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Studies on the Constituents of *Artemisia argyi* LÉVL *et* VANT

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Twelve compounds were isolated from *Artemisia argyi*, LÉVL *et* VANT (Compositae) collected in China: ethyl palmitate, ethyl oleate, ethyl linoleate, lupenone, lupenyl acetate, α-amyrin acetate, β-amyrin acetate, glutinone, fernenone, 24-methylene-cycloartanone, simiarenol and *trans*-phenylitaconic acid. This is the first report of the isolation of *trans*-phenylitaconic acid from a natural souce.

Keywords——Compositae; *Artemisia argyi*; fatty acid ethyl ester; triterpene; *trans*-phenylitaconic acid

Artemisia argyi LÉVL *et* VANT has been used as an antitumor agent and for the treatment of hemorrhoids in China. Chinese chemists[1] have already isolated α-phellandrene, camphene, α-cedrene, borneol acetate, elemol, isoborneol, carvone and two antiasthmatic principles, *trans*-carveol and α-terpineol, from the essential oil of the leaves of *A. argyi*. During the course of our search for biologically active substances from the whole plant, we isolated twelve compounds (**1**—**12**). In the present paper, we describe the isolation and identification of these compounds.

Air dried whole plants of *A. argyi*, collected from chong-ming prefecture, Jiang-su province of China, were extracted with ethanol. The ethanol extract was chromatographed on silica gel to give five fractions (A—E) as shown in Chart 1. Fraction A was separated by

