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Recent advances in chemical studies on the active principles from plants for fertility regulation

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Abstract - Recent results of studies on the active principles from Chinese medicinal plants for fertility regulation in our Institute will be presented. These include abortion principles tanguticacin, daphnegiraldicin, daphnegiraldin and 15,16-dihydrodaphnetoxin from some Daphne species; early pregnancy-terminating components pseudolaric acid type compounds from Pseudolarix kaempferi and cycloartane triterpenic acid from Gardenia jasminoides. It also includes separation of optical active d- and l-gossypol—a male's antifertility agent. The chemical transformation and structure elucidation of these principles will be discussed.

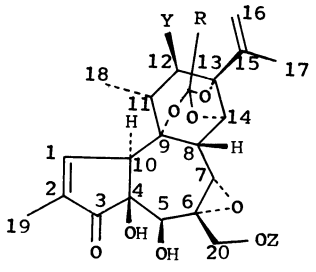
INTRODUCTION

The problem of population is a universal one. During the past 34 years, from 1950 - 1984, the world population has increased by 2.3 billion or two times. The World Bank predicted that by the end of this century another 1.5 billion will be added and by 2050 the population will be 9.8 billion. That means in one hundred years period (1950 - 2050), the world population will be quadrupled. China has nearly 1/5 of the world population, but only 10% of the cultivated land. In order to improve the quality of life and to synchronize the rate of population growth with the economical development, the Chinese government pays much attention to the promotion of family planning work and to study on the methods of contraception. From ancient time the plants using for fertility reguration were described in "Ben-Cao-Gang-Mu" (or "Compendium of Materia Medica") in 1590 and in other medical books as emmenagogues, abortificients or contraindications to pregnancy. There were also some medicinal herbs and special prescriptions used in folk medicine. But all of these need further study, exploration, systematization and improvement. Since the requirement for antifertility drugs is strict in respect of having high efficiency but very low or nearly none toxicity, and the reliability of the folk prescriptions is so low that brought much difficulty to the research work. After hard work in the past few years in whole China some promising agents are have been put into application and some are under studying. Here the recent results of study in Shanghai Institute of Materia Medica are additionally reviewed (ref. 1).

DAPHNETOXIN TYPE COMPOUNDS

It was reported previously the ortho-ester diterpene yuanhuacin 1, isolated from the roots of a Chinese medicinal plant Daphne genkwa, is an abortive principle and has been used in clinic with 70-80 μg per pregnancy woman by intra-amniotic injection since 1977 (ref. 2). It is the same compound as gnidilatidin, isolated from Gnidia latifonia by Kupchan (ref. 3). In continuous study of this direction several other new daphnetoxin type components were isolated from the plant and other Daphne species. These include yuanhuadin 2 and yuanhuafin 3 from Daphne genkwa (ref. 4,5); daphnegiraldicin 4 and daphnegiraldin 5 from D. giraldii (ref. 6); tanguticacin 6 and 15,16-dihydrodaphnetoxin 8 from D. tangutica (ref. 7). All of these compounds have the same skeleton but differ each from other on the ester group at C-12, side chain at ortho-ester carbon C-21 and a few on the ester group at C-20 (Table 1). Their structures can be elucidated by different conditions of hydrolysis and spectral analyses, e.g. acetonide of yuanhuadin 2 is the same product when 1 hydrolysed with sodium methylate,

TABLE 1. New daphnetoxin type compounds from Daphne species



No.	Compound	M	Y	R	Z
1	Yuanhuacin	648	C_6H_5COO-	$C_5H_{11}CH=CHCH=CH-$	H
2	Yuanhuadin	586	CH_3COO-	$C_5H_{11}CH=CHCH=CH-$	H
3	Yuanhuafin	540	CH_3COO-	C_6H_5-	H
4	Daphnegiraldicin	608	$CH_3(CH_2)_3CH=CHCOO-$	C_6H_5-	H
5	Daphnegiraldin	680	$CH_3(CH_2)_{10}COO-$	C_6H_5-	H
6	Tanguticacin	884	$CH_3(CH_2)_2(CH=CH)_2CH=CHCOO-$	C_6H_5-	$n-C_{15}H_{31}CO$
7	Gniditrin	646	"	C_6H_5-	H
8	15,16-Dihydro-daphnetoxin	484	H	C_6H_5-	H

