化合物Ⅳ：黄色粉末，mp 285～289 ℃，分子式 $C_{12}H_{12}N_2O_2$。EI-MS、^{1}H-NMR和^{13}C-NMR数据与文献报道[2]完全一致，且氢谱与标准图谱吻合很好，故此化合物鉴定为 2,3,4,9-四氢-1*H*-吡啶骈[3,4-b]吲哚-3-羧酸。

4 讨论

本实验分离得到的生物碱因其量较低而没有对它们进行来源分析。笔者初步认为它们应该来源于单味药材。因生物碱类化合物水溶性较好，在复方各单味药合煎时溶出率较高，且合煎时不同类物质之间存在相互促溶作用。这一观点基本上符合中医复方思想，同时也说明单味药醇提物较难分离得到以上单体化合物。

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Non-alkaloids in *Hippeastrun vittatum*

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Abstract: **Objective** To investigate the non-alkaloid constituent of *Hippeastrun vittatum* (Amaryllidaceae). **Methods** Solvent extraction and column chromatography were used to isolate the non-alkaloid constituents, and physicochemical constants and spectroscopic analysis were employed for structural elucidation. **Results** Five glycosphingosilipids were isolated, and their structures were elucidated to be (2*S*, 3*R*, 4*E*, 8*Z*)-2-[(2*R*-2-hydroxyhexadecanoyl) amido]-4, 8-octadecadiene-1, 3-diol 1-*O*-β-*D*-glucopyranoside (Ⅰ), (2*S*, 3*R*, 4*E*, 8*E*)-2-[(2*R*-2-hydroxyhexadecanoyl) amido]-4, 8-octadecadiene-1, 3-diol 1-*O*-β-*D*-glucopyranoside (Ⅱ), (2*S*, 3*R*, 4*E*, 8*Z*)-2-[(2*R*-2-hydroxyoctadecanoyl) amido]-4, 8-octadecadiene-1, 3-diol 1-*O*-β-*D*-glucopyranoside (Ⅲ), (2*S*, 3*R*, 4*E*, 8*E*)-2-[(2*R*-2-hydroxyoctadecanoyl) amido]-4, 8-octadecadiene-1, 3-diol 1-*O*-β-*D*-glucopyranoside (Ⅳ), (2*S*, 3*R*, 4*E*, 8*Z*)-2-[(2*R*-2-hydroxyeicosadecanoyl) amido]-4, 8-octadecadiene-1, 3-diol 1-*O*-β-*D*-glucopyranoside (Ⅴ), respectively. **Conclusion** They are all isolated from the fresh bulbs of *H. vittatum* for the first time.

Key words: Amaryllidaceae; *Hippeastrun vittatum* (L'Herit.) Herb.; glycosphingosilipids

花朱顶红中非生物碱类成分研究

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摘 要：**目的** 研究石蒜科植物花朱顶红中非生物碱类成分。**方法** 采用大孔树脂、硅胶、ODS柱色谱分离纯化，经理化常数、光谱学方法鉴定结构。**结果** 分离得到了5个葡糖鞘脂类成分，其结构分别鉴定为 (2*S*,3*R*,4*E*,8*Z*)-2-[(2*R*-2-羟基十六酰)氨基]-4,8-十八二烯-1,3-二醇 1-*O*-β-*D*-吡喃葡萄糖苷 (Ⅰ)、(2*S*,3*R*,4*E*,8*E*)-2-[(2*R*-2-羟基十六酰)氨基]-4,8-十八二烯-1,3-二醇 1-*O*-β-*D*-吡喃葡萄糖苷 (Ⅱ)、(2*S*,3*R*,4*E*,8*Z*)-2-[(2*R*-2-羟基十八酰)氨基]-4,8-十八二烯-1,3-二醇 1-*O*-β-*D*-吡喃葡萄糖苷(Ⅲ)、(2*S*,3*R*,4*E*,8*E*)-2-[(2*R*-2-羟基十八酰)氨基]-4,8-十八

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二烯-1,3-二醇 1-*O*-β-*D*-吡喃葡萄糖苷(Ⅳ)、(2*S*,3*R*,4*E*,8*Z*)-2-[(2*R*-2-羟基二十酰)氨基]-4,8-十八二烯-1,3-二醇 1-*O*-β-*D*-吡喃葡萄糖苷(Ⅴ)。**结论** 它们均为首次从花朱顶红中分得。

