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SOME PROGRESS ON THE CHEMISTRY OF NATURAL BIOACTIVE TERPENOIDS FROM CHINESE MEDICINAL PLANTS

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(1) Pseudolaric acids – Novel diterpenes, Pseudolaric acid A, B, C and D were isolated from Pseudolarix kaempferi *Gorden (pinaceae). Their structures were assigned by spectroscopic data and chemical correlations. In the continueous studies, the absolute configurations, the conformations in the solutions, the fragmentation mechanisms of MS and assigments of all NMR spectral signals were also reported. They showed the antifungal and cytotoxic activities. (2) – Daphnane diterpenes – In the further studies on the plants of Thymelaeaceae, besides 10 known diterpenes, 16 new daphnane diterpenes were isolated from* Daphne genkwa, D. tangutica, D. giraldii, Wikstroemie chamaedaphne. *They showed the antifertilities activities. (3)* Tripterygium *diterpenes – 14 new diterpenes were isolated from* Triperygium wilfordii, T. regeli *and* T. hypoglaucum. *Some of them showed the antitumour activities. The CD spectra showed that A/B ring of all compounds have trans configuration as same as tripdiolide and triptolide determined by X-ray diffraction. (4) Pregnane glycosides from* Marsdenia koi – *Two new pregnane glycosides marsdenikoiside A and marddenikoiside B which can terminate the early pregnancy were isolated from* Marsdeia koi. *Their structures were elucidated by hydrolysis and spectroscopic methods.*

Key words: pseudolaricie acids – daphnane diterpenes – tripterygium diterpenes – pregnan glycosides – structures – pharmacology

China is a country with abundant resources of medicinal plants for treatment of various kinds of human diseases for thousands years and with the rich experiences in Chinese folk medicine. The studies on the Chinese herbs play a very important role in the development of new drugs. This paper is dealing with the bioactive terpenes isolated from *Pseudolarix*, *Tripterygium*, *Daphne* and *Marsdenia* species.

1. PSEUDOLARIC ACIDS FROM *PSEUDOLARIX KAEMPFERI*

The bark of *Pseudolarix kaempferi* Gordon (Pinaceae), its Chinese name: Tu-Jin Pi, has been used in Chinese folk medicine as a fungicide for a long time. The active compounds, pseudolaric acid A, B, C and D were isolated from acidic fraction by silica gel chromatography. Li et al. (1982) and Zhou et al. (1983) reported the structure elucidation of pseudolaric acid B by its ^{1}H NMR, ^{13}C NMR and MS spectra data and the spectral data of dehydroproduct 5.

Pseudolaric acid A was identified by chemical correlation (Figs 1, 2).

In the further studies, the absolute configurations were determined by the exciton chirality method as 3S, 4S, 10R and 11R (Ying, 1988) (Fig. 3).

For the conformational analysis, the five membered ring was envelop form and the seven membered ring was chair-like form. The orientation of the unsaturated side chain significantly influenced the conformation of the lacton ring. The nOe experiments of pseudolaric acid B revealed that the protons at C-3, C-12 and C-13 closed to each other, but far from the protons at C-5. This results coincided in the conformational calculation by MMFF Option of Chemlab II Program. The conformational structure of *6* with the lowest steric energy of 68.465 Kcal/Mole showed the lacton ring was planar, neither boat-like nor chair-like.

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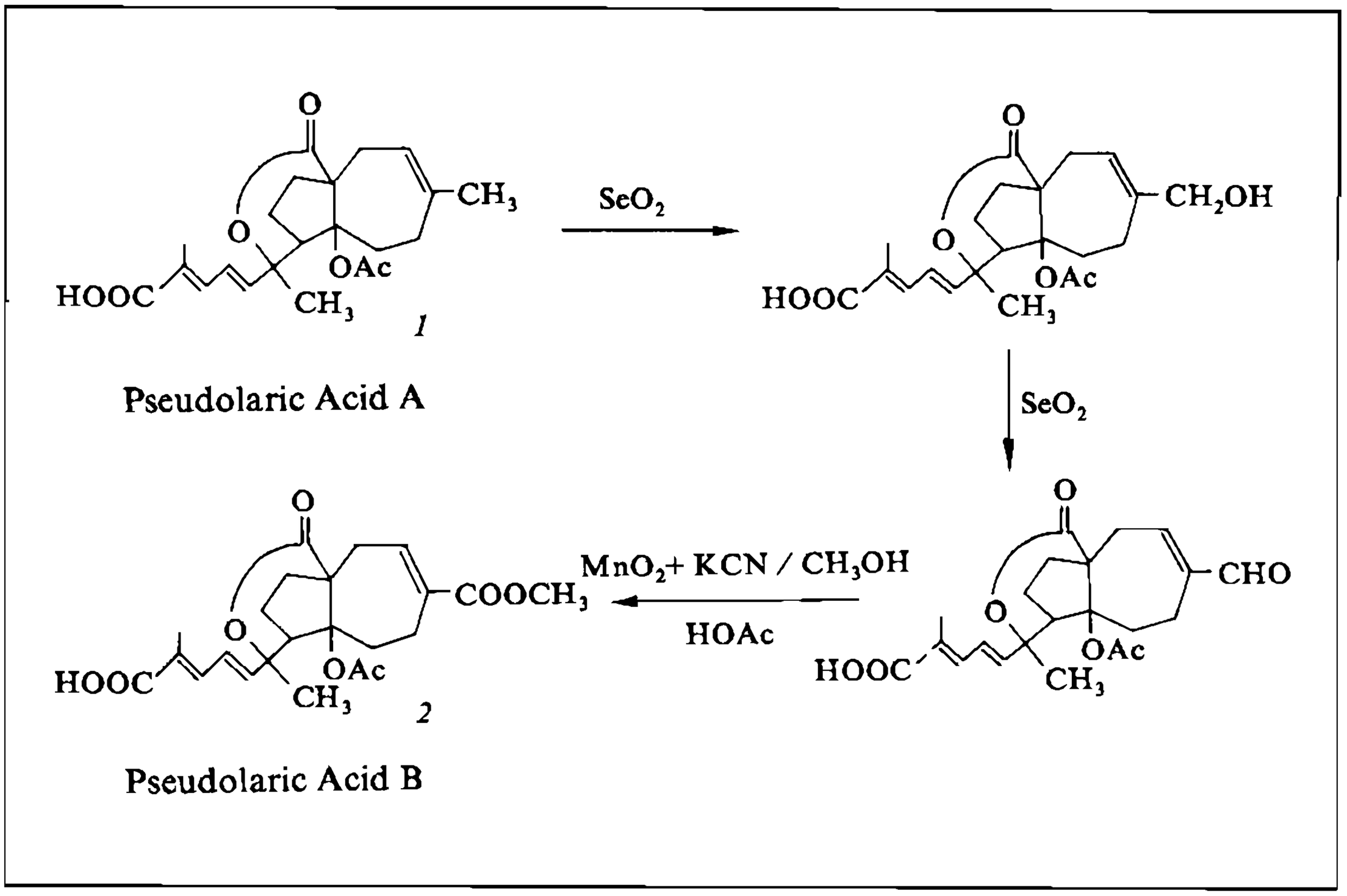