then acetonized with acetone and p-toluenesulfonic acid and acetylated with acetic anhydride and pyridine (Fig. 1). When tanguticacin 6 was hydrolysed with sodium methylate 12-hydroxydaphnetoxin and two acids were obtained. After methylation the acids were identified as methyl ester of palmitic acid and 2,4,6-decatrieneoic acid which were detected by GC-MS. At the same time tanguticacin can be synthesized by gniditrin 7 and palmityl chloride. But 6 could not form acetonide due to esterification of C-20 hydroxyl group taking place (Fig. 2).

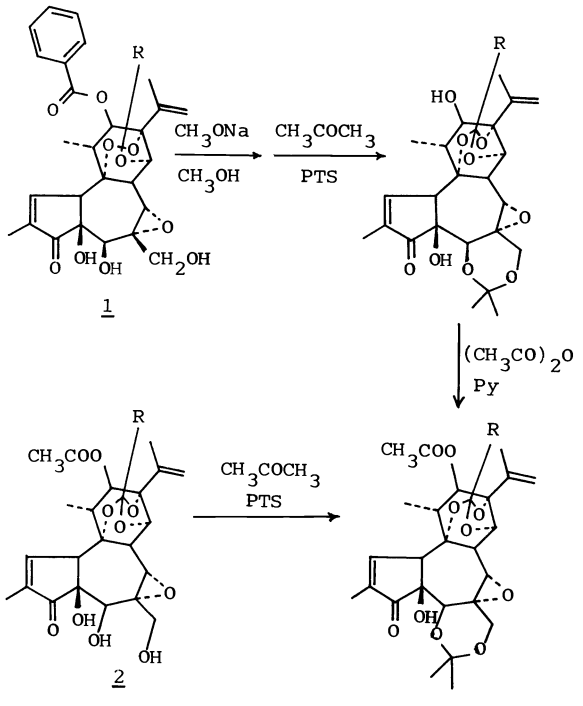


Fig. 1

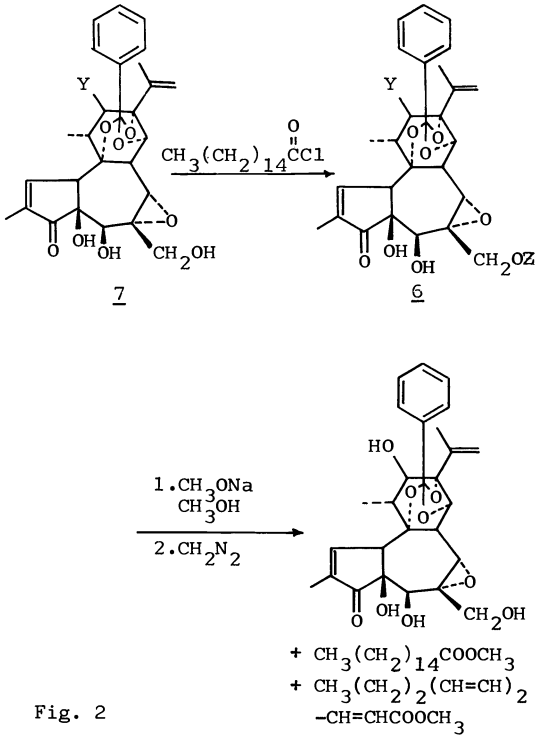


Fig. 2

The high resolution MS and determination of their metastable ions showed two different type of fragmentations of the daphnetoxin type compounds could be distinguished depending on C-12 substitute. All C-12 oxygenated compounds have a pair of important ions [M-R'COO·] and [M-R'COOH] due to direct fission of 12-OCOR' or by elimination of McLafferty rearrangement. The removal of orthoester unit could take place simultaneously with formation of less aboundant [M-RCOOH] ion. m/z 358 [M-HY-RCOOH] was a key intermediate ion, representing the basic skeleton of the C-12 oxygenated series. m/z 340 ($358-H_2O$), 327 ($358-{}^{\circ}CH_2OH$), 317 ($358-{}^{\circ}C_3H_5$) and 311 ($358-CH_2O-OH$) are the subsequently produced ions. They comprised characteristic ion series for C-12 oxygenated compounds. The ester parts could be identified by fragmentation ion in higher mass range and confirmed by the strong $RC{\equiv}O^+$ (Fig. 3). All C-12 unsubstituted compounds have important fragment m/e 360 [M-RCOOH] due to direct removal of the ortho-ester unit from the molecular ion. Subsequent rejection of H_2O, ${}^{\circ}CH_2OH$, ${}^{\cdot}C_3H_5$ ... resulted ion series m/z 342, 329,324, 319, 313 — a mass increment of 2 units in comparison with corresponding ion series of C-12 oxygenated compounds (Fig. 4) (ref. 8). According to the results of pharmacological test all of these daphnetoxin type compounds have abortive activity in rats.