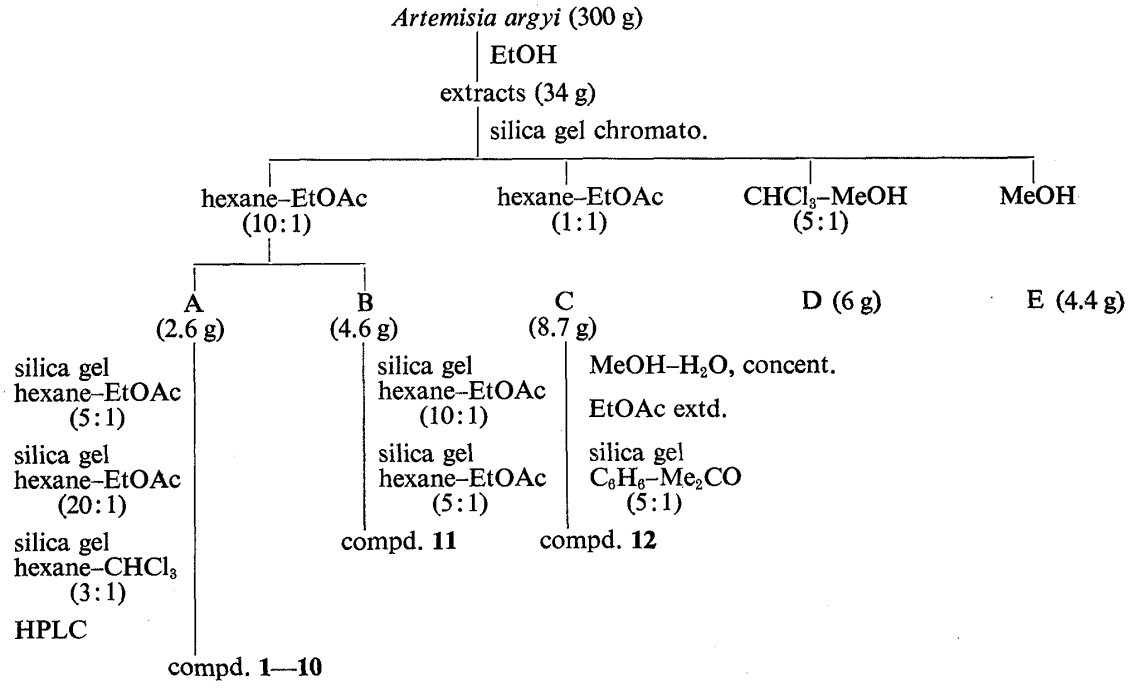


Chart 1

1 $CH_3(CH_2)_{14}COOC_2H_5$
ethyl palmitate

2 $CH_3(CH_2)_7-CH=CH-(CH_2)_7COOC_2H_5$
ethyl oleate

3 $CH_3(CH_2)_4-CH=CH-CH_2-CH=CH(CH_2)_7COOC_2H_5$
ethyl linoleate

Fig. 1

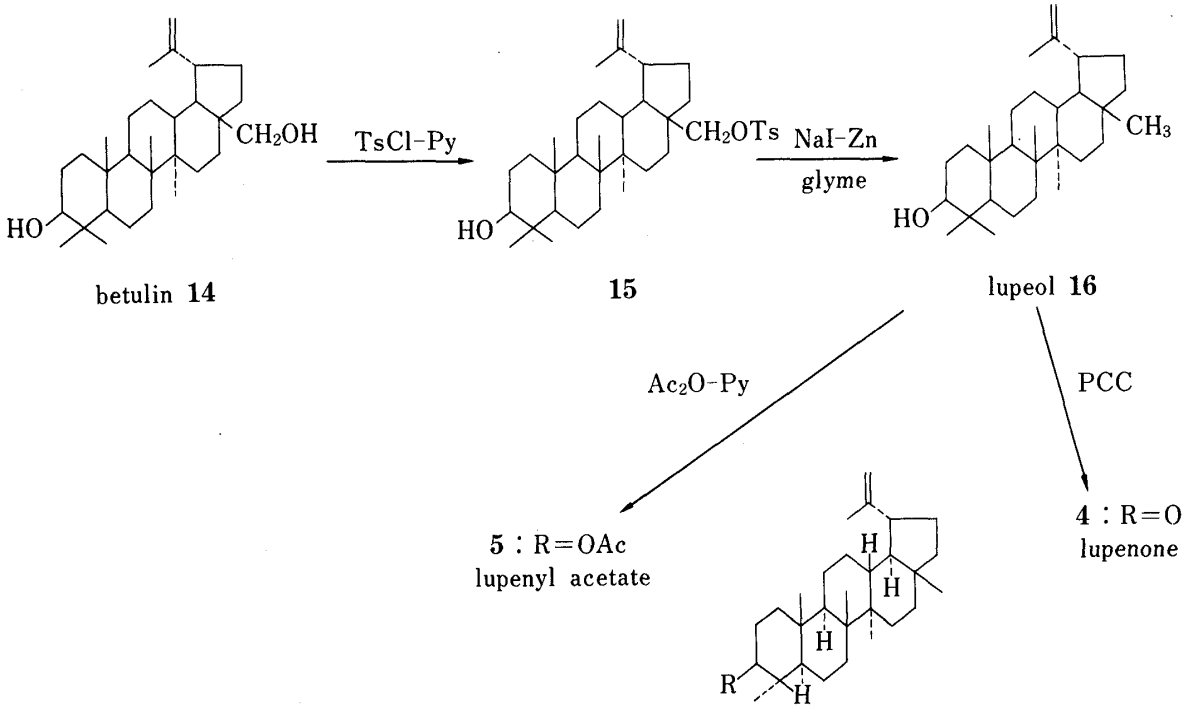


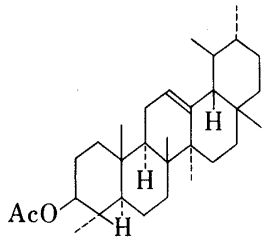
Fig. 2

repeated chromatography on silica gel using a variety of solvent systems (Chart 1), and then high-pressure liquid chromatography (HPLC) of each fraction gave ten compounds **1**—**10**. Repeated silica gel column chromatography of Fr. B gave compound **11**. Water was added to the methanol solution of Fr. C, then the solution was concentrated and extracted with EtOAc. The EtOAc extract was concentrated to leave an oil, which was subjected to silica gel chromatography to give compound **12**.