Fig. 1 : the structures of pseudolaric acid B, C and D.

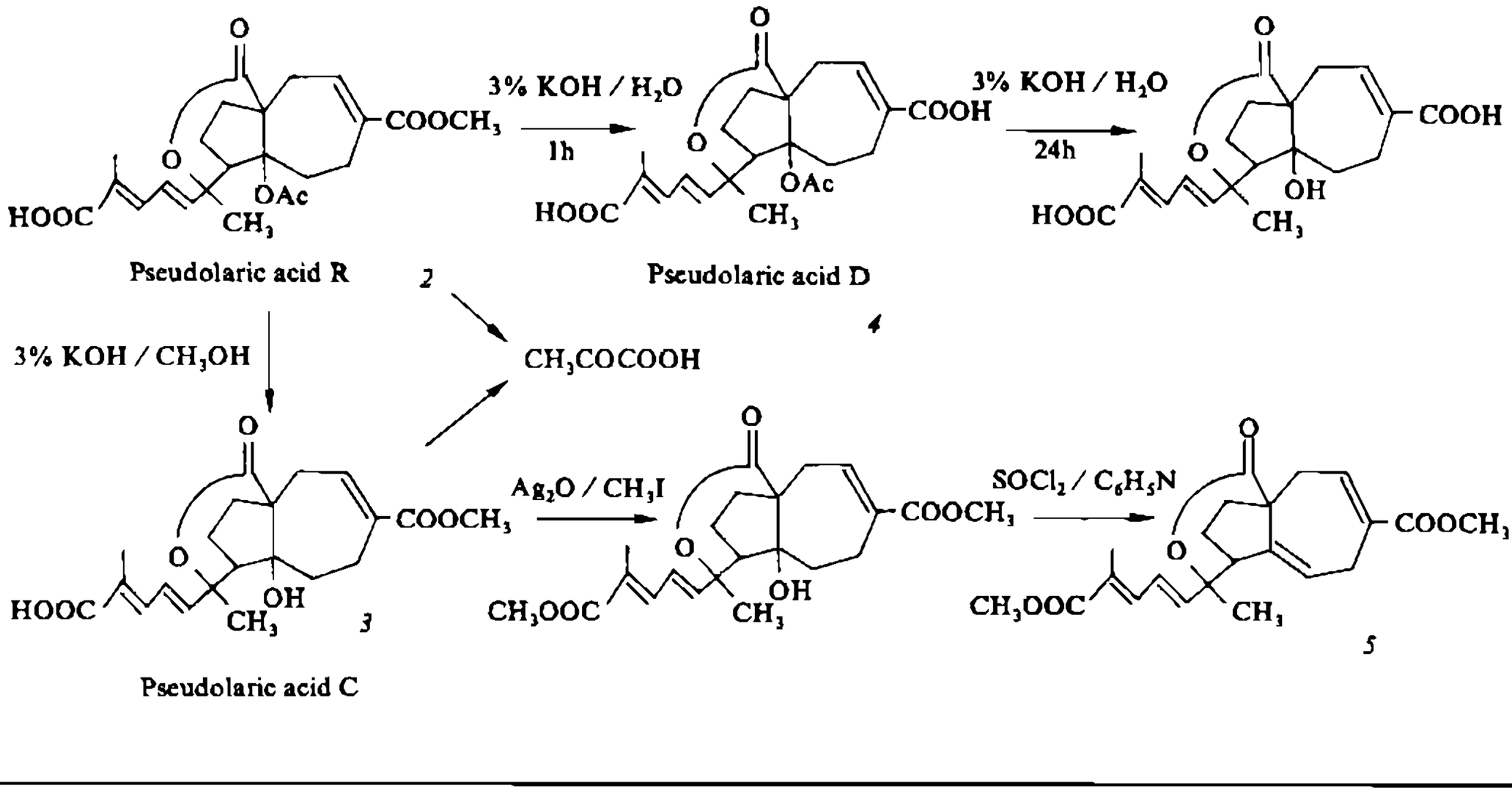


Fig. 2: chemical correlation between pseudolaric acid A and B.

All of the NMR parameters of *2* were unambiously assigned by HETCOR and selective INEPT experiments.

Pseudolaric acid A *1* and B *2* showed the anti-fungous activities. Recently, we found pseudolaric acid B was a general cytotoxic agent against P-388 lymphocytic leukemia, KB carcinoma of the naspharynx, HT-1080 fibrosarcoma, HOO 578T breast cancer, human melanoma, a human lung cancer and a human colon cancer cell lines (Fig. 5).