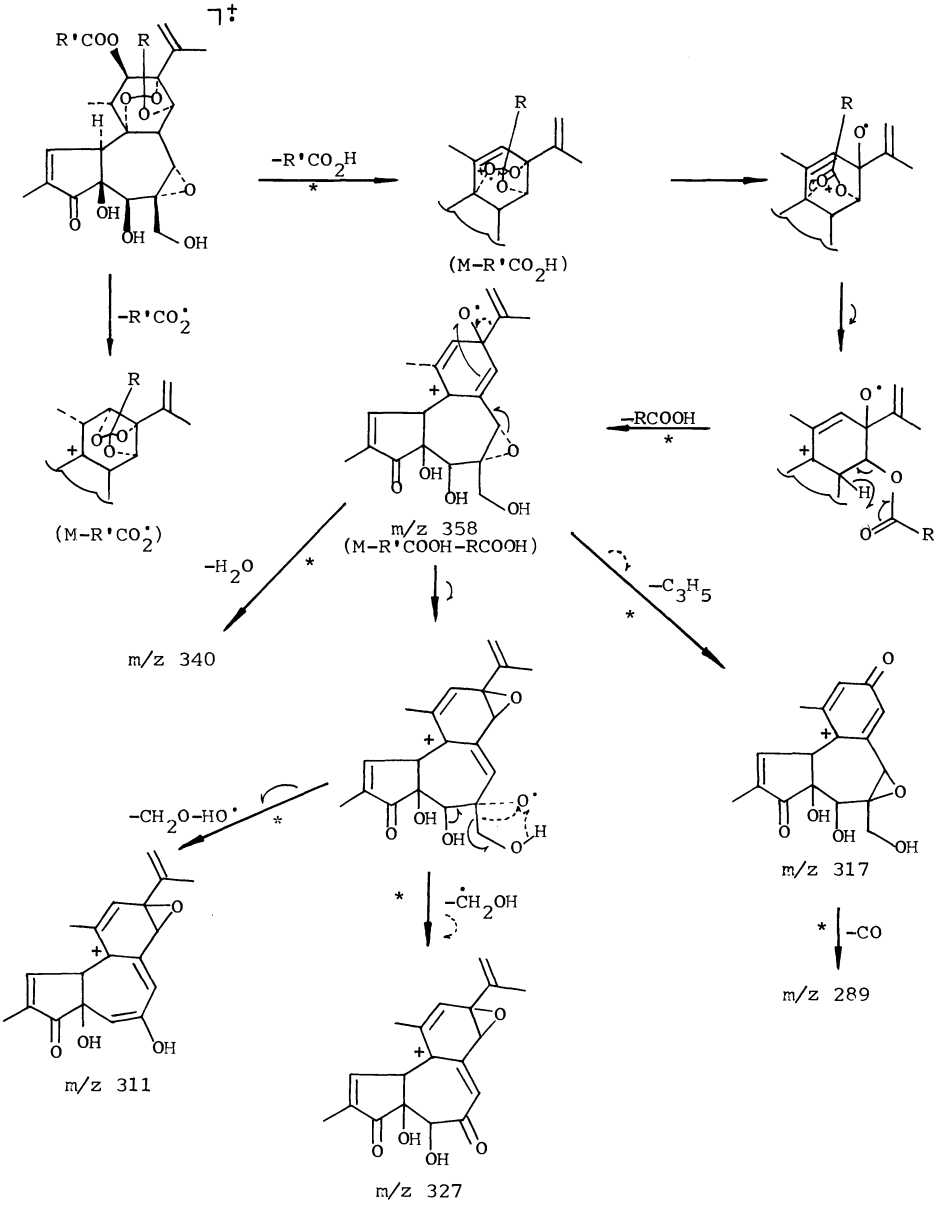


Fig. 3 MS fragmentation of C-12 oxygenated compounds

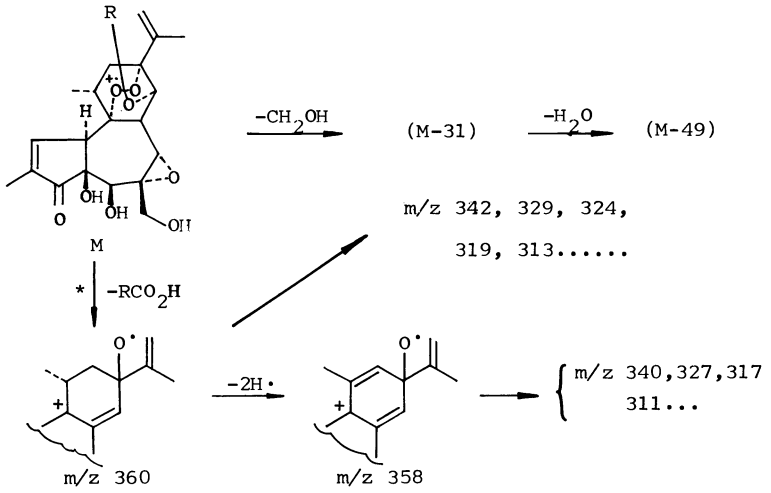


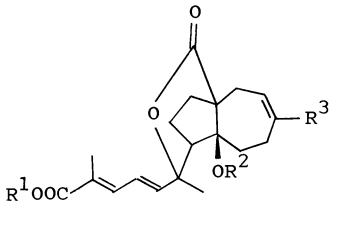
Fig. 4 MS fragmentation of C-12 unsubstituted compounds

PSEUDOLARIC ACIDS

Tu-Jin-Pi—the root barks of Pseudolarix kaempferi is a traditional Chinese medicine used for treatment of some kinds of fungus diseases such as tinea pedis (athlate's foot). The antifungus active principles were found as diterpenic acids, namely pseudolaric acids A, 9; B, 10; C, 11 and C_2, 12. The structures were determined by spectral analyses and confirmed by X-ray diffraction. All of them have the same new skeleton (ref. 9, 10, 11).

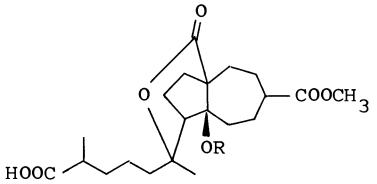
Pharmacological screening test showed that pseudolaric acid B, 10, either in CMC or in sodium bicarbonate solution indicated significant effect on terminating early pregnancy in rats, rabbits or dogs at dosage 10-40 mg/kg (ref. 12). In studying the relationship between activities and functional groups a series of analog compounds was synthesized (Fig. 5, compounds 13-28), but neither substitution of R^1, R^2 and R^3 (13-23) nor saturation of double bonds (24-26), none is more active than pseudolaric acid B, 10. It seems that the structure is quite specific for the activity and the -OAc at C-4 is necessary for the activity (ref. 13), since 10 loses its activity after deacetylation.

