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Research Paper

Study of steroidal saponins in *Dioscorea Zingiberensis* C.H.Wright

Yinhong Wang[1], Daowan Lai[2], Yongmin Zhang[3], Along Kang[4], Yongxiao Cao[5], and Wenji Sun[1,*]

[1]Biology & Medicine Key Laboratory of Shaanxi Province, Xi'an, 710069, China.

[2]State Key Laboratory of Natural & biomimetic Drugs, Peking University, Beking, 100083, China.

[3]Université Pierre & Marie Curie-Paris 6, Institut Parisien de Chimie Moléculaire (UMR 7201), 4 place Jussieu, 75005 Paris, France.

[4]451 Military Hospital of China, Xi'an, 710055, China.

[5]Dept. of Physiology & Pathophysiology, Xi'an Jiaotong Uni., College of Medicine, Xi'an, China.

[*]Corresponding author

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ABSTRACT

Six steroidal saponins, isolated by a series of chromatographic separations from the rhizomes of *Dioscorea zingiberensis* C.H.Wright, were characterized as dioscin (**1**), gracillin (**2**), Zingiberensis Newsaponin (**3**), β-sitosterol-3-O-β-D-glucoside (**4**), (25R)-26-o-{β-D-glucopyranosyl}- furost-5, 20(22)- dienyl-3-o-β-D-glucopyranosyl(1→4) -[α-L-rhamnopynosyl(1→2)]- β-D-galactopyranoside (named as Huangjiangsu A, **5**), 3-o-{[β-D-glucopyranosyl(1→3)]-[α-L-rhamnopynosyl(1→2)]-(6′-o-Δ9,12-octadecadienoic acid ester)- β-D-glucopyranosyl}-diosgenin (named as Huangjiangsu B, **6**) based on the extensive spectroscopic analysis (including IR, ^{1}H NMR, ^{13}C NMR and MS data). Among them, compound 5 and 6 were new compounds. The purification and the structure characterization of these compounds were described. The isolated compounds and the total saponins were evaluated for their activity *in vitro* of relaxation to the blood vessel, and the results indicated that their pharmacologic activities *in vitro* were statistically significant. The relaxation rate of compound **5** is 35.4%±9.2%, which is the strongest among all the compounds. The experimental data and analytic results were described.

Keywords: *Dioscorea zingiberensis*; Steroidal saponins; Determination; Pharmacology; Relaxation.

INTRODUCTION

Dioscorea is an important genus in vegetable kingdom, which has been widely distributed all over the world. The modern pharmacologic study on dioscorea found that diosgenin was raw material to synthesize kinds of hormonal medicines (Nie, et al., 2004), and steroid saponins had effects on resisting arthritis (Fang, et al., 1982; Liu, et al., 1983), reducing the level of cholesterin (Ma, et al., 2002), curing arteriosclerosis and coronary heart disease (Tang, et al., 1983), which revealed that dioscorea had favorable clinic application.

Dioscorea zingiberensis C.H.Wright, is a perennial herb, and distributes widely in south of Shaanxi, Henan, Hubei, and Sichuan province of People's Republic of China (Huai, et al., 1989; Ding, et al., 1991). As an important species of dioscorea, it has also attracted much attention because of its high contents of diosgenin and steroid saponins, as well as smartable pharmacologic action. It is used to treat cough with lung heat, pyretic stranguria, anthracia, swelling, ulcer and sprain. Since several decades, a number of contemporary studies on steroidal saponins in *D. zingiberensis* have been reported in literatures (Cheng, et al., 2008; Yang, et al., 2007; Xu, et al., 2007; Sun, et al., 2003; Qian, et al., 2006; Liu, et al, 1985; Tang, et al., 1987). Scientists found that the content of diosgenin in *D. zingiberensis* was the highest following the study of diosgenin content of 9 species of dioscorea (Xiao, et al., 2006), which indicated that *D. zingiberensis* has promising application foreground. Contemporary clinical application demonstrated that diosgenin could significantly reduce the content of cholesterol in blood of rats (Ma, et al., 2002), which can indirectly reduce the probability of suffering from coronary artery disease (CAD) and the total steroidal saponins of it had excellent efficiency to treat CAD, decrease stenocardia and regulate metabolism, and no toxic and adverse reactions have been found (Tang, et al., 1983). Furthermore, its total steroidal saponins could treat artherosclerosis, high blood-fat, wheeze, inflammation and tumor (Fang, et al., 1982; Liu, et al., 1983).

