2018 International Conference on Zoology, Botany and Ecology (ICZBE 2018)

The study of chemical constituents of Hippeastrum vittatum

Xin Guosong[1,2], He Jiaxin[1,2], Yu Wenjing[1,2], Ji Yubin[1,2*]

[1] Harbin university of commerce medicine postdoctoral workstation; Heilongjiang Harbin, 150076

[2] National ministry of education anti-tumor natural drug engineering research center; Heilongjiang Harbin, 150076

Keywords: Hippeastrum; Lycoris; Chemical composition; Structure identification

Abstract: Objective aiming to study the chemical composition of hippeastrum dry leaves. **Methods** The separation techniques of silica gel column chromatography, ODS column chromatography, HPLC analysis and preparation of liquid phase were adopted, and the methods of MS, NMR and other methods of spectroscopy were used to identify the structure of the compound. **Results** Four monomer compounds were obtained by separation and purification, according to the physicochemical properties of the obtained compounds, and the structure of the compounds was identified by the spectra of ^{1}H-NMR, ^{13}C-NMR and MS, namely(2S)-7,3'-dihydroxy-4'-methoxy-flavan and (2S)-7-hydroxy-3'',4'-dimethoxy-flavan. In the dichloromethane partial extract, compound 3 was prepared as 4'-dihydroxy-7,3'-methoxy-flavan and the No.4 compound 7,4'-dihydroxy-3'-methoxy-flavan finally. **Conclusion** This four compounds is isolated from Hippeastrum. Compound 1 and compound 4 have strong anti-tumor activity, compound 2 and compound 3 antitumor activity.

1. Introduction

Hippeastrum vittatum is a perennial evergreen herb belonging to Hippeastrum, Amaryllidaceae, Monoeotyledonae[1-3]. Existing literatures show that the Hippeastrum vittatum has extensive pharmacological activities like anti-tumor, anti-virus, anti-inflammatory, anti-malaria and other pharmacological activities, and has good effects on cardiovascular and nervous system. According to existing literature, although there are many active constituents in the bulbs of Hippeastrum vittatum, little is known about the chemical constituents in the leaves. To confirm therapeutic material basis of Hippeastrum vittatum, the present study aims to separate and identify chemical constituents from leaves of Hippeastrum vittatum, then screen active ingredients, providing theoretical basis for further development and utilization of Hippeastrum vittatum.

2. The main instruments and reagents

2.1 The main instrument

EYELA N-1100 Rotary evaporator(Tokyo Physical and Chemical Instruments Co., Ltd.); BS110S electronic analytical balance(Beijing Saidolis Balance Co., Ltd.); Electrothermal constant temperature blast dryer DHG-9140A (Shanghai Yiheng Technology Co., Ltd.); Ultrasound machine KQ2200DE(Jiangsu Kunshan Ultrasound Instrument Co., Ltd.); Multi-purpose Vacuum Pump for Circulating Water (Zhengzhou Great Wall Technology Industry and Trade Co., Ltd.).

2.2 Reagents and samples

Hippeastrum vittatum provided by Life science and environmental science research center of Harbin university of commerce; Human hepatoma hepg-2 was purchased from Institute of cell of Chinese academy of medical science;absolute ethyl alcohol(analytically pure,Batch number: 20121008); methylene chloride(analytically pure, Batch numbe: 20140516); methyl alcohol(analytically pure, Batch numbe: 20140708); Petroleum ether(analytically pure, Batch numbe: 20130423); Ethyl acetate(analytically pure ,Batch numbe: 20131014); N-butyl alcohol(analytically pure, Batch numbe: 20130301).