Fig. 5 Pseudolaric acids and their derivatives

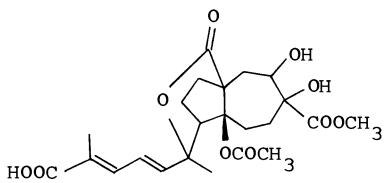


9 - 23

	R^1	R^2	R^3
9	H	CH_3CO	CH_3
10	H	CH_3CO	COOMe
11	H	H	COOMe
12	H	CH_3CO	COOH
13	H	H	COOH
14	H	H	COOEt
15	H	CH_3CO	COOEt
16	H	H	$COOCH_2CH_2CH_3$
17	H	CH_3CO	$COOCH_2CH_2CH_3$
18	H	H	$COOCH_2CH_2CH_2CH_3$
19	H	CH_3CO	$COOCH_2CH_2CH_2CH_3$
20	$(CH_3)_2CH$	CH_3CO	COOMe
21	$(CH_3)_2CH$	H	COOH
22	$(CH_3)_2CH$	CH_3CO	COOH
23	$(CH_3)_2CH$	H	COOMe



24 R=CH_3CO 25 R=H



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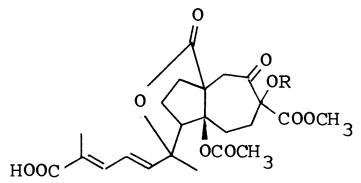


27 R=H 28 R=CH_3CO

CYCLOARTANE TRITERPENIC ACIDS

The flower of Gardenia jasminoides was used in folk medicine in Yunnan Province of China for birth control. It was found that the ethyl acetate extract of the flower showed significant effect on terminating early pregnancy in rats. Flash column chromatography separation afforded two active compounds, namely gardenic acid and gardenolic acid B. The structure of gardenic acid 29, $C_{39}H_{54}O_8$(M^+=650), m.p. 94°C, was reported previously (ref. 1). Recently the structure of gardenolic acid B 30 has been deduced by spectral analyses.

Gardenolic acid B, m.p. 214-216°C; $[\alpha]_D^{17}$ 51.2°(c 0.168, MeOH); $C_{30}H_{46}O_5$(M^+ 486.3339). Its diacetate 31 $C_{34}H_{50}O_7$, m.p. 114-117°C; The ^1H NMR spectrum of 31 showed two doublet at δ 0.51 and δ 0.80 (each d, J= 4 Hz), characteristic of a methylene group in a cyclopropyl ring system. The signal at δ 5.51 (dd, $J_{ax,eq}$= 4 Hz; $J_{ax,ax}$= 12 Hz) indicated a 3α-axial proton. Therefore one of the acetoxy group is located at 3β-position of the cycloartane triterpene. The another acetoxy group was proposed to be at 1α on the basis of the broad singlet proton signal at higher δ 4.69. The UV (236 nm, log ε 4.02) and IR (1617, 1686 cm^{-1}) indicated the presence of an α,β-unsaturated ketone in its side chain. The double bond was at C-24(25) due to the ^1H NMR enolic proton signal at δ 6.05(s), which coupled with two terminal enolic methyl at δ 1.90 and 2.15 (each s). Besides there were two protons at δ 2.40 (d,J= 13 Hz) and δ 2.66 (dd, J= 4, 13 Hz) adjacent to the ketone group and coupled with C-19 methyl (δ 0.87, d, J= 5 Hz). Finally the carboxyl group was proposed locating at C-29 because of the C-30 methyl signal was in rather low field (δ 1.20). All stereoconfigurations of 30 were proposed on the basis of biogenetic reason. The proposed structure was further proved by high resolution mass spectral and ^{13}C NMR analyses (Fig. 6).

