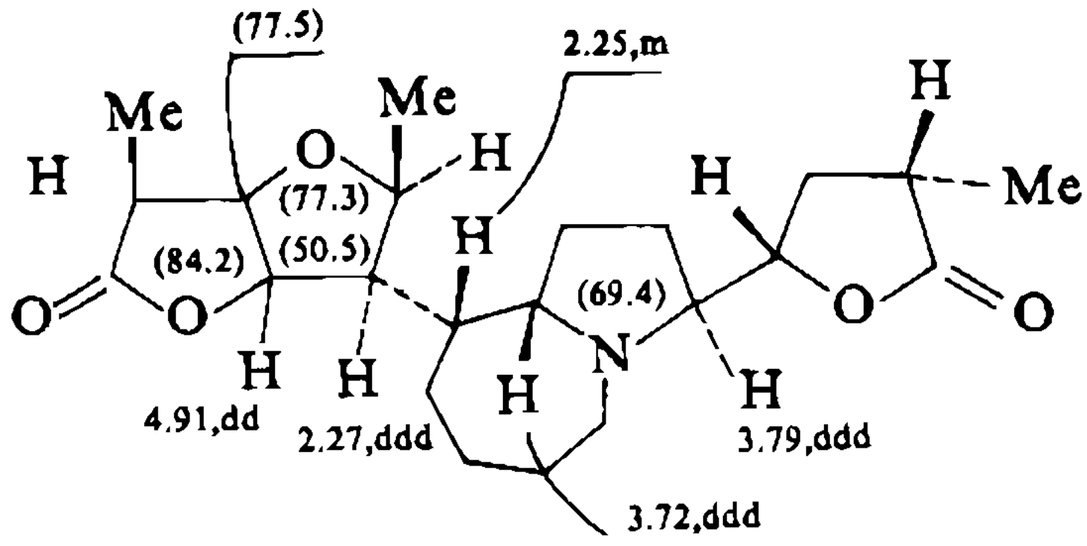
3 *Anomallotuside*

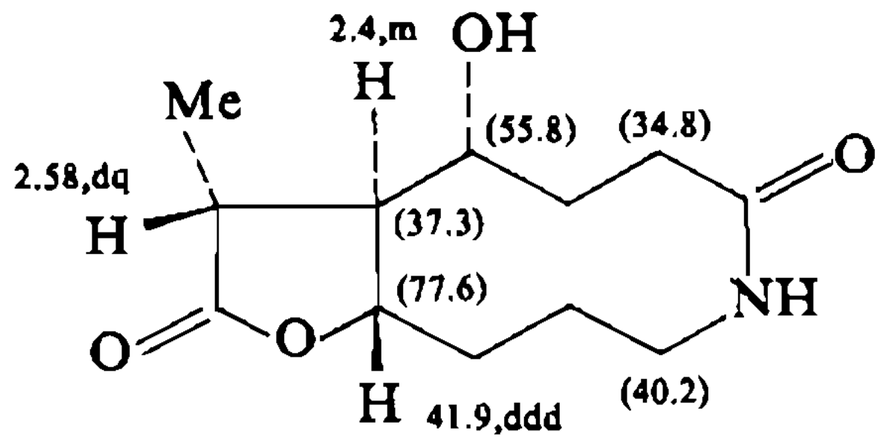
The NMR spectra of these compounds showed they had characteristic C-20 methyl group at the high field. For anomallotusin: C-20 methyl at δc 14.79 ppm., its H-10 signal 2.17 (dd, J = 13.0; 2.5 Hz) couples only with H-1. Two geminate protons located at C-11 2.40 and 2.55 (each d, J = 13.9 Hz) do not couple with other protons. All of their configurations were determined by 2D NMR techniques including mono- and hetero-cosy, noesy and long range cosy spectral analyses. Their basic data are cited in Fig. 4.

Isoanomallotusin showed high inhibition effect on P_{388} cell but inactive *in vivo* test.