The present paper describe the isolation and structural elucidation of six steroidal saponins (1—6) from the aqueous ethanol (70%) extract of dried rhizome of *D. zingiberensis*. And their pharmacologic activities of curing cardiovascular disease were also investigated.

MATERIALS AND METHODS

***Chemicals*:** Compounds **1-6** and the total steroidal saponins, isolated from *D. zingiberensis* (purchased from Ankang city), were dissolved in a mixture of DMSO and water (1:7). All of their concentrations are 1E-2mol/L. Verapamil was purchased from 451 Military Hospital of China and dissolved in water to 2E-4mol·L^{-1}. All other chemicals were purchased from commercial sources and used as received, water was doubly distilled in the laboratory.

Buffer solution containing (g/L): NaCl 6.954, $NaHCO_3$ 1.260, KCl 0.343, $MgCl_2$

0.244, NaH_2PO_4 0.187, $CaCl_2$ 0.166 and glucose 1.090.

***Animals*:** Sprague-Dawley rats (weighing 200–300 g) were from the Animal Center of Xi'an Jiaotong University College of Medicine. The animals were housed in Animal house, Department of Pharmacology and Physiology, in polycarbonate cages, in a room maintained under controlled room temperature 25±2°C, relative humidity 60-70% and provided with food and water. All the experimental procedures involving animals were in accordance with the Regulations of Experimental Animal Administration issued by the state committee of Science and Technology of People's Republic of China (1993). The animals were kept in normal before experimentation.

Extraction and isolation: The dried rhizomes of *D. zingiberensis* which were crushed and passed through 20 mesh screen were mixed by 70% aqueous ethanol and soaked for one night at room temperature. The percolate were condensed in vacuum to get brown residues which were suspended in water, and extracted by acetic ether, n-butanol saturated with water, successively. The n-butanol portion was chromatographed over silica gel (160-200 mesh) using dichlormethane-methanol-water (65:35:10) as mobile phase to give four fractions (Fr.1-4). Fr. 2 was repeatly chromagraphed over silica gel using dichlormethane-methanol-water (70:30:10 and 65:35:10, respectively) to obtain compound **3** and **5**. Fr. 3 was repeatly chromagraphed over silica gel using dichlormethane-methanol-water (75:25:10 and 70:30:10, respectively) to obtain compound **1** and **2**. And Fr. 4 was also repeatly chromagraphed over silica gel using dichlormethane-methanol-water (75:25:10) to obtain compound **4** and **6**.

Identification: These six compounds, characterized by 1D (^{1}H-NMR, ^{13}C-NMR), and 2D NMR(Incluing INEPT, ^{1}H–^{1}H COSY, HMQC, HMBC and ROESY), and MS(TOF-MS, ESI-MS) were defined as dioscin(1), gracillin(2), Zingiberensis Newsaponin(3), β- sitosterol-β-D- glucoside(4), (25R)-26-o-{β-D-glucopyranosyl }-furost-5, 20(22)- dienyl-3-o-β-D-glucopyranosyl(1→4) -[α-L-rhamnopynosyl(1→2)]-β-D-galactopyranoside (named as Huangjiangsu A, 5) and 3-o-{[β-D-glucopyranosyl(1→3)]-[α-L-rhamnopynosyl(1→2)]-(6′-o-Δ9,12-octadecadienoic acid ester) - β-D-glucopyranosyl}-diosgenin(named as Huangjiangsu B, 6), respectively.