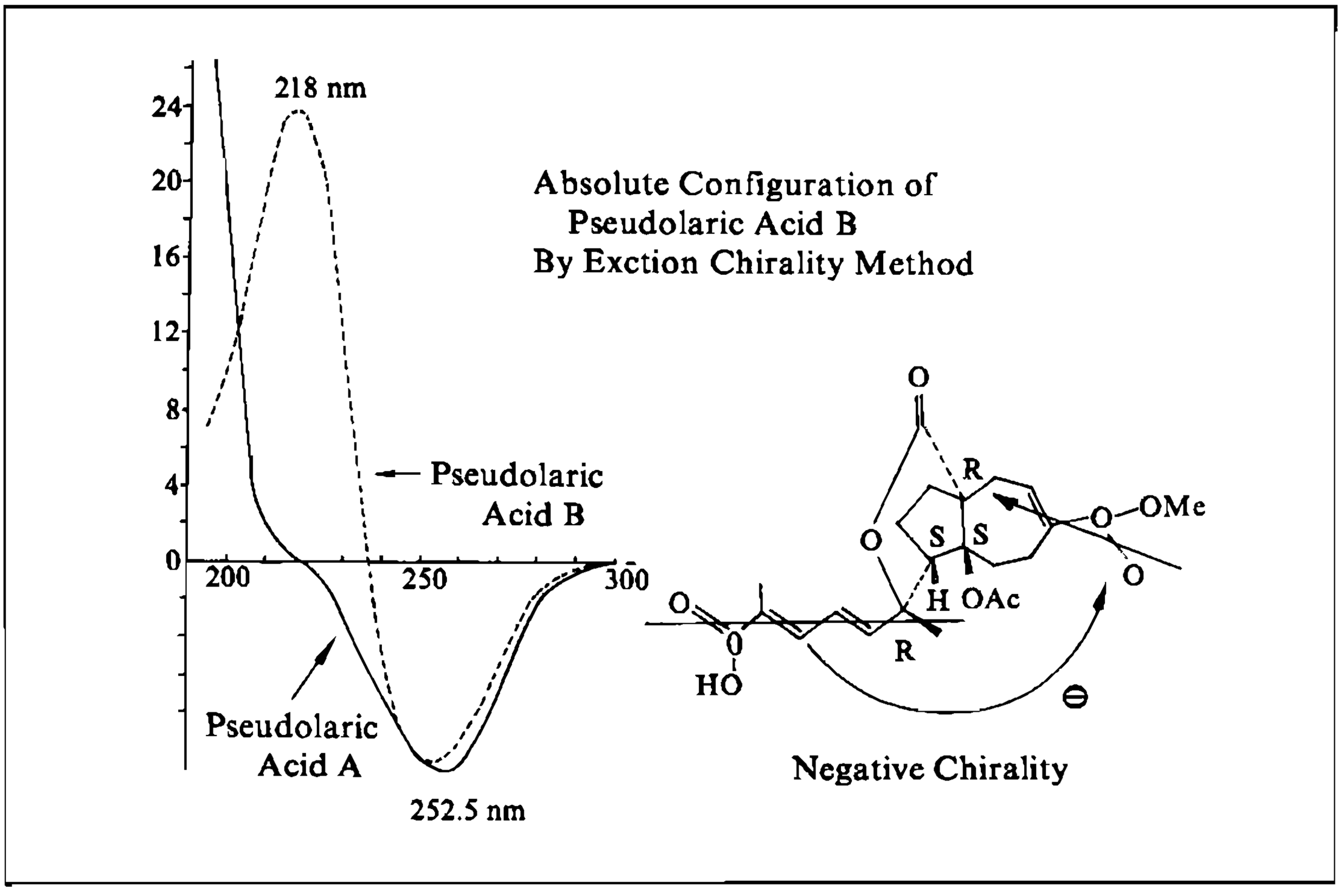


Fig. 3: absolute configuration of pseudolaric acid B by exciton chirality method.