DOI: 10.25236/iczbe.2018.003

3. Experimental method

3.1 Chemical constituent’s extraction

The fresh leaves of Hippeastrum vittatum were sliced and dried, crushed, sieved to debris through 20-30 mesh for being used later. 3 kg dried red leaves of Hippeastrum red were weighed, placed in a round-bottom flask with 70% ethanol solvent inside. The ratio of the material to liquid was 1:12. The mixture was heated and refluxed at 90℃ for 3 times and for 3 hours each time, and the extracts were mixed. The reagent was distilled off under reduced pressure by a rotary evaporator, the product was concentrated to a total extract. The primary extract was mixed with a small amount of water to suspend and then successively extracted with light petroleum, dichloromethane, ethyl acetate and n-butyl alcohol three times each, and the extracts were mixed respectively. The concentrated recovery reagent contained 84.9g light petroleum, 17g dichloromethane, 23.8g ethyl acetate, and 140.4g n-butyl alcohol.

3.2 Separation and purification

The n-butyl alcohol and the dichloromethane were further separated. The n-butanol fraction was repeatedly separated by a silica gel column, and the ratio of dichloromethane-methanol was 100:0, 100:1, 80:1, 60:1, 50:1, 40:1, 30:1, 20:1, 15:1, 10:1, 8:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, and used the 0:100 one for the column, the obtained fraction was tested by TLC after the combination, the compound 1 (10.6 mg) and compound 2 (9.3 mg) were obtained by repeated purifications on an OSD column. The dichloromethane-methanol adsorption chromatography method used a dichloromethane-methanol elution system in the order of 200:0, 200:1, 100:1, 80:1, 60:1, 50:1, 40:1, 30:1, 20:1, 15:1, 10:1, 8:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, and used the 0:100 one for the column, the obtained fractions were tested and combined by TLC, and the monomer compound obtained by the purification on the OSD column was compound 3 (25.6 mg), compound 4 (12.3 mg), and compound 5 (10.9 mg).

4. Structure identification According to the physicochemical properties of the compound (such as the crystal form, melting point, physicochemical detection and chromatographic identification) and the spectral data (^{1}H-NMR, ^{13}C-NMR), the structure of the compound can be identified and analyzed by comparing the separated monomers with previous literature and the spectrum library.

Compound 1: Light yellow cubic crystal (CH_3OH), $[\alpha]_D^{20}$= - 45.5 (MeOH, 0.30). Positive ions EI-MS m/z showed: 272[M+H]$^+$. The speculative molecular formula was $C_{16}H_{16}O_4$. In the spectral of ^{1}H-NMR (CD_3OD, 600 MHz), δ_H: 6.89 (1H, d, J = 1.8 Hz), 6.86 (1H, d, J = 8.4 Hz), 6.81 (1H, dd, J = 8. 4, 1.8 Hz), 6.82 (1H, d, J = 8.4 Hz), 6.32 (1H, dd, J = 8.4, 2.4 Hz), and 6.27 (1H, d, J = 2.4 Hz), including two ABX coupled systems, where 3.83 (6H, s) is the signal of a methoxy group, δ_H 2.8-2. 61 and 2.10-1. 92 represent the characteristic signal of C-ring proton of the flavonoid. In the spectral of ^{13}C-APT (CD_3OD, 150MHz), δ_C: 157.6 (C-9), 157.1 (C-7), 148.6 (C-4′), 147.5 (C-3′) were the carbon signals that locates in benzene ring connected to O atom, 136.4 (C-1′), 131.0 (C-5) were signals of quaternary carbon of benzene ring, 118.6 (C-6′), 114.4 (C-10), 114.3 (C-5′), 112.7 (C-2′), 109.1 (C-6), 104.1 (C-8) were C signals of two ABX systems in benzene, 78.7 (C-2) was carbon signal in C-ring with connecting to O, 56.9 (4′-OCH_3) represented there was a methoxy group, 31.6 (C-4), 25.6 (C-3) represented the secondary carbon was in C-ring.

The aforementioned data is consistent with the ref. reported. Therefore, the compound was identified to be (2S)-7,3'-dihydroxy-4'-methoxy-flavan, with the molecular formula $C_{16}H_{16}O_4$ and molecular weight 272.30. Fig.1-1 and Fig.1-2 show the ^{13}C-NMR and ^{1}H-NMR respectively.