关键词:石蒜科;花朱顶红;葡糖鞘脂类
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The plants of Amaryllidaceae, widely distributed from the tropics to the subtropics, have 83 genus and around 1 000 species in the world[1]. *Hippeastrun vittatum* (L'Herit.) Herb. is one of these plants of *Hippeastrum* Herb. The constituent investigations of this family plants have been concentrated on alkaloid constituents, known as Amaryllidaceae alkaloids because of their special structures, and 120 alkaloids were isolated by now and some of them had been studied on bioactivity. Generally, various kinds of constituents exist in plants, accounting for their bioactivities, and one type of constituents can not be responsible for all the bioactivities. Similarly, plants of *Hippeastrum* Herb. also have various bioactivities, such as anti-tumor, anti-inflammatory, *etc*, and only alkaloid constituents can not be enough to explain their bioactivities. Apparently, the investigation of the non-alkaloid constituents is also necessary. However, such kind of research work had seldom reported. As one part of Amaryllidacea plants' researches, the present paper reports the non-alkaloid constituents of *H. vittatum*.

1 Experiments

Optical rotations were measured with a JASCO DIP-140 polarimeter. NMR spectra were recorded on a JEOL Lambda 500 spectrometer. Mass spectra were obtained on a JEOL JMS SX-102A spectrometer. HPLC was carried out with TOSOH CCPM-11 UV-8020 and TOSOH CCPP-M UV 8011 chromatographs and TOSOH TSK gel ODS-120A (25 cm × 4.6 mm and 30 cm × 21.5 mm) column. Column chromatography was performed on Kieselgel 60 H (Merck, 5-40 μm), Chromatorex ODS (DM-1020T, Fuji-Silysia), and DIAION HP-20 (Mitsubishi Chem. Ind. Co. Ltd.). TLC was carried out on HPTLC-Fertigplatten Kiesel gel 60 F_{254} (Merk) and HPTLC-Fertigplatten RP-18 $WF_{254}s$ (Merk).

1.1 Isolation of compound Ⅰ-Ⅴ

The fresh bulbs of *H. vittatum* (identified by professor Zhang Jing-min in Pharmacognosy Department of Pharmacy College, Jilin University) (20 kg) were cut into small pieces and extracted with methanol for three times at room temperature. Removal of the solvent from the combined methanol solution under reduced pressure gave the methanol extract (1 030 g). The methanol extract was chromatographed on DIAION HP-20, eluting with H_2O (928.00 g), 10% MeOH (28.18 g), 30% MeOH (19.59 g), 50% MeOH (12.30 g), 70% MeOH (9.22 g), MeOH (22.16 g). The MeOH eluate was chromatographed on silica gel with MeOH-$CHCl_3$-EtOAc-H_2O (2∶2∶4∶1, lower layer) to give six fractions: fr.1 (12.10 g), fr.2 (5.55 g), fr.3 (0.50 g), fr.4 (1.16 g), fr.5 (0.73 g), fr. 6 (0.84 g). Fr.2 was further chromatographed on silica gel with $CHCl_3$-CH_3OH-H_2O (6∶1∶0.1, homogeneous) to give two fractions: fr. 2-1 (4.60 g), fr. 2-2 (0.46 g). Fr. 2-2 was further subjected to ODS chromatography with 95% MeOH to give four fractions: fr. 2-2-1 (80 mg), fr. 2-2-2 (44 mg), fr. 2-2-3 (39 mg), fr. 2-2-4 (262 mg). Fr. 2-2-1, fr. 2-2-2, and fr. 2-2-3 were further subjected to HPLC (solvent MeOH, flow rate 10 mL/min) to afford Ⅰ (50 mg), Ⅱ (25 mg) from fr. 2-2-1, Ⅲ (24 mg), Ⅳ (19 mg) from fr. 2-2-2, Ⅴ (25 mg) from fr. 2-2-3.

Compound Ⅰ: A white powder, $[\alpha]_D^{25}$ +3.7 (c=1.0, MeOH), FAB-MS *m/z*: 737 $[M+Na]^+$, 697 $[(M+H)-H_2O]^+$, 535 $[(M+H)-H_2O-162]^+$. ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (CD_3OD) see Table 1.