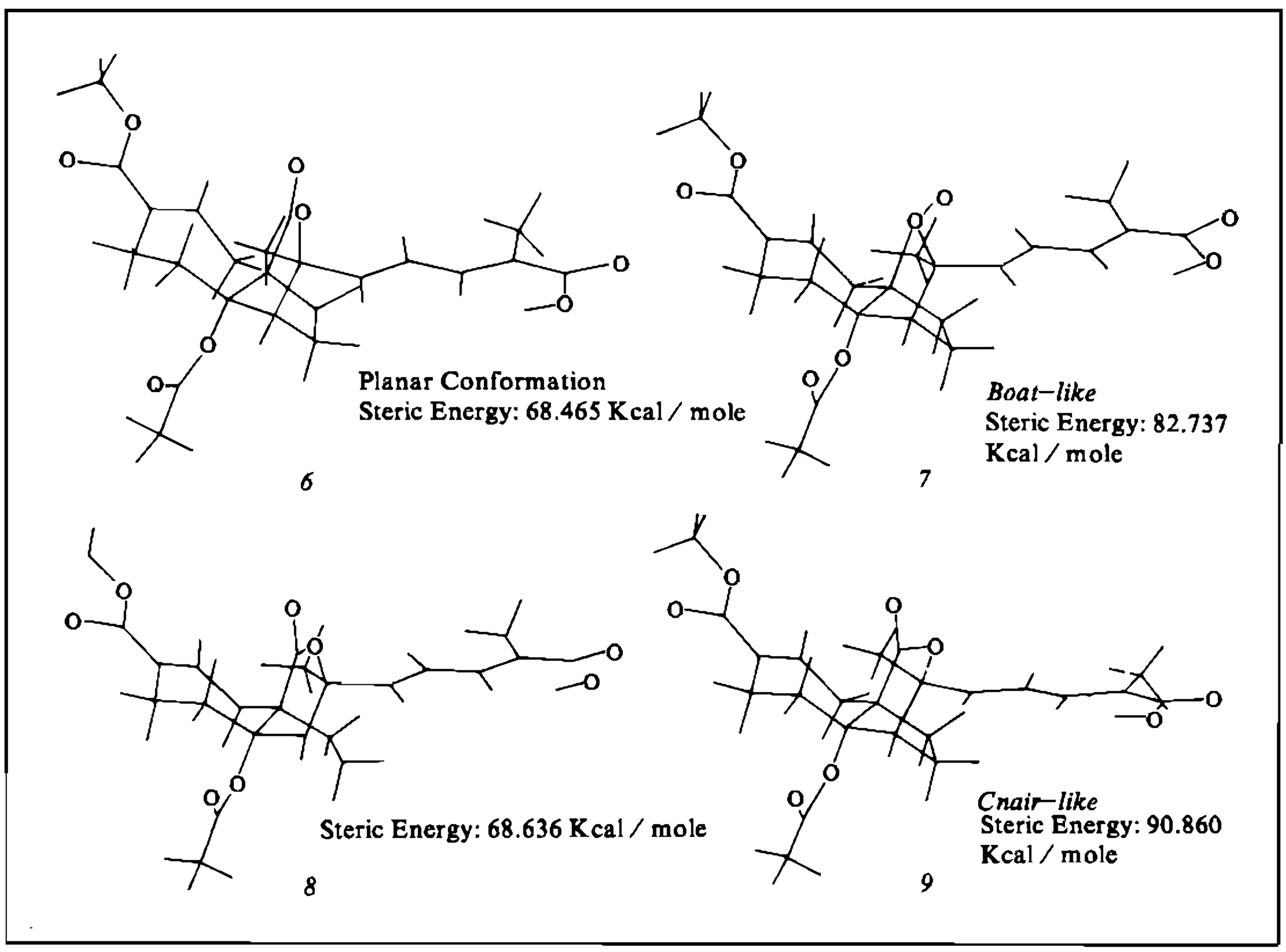


Fig. 4: Calculated conformation of pseudolaric acid B (by MMFF Option of Chemlab II Program).

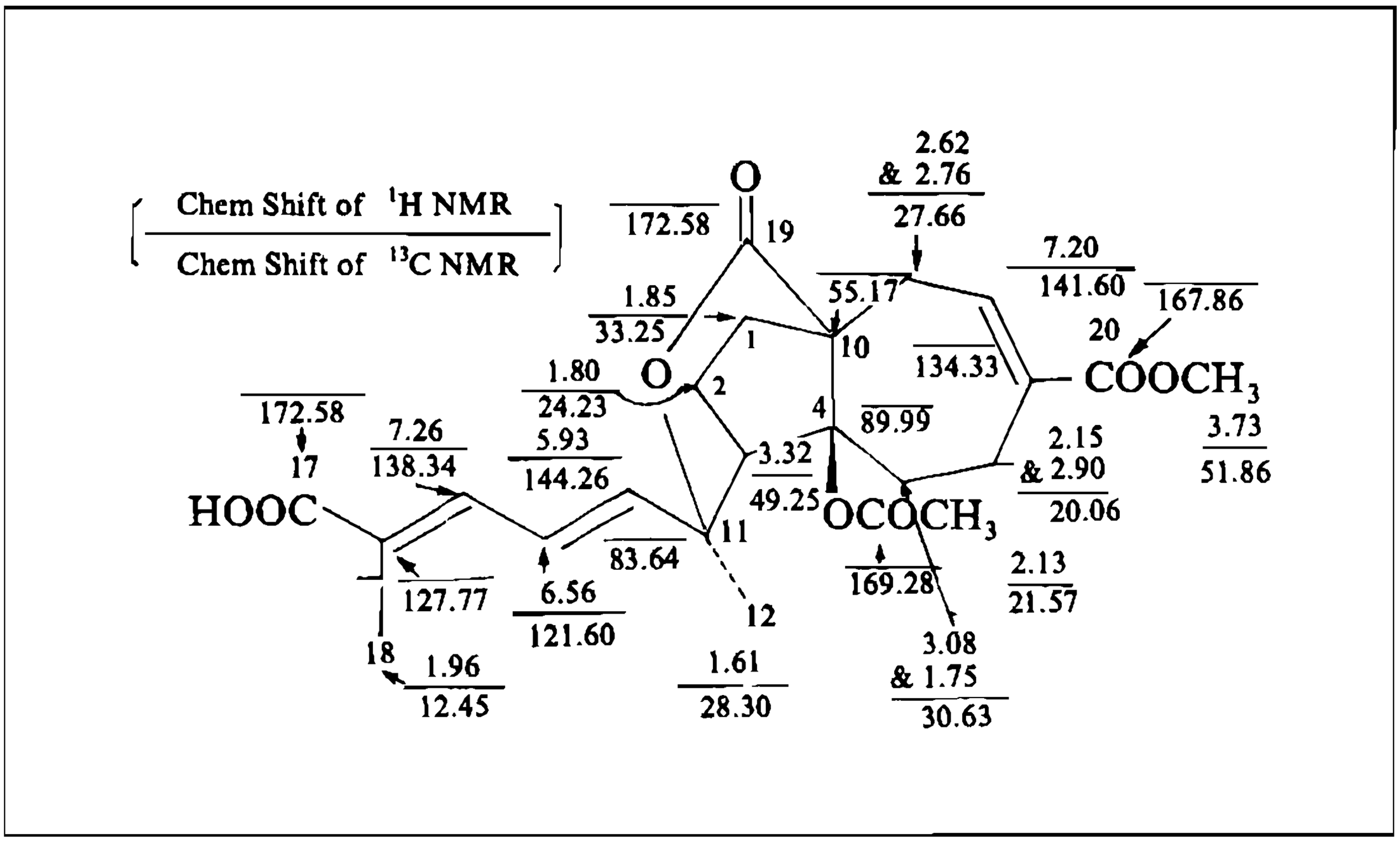


Fig. 5: NMR spectral parameters for pseudolaric acid B.