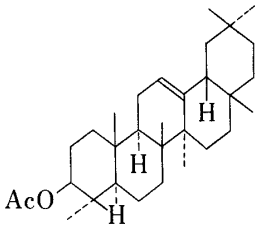
Compounds **1**—**3** were characterized as ethyl palmitate, ethyl oleate and ethyl linoleate by gas chromatography-mass spectroscopy (GC-MS) and proton magnetic resonance (^{1}H-NMR, 400 MHz) spectroscopy in comparison with authentic samples (Fig. 1).

It was presumed from the mass and ^{1}H-NMR spectra that **4** and **5** might be lupenone and lupenyl acetate, respectively, which are lupane derivatives possessing a double bond at position 20 (29).

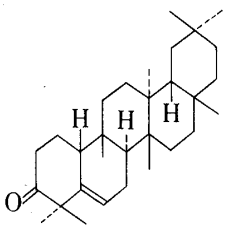
In order to compare **4** and **5** with authentic lupenone and lupenyl acetate, we synthesized these compounds from betulin (**14**) isolated from *Sparganium stoloniferum* in our laboratory. Thus, betulin (**14**) was treated with *p*-toluenesulfonyl chloride to give a tosylate (**15**), which was then reduced to lupeol (**16**) with NaI–Zn in glyme.[2] Lupeol (**16**) was oxidized with pyridinium chlorochromate to afford lupenone, while acetylation of **16** with Ac_2O–Py gave



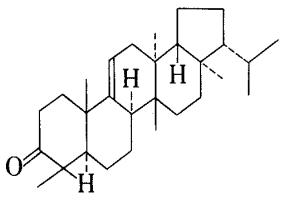
6 α-amyrin acetate



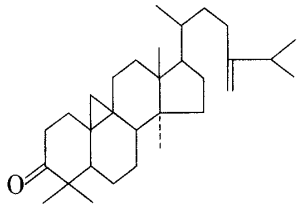
7 β-amyrin acetate



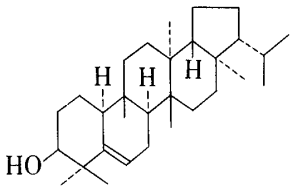
8 glutinone



9 fernenone

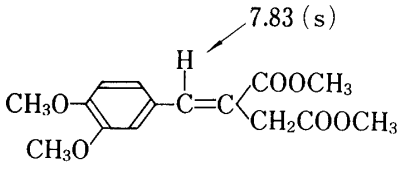


10 24-methylene-cycloartanone

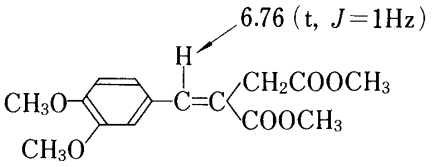


11 simiarenol

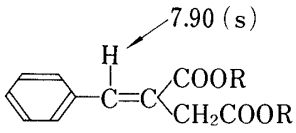
Fig. 3



13A *trans*-



13B *cis*-



12 : *trans*-phenylitaconic acid, R=H
12A : methyl ester, R=CH_3

Fig. 4

lupenyl acetate. The ^{1}H-NMR (400 MHz) spectra of lupenone and lupenyl acetate synthesized from betulin (**14**) were identical with those of **4** and **5**, respectively (Fig. 2).

Compounds **6**—**11** were identified as α-amyrin acetate,[3] β-amyrin acetate,[3] glutinone,[4] fernenone,[5] 24-methylene-cycloartanone[6] and simiarenol[7] by comparison of their ^{1}H-NMR spectra with those of authentic samples (Fig. 3).

Compound **12**, mp 186—188 °C, $C_{11}H_{10}O_4$, gave a molecular ion peak at m/z 206 in the FD-MS. The ^{1}H-NMR spectrum of **12** showed signals due to methylene protons (δ 3.48, s), five aromatic protons (δ 7.39, s) and an olefinic proton (δ 7.88, s). The infrared (IR) spectrum of **12** indicated the presence of unsaturated carboxylic acid (1600—1700 cm^{-1}). Methylation of **12** with diazomethane gave a dimethyl ester (**12A**). These chemical and spectral properties of **12** and **12A** showed that **12** is phenylitaconic acid[8] (Fig. 4).