Compound Ⅱ: A white powder, $[\alpha]_D^{25}$ +3.6 (c=0.42, MeOH), FAB-MS *m/z*: 737 $[M+Na]^+$, 715 $[M+Na]^+$, 697 $[(M+H)-H_2O]^+$, 535 $[(M+H)-H_2O-162]^+$. ^{1}H-NMR (CD_3OD) δ: 0.90 (6H, t, H-18, 16′), 1.29 (36H, m, H-

Table 1　^{1}H-NMR and ^{13}C-NMR data of compound Ⅰ (CD_3OD)

Position	^{1}H-NMR, δ (m, J in Hz)	^{13}C-NMR, δ	H-H COSY	HMBC (C-H)
1a	3.72 (dd, 10.1, 3.4)	69.7	1b, 2	1C-1″H, 3H; 1H-1″C, 3C
1b	4.11 (dd, 10.1, 5.5)	69.7	1a, 2	1C-1″H, 3H; 1H-1″C, 3C
2	3.99 (m)	54.6	1a, 1b, 3	2C-4H; 2H-1′C
3	4.14 (m)	72.8	2, 4	3C-1H, 5H; 3H-1C, 5C
4	5.49 (dd, 15.3, 7.3)	131.3	3, 5	4C-2H, 6H; 4H-2C, 6C
5	5.74 (dtd, 15.3, 5.9, 0.6)	134.4	4, 6	5C-3H, 7H; 5H-3C, 7C
6	2.09 (m)	33.7	5, 7, 4	6C-4H, 8H; 6H-8C, 4C
7	2.12 (m)	27.9	6, 8, 9	7C-5H, 9H; 7H-9C, 5C
8	5.38 (t, 4.8 Hz)	129.8	7	8C-10H, 6H; 8H-6C, 10C
9	5.38 (t, 4.8 Hz)	131.4	10	9C-7H; 9H-7C
10	2.04 (m)	28.3	9, 11	10C-8H; 10H-8C
11—15	1.29 (m)	30.4—30.9		
16	1.29 (m)	33.1		
17	1.29 (m)	23.7	18	
18	0.90 (t-like)	14.5	17	
1′		177.2		1′C-2H, 3′H
2′	3.99 (m)	73.1	3′a, 3′b	
3′a	1.54 (m)	35.4	3′b, 2′, 4′	
3′b	1.71 (m)	35.4	3′a, 2′, 4′	
4′	1.42 (m)	26.2	3′a, 3′b, 5	
5′—13′	1.29 (m)	30.4—30.9		
14′	1.29 (m)	33.1		
15′	1.29 (m)	23.7	16′	
16′	0.90 (t-like)	14.5	15′	
1″	4.27 (d, 7.6 Hz)	104.7	2″	1″C-1H; 1″H-1C, 3″C, 5″C
2″	3.19 (dd, 7.6, 9.1)	75	1″, 3″	
3″	3.36 (m)	77.9	2″, 4″	
4″	3.30 (m)	71.6	3″, 5″	
5″	3.28 (m)	78	4″, 6″a, 6″b	
6″a	3.67 (dd, 5.2, 11.9)	62.7	5″, 6″b	
6″b	3.87 (dd, 11.9, 1.8)	62.7	5″, 6″a	

11—17, H-5′—15′), 1.41 (2H, m, H-4′), 1.56 (1H, m, H-3′a), 1.71 (1H, m, H-3′b), 1.98 (2H, m, H-10), 2.07 (4H, s, H-6, 7), 3.71 (1H, dd, J=10.3, 3.7 Hz, H-1a), 4.11 (1H, dd, J=10.3, 5.5 Hz, H-1b), 3.99 (1H, m, H-2, 2′), 4.14 (1H, m, H-3), 5.48 (1H, dd, J=15.3, 7.3 Hz, H-4), 5.73 (1H, dtd, H-5), 5.43 (2H, t, H-8, 9); glucose residue, 4.27 (1H, d, J=7.6 Hz, H-1″), 3.19 (1H, dd, J=7.6, 9.1 Hz, H-2″), 3.36 (1H, m, H-3″), 3.30 (1H, m, H-4″), 3.28 (1H, m, H-5″), 3.67 (1H, dd, J=5.5, 11.9 Hz, H-6″a), 3.87 (1H, dd, J=11.9, 1.5 Hz, H-6″b). ^{13}C-NMR (CD_3OD) δ: 177.2 (C-1′), 134.4 (C-5), 131.2 (C-4), 132.0 (C-9), 130.7 (C-8), 73.1 (C-2′), 72.9 (C-3), 69.7 (C-1), 54.6 (C-2), 35.9 (C-3′), 33.7 (C-6), 33.6 (C-10), 33.3 (C-7), 33.1 (C-16, 14′), 30.8—30.3 (C-11—15, 5′—13′), 26.2 (C-4′), 23.7 (C-17′, 15′), 14.5 (C-18, 16′); glucose residue, 104.7 (C-1″), 75.0 (C-2″), 77.9 (C-3″), 71.6 (C-4″), 78.0 (C-5″), 62.7 (C-6″).