2. THE TERPENOIDS FROM *TRIPTERYGIUM*

Tripterygium wilfordii was used as an anti-cancer drug, male contraceptives and immunodepressant in chinese folk medicine for a long time. Kupchan et al. (1972) reported triptolide, tripdiolide and triptonide were isolated with significant anti-tumor activities, but the high toxicities restricted its application in clinics. It encouraged us to study the bioactive ingredients with lower toxicities from *Tripterygium wilfordii, T. hypoglaucum, T. regeli* and *T. forrettii.* In addition to 10 known triterpenes – celastrol, 3-oxo-friedelan-28-oic acid, 3-oxo-friedelan-28-al, wilforlide A, wilforlide B, 3-epi-katonic acid, 3β,22α-dihydroxy-Δ^{12}-oleanane-29-oic acid, 3,24-dioxo-friendelan-29-oic acid, orthosphenic acid and salaspermic acid, 15 new diterpenes (*12-26*) were isolated from the plants of *Tripterygium* by silica gel chromatography and elucidated by ^{1}H-NMR, ^{13}C NMR, HETCOR, selective INEPT and chemical correlations. *20* was confirmed by X-ray diffraction (Fig. 6).

As in Fig. 7, the α,β-unsaturated lactone compounds showed the negative Cotton effect at 240-250 nm and all of the 3-ketone compounds showed the positive Cotton effect around 290 nm. By the octant rule, it was proved that the A/B ring of all diterpenes were trans as in triptolide which configurations has been determined by X-ray diffraction. The celastrol demonstrated the immunodepressive activities and compounds *20* and *26* showed the anti-tumour activities against P-388 *in vitro*.

3. BIOACTIVE PREGNANE GLYCOSIDES FROM *MARSDENIA KOI*

By random screening, the methanol extract of *Marsdenia koi* showed the anti-fertility activities. Monitored by bioassay, two pregnane glycosides, marsdenikoiside A *27* and marsdenikoiside B *28*, were isolated from ethanol extracts by SiO_2 chromatography and lowbar chromatography on reverse phase column (RP-8). By hydrolysis in alkali medium, *27* and *28* converted to same deacyl glycoside *29*. After hydrolysis of *29* in acidic medium, the genin was identified as dihydrosarcostin *30* by NMR, MS and physical constants and the sugar parts after separation and purification were elucidated as D-cymarose and D-pachybiose separatively by ^{1}H-NMR and HPLC (sugar park column) compared with authenic samples. The sequences of the sugar part in *27*, *28* and *29* was determined by FD-MS and the location of the acyl groups was confirmed by decoupling techniques in ^{1}HNMR (Fig. 8).