The stereochemistry of the double bond of **12** was determined from the ^{1}H-NMR spectral data. Momose and coworkers[8] reported that an olefinic proton having *E*-configuration with respect to a methylene group, such as in **13A**, appeared at δ 7.83 as a singlet, whereas the olefinic proton in **13B**, which has *Z*-configuration, appeared at δ 6.76 as a triplet because of allylic coupling. In the ^{1}H-NMR of **12A**, the olefinic proton signal appeared at δ 7.90 as a singlet. Thus, the olefinic proton and the methylene group of **12** were confirmed to be in the *E*-configuration, as shown in Fig. 4.

This is the first report of the isolation of *trans*-phenylitaconic acid from a natural source.

Experimental

Melting points were determined on a Yanagimoto micro-melting points apparatus and are uncorrected. Spectral data were obtained on the following instruments; IR on a Shimadzu IR-430 in KBr; ^{1}H-NMR on a JEOL FX-400 (400 MHz) in $CDCl_3$ solution; mass spectra (MS) on a Hitachi RMU-6M. High performance liquid chromatography (HPLC) was carried out on an 8 × 300 mm column using a Hitachi pump and a Shodex RI SE-11 detector.

Extraction and Isolation——Air-dried whole plants (300 g) of *Artemisia argyi* LÉVL *et* VANT, collected in Chongming prefecture, Jiang-su province of China, were extracred with EtOH. The ethanol extract was concentrated and the residue (34 g) was chromatographed on silica gel with hexane–EtOAc (10 : 1) to give Fr. A (2.6 g) and Fr. B (4.6 g), with hexane–EtOAc (1 : 1) to give Fr. C (8.7 g), with $CHCl_3$–MeOH (5 : 1) to give Fr. D (6 g), and with MeOH to give Fr. E (4.4 g).

Fraction A was chromatographed repeatedly on silica gel with a variety of solvents (hexane–EtOAc (5 : 1), hexane–EtOAc (20 : 1) and hexane–$CHCl_3$ (3 : 1)), followed by HPLC (Nucleosil 50-5, hexane–EtOAc (300 : 1) and ODS-2, MeOH–H_2O (98 : 2)) to isolate compounds **1** (210 mg), **2** (50 mg), **3** (100 mg), **4** (180 mg), **5** (160 mg), **6** (18 mg), **7** (16 mg), **8** (2.6 mg), **9** (5.5 mg) and **10** (1 mg).

Fraction B was chromatographed repeatedly on silica gel with hexane–EtOAc (10 : 1 and 5 : 1) to give **11** (45 mg). Water (58 ml) was added to the methanol solution (29 ml) of Fr. C and the mixture was filtered. The filtrate was concentrated *in vacuo* and then water (17 ml) was added. The whole was extracted with EtOAc. The EtOAc extract was concentrated to leave an oily material, which was then subjected to chromatography on silica gel. Elution with C_6H_6–Me_2CO (5 : 1) afforded **12** (60 mg).

Compound 1 (Ethyl Palmitate)——^{1}H-NMR δ: 1.26 (3H, t, J=7 Hz, $OCH_2C\underline{H}_3$), 4.12 (2H, q, J=7 Hz, $OC\underline{H}_2CH_3$), MS m/z: 284 (M^+), 255, 157. This was identical with an authentic sample on the basis of GC-MS and ^{1}H-NMR spectral comparisons.

Compound 2 (Ethyl Oleate)——^{1}H-NMR δ: 0.88 (3H, t, J=6 Hz, $C\underline{H}_2CH_3$), 1.23 (3H, t, J=7 Hz, $OCH_2C\underline{H}_3$), 4.12 (2H, q, J=7 Hz, $OC\underline{H}_2CH_3$), 5.35 (2H, m, –CH=CH–), MS m/z: 310 (M^+), 284, 264, 157. This was identical with an authentic sample on the basis of GC-MS and ^{1}H-NMR spectral comparisons.