Compound Ⅲ: A white powder, $[\alpha]_D^{25}$ +3.6 (c=1.0, MeOH), FAB-MS m/z: 743 $[M+H]^+$, 725 $[(M+H)-H_2O]^+$, 563 $[(M+H)-H_2O-162]^+$. ^{1}H-NMR (CD_3OD) δ: 0.90 (6H, t, H-18, 18′), 1.29 (40H, m, H-11—17, H-5′—17′), 1.42 (2H, m, H-4′), 1.56 (1H, m, H-3′a), 1.72 (1H, m, H-3′b), 2.04 (2H, m, H-10), 2.09 (2H, m, H-6), 2.12 (2H, m, H-7), 3.72 (1H, dd, J=10.4, 3.7 Hz, H-1a), 4.11 (1H, dd, J=10.4, 5.8 Hz, H-1b), 3.99 (1H, m, H-2, 2′), 4.14 (1H, m, H-3), 5.49 (1H, dd, J=15.3, 7.3 Hz, H-4), 5.74 (1H, dtd, J=15.3, 5.9, 0.9 Hz, H-5), 5.38 (2H, t, J=4.6 Hz, H-8, 9); glucose residue, 4.27 (1H, d, J=7.6 Hz, H-1″), 3.19 (1H, dd, J=7.6, 9.2 Hz, H-2″), 3.36 (1H, m, H-3″), 3.30 (1H, m, H-4″), 3.28 (1H, m, H-5″), 3.67 (1H, dd, J=5.5, 11.9 Hz, H-6″a),

3.87 (1H, dd, J= 11.9, 1.6 Hz, H-6″b). ^{13}C-NMR (CD_3OD) δ: 177.2 (C-1′), 134.4 (C-5), 131.3 (C-4), 131.4 (C-9), 129.9 (C-8), 73.1 (C-2′), 72.9 (C-3), 69.7 (C-1), 54.6 (C-2), 35.9 (C-3′), 33.7 (C-6), 33.1 (C-16, 16′), 30.9－30.4 (C-11－15, 5′－15′), 28.3 (C-10), 27.9 (C-7), 26.2 (C-4′), 23.7 (C-17, 17′), 14.5 (C-18, 18′); glucose resideue, 104.7 (C-1″), 75.0 (C-2″), 77.9 (C-3″), 71.6 (C-4″), 78.0 (C-5″), 62.7 (C-6″).

Compound Ⅳ: A white powder, $[\alpha]_D^{25}$ +3.6 (c=0.40, MeOH-$CHCl_3$, 1 : 1), FAB-MS m/z: 743 $[M+H]^+$, 725 $[(M+H)-H_2O]^+$, 563 $[(M+H)-H_2O-162]^+$. ^{1}H-NMR (CD_3OD) δ: 0.90 (6H, t, H-18, 18′), 1.29 (40H, m, H-11－17, H-5′－17′), 1.41 (2H, m, H-4′), 1.56 (1H, m, H-3′a), 1.71 (1H, m, H-3′b), 1.98 (2H, m, H-10), 2.07 (4H, s, H-6, 7), 3.73 (1H, dd, J= 10.4, 3.7 Hz, H-1a), 4.10 (1H, dd, J= 10.4, 5.5 Hz, H-1b), 3.99 (1H, m, H-2, 2′), 4.14 (1H, m, H-3), 5.49 (1H, dd, J=15.3, 7.0 Hz, H-4), 5.73 (1H, dtd, H-5), 5.43 (2H, t, H-8, 9); glucose residue, 4.27 (1H, d, J=7.6 Hz, H-1″), 3.19 (1H, dd, J=7.6, 9.2 Hz, H-2″), 3.36 (1H, m, H-3″), 3.30 (1H, m, H-4″), 3.28 (1H, m, H-5″), 3.67 (1H, dd, J=5.5, 11.9 Hz, H-6″a), 3.87 (1H, dd, J= 11.9, 1.5 Hz, H-6″b). ^{13}C-NMR (CD_3OD) δ: 177.2 (C-1′), 134.4 (C-5), 131.2 (C-4), 132.0 (C-9), 130.7 (C-8), 73.2 (C-2′), 72.9 (C-3), 69.7 (C-1), 54.7 (C-2), 35.9 (C-3′), 33.7 (C-6), 33.6 (C-10), 33.3 (C-7), 33.0 (C-16, 16′), 30.8－30.3 (C-11－15, 5′－15′), 26.1 (C-4′), 23.7 (C-17, 17′), 14.5 (C-18, 18′); glucose resideue, 104.7 (C-1″), 75.0 (C-2″), 77.9 (C-3″), 71.6 (C-4″), 78.0 (C-5″), 62.7 (C-6″).