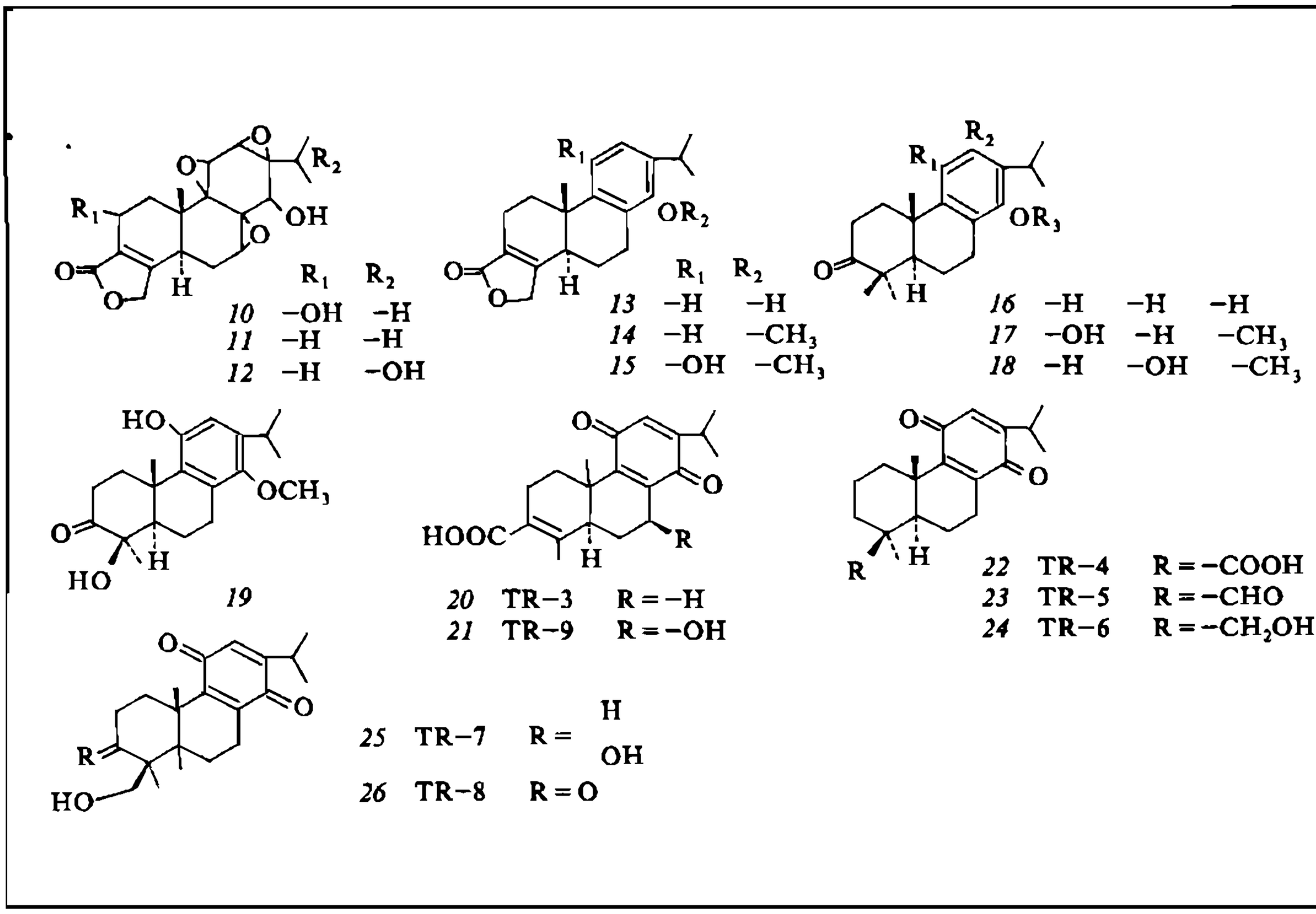


Fig. 6: the diterpenes isolated from *Tripterygium.*

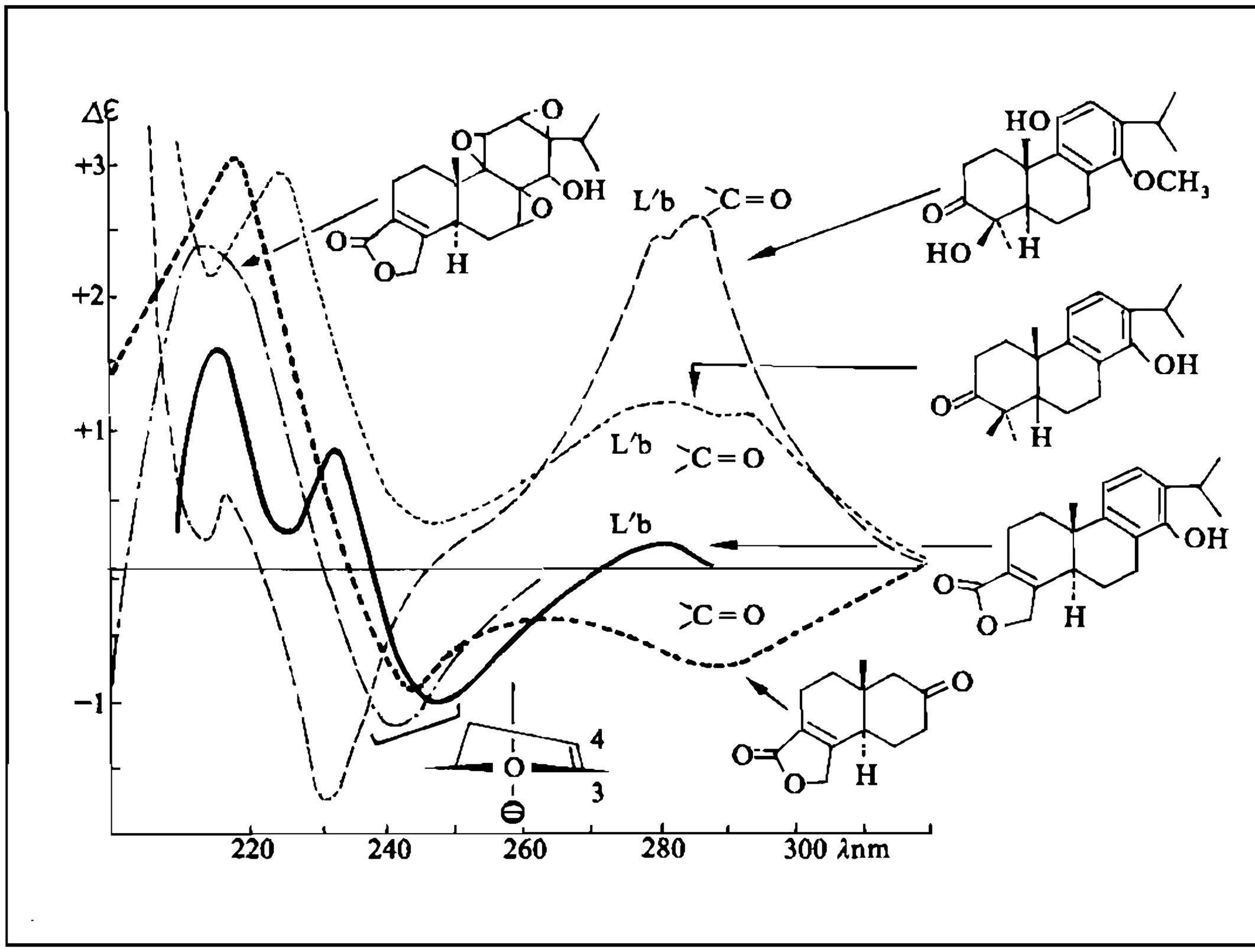


Fig. 7: the CD spectra and stereochemistry of Tripteryguim diterpenes.