Compound 3 (Ethyl Linoleate)——^{1}H-NMR δ: 1.29 (3H, t, J=7 Hz, $OCH_2C\underline{H}_3$), 2.79 (2H, m, CH=CH–$C\underline{H}_2$–CH=CH), 4.12 (2H, q, J=7 Hz, $OC\underline{H}_2CH_3$), 5.37 (4H, m, 2 × CH=CH). This was identical with an authentic sample on the basis of GC-MS and ^{1}H-NMR spectral comparisons.

Compound 4 (Lupenone)——mp 165—167 °C, IR ν_{max}^{KBr} cm^{-1}: 1690. ^{1}H-NMR δ: 0.79—1.07 (6 × CH_3), 1.68 (3H, s, CH_3–C=C), 2.44 (2H, m, $COCH_3$), 4.57, 4.69 (2H, br s, C=CH_2), MS m/z: 424.3698 (M^+, Calcd for $C_{30}H_{48}O$ 424.3702), 409, 381, 205 (100), 189.

Compound 5 (Lupenyl Acetate)——mp 213—215 °C, IR ν_{max}^{KBr} cm^{-1}: 1725, ^{1}H-NMR δ: 0.78—1.02 (6 × CH_3), 1.67 (3H, s, CH_3–C=C), 2.03 (3H, s, OAc), 4.47 (1H, m, C$\underline{H}$OAc), 4.55, 4.69 (2H, br s, C=CH_2), MS m/z: 468.3955 (M^+, Calcd for $C_{32}H_{52}O_2$ 468.3965), 409, 218, 205, 129 (100).

Syntheses of Lupenone and Lupenyl Acetate from Betulin (14)——*p*-Toluenesulfonyl chloride (232 mg) was added to a solution of betulin (**14**) (455 mg) in pyridine (4 ml) at room temperature, and stirring was continued for 10 h. The reaction mixture was poured into ice-cold water and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with 2% HCl, dried over sodium sulfate and concentrated. The residue was chromatographed on silica gel with C_6H_6–Me_2CO (15 : 1) to give **15** (320 mg). A mixture of **15** (320 mg), NaI (653.5 mg), Zn (610.2 mg), glyme (6 ml) and hexamethylphosphoric triamide (5 ml) was refluxed for 3 h under stirring. The mixture was filtered and the filtrate was extracted with $CHCl_3$. The organic layer was dried and concentrated to leave an oil which was chromatographed on silica gel to give 73 mg of lupeol (**16**). Pyridinium chlorochromate (60 mg) and Synthetic Zeolite A-3 (140 mg) were added to a stirred solution of lupeol (**16**) (30 mg) in CH_2Cl_2 (4 ml). After 0.5 h, the reaction mixture was subjected to chromatography on silica gel with hexane–C_6H_6 (2 : 1), followed by HPLC (Nucleosil 50-5, hexane–EtOAc = 20 : 1) to give 13 mg of lupenone, which was identical with **4** (^{1}H-NMR). Lupeol (**16**) (43 mg) was treated with Ac_2O (100 μl) and pyridine (0.5 ml) at room temperature for 24 h. Usual work-up gave an oily material, which was purified by HPLC (Nucleosil 50-5, hexane–EtOAc = 30 : 1). The product was identical with **5** (^{1}H-NMR).

Compound 6 (α-Amyrin Acetate)——mp 200—201 °C, IR ν_{max}^{KBr} cm^{-1}: 1730, ^{1}H-NMR δ: 0.80—1.07 (8 × CH_3), 2.05 (3H, s, OAc), 4.52 (1H, m, C$\underline{H}$OAc), 5.12 (1H, t, J=3 Hz, CH_2–C$\underline{H}$=C), MS m/z: 468.3994 (M^+, Calcd for $C_{32}H_{52}O_2$ 468.3965), 218 (100), 203, 189. This was identical with an authentic sample on the basis of ^{1}H-NMR spectral comparison.