Tissue preparation: Sprague-Dawley rats (body weight 250–300 g) were executed by vertebrae cervicales dislocation. The superior mesenteric artery (0.5–1 mm in diameter) was removed gently, immersed in cold buffer solution and dissected free of adhering tissue under a light microscope. The vessels were then cut into 1-mm-long cylindrical segments, used directly. The arterial segments were placed in a buffer solution and mounted on two L-shaped metal prongs, one of which was connected to a force displacement transducer for continuous recording of the isometric tension, and the other to a displacement device. The mounted arterial segments were immersed in temperature controlled (37°C) tissue baths containing the buffer solution. The solution was continuously gassed with 5% CO_2 in O_2 resulting in a P^H of 7.4. The artery segments were allowed to stabilize at a resting tension of 3 mN for 2 h before the experiments were started.

In vitro pharmacology: After equilibration, a vasoconstrictor was added to the bath. Once the sustained tension was obtained, the six compounds, main saponins, control group and positive control group were added to the baths respectively after pre-contraction with penylephrine(1E-6mol/L), and the concentration-response relation of the vasoconstrictor were constructed.

Data analysis: All data are expressed as Mean ± S.E.M. Unpaired Student's t-test was used to compare two sets of data and one-way for comparisons of more than two data sets. A P-value of <0.05 was considered to be significant. Relaxation responses in each segment are expressed as a percentage of the penylephrine-induced contraction.

RESULTS

Identification of compounds 1-6: Six compounds were obtained from the n-butanol layer of *D. zingiberensis* after a series of chromatographic separations. Compounds **1**—**4** are known steroidal saponins and their structures were identified as 3-o-{α-L-rhamnopyranosyl (1→4)-[α-L-rhamnopyranosyl (1→2)]-β-D-glucopyranosyl}-diosgenin(dioscin, **1**), 3-o-{β-D-glucopyranosyl(1→3)-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranosyl}-diosgenin(gracillin,**2**),3-o-[β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→4)]-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranosyl-diosgenin(ZingiberensisNewsaponin, **3**), β-sitosterol-3-O-β-D- glucoside(**4**), respectively. The physical and spectral data of these compounds were consistent with those reported in the literature. **5** and **6** are new compounds and their structures were characterized as (25R)-26-o-{β-D-glucopyranosyl}-furost-5,20(22)-dienyl-3-o-β-D-glucopyranosyl(1→4) -[α-L-rhamnopynosyl(1→2)]- β-D-galactopyranoside (named as Huangjiangsu A, **5**) and 3-o-{[β-D-glucopyranosyl(1→3)]-[α-L-rhamnopynosyl(1→2)]-(6′-o-Δ9,12-octadecadienoic acid ester) - β-D-glucopyranosyl}-diosgenin (named as Huangjiangsu B, **6**). Their structures are shown in Fig.1.

***Compound* 1:** dioscin, $C_{45}H_{72}O_{16}$, white crystal, mp: 294-296°C; IRυ(KBr): 3398(υ_{OH}), 2937(υ_{CH}), 1654($\upsilon_{C=CH}$), 1454, 1377, 1135. Both its spot colour and Rf value were accorded with those of dioscin by TLC. ^{13}C NMR(CD_3OD, 100MHz) δ141.9(C, C-5), δ122.6(CH, C-6), δ110.6(C, C-22), δ102.8(CH, C-1‴, Glc), δ101.8(CH, C-1″, Rha), δ100.3(CH, C-1′, Gal), δ82.2(CH, C-16), δ79.2(CH, C-3), δ67.4(CH_2, C-26), δ63.7(CH, C-17), δ57.8(CH, C-14), δ49.5(CH, C-9), δ42.9(CH, C-20), δ41.4(C, C-13), δ40.9(CH_2, C-12), δ39.5(CH_2, C-4), δ38.0(C, C-10), δ31.4(CH_2, C-23), δ32.4(CH_2, C-7), δ31.4(CH_2, C-8), δ32.8(CH_2, C-15), δ38.0(CH_2, C-1), δ30.7(CH_2, C-2), δ29.9(CH_2, C-24), δ21.9(CH_2, C-11), δ19.9(CH_3, C-19), δ18.4(CH_3, C-6″, Rha), δ17.5(CH_3, C-27), δ16.8(CH_3, C-18), δ14.9(CH_3, C-21), both ^{13}C and ^{1}H NMR data are consistent with the literature(Chen, et al., 1995; Kang, et al., 2005).