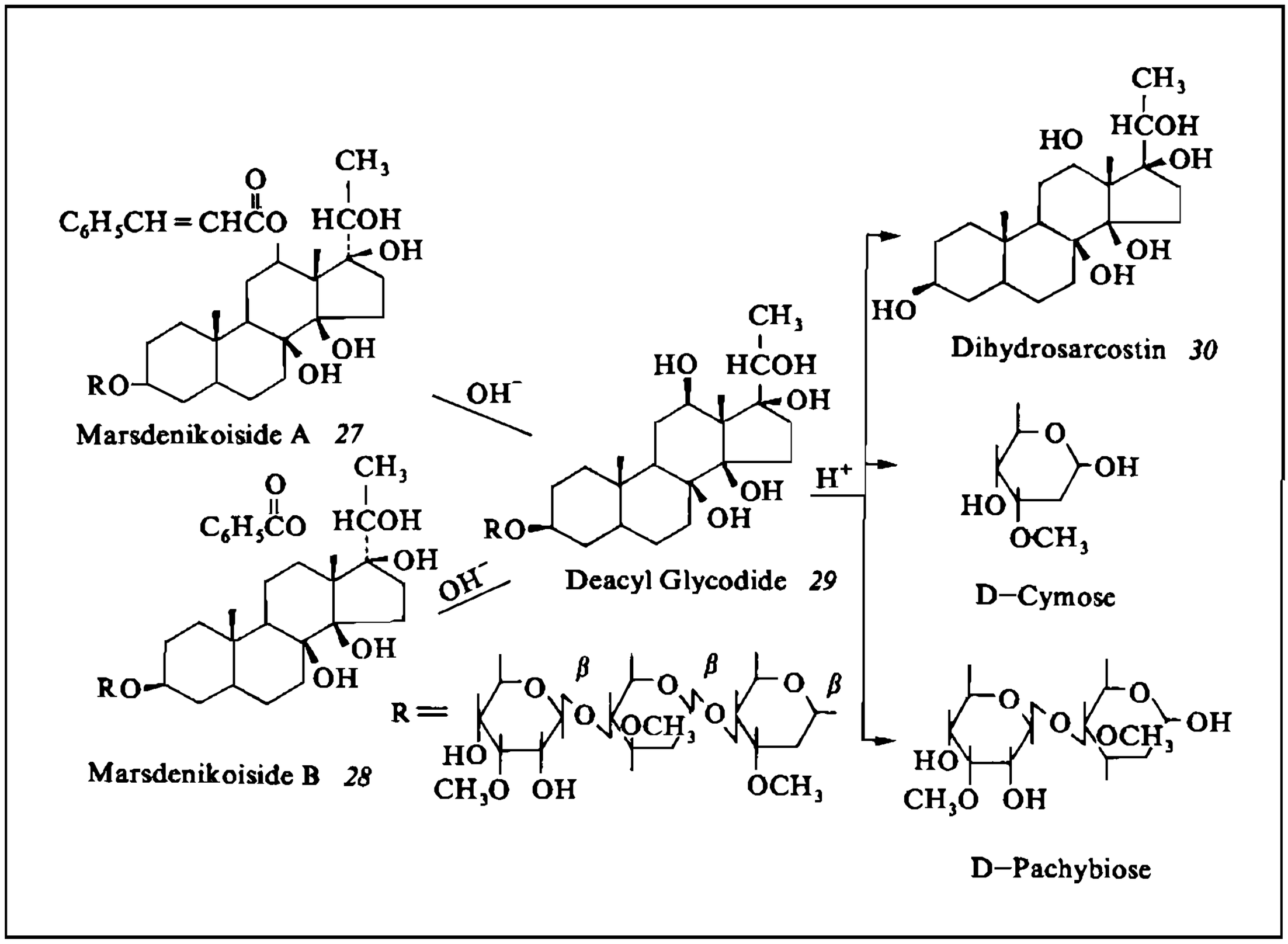


Fig. 8: the structure determination of marsdenikoiside A *27* and B *28*.

27 showed the anti-fertility activities *in vivo* without any estrogenic effects. The studies on bioactivities of *28* was in progress.

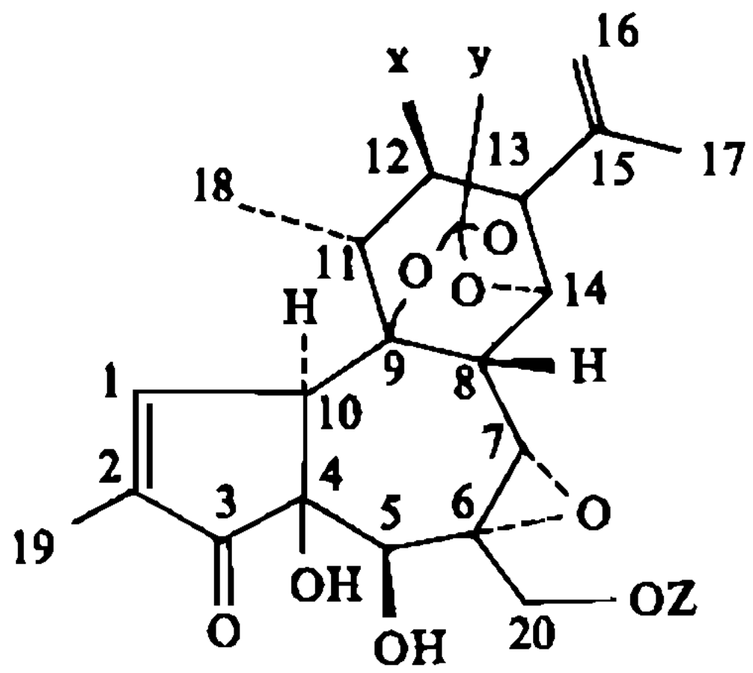
4. DAPHNANE DITERPENES FROM THE PLANTS OF *THYMALAEACEAE*

In previous paper, it was reported that a series daphnane diterpenes such as yuanhuacin and yuanhuadin with abortifacient acitivites have been isolated from *Daphne genkwa* in our group (Ying et al., 1977; Wang et al., 1981) and they were used in the clinics as an abortifacient by intra-ammiotic injection at the dose of 70-80 μg/case with the effectiveness more than 98% (Lin et al., 1980). Their structures were elucidated by the fragmentation of MS, assignment of ^{1}H-NMR, the formation of acetonide and the identification of the acid parts after the hydrolysis in alkali or in acidic medium.

The mechanisms of their abortifacient activities were the release of endogenous prostaglandins resulting from degeneration and necrosis of decidual cells.

In the continuous studies, besides 6 known compounds, 17 new daphnane diterpenes were isolated from *Daphne genkwa, D. Tangutica, D. giraldii, Wikstroemia chamaedaphne* and *W. pilosa* Fig. 9).

Their structures were elucidated by ^{1}H-NMR, ^{13}C-NMR, MS and hydrolysis in acidic and in alkali medium. The studies on the mechanisms of the MS fragmentation by defocusing techniques and high resolution mass sprectroscopy (Huang et al., 1985) found that the daphnane diterpenes with C-12 oxygenated group showed the fragmentation peak of m/z 358 corresponding to the skeleton after loosing the acyl group, but the C-12 unsubstituted series gave the fragmentation peak of m/z 360. The acyl group was identified by the base peak in low mass range of MS and confirmed by comparison with authenic samples by GC-MS after hydrolysis in alkali or acidic medium and methylation.