Compound Ⅴ: A white powder, $[\alpha]_D^{25}$ +3.7 (c=1.0, MeOH), FAB-MS m/z: 771 $[M+H]^+$, 755 $[(M+H)-H_2O]^+$, 591 $[(M+H)-H_2O-162]^+$. ^{1}H-NMR (CD_3OD) δ: 0.83 (6H, t, H-18, 20′), 1.21 (44H, m, H-11－17, H-5′－19′), 1.35 (2H, m, H-4′), 1.49 (1H, m, H-3′a), 1.71 (1H, m, H-3′b), 1.96 (2H, m, H-10), 2.05 (4H, m, H-6, 7), 3.70 (1H, dd, J=10.4, 3.7 Hz, H-1a), 3.99 (1H, m, H-1b), 3.97 (1H, m, H-2, 2′), 4.07 (1H, m, H-3), 5.43 (1H, dd, J=15.3, 7.0 Hz, H-4), 5.68 (1H, dtd, J= 15.3, 5.8, 0.6 Hz, H-5), 5.31 (2H, m, H-8, 9); glucose residue, 4.22 (1H, d, J=7.6 Hz, H-1″), 3.19 (1H, H-2″), 3.35 (1H, m, H-3″), 3.31 (1H, m, H-4″), 3.24 (1H, m, H-5″), 3.70 (1H, dd, m, H-6″a), 3.81 (1H, dd, J=12.4, 2.4 Hz, H-6″b). ^{13}C-NMR (CD_3OD) δ: 176.1 (C-1′), 133.9 (C-5), 129.3 (C-4), 130.8 (C-9), 128.7 (C-8), 72.3 (C-2′), 72.1 (C-3), 68.5 (C-1), 53.4 (C-2), 34.7 (C-3′), 32.6 (C-6), 32.1 (C-16, 18′), 29.9－29.5 (C-11－15, 5′－17′), 27.4 (C-10), 26.9 (C-7), 25.4 (C-4′), 22.8 (C-17, 19′), 14.1 (C-18, 20′); glucose resideue, 103.2 (C-1″), 73.6 (C-2″), 76.5 (C-3″), 70.0 (C-4″), 76.4 (C-5″), 61.5 (C-6″).

1.2 Acid hydrolysis of compounds Ⅰ－Ⅴ

A solution of each sample (0.5 mg) in dioxane (0.3 mL) was treated with 2 mol/L HCl in dioxane-H_2O (1 : 1) (0.1 mL) and the whole was sealed and heated at 70 ℃ for 1 h. The reaction mixture was diluted with methanol and then subjected to HPLC, column: TKS gel ODS-120A, solvent: 0.5% TFA methanol, flow rate: 0.5 mL/min. The obtained aliphatic acid was analyzed on MS. The reaction mixtures were also subjected to TLC and compared with *D*-glucose. The aliphatic acid obtained from compounds Ⅰ and Ⅱ was identical to 2-hydroxypalmitic acid, t_R 12.8 min, $[M-H]^-$ 271.0, those from compounds Ⅲ and Ⅳ was 2-hydroxyoctadecanoic acid, t_R 14.6 min, $[M-H]^-$ 299.1, and that from compound Ⅴ was 2-hydroxyeicosanoic acid, t_R 18.9 min, $[M-H]^-$ 327.2.

1.3 Methanolysis of compounds Ⅰ－Ⅴ

A solution of each sample (10 mg) in methanol, to which concentrated HCl (0.2 mL) was added, was heated at 80 ℃ for 2 h. The reaction mixture was subjected to silica gel column, eluted with hexane-EtOAc (6 : 1), and the fatty acid methyl ester was obtained. Methyl 2-hydroxypalmitate was from compounds Ⅰ and Ⅱ, $[\alpha]_D^{26}$－