***Compound* 2:** gracillin, $C_{45}H_{72}O_{17}$, white acicular crystal, mp: 290-291°C, both its spot colour and Rf value were accorded with those of gracillin by TLC. ^{1}H NMR(pyridine-d_5, 500MHz) δ6.36(1H, s, H-1″, Rha), δ5.08(1H,d,J=7.5HzH-1‴, Glc), δ4.91(1H, H-1′, Glc), δ5.34(1H, s, H-6), δ0.82(3H, s, H-18), δ1.05(3H, s, H-19), δ1.63(3H, s, H-21), δ0.69(3H, s, H-27), ^{13}C NMR(pyridine-d_5, 125MHz) δ140.8(C,C-5), δ121.8(CH, C-6), δ109.3(C,C-22), δ104.5(CH, C-1‴, Glc), δ102.2(CH, C-1″, Rha), δ100.1(CH, C-1′, Glc), δ81.1(CH, C-16), δ77.9(CH, C-3),

δ66.9(CH_2, C-26), δ62.9(CH, C-17), δ56.7(CH, C-14), δ50.3(CH, C-9), δ42.0(CH, C-20), δ40.5(C, C-13), δ39.9(CH_2, C-12), δ38.8(CH_2, C-4), δ37.2(C, C-10), δ32.4(CH_2, C-23), δ32.2(CH_2, C-7), δ31.9(CH_2, C-8), δ31.7(CH_2, C-15), δ30.6(CH_2, C-1), δ30.1(CH_2, C-2), δ29.3(CH_2, C-24), δ21.1(CH_2, C-11), δ19.4(CH_3, C-19), δ18.7(CH_3, C-6″, Rha), δ17.3(CH_3, C-27), δ16.3(CH_3, C-18), δ15.0(CH_3, C-21), ^{13}C and ^{1}H NMR data are consistent with the literature(Chen, et al., 1995; Agrawal, et al., 1985; Kang, et al., 2005).

***Compound* 3:** Zingiberensis Newsaponin, $C_{51}H_{82}O_{22}$, white scobicular crystal, mp276-278°C; IRυ(KBr)cm^{-1}: 3419(υ_{OH}), 2929(υ_{CH}), 1646($\upsilon_{C=CH}$), 1452, 1372, 1059(υ_{c-o}). Both its spot colour and Rf value were accorded with those of Zingiberensis Newsaponin by TLC. ^{1}H NMR(pyridine-d_5, 500MHz) δ4.90(1H, s, H-1′, Glc), δ6.18(1H, s, H-1″, Rha), δ5.05(1H, d, J=7.5, H-1‴, Glc), δ5.23(1H, d, J=7.5,H-1⁗, Glc), δ5.27(1H, s, H-6), δ0.81(3H, s, H-18), δ1.03(3H, s, H-19), δ1.12(3H, s, H-21), δ0.68(3H, s, H-27), ^{13}C NMR(pyridine-d_5, 125MHz) δ140.8(C, C-5), δ121.8(CH, C-6), δ109.2(C, C-22), δ105.8(CH, C-1⁗, Glc), δ104.5(CH, C-1‴, Glc), δ101.7(CH, C-1″, Rha), δ100.0(CH, C-1′, Glc), δ81.1(CH, C-16), δ78.2(CH, C-3), δ66.9(CH_2, C-26), δ62.9(CH, C-17), δ56.6(CH, C-14), δ50.3(CH, C-9), δ42.0(CH, C-20), δ40.5(C, C-13), δ39.9(CH_2, C-12), δ38.9(CH_2, C-4), δ37.1(C, C-10), δ31.8(CH_2, C-23), δ32.3(CH_2, C-7), δ31.7(CH_2, C-8),δ32.2(CH_2, C-15), δ37.5(CH_2, C-1), δ30.1(CH_2, C-2), δ29.3(CH_2, C-24), δ21.1(CH_2, C-11), δ19.4(CH_3, C-19), δ18.6(CH_3, C-6″, Rha), δ17.3(CH_3, C-27), δ16.3(CH_3, C-18), δ15.0(CH_3, C-21), ^{13}C NMR and ^{1}H NMR data are consistent with the literature(Jain, 1987).