Compound	X	Y	Z
Yuanhuacin *	C_6H_5COO-	$CH_3(CH_2)_4(CH=CH)_2-$	H–
Yuanhuadin *	CH_3COO-	$CH_3(CH_2)_4(CH=CH)_2-$	H–
Yuanhuafin *	CH_3COO-	C_6H_5-	H–
Yuanhuatin * +	C_6H_5COO-	C_6H_5	H–
12–Benzoyl–daphnetoxin *	C_6H_5COO-	C_6H_5-	H–
Gniditrin	$CH_3(CH_2)(CH=CH)_3COO-$	C_6H_5-	H–
Gnidicin	$C_6H_5CH=CHCOO-$	C_6H_5-	H–
Excoecariatoxin	H–	$CH_3(CH_2)_4(CH=CH)_2-$	H–
Daphnetoxin	H–	C_6H_5-	H–
1,2–Dihydro–daphnegiraldifin *	H–	C_6H_5-	$CH_3(CH_2)_{14}CO-$
12–Hydroxyl–daphnetoxin	HO–	C_6H_5-	H–
Simplexin	H–	$CH_3(CH_2)_7CH_2-$	H–
Tanguticacin *	$CH_3(CH_2)_2(CH=CH)_3COO-$	C_6H_5-	$CH_3(CH_2)_{14}CO-$
Tanguticadin *	$CH_3(CH_2)_2(CH=CH)_3COO-$	C_6H_5-	5,20–Acetonide
Tanguticafin *	$C_6H_5CH=CHCOO-$	C_6H_5-	5,20–Acetonide
Tanguticagin *	$C_6H_5CH=CHCOO-$	C_6H_5-	$CH_3(CH_2)_{14}CO-$
Tanguticahin *	= 15,16–Dihydro–daphnetoxin		
Tanguticakin *	= 1,2–Dihydro–daphnetoxin		
Tanguticalin *	H–	C_6H_5-	$CH_3(CH_2)_{16}CO-$
Tanguticamin *	H–	C_6H_5-	$CH_3(CH_2)_{14}CH=CHCO-$
Daphnegiraldicin *	$CH_3(CH_2)_{10}COO-$	C_6H_5-	H–
Daphnegiraldin *	$CH_3(CH_2)_3CH=CHCOO-$	C_6H_5-	H–
Daphnegiraldifin *	H–	C_6H_5-	$CH_3(CH_2)_{14}CO-$

Note: Above compounds were isolated in our Lab.
* New compound.
\+ 1,2–Dihydroderivative.

Fig. 9: the daphnane diterpenes from the plants of *Thymelaeaceae*.

Some compounds were assigned by acetonization and correlation.

The preliminary pharmacological studies showed that the toxicity and effectiveness both decreased significantly when the acetonization was taken place on C-5 and C-20. But the esterification on C-20 with a long chain fatty acid decreased the toxicity obviously and still keep the effectiveness. It mentioned us it was possible to find the compound with lower toxicity and keep the efficacy through derivatization.

REFERENCES

HUANG, Z. H.; YANG, Y. M.; DAI, M. L. & WANG, C. R., 1985. Studies on the mass spectra of diterpene orthoesters from *Thymelaeaceae*. *Acta Chem. Sin.*, *43:* 529-538.

KUPCHAN, S. M.; COURT, W. A.; DAILEY, R. G.; GILMORE, Jr. C. J. & BRYAN, R. F., 1972. Triptolide and tripdiolide, novel anti-leukemic diterpenoid triepoxides from Tripterygium wilfordii. *J. Amer. Chem. Soc., 95:* 7194-7195.

LI, Z. L.; PAN, D. J.; HU, C. Q.; WU, Q. L.; XU, G. Y.; ZHOU, B. N.; YING, B. P.; SUN, G. J.; CHEN, Z. X.; HAN, J. & YUE, Y. F., 1982. Studies on the anti-fungal constituents of tu-jin-pi – The structures of novel diterpenes, pseudolaric acid A, B, C and D, p. 150-154. In *Chemistry of Natural Products – The Proceedings of Sino-American Symposium on Chemistry of Natural Products.* Science Press, Beijing, and Bordon & Breach, Science Publishers, Inc., New York.

LIN, Z. M.; ZHU, M. K.; PANG, D. W.; JIANG, X. H.; LIU, M. Z.; DING, G. S. & YANG, B. Y., 1980. Some Pharmacological studies of two abortifacient diterpenoids, yuanhuacin and yuanhuadin. In *Recent Advances in Fertility Regulation.* Atar S. A., Geneva.

WANG, C. R.; CHEN, Z. X.; YING, B. P.; ZHOU, B. N.; LIU, J. S. & PAN, B. C., 1981. Studies on the active principles of the root of yuanhua *(Daphne genkwa)*. II. Isolation and structure of a new antifertile diterpene yuanhuadin. *Acta Chem. Sin., 39:* 421-426.

YING, B. P.; WANG, C. R.; ZHOU, B. N.; PAN, P. C. & LIU, J. S., 1977. Studies on the active principles of the root of yuanhua *(Daphne genkwa)*. 1. Isolation and structure of yuanhuacin. *Acta Chem. Sin., 35:* 103-108.

YING, B. P.; XU, R. S.; MI, J. F. & HAN, J., 1988. Absolute configuration of pseudolaric acid B. *Acta Chem. Sin., 46:* 85-86.

ZHOU, B. N.; YING, B. P.; SONG, G. Q.; CHEN, Z. X.; HAN, J. & YAN, Y. F., 1983. Pseudolaric acids from *Pseudolarix kaempferi*. *Planta med., 47:* 35-38.