5.8 (c = 0.27), FAB-pos-MS: 287 $[M+H]^+$, ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7.0 Hz), 1.25 (24H, m), 1.64 (1H, m), 1.78 (1H, m), 3.79 (3H, s), 4.19 (1H, ddd, J=4.3, 5.8, 9.9 Hz), ^{13}C-NMR ($CDCl_3$) δ: 175.9, 70.5, 52.5, 34.4, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 24.7, 22.7, 14.1. Methyl 2-hydroxyoctadecanate was from compounds Ⅲ and Ⅳ. $[\alpha]_D^{25}$ −16.5 (c=0.13, $CHCl_3$), FAB-pos-MS: 315 $[M+H]^+$, ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J= 7.0 Hz), 1.25 (28H, m), 1.64 (1H, m), 1.78 (1H, m), 3.79 (3H, s), 4.19 (1H, dd, J= 4.3, 7.0 Hz), ^{13}C-NMR ($CDCl_3$) δ: 175.3, 70.5, 52.5, 34.4, 31.9, 29.7, 29.6, 29.5, 29.3, 24.7, 22.7, 14.1. Methyl 2-hydroxyeicosanate was from compound Ⅴ: $[\alpha]_D^{25}$ −23.7 (c=0.17, $CHCl_3$), FAB-pos-MS: 343 $[M+H]^+$, ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J= 7.0 Hz), 1.25 (32H, m), 1.64 (1H, m), 1.78 (1H, m), 3.79 (3H, s), 4.19 (1H, dd, J= 4.3, 7.2 Hz), ^{13}C-NMR ($CDCl_3$) δ: 175.9, 70.5, 52.5, 34.4, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 24.7, 22.7, 14.1.

1.4 The absolute configuration of carbon-2 of fatty acid

Fatty acid methyl esters were reacted with (−)-MTPA (methoxytrifluoromethylphenylacetic acid chloride) and (+)-MTPA, respectively, on modified Moshers method. The reaction products were measured on NMR.

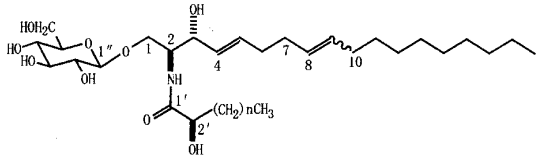
1.4.1 (−)-MTPA-methyl-2-hydroxypalmitate, ^{1}H-NMR ($CDCl_3$) δ: 5.18 (1H, t, J=6.4 Hz, H-2), 3.75 (3H, s, $COOCH_3$), 1.91 (2H, m, H-3), 1.39 (2H, m, H-4), 1.26 (m), 0.88; (+)-MTPA-methyl-2-hydroxypalmitate, ^{1}H-NMR ($CDCl_3$) δ: 5.16 (1H, t, J=6.7 Hz, H-2), 3.79 (3H, s, $COOCH_3$), 1.85 (2H, m, H-3), 1.30 (2H, m, H-4), 1.26 (m), 0.88. $\Delta\delta$ (COOMe) = −0.04, $\Delta\delta$ (H-2) = +0.02, $\Delta\delta$ (H-3) = +0.06, $\Delta\delta$ (H-4) = +0.09, according to modified Moshers method rules[2], the absolute configuration of carbon-2 of methyl-2-hydroxypalmitate was *R*-configuration.

1.4.2 (−)-MTPA-methyl-2-hydroxyoctadecanate, ^{1}H-NMR ($CDCl_3$) δ: 5.17 (1H, t, J=6.4 Hz, H-2), 3.75 (3H, s, $COOCH_3$), 1.91 (2H, m, H-3), 1.40 (2H, m, H-4), 1.26 (m), 0.88; (+)-MTPA-methyl-2-hydroxyoctadecanate, ^{1}H-NMR ($CDCl_3$) δ: 5.16 (1H, t, J=6.7 Hz, H-2), 3.79 (3H, s, $COOCH_3$), 1.86 (2H, m, H-3), 1.30 (2H, m, H-4), 1.26 (m), 0.88. $\Delta\delta$ (COOMe) = −0.04, $\Delta\delta$ (H-2) = +0.01, $\Delta\delta$ (H-3) = +0.05, $\Delta\delta$ (H-4) = +0.10, according to modified Moshers method rules[2], the absolute configuration of carbon-2 of methyl-2-hydroxyoctadecanate is also *R*-configuration.

1.4.3 (−)-MTPA-methyl-2-hydroxyeicosanate, ^{1}H-NMR ($CDCl_3$) δ: 5.17 (1H, t, J=6.4 Hz, H-2), 3.75 (3H, s, $COOCH_3$), 1.91 (2H, m, H-3), 1.40 (2H, m, H-4), 1.26 (m), 0.88; (+)-MTPA-methyl-2-hydroxyeicosanate, ^{1}H-NMR ($CDCl_3$ solvent) δ: 5.16 (1H, t, J=6.7 Hz, H-2), 3.78 (3H, s, $COOCH_3$), 1.85 (2H, m, H-3), 1.30 (2H, m, H-4), 1.26 (m), 0.88. $\Delta\delta$ (COOMe) = −0.03, $\Delta\delta$ (H-2) = +0.01, $\Delta\delta$ (H-3) = +0.06, $\Delta\delta$ (H-4) = +0.10, according to modified Moshers method rules[2], the absolute configuration of carbon-2 of methyl-2-hydroxyeicosanate is also *R*-configuration.