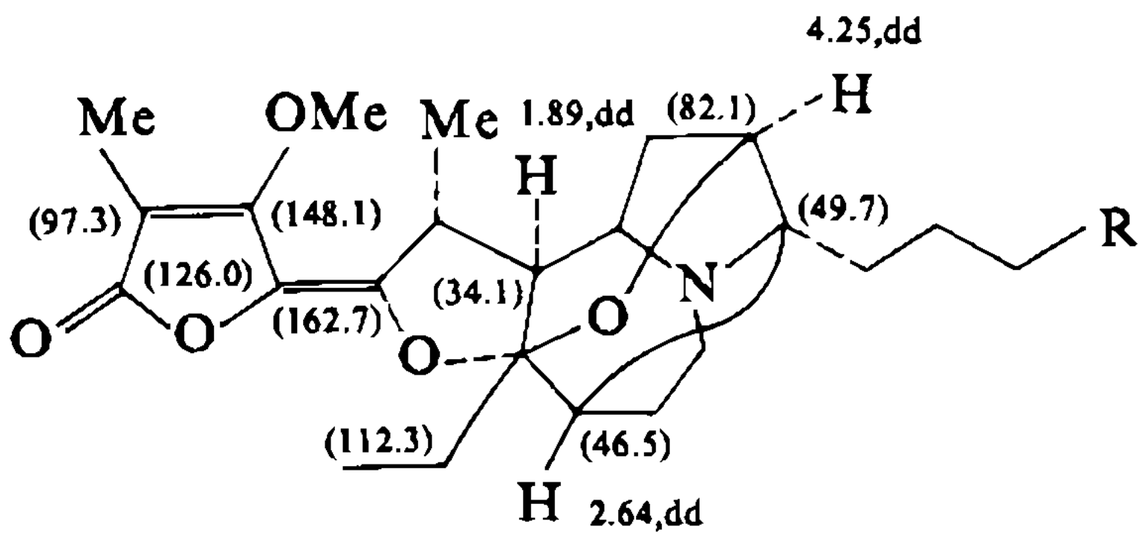
Stemona parviflora C. H. Wright

It belongs to the family stemonaceae. It is an another special plant growing in Hainan Island. The roots of *Stemona* plants are used in Chinese traditional medicine as anticough agent and insectiside. It was reported the active components are alkaloids (Sakata et al., 1978). Almost all structures of *Stemona* alkaloids (about 8) were determined by X-ray diffraction method due to their unstability and complexity (Gotz & Strunz, 1975). Most of theirstructures are quite different. In 1984 we first determined the structure of stemotinine and isostemotinine isolated from *Stemona tuberosa*, growing in





Parvistemonine *Parvistemoamide*



$R = CH_3$ *Parvistemonine* $R = CH_2OH$ *Parvistemoninol*

4

Yunnan province, by spectral analyses (Xu et al., 1982) and later the structure of stemotinine was proved by X-ray idffraction method (Xu et al., 1986). At the same time we discovered a ^{1}H-HMR line broadening effect, i.e. addition of a trace quantity of DCl resulted in extensive broadening of the signals due to protons spatially closed to the nitrogen lone pair. The amount of DCl should be that the customary down-field shift accompanying with the formation of ammonium ions was as yet not observable. Later we found the most suitable amount of DCl is one sixth mole and the distinguished line broadening effect appeared when protons in the molecule were fare in chain linkage but spatially closed to the nitrogen lone pair. The protons neighboured with nitrogen atom can be recognized according to the calculated shifed data after adding the DCl: *cis* protons to the nitrogen lone pair are more shifted than *trans*. This effect was helpful to clarify subtle structural differences of the alkaloids especially in the structure elucidation of the *Stemona* alkaloids. (He et al., 1990).

Four new alkaloids were separated from the roots of *St. parviflora* by using flash chromatography method:

parvistemonine; $C_{22}H_{33}NO_5$; m.p. 295-296 °C; $[\alpha]_D$ 26.6 ° (MeOH)

parvistemoamide; $C_{12}H_{19}NO_4$; m.p. 197-198 °C

parvistemoninine; $C_{22}H_{23}NO_5$; m.p. 76-78 °C; $[\alpha]_D$ −256.6 ° (MeOH)

parvistemoninol; $C_{22}H_{29}NO_6$; m.p. 224-226 °C; $[\alpha]_D$ 106° (MeOH)

Their structures were elucidated by spectral analyses including 2D NMR techniques; mono- and hetero-nuclear cosy, noesy and coloc as well as the ^{1}H-NMR line broadening effect. The parvistemoninine is optical antipode of stemofoline (Irie et al., 1970) and the structures of parvistemonine have been confirmed by X-ray crystalographical method.

The bioassay of these new alkaloids are under progress.

Continued research on bioactive components from plants of Hainan island will hopefully bring us the beneficial inspiration in discovery of new drugs (Fig. 4).

REFERENCES

GOTZ, M. & STRUNZ, G. M., 1975. Tuberostemonine and related compounds: the chemistry of Stemona alkaloids, p. 143-160. In K. Wiesner, *Alkaloid*. Butternorth & Co., London.

HE, X.; LIN, W. H.; XU, R. S. & ZHONG, Q. X., 1990. The ^{1}H-nmr line broadening effect and its application in determination of some alkaloid configurations. *Acta Chim. Sin.*, *48*: 691-699.

IRIE, M.; MASAKI, N.; OHON, K.; OSAKI, K.; TAGA, T. & UYEO, S., 1970. The crytal structure of a new alkaloid, stemofoline, from Stemona. *Japonica, Chem. Commun.*, p. 1066.

LIN, W. H.; YING, B. P.; TANG, Z. J.; XU, R. S. & ZONG, Q. X., 1990. The structure of parvistemonine. *Acta Chim. Sin.*, *48*: 811-814.

SAKATA, K.; KATSUYUKI, A.; HANG, C. F.; SAKURAI, A.; TAMURA, S. & MURAKOSHI, S., 1978. Stemospironine, a new insecticidal alkaloid of Stemona iaponica Miq. isolation structural determination and activity. *Agric. Biol. Chem.*, *42*: 457-463.

XU, R. S.; LIU, Y. W.; YAO, J. X. & MAK, J. C. W., 1986. Crystal and molecular structure of Stemotinine. *J. Struct. Chem.*, *5*: 97-100.

XU, R. S.; LU, Y. J.; CHU, J. X.; IWSHITA, T.; NAOKI, H.; NAYA, Y. & NAKANISHI, K., 1982. Studies on some new Stemona alkaloids. *Tetrahedron*, *38*: 2667-2670.

XU, R. S.; ZHU, Q. Z. & XIE, Y. Y., 1985. Recent advances in studies on Chinese medicinal herbs with physiological activity. *J. Ethnopharmacol.*, *14*: 223-253.