Compound 7 (β-Amyrin Acetate)——mp 218—220 °C, IR ν_{max}^{KBr} cm^{-1}: 1730, ^{1}H-NMR δ: 0.83—1.13 (8 × CH_3), 2.04 (3H, s, OAc), 4.52 (1H, m, C$\underline{H}$OAc), 5.16 (1H, t, J=3 Hz, CH_2C$\underline{H}$=C), MS m/z: 468.3962 (M^+, Calcd for $C_{32}H_{52}O_2$ 468.3964), 249, 218 (100), 205, 203, 189. This was identical with an authentic sample on the basis of ^{1}H-NMR spectral comparison.

Compound 8 (Glutinone)——mp 245—247 °C, IR ν_{max}^{KBr} cm^{-1}: 1700, ^{1}H-NMR δ: 0.82—1.23 (8 × CH_3), 2.41 (2H,

m, $COCH_2$), 5.68 (1H, m, $CH_2C\underline{H}=C$), MS *m/z*: 424.3738 (M^+, Calcd for $C_{30}H_{48}O$ 424.3703), 274, (100), 259, 218. This was identical with an authentic sample on the basis of ^{1}H-NMR spectral comparison.

Compound 9 (Fernenone)——mp 191—194 °C, IR ν_{max}^{KBr} cm^{-1} 1700, ^{1}H-NMR δ: 0.75—1.31 ($8\times CH_3$), 2.78 (2H, m, $COCH_2$), 5.37 (1H, m, $CH_2C\underline{H}=C$), MS *m/z*: 424.3669 (M^+, Calcd for $C_{30}H_{48}O$ 424.3702), 409, 257 (100), 245. This was identical with an authentic sample on the basis of ^{1}H-NMR spectral comparison.

Compound 10 (24-Methylene-cycloartanone)——mp 111—112 °C, ^{1}H-NMR δ: 0.59, 0.79 (each 1H, d, $J=4$ Hz, cyclopropane), 0.90—1.10 ($7\times CH_3$), 2.17 (2H, m, $COCH_2$), 4.67, 4.71 (each 1H, br s, $C=CH_2$), MS *m/z*: 438.3813 (M^+, Calcd for $C_{31}H_{50}O$ 438.3858) (100), 425, 395, 355, 340, 313, 300. This was identical with an authentic sample on the basis of ^{1}H-NMR spectral comparison.

Compound 11 (Simiarenol)——mp 212—215 °C, IR ν_{max}^{KBr} cm^{-1}: 3500, 1640, ^{1}H-NMR: 0.83, 0.88 (each 3H, $J=$ 6.8 Hz, $CH(C\underline{H}_3)_2$), 0.78—1.14 ($6\times CH_3$), 3.47 (1H, m, $C\underline{H}OH$), 5.61 (1H, m, $C=C\underline{H}CH_2$), MS *m/z*: 426.3857 (M^+, Calcd for $C_{30}H_{50}O$ 426.3859), 274 (100), 259, 231. This was identical with an authentic sample on the basis of ^{1}H-NMR spectral comparison.

Compound 12 (*trans*-Phenylitaconic Acid)——mp 186—188 °C, IR ν_{max}^{KBr} cm^{-1}: 2900, 1660, 1420, ^{1}H-NMR (CD_3OD) δ: 3.48 (2H, s, $C\underline{H}_2COOH$), 7.39 (5H, s, C_6H_5), 7.88 (1H, s, $CH=C$), MS *m/z*: 206 (M^+, FD-MS), 188, 162, 116.

Methylation of 12——Diazomethane in Et_2O was added to a solution of **12** in MeOH, and the mixture was concentrated to give the dimethyl ester (**12A**).

Compound 12A——^{1}H-NMR δ: 3.54 (2H, s, $C\underline{H}_2COOCH_3$), 3.73, 3.83 (each 3H, s, $2\times COOCH_3$), 7.36 (5H, s, C_6H_5), 7.90 (1H, s, $CH=C$), MS *m/z*: 234.0886 (M^+, Calcd for $C_{13}H_{14}O_4$ 234.0891), 202, 174, 115.

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