2 Results and Discussion

Fresh bulbs of *H. vittatum* were extracted with methanol and the extracts were subjected to Diaion column, silica gel column, and ODS column chromatography. Further purification on HPLC resulted in the isolation of five compounds. On the basis of analysis of NMR data, compounds Ⅰ − Ⅴ belonged to sphingo-lipid glycosides. The structures were seen in Fig. 1.



Ⅰ 8*Z*, *n*=13 Ⅱ 8*E*, *n*=13 Ⅲ 8*Z*, *n*=15
Ⅳ 8*E*, *n*=15 Ⅴ 8*Z*, *n*=17

Fig. 1 Structures of compounds Ⅰ − Ⅴ

Compound Ⅰ: White powder, had $C_{40}H_{75}NO_9$ based on the analysis of NMR and MS data. The ^{1}H-NMR and ^{13}C-NMR data of compound Ⅰ sug-

gested the presence of a sugar residue, an amide linkage and aliphatic long chains. On acid hydrolysis compound Ⅰ yielded glucose. ^{13}C-NMR spectrum appeared the signals δ 102.7, 75.0, 77.9, 71.6, 78.0, and 62.7, which were similar to that of glucose, the proton signals assignable to these carbons in the HMQC NMR spectra, δ 4.27, 3.19, 3.36, 3.30, 3.28, 3.67, and 3.87 were correlated in turn in the H-H COSY NMR spectra, and the coupling constants of anomeric proton (δ 4.27) was 7.6 Hz, suggesting that the configuration of anomeric carbon is β-configuration. From the above information, the sugar residue was induced to be β-*D*-glucopyranosyl group. In the products of acid hydrolysis, one fatty acid was isolated with HPLC, which was identical to 2-hydroxypalmitic acid by MS data in comparison with authentic sample. Methanolysis of compound Ⅰ liberated methyl 2-hydroxypalmitate, which reacted with (−)-MTPA and (+)-MTPA. The ^{1}H-NMR data of the products proved that the absolute configuration of carbon-2 of 2-hydroxypalmitate was *R*-configuration based on modified Moshers method rules[2]. In the NMR of compound Ⅰ, the signals δ 3.99 (H-2′), 1.72 and 1.55 (H-3′) in the ^{1}H-NMR, δ 177.2 (C-1′), 73.1 (C-2′), and 35.9 (C-3′) were assignable to 2-hydroxypalmitic residues. Apart from the signals of glucose residue and 2-hydroxypalmitic residue, the remained signals indicated sphingosine residue[3]. The first double bond of sphingosine residue was found to be *trans*, as evidenced by the large vicinal coupling constant (J=15.3 Hz). While the second double bond at C-8 and C-9 was determined to be *cis* by the chemical shifts of the carbons attached the double bond, δ 27.9 (C-7), 28.3 (C-10), on basis of that the signals of carbons next to a *trans* double bond appeared at δ 32 and 33, while those of a *cis* double bond appear at δ 27 and 28[3]. Furthermore, the signal δ 3.99, which was assignable to the proton attached to C-2, δ 54.6, was correlated to that of a carbonyl group δ 177.2 (C-1′) through nitrogen in the HMBC NMR spectrum, that is, and amide group existed, and the correlation between the carbon signal δ 102.7 (C-1″) and the proton signals δ 3.72 (H-1a) and 4.11 (H-1b) in the HMBC NMR spectrum proved that the sugar residue attached to C-1. Finally, on the basis of comparison of NMR data with the reported values[4], the structure of compound Ⅰ was identified as (2*S*, 3*R*, 4*E*, 8*Z*)-2-[(2*R*-2-hydroxyhexadecanoyl) amido]-4, 8-octadecadiene-1, 3-diol 1-*O*-β-*D*-glucopyranside.

Compound Ⅱ: White powder, had the same molecule formula as that of compound Ⅰ, $C_{40}H_{75}NO_9$. On acid hydrolysis compound Ⅱ yielded glucose and one fatty acid which was identical to 2-hydroxypalmitic acid by MS data in comparison with authentic sample. Methanolysis of compound Ⅱ liberated methyl 2-hydroxypalmitate, which was proved to be *R*-configuration through the same method as the above. It had similar NMR spectra to those of compound Ⅰ except for the signals of two methylene carbons attached to double bond. In the ^{13}C-NMR spectra of compound Ⅰ, the two methylene carbons′ chemical shifts were δ 27.9 (C-7) and 28.3 (C-10), which confirmed the *cis* geometry of the second double bond, while in the ^{13}C-NMR spectra of compound Ⅱ, they appeared at δ 33.3 (C-7) and 33.6 (C-10), which indicated that the geometry of the second double bond is *trans*. Therefore the structure of compound Ⅱ was identified as (2*S*, 3*R*, 4*E*, 8*E*)-2-[(2*R*-2-hydroxyhexadecanoyl) amido]-4, 8-octadecadiene-1, 3-diol 1-*O*-β-*D*-glucopyranside.