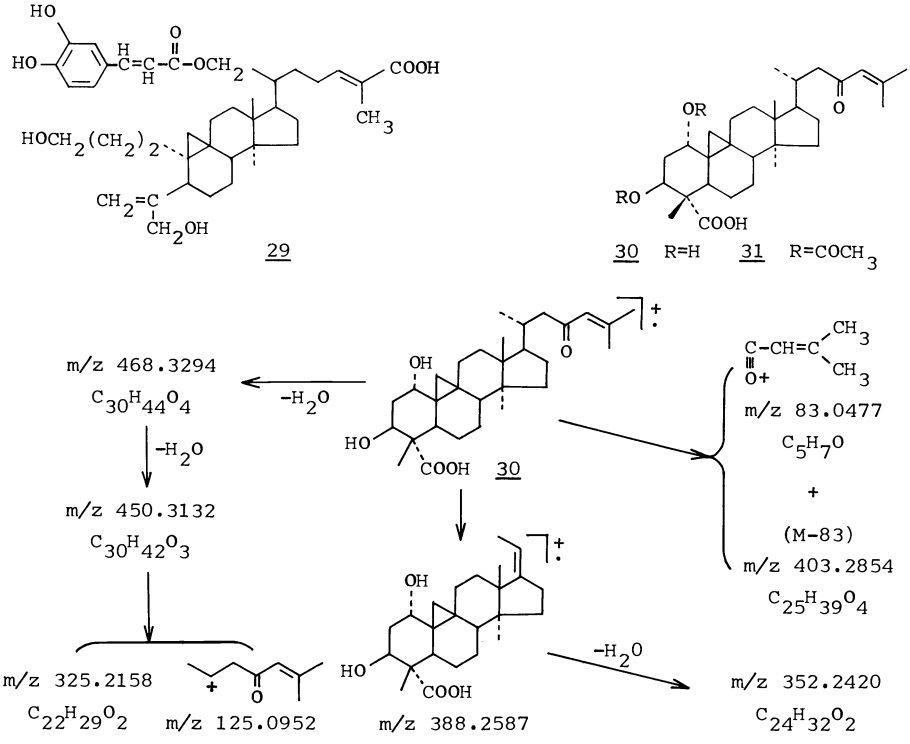


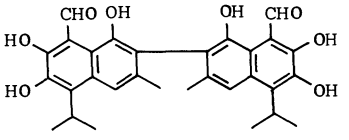
Fig. 6 Mass fragmentation of gardenolic acid B

All ^{13}C NMR signal assignments of 31 are based on the literature data (ref. 14): δ 74.9(d, C-1), 38.3(t, C-2), 73.2(d, C-3), 52.4(s, C-4), 45.3(d, C-5), 22.0(t, C-6), 28.1(t, C-7), 46.1(d, C-8), 21.3(s, C-9), 27.9(s, C-10), 26.1 (t, C-11), 35.3(t, C-12), 45.3(s, C-13), 49.0(s, C-14), 32.6(t, C-15), 24.5 (t, C-16), 52.4(d, C-17), 17.6(q, C-18), 30.6(t, C-19), 33.4(d, C-20), 19.4 (q, C-21), 51.7(t, C-22), 201.4(s, C-23), 124.4(d, C-24), 154.5(s, C-25), 27.6(q, C-26), 20.6(q, C-27), 18.9(q, C-28), 178.9(s, C-29), 9.5(q, C-30), 170.2(2xs, 2xCH_3CO), 20.9(2xq, 2xCH_3CO).

Thus the structure of gardenolic acid B is 1α,3β-dihydroxy-23-oxo-24(25)-en-cycloartan-3-olic acid. The cycloartane triterpenic acid with the side chain is rarely reported previously (ref. 15). The structure is going to be confirmed by X-ray diffraction.

d- AND l-GOSSYPOL *32*

Gossypol is a main component found in cottonseed. Its structure was proved to be a phenolic di-naphthaldehyde, 2,2'-bis[8-formyl-1,6,7-tri-hydroxy-5- isopropyl-3-methylnaphthalene]. The four ortho substituents of gossypol are bulky enough to restrict rotation. Therefore it exists with two optical isomers. But in nature it occurs as a racemate (ref. 16).



32

In the years of sixties when the investigation around districts where cotton are cultivated in abundance, it was found that the cotton oil cause sterility of the males, and active principle is gossypol. Since then much work have been done on pharmacological experiments and clinical observations (ref. 17). In 1981 a (+)-gossypol was separated from Thesoesia populnea (Malvaceae) with $[\alpha]_D$ +457° (benzene)(ref. 18). It showed negative effect in pharmacological test. It seems reasonable to assume that (-)-gossypol would be the effective enantiomer. Recently resolution of gossypol was successful and the both pure optically active (-)-gossypol and (+)-gossypol were obtained in our Institute by Cai et al. The specific rotations were found to be -468° (benzene) and +461° (benzene) respectively. NMR, MS, IR and HPLC of (-)-gossypol and (+)-gossypol were identical with those of natural racemic gossypol (ref. 19).

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