***Compound* 4:** β-Sitosterol-3-O-β-D-glucoside, $C_{35}H_{60}O_6$, mp: 290-291°C, IR (KBr) cm^{-1}: 3427 (OH), 1632 (C=C), 1462, 1367, 970 , ^{1}H NMR(pyridine-d_5, 500MHz) δ5.34(1H, s, H-6), δ5.05(1H, d, J=7.5, H-1′), δ0.65(3H, s, H-28), δ0.67(3H, s, H-29), δ0.83(3H, s, H-18), δ0.93(3H, s, H-26), δ1.03(3H, s, H-19), δ1.13(3H, s, H-21), ^{13}C NMR(pyridine-d_5, 125MHz) δ141.0(CH_2, C-8), δ121.9(CH_2, C-7), δ102.6(CH, C-1′, Glc), δ78.6(CH, C-3), δ56.3(CH, C-17), δ56.2(CH, C-14), δ51.5(CH_2, C-24), δ50.4(CH, C-9), δ42.5(C, C-13), δ42.0(CH, C-20), δ39.9(C, C-5), δ39.4(CH_2, C-4), δ39.4(CH_2, C-12), δ37.0(CH_2, C-1), δ34.3(C, C-22), δ34.2(C, C-10), δ32.1(CH_2, C-25), δ31.5(CH_2, C-2), δ29.3(CH, C-6), δ28.6(CH, C-16), δ26.5(CH_2, C-23), δ24.6(CH_2, C-28), δ23.4(CH_2, C-15), δ21.3(CH_2, C-11), δ20.0(CH_3, C-21), δ19.2(CH_3, C-27), δ19.1(CH_2, C-26), δ12.5(CH_2, C-29), δ12.2(CH_3, C-19), δ12.0(CH_3, C-18), ^{13}CNMR and ^{1}HNMR data are consistent with the literature (Wei, et al, 1997).

***Compound* 5:** Obtained as white scobicular crystal and named as Huangjiangsu A. mp: 274-276°C, IRυ3420(υ_{OH}), 2930(υ_{CH}), 1645($\upsilon_{C=CH}$), 1455, 1375, 1075(υ_{c-o}). The HREI-MS showed a molecular ion peak [M-1] at 1045.5189 indicating the molecular formula $C_{51}H_{82}O_{22,}$ which was supported by the ^{13}C NMR spectrum. The ^{13}C NMR spectrum showed 51carbon signals, of which 27 were assigned to the aglycone, and 24 to the sugar moieties (Table 1). Acid hydrolysis of **5** with 1 M HCl indicated the presence of D-galactose, L-rhamnose and D-glucose, with a ratio of 1:1:2 quantified by TLCS. And the ^{1}H and ^{13}C NMR spectra also showed four anomeric proton signals at δ 4.95(d, J = 7.4 Hz), 6.25 (br s), 4.83 (d, J = 7.7 Hz) and 5.12 (d, J = 7.7Hz) and the corresponding carbon signals at δ 99.9, 101.8, 104.9 and 105.2, respectively.

Besides, its ^{1}H NMR spectrum showed signals for four methyl groups at 0.71(br s), 1.04 (s), 1.63 (s), 1.01(d, J = 5 Hz), and an olefinic proton at δ 5.27 (br d, J

= 4.2 Hz) derived from the steroidal skeleton (Hann, et al., 1975). The significant lower field shift(+8.2) of C-26 (δ 74.9) compared with that of diosgenin(at δ 66.7)(Hann, et al., 1975) indicated its circle F was ruptured and a glycoside was formed. Together with the chemical shifts of δ 78.0 (C-3) revealed that **5** was a bisdesmosidic glycoside.And C-20 and C-22 resonated at δ 104.9 and 152.4 respectively, which were also remarkablely downfield compared with that of diosgenin(δ 41.6 of C-20, and 109.1 of C-22) indicating a double bond connecting them. Thus, the aglycone was established which were similar to those of the aglycone of zingiberenin G (Yang, et al., 2008).