Compound Ⅲ: White powder, had $C_{42}H_{79}NO_9$ based on the analysis of NMR and MS data. The ^{1}H-NMR and ^{13}C-NMR data of compound Ⅲ suggested the presence of a sugar residue, amide linkage, and aliphatic long chains. On acid hydrolysis compound Ⅲ yielded glucose and one fatty acid which was identical to 2-hydroxyoctadecanoic acid by MS data in comparison with authentic sample. Methanolysis of compound Ⅲ liberated methyl 2-hydroxyoctadecanate, which was proved to be *R*-configuration through the same method as the above. Compound Ⅲ had similar ^{1}H-NMR and ^{13}C-NMR spectra to those of compound Ⅰ. But

there are 36H at δ 1.29 (36H, m, H-11—17, 5′—15′) in the ^{1}H-NMR of compound Ⅰ, while there are 40H at δ 1.29 (40H, m, H-11—17, 5′—17′) in the ^{1}H-NMR of compound Ⅲ, which are attributed to the substitution of 2-hydroxyoctadecanoic acid for 2-hydroxypalmitic acid. Finally, on the basis of comparison of NMR data with the reported values[4], the structure of compound Ⅲ was elucidated to be (2*S*, 3*R*, 4*E*, 8*Z*)-2-[(2*R*-2-hydroxyoctadecanoyl) amido]-4, 8-octadecadiene-1, 3-diol 1-*O*-β-*D*-glucopyranside.

Compound Ⅳ: White powder, had the same molecule formula as that of compound Ⅲ, $C_{42}H_{79}NO_9$. Acid hydrolysis and methanolysis confirmed that compound Ⅳ also have 2*R*-2-hydroxyoctadecanoyl residue. It had similar NMR spectra to those of compound Ⅲ except for the signals of two methylene carbons attached to double bond, which confirmed the geometry of the second double bond is *trans*. It also showed compound Ⅲ had similar ^{1}H-NMR and ^{13}C-NMR spectra to those of compound Ⅱ except for the signals at δ 1.29, which are attributed to the substitution of 2-hydroxyoctadecanoic acid for 2-hydroxypalmitic acid. Thus, the structure of compound Ⅳ was elucidated to be (2*S*, 3*R*, 4*E*, 8*E*)-2-[(2*R*-2-hydroxyoctadecanoyl) amido]-4, 8-octadecadiene-1, 3-diol 1-*O*-β-*D*-glucopyranside.

Compound Ⅴ: White powder, had $C_{44}H_3NO_9$ based on the analysis of NMR and MS data. The ^{1}H-NMR and ^{13}C-NMR data of compound Ⅴ also showed the presence of a sugar residuce, an amide linkage, and aliphatic long chains. Acid hydrolysis and methanolysis confirmed that compound Ⅴ also have 2*R*-2-hydroxyeicosadecanoyl 1 residue through the same method as the above. Compound Ⅴ had similar ^{1}H-NMR and ^{13}C-NMR spectra to those of compounds Ⅰ and Ⅲ except for the signals at δ 1.29, which are attributed to the substitution of 2-hydroxyeicosadecanoic acid. In conclusion, the structure of compound Ⅴ was elucidated to (2*S*, 3*R*, 4*E*, 8*Z*)-2-[(2*R*-2-hydroxyeicosa-decanoyl) amido]-4, 8-octadecadiene-1, 3-diol 1-*O*-β-*D*-glucopyranside.

All these glycosphingosilipids are first isolated from Amaryllidacea plants. Glycosphingosilipids have the effects of anti-ulcerogenic, anti-hepatotoxic activity, inhibitory activity against proteinkinase C, and participate clarifies in antigen-antibody reactions and transmission[4]. The existence of glycosphingosilipids in this plant further clarifies the bioactivities of this plant. In spite of the isolation from other family plants, the absolute configuration elucidations of aliphatic acid in glycosphingosilipids were not satisfactory. Some compounds were confirmed with specific optical rotation, and their value and measure concentration were too small, while others were not proved. In this paper, this problem has been settled down by using the modified Moshers method.

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