Compound 2: Canary yellow cubic crystal (CH_3OH), optical rotation was $[\alpha]_D^{20}$= +8.33(MeOH, 0.30), Cation ions EI-MS m/z display: 286[M+H]$^+$, speculative molecular formula: $C_{17}H_{18}O_4$. In ^{1}H-NMR (CD3OD,600 MHz) Spectrum, δ_H: 7.03 (1H, d, J= 1.8 Hz), 6.95 (1H, d, J= 8.4 Hz), 6.96 (1H, dd, J= 8.4, 1.8 Hz) and 6.85 (1H, d, J= 8.4 Hz), 6.32 (1H, dd, J= 8.4, 2.4 Hz), 6.27 (1H, d, J= 2.4 Hz),including 2 ABX coupled systems, where 3.83(6H, s) and 3.84(6H, s) were both the signals of a methoxy group, δ_H2.8-2.61 and 2.10-1.92 represent the characteristic signal of C-ring proton of the flavonoid. In ^{13}C-APT (CD_3OD, 150 MHz) Spectrum, δ_C: 157.8 (C-9), 157.3 (C-7), 150.6 (C-4′), 150.2 (C-3′) were the carbon signals locates in benzene ring connected to O atom, 136.5 (C-1′), 131.2 (C-5) were signals of quaternary carbon of benzene ring, 119.9 (C-6′), 114.5 (C-10), 113.0 (C-5′), 111.3 (C-2′), 109.4 (C-6), 104.2 (C-8) were C signals of two ABX systems in benzene, 79.0 (C-2) was carbon signal in C-ring with connecting to O, 56.9 and 56.6 were both the signals of a methoxy group, 31.6 (C-4), 25.6 (C-3) represented the secondary carbon was in C-ring.

On the basis of comparison of ^{1}H NMR and ^{13}C-APT spectral data with the available in literature[20-21], compound 2 was assigned as(2S)-7-hydroxy-3', 4'-dimethoxy-flavan, with the molecular formula $C_{17}H_{18}O_4$ and molecular weight 286.12. Fig.1-3 and Fig.1-4 show the ^{13}C-NMR and ^{1}H-NMR respectively.

Compound 3: Yellow rod-shaped crystal(CH_3OH). The IR spectrum showed dark spot at 254nm. In ^{1}H-NMR Spectrum, the presence of a benzene ring for 6H was observed at δ_H6.89 (d,J = 8.2 Hz,1H), 6.83 (m,2H), 6.77 (d,J=8.1 Hz,1H), 6.27 (dd,J=8.1,1.7 Hz,1H) and 6.19 (s, 1H), a signal at δ_H4.88(d,J=9.2 Hz,1H) is due to the presence of hydroxyl group. Two methoxy group were observed at δ_H3.75 (s, 3H), δ_H3.38 (s, 3H) whereas a saturated carbon at δ_H2.78 (m, 1H), 2.57 (m, H), 2.37 (s, 1H), 2.03 (d, J=13.3 Hz, 1H). The corresponding ^{13}C-NMR spectrum included eight carbon signals on dihydrobenzopiperan at δ_C156.84(connecting to methoxy), δ_C155.75(on benzene ring, connecting to O), δ_C130.22, δ_C108.69, δ_C103.12(all above three were on benzene ring), δ_C76.97(connecting to benzene ring), δ_C29.80 and δ_C24.07, seven carbon signals on benzene ring at δ_C147.52(connecting to methoxy), δ_C 146.76(connecting to hydroxyl), δ_C134.71(connecting to dihydrobenzopiperanl), δ_C113.81(common to the benzene ring and the epoxy six ring), δ_C118.69, δ_C111.89, 110.39, in addition to two carbon signals on methoxy.