Starting from the anomeric protons of each sugar unit, all the hydrogens within each spin system were assigned using COSY with the aid of NOESY experiments, while the carbons were assigned by HMQC and further confirmed by HMBC experiments. On comparison of the ^{13}C NMR data between **5** and zingiberenin G, the similar C-26 sugar chain was established which was further confirmed by the HMBC correlations between H-1 of Glc′(δ 5.12) and C-26 (δ 74.9). The linkage of the sugar units at C-3 of the aglycone was established from the following HMBC correlations: H-1 of Rha (δ 6.25) with C-2 of gal (δ 77.2), H-1 of Glc (δ 4.83) with C-4 of gal (δ 82.1), H-1 of Gal (δ 4.95) with C-3 of the aglycone (δ 78.0). The same conclusion with regard to the sugar sequence was also drawn from the NOESY experiment. From the above evidence, the structure of Huangjiangsu A (**5**) was elucidated as (25R)-26-O-{β-D-glucopyranosyl}- furost-5, 20(22)- dienyl 3-O-β-D-glucopyranosyl(1→4) -[α-L-rhamnopynosyl(1→2)]- β-D-galactopyranoside. ^{13}C NMR and ^{1}H NMR data are given in Table 1.

***Compound* 6:** Obtained as white powder and named as Huangjiangsu B. mp: 244-246°C, IRυ(KBr)cm^{-1}: νmax 3435(υ_{OH}), 2924(υ_{CH}), 2854, 1739, 1459, 1376, 1165, 1048($\upsilon_{c\text{-}o}$), 900 cm^{-1}. The positive-ion MALDITOF-MS and HRESI-MS of **6** showed an psuedomolecular $[M+Na]^+$ ion peak at *m/z* 1169.29, 1169.4970, respectively, indicating the molecular formula of $C_{63}H_{102}O_{18}$.

The presence of three anomeric protons signals atδ4.90 (br s), 6.36 (br s) and 5.07 (d, J =8.0Hz), δ6.36 (*br* s), δ 5.07 (*br* s), δ 4.90 (*br* s) suggested the presence of three monosaccharides. (Table-1). Acid hydrolysis and gas chromatographic analysis of the peracetylated derivatives of the sugars indicated the presence of rhamnose and glucose in a 1:2 ratio.

In comparison the ^{1}H and ^{13}C spectra of compound **6** with literature (Hann, et al., 1975; Agrawal, et al., 1985), the aglycone was establish as diosgenin. The chemical shift of C-3(δ 78.5) revealed a sugar chain was linked, and the connectivity of the sugar units was deduced from the HMBC spectrum. The presence of cross-peaks between the proton signals at δ 4.90 (H-1 of Glc), δ 4.18 (H-2 of Glc), δ 4.14 (H-2 of Glc) and the carbon signals at δ 78.5 (C-1, aglycone), δ 102.3 (C-1of Rha) and δ 104.7 (C-1 of Glc′), respectively, indicated a 1,2,3-tri-substituted glucopyranose, which was common to the collection of gracillin (Chen, et al., 1995; Agrawal, et al., 1985; Kang, et al., 2005).