The aforementioned data is consistent with the ref. reported. Therefore, the compound was identified to be 4'-dihydroxy-7,3'-methoxy-flavan, with the molecular formula $C_{17}H_{18}O_4$ and molecular weight 286.12. Fig.1-5 and Fig.1-6 show the ^{13}C-NMR and ^{1}H-NMR respectively.

Compound 4: transparent oily product(CH_3OH). The IR spectrum showed dark spot at 254nm. In ^{1}H-NMR Spectrum, the presence of a benzene ring for 6H was observed at

δ_H6.98 (s,1H), 6.92 (t, *J*=8.7 Hz,2H), 6.81 (d,*J*=8.4 Hz,1H), 6.26(dd, *J* = 8.2, 2.1 Hz, 1H), 6.18 (d, *J*=2.1 Hz,1H), a signal at δ_H4.93(dd, *J*=9.9,1.3 Hz,1H) was due to the presence of hydroxyl group, a signal at δ_H3.74 (d, *J*=4.7 Hz,3H) indicated the presence of methoxy group. δ_H2.88 (m,1H), 2.79 (m,1H), 2.05 (m,1H), 1.91(m,1H) were characteristic signals of protons in the saturated carbon. The corresponding ^{13}C-NMR spectrum included seven carbon signals on dihydrob -enzopiperan at δ_C157.68(connecting to O), δ_C155.76(connecting to hydroxyl), δ_C130.11, δ_C108.69, δ_C103.30(all above three were observed on benzene ring), δ_C77.16(connecting to benzene ring), δ_C55.92 and δ_C55.85, seven carbon signals on benzene ring at δ_C148.68(connecting to methoxy), δ_C149.03(connecting to hydroxyl), δ_C134.63 (connecting to dihydrobenzopiperanl), δ_C111.99(common to the benzene ring and the epoxy six ring), δ_C118.69, δ_C111.89, 110.39, in addition to two carbon signals on methoxy.

On the basis of comparison of ^{1}H NMR and ^{13}C NMR spectral data with the available in literature, compound 4 was assigned as 7,4'-dihydroxy-3'-methoxy-flavan, with the molecular formula $C_{16}H_{16}O_4$ and molecular weight 272.10. Fig.1-7 and Fig.1-8 show the ^{13}C-NMR and ^{1}H-NMR respectively.

5. Conclusion

In the present study, petroleum ether, dichloromethane, ethyl acetate and n-butyl alcohol were extracted from 3 kg of dried leaves of Hippeastrum vittatum with reflux method, and the weights of them were 84.9 g, 17.0 g, 23.8 g and 140.4 g, respectively.

Furthermore, four monomeric compounds with higher purity were obtained from n-butyl alcohol and dichloromethane. After spectroscopic analysis of the structure of compound, the structures of four compounds were identified as (2S)-7,3'-dihydroxy-4'-methoxy-flavan, (2S)-7-hydroxy-3'',4'-dimethoxy-flavan, 4'-dihydroxy-7,3'-methoxy-flava and 7, 4'-dihydroxy-3'-methoxy-flavan, respectively. These four compounds were flavonoid compounds isolated from Hippeastrum vittatum for the first time.

Acknowledgments

This work was supported by Harbin municipal science and technology bureau project (2016RQQXJ124, 2016RAXXJ064); Innovation talent project of education department of Heilongjiang province(UNPYSCT-2017208); Harbin university of commerce campus project(17XN008).

References

[1] Wang xian, xiong min, wei zunzheng, et al. Research review of Hippeastrum vittatum [J]. Agricultural science and technology and information (modern gardening),2014,11(08):49-54.

[2] Wu yongpeng, yuan yaling, li shujuan, et al. A study on the relationship between bulb larvae and scales of Hippeastrum vittatum bulb [J]. Journal of Shanxi normal university (natural science),2017,45(01):78-81.

[3] Zhang Lin, cheng haizhong, zhou yuzhen, et al. Research progress of Hippeastrum vittatum [J]. Jiangsu agricultural science,2011,39(05):225-228.