In the ^{13}C NMR spectrum, the carbon signal at δ173.4 demonstrated the

presence of a carbonyl group, and the profuse chemical shift between 14-40 ppm demonstrated the existence of an aliphatic chain which contained two double bonds (δ 128.41, 128.46, 130.42 and 130.48). Furthermore, the carbon signal of C-6′ of Glc(δ 64.2) was shifted to lower field compared with gracillin(δ 61.4), and HMBC experiment demonstrated that H-6′(δ 4.81,4.66 of H-6a′ and H-6b′,respectively) correlated with the carbon signal of δ173.4 ppm. Above evidence indicated that C-6′(Glc) connected with a long aliphatic chain through an ester bond. The fatty acid chain had a molecular formula $C_{18}H_{31}O_2$, determined from its fragment ion peak at *m/z* 318.2271 ($[C_{18}H_{31}O_2+K]^+$) in HRESI-MS(Positive). The ion peak at *m/z* 274.08 in MALDITOF-MS(positive) and 274.2108 in HRESI-MS, in accordance with the molecular formula $[C_{18}H_{31}O_2\text{-}CO_2+K]^+$ which arised from decarboxylation of the fatty acid chain($C_{18}H_{31}O_2$) through α-cleavage. The ion peaks (m/z) in MALDITOF-MS: 136.91($[C_7H_{14}+K]^+$), 176.85($[C_{10}H_{18}+K]^+$) indicated that the double bonds positioned at ω-6 and 9. Thus, the fatty acid was an determined to be Δ9,12-octadecadienoic acid. Compared the ^1H and ^{13}C NMR spectra of **6** with those of 3-O-[α-L-rhamnopyranosysyl(1→2)-{α-L-rhamnopyranosysyl(1→4)} -(6′-O-hexadecanoyl)-β-D-glucopyranosyl]-25(R)-spirost-5-en-3β-ol.(Shu, 2006) and found that their spectroscopic datas were similar except a few differences in the fatty chain.

Compound **6** was thus identified as 3-o-{[β-D-glucopyranosyl(1→3)] -[α-L-rhamnopynosyl(1→2)]-(6′-o-Δ9,12-octadecadienoic acid ester)-β-D-glucopyranosyl}-diosgenin(its chemical shifts were summarized in Table 1).

Pharmacologic effect in vitro: Table-2, shows the relaxative effect of compounds **1-6** and main saponins on mesenteric artery per-contracted by penylephrine. The relaxation rate of huangjiangsu A is 35.4%±9.2%, which is the strongest among all the compounds, and the relaxation of the total saponins is least, suggesting that compounds may counteract the relaxation among the total saponins.

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Table- 1: ^{13}C NMR(125 MHz) spectral data of compounds 5 and 6(in pyridine-d_5)

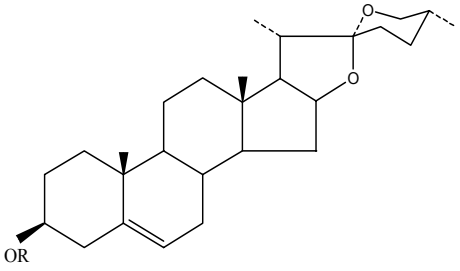
Agly cone	**Compound 5**		**Compound 6**		**Sugars chain**	**Compound 5**		**Compound 6**	
	δ_c	δ_H	δ_c	δ_H		δ_c	δ_H	δ_c	δ_H
1	37.5	0.96, 1.73	30.6	1.57, 0.86		3-O-Gal		3-O-Glc	
2	30.1	1.86, 2.09	30.0	1.90, 2.15	1′	99.9	4.95	100.4	4.90
3	78.0	3.86	78.5	3.93	2′	77.2	4.23	77.0	4.18
4	38.9	2.74	38.9	2.80	3′	77.7	4.20	78.7	4.14
5	140.7		140.9		4′	82.1	4.23	82.9	4.15
6	121.8	5.27	121.9	5.36	5′	76.2	3.85	74.4	3.87
7	32.4	1.85	32.3	1.45	6′	61.5	4.46, 4.52	64.2	4.81, 4.66
8	31.4	1.84	31.8	1.67		Rha(1→2)		Rha(1→2)	
9	50.3	0.88	50.4	0.92	1″	101.8	6.25	102.3	6.36
10	37.1		37.2		2″	72.5	4.74	72.4	4.87
11	21.2	1.43	21.2	1.48	3″	72.7	4.58	72.8	4.56
12	39.6	1.14, 1.72	39.9	1.74 ,1.10	4″	74.1	4.34	74.1	4.30
13	43.4		40.5		5″	69.5	4.94	69.6	4.89
14	54.9	0.85	56.7	0.84	6″	18.7	1.76	18.7	1.74
15	34.5	2.11, 1.50	32.1	2.05 ,1.45		Glc(1→4)		Glc(1→3)	
16	84.4	4.78	81.1	4.57	1‴	104.9	4.83	104.7	5.07
17	64.5	2.43	63.0	1.82	2‴	75.2	4.04	75.0	4.01
18	14.1	0.71	16.4	0.84	3‴	78.6	4.24	78.7	4.03
19	19.4	1.04	19.4	1.06	4‴	71.7	4.24	71.5	4.11
20	104.9		42.0	1.96	5‴	78.5	3.96	78.5	3.95
21	11.8	1.63	15.0	1.14	6‴	62.8	4.40, 4.57	62.4	4.26, 4.56
22	152.4		109.3			26-O-Glc		Fatty acid chain	
23	23.7	2.22	32.4	2.05	1	105.2	5.12	173.4	
24	31.4	1.48	29.3	1.57	2	75.0	4.04	34.4	2.36
25	33.5	1.94	30.2	1.88	3	78.6	4.24	27.6	1.62
26	74.9	3.60, 3.94	66.9	3.58, 3.49	4	71.2	4.27		
27	17.3	1.01	17.3	0.69	5	78.5	3.96		
					6	61.9	4.47, 4.34		
					8			32.3	2.10
					9,10,12,13			128-130	5.4-5.5
					11			37.6	2.92
					14			31.7	2.10

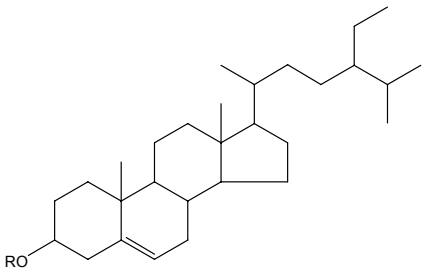
	17	22.8	
	18	14.2	0.86
	other	29-31	

Table-2: The relaxation rate of compounds and the total saponins isolated from *dioscorea zingiberensis* on mesenteric artery of rats per-contracted by penylephrine.

Name	n	Relaxation rate (%)
Control	8	4.7±3.3
Total saponins	8	19.5±4.8*
Dioscin	8	22.2±5.5*
Gracillin	8	20.8±3.2*
Zingiberensis newsaponin	8	28.0±11.2*
Huangjiangsu A	8	35.4±9.2*
β-sitosterol-3-O-β-D- glucoside	8	26.8±3.9*
Huangjiangsu B	8	19.5±3.1*
positive control	8	73.9±10.4*

* represented P<0.05



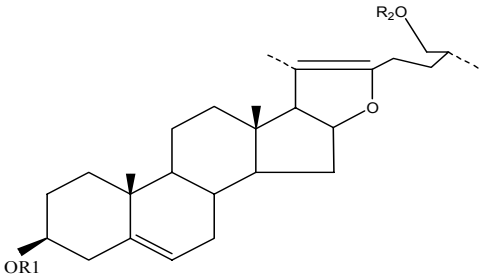


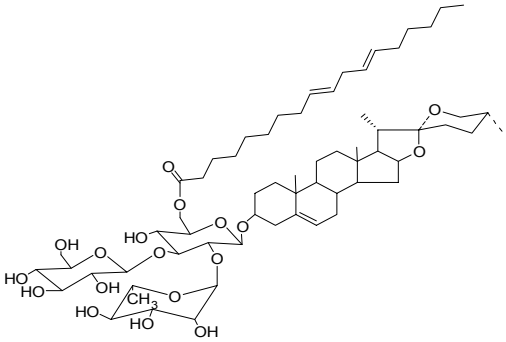
1 R= -glc-[(2→1)rha]-(4→1)rha

2 R= -glc-[(2→1)rha]- (3→1)glc

3 R= -glc-[(2→1)rha]-[(4→1)glc-(3→1)glc]

4 R= -glc





5 R_1= -gal-[(2→1)rha]-(4→1)glc

6 R_2= -glc

Figure-1: Structures of compounds 1-6 isolated from rhizome of *Dioscoorea zingiberensis*.

