JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2013. Vol. **7**(Spl. Edn.), p. 329-333

Chemical Constituents of the Root of *Mirabilis jalapa*

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(Received: 03 March 2013; accepted: 14 April 2013)

To investigate the chemical constituents of the roots of *Mirabilis jalapa*. Componds were extracted by petroleum ether and then purified by silica gel column chromatography. Their structures were characterized by NMR. Three chemical compounds were respectively β-sitosterol (I), isopropyl(4-(hydroxymethyl)phenyl) carbamate(II), (*E*)-methyl 2-(3-methoxypropyl)hexadec-14-enoate (III). Conclusion isopropyl (4-(hydroxymethyl) phenyl) carbamate(II) was first reported in this plant.

Key words: *Mirabilis jalapa*; chemical constituents; chrysophanol; stigmasterol.

Heroin dependence has become a serious Societal public health problem in recent years, it has influenced on all classes in many countries, even including women and children, cause tremendous harm to society. Controlling and treating heroin dependence became an important link for Drug - Control Strategy. Opioid drug for long term usage may lead endurance and addiction, in clinic treatment aspect, psychological dependence and strong irrepressible heroin craving after withdrawal syndrome lead to revert to take drugs, at the present time we needed an effective way to cure withdrawal syndrome of heroin dependence.

Heroin abusing in china rapidly spread, Hospital everywhere and drug rehabilitation center used traditional chinese medicine one after another, to treat withdrawal syndrome of heroin dependence, got certain effects in clinic(Mao *et al*.,1992; Guo *et al*.,1995), traditional chinese medicine had distinctive quality at symptomatic treatment and adjusting the body's function.

Extract of Mirablis jalapa Root had definite central nervous action, for example, calm, hypnogenesis and analgesic(Wang, *et al*.,2002; Li *et al*.,2001).The r[l]oots of Mirabilis jalapa alternate name "urderground mouse","*pollen head", "Gouache head", "*rouge flower head", was dried root of Mirabilis jalapa of mirabilis plants of Nyctaginaceae. medicinal value of the roots of Mirabilis jalapa began to see in "A Supplement to the Compendium of Materia Medica". According to bibliographical informationÿthe roots of Mirabilis jalapa had anticancer, antibacterium, conception control(Li *et al*., 1994).. resist human immunodeficiency virusÿand so on.

Herb root artifactitious product, conico-acuminate or cylindrical, straight or slightly curved, length was 5~15cm, width was 1~4cm, thickness was 0.8~1.5cm, surface was pale color or grey yellow, longitudinal furrow and mark of fibrous root, peak had varying length stem base or trace, texture is hard, difficult to snap, section was keroid, concentric texture; odourlessness , lightly seasoned, chewing had tingled to laryngeal.

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At present study of the roots of Mirabilis jalapa mainly focus on Mirabilis jalapa proteinÿbut chemical constituents was research less. By Improving the experimental instrument, test result could reach relative quite accurate effect, chemical composition of root of Mirabilis jalapa was studied once again(He *et al* ,1996),they applied Capillary gas chromatography mass spectrometry to analyze systematically unstuousness chemical composition in ethanol extract, and identify to get seventeen components including ethyl oleate, 3A,4,7A-tetrahydroendo-4,7-methanoindene 0dicyclopentadiene alcohol and so on.After dry product of root of Mirabilis jalapa (9.35 kg) was grinded into coarse powder(Wei *et al*, 2003) , coldly leaching three times by 75% ethanol , merge extracting solution, filter, Concentrate to obtain total extraction solution. This extraction solution was 2400mL, in turn leach by acetic ether and normal butanol ,concentrate to get acetic ether and normal butanol extract. Separate and identify four chemical compound, allantoin llantoin, 23C-fatty acid glycerol ,monoester, boeravinone, β-sitosterol.

This paper separated and got three chemical compound from petroleum ether site of 95% alcohol extract of the roots of Mirabilis jalapa, Three compounds were identified as β-sitosterol (I), isopropyl(4-(hydroxymethyl) phenyl) carbamate (II), (*E*)-methyl 2-(3-methoxypropyl)hexadec-14-enoate (III),chemical compound II and III was firstly found in this plant.

MATERIALS

Instrument

X26 micro melting point apparatus, BrukerAV400MHz NMR spectrometer with superconducting magnet, column chromatogram and Thin-layer chromatography silica gel was produced by Qingdao ocean chemical plant. petroleum ether(60~90°C), chloroform, ethyl acetate, acetone and normal butanol were all analytically pure. crude drugs purchased from Bozhoou city corporation of medical herbs, they were identified as dried root of Mirabilis jalapa by professor Lei Juntao.

Extraction and separation

To weigh dried root of Mirabilis jalapa 20 kg and smash, using 95% alcohol(volume fraction) Percolation extracting, merge extract extracting solution , condense, obtain extract 2. 025 kg. using a little hot water to dissolve extract, in correct order leach by petroleum ether, chloroform, ethyl acetate and normal butanol. petroleum ether extract 800g was chromatographed over *silica gel* column chromatogram, by ethyl acetate (60~90!)- acetone gradient elution, and got chemical compound (‘!)-(b!).Structural identification chemical compound ‘! is colorless crystal; Molecular formula is $C_{29}H_{50}O$, Easily dissolving in organic solvent such as chloroform, ethyl acetate, Hardly soluble in methanol, ethanol. Lieber_mann-Burchard reaction was positive.

The EI-MS fragments at m/z 414[M⁺,46] 399[M⁺cþCH$_3$, 23],396[M⁺cþH$_2$Oÿ30] 255 (55), 213(69),159(72),145 (98), 107(83),91(69),55(76).The ‘H-NMR(CDC1$_3$, 400 MHz) spectrum of I showed the signals of six Me (Table 1) groups at ä(H) 1. 04(3H,s, CH$_3$-19),0. 94(3H,d,J=4. 4 H z,CH$_3$-21),0. 84(3H, t, J=6. 8 Hz, CH$_3$-29),0. 78(3H,d,J=7. 2 Hz, CH$_3$-27) ,0. 73(3H,d,J=7. 2Hz,CH$_3$-26),0.61(3H,s,CH$_3$-18),as well as two aromatic resonances at δ(H) 5. 28(1H, t, J= 5. 2 Hz, H-6),3. 46(1H, m, H-3). The figures above are in accord with the literature report(Lin *et al*., 2008). It was mixed with known β-sitosterol and its meltingpoint didn’t drop, and Rf value of thin-layer chromatography is according. The Compound I s identified as β-sitosterol (Fig.1). Data of I corresponding arbitrary atom numbering see Fig.2.

Compound II,white needle crystal (Petroleum ether:acetone,10:2) The ‘H-NMR (acetone2d6) spectrum of II (Table 2)showed the signals of four aromatic resonances at δ(H) 7. 61(1H,dd),7.59,1H,dd,7.26 (1H,dd, H-2),7.25 (1H,dd, H-2), as well as five aromatic resonances at δ(H) 1.34(6H, m),8. 52 (1H, s), 5. 06 (1H, m), 3. 99(1H,

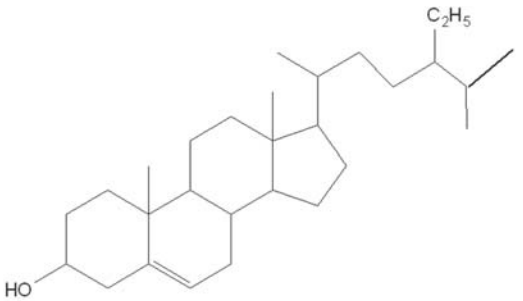

Fig. 1. Compound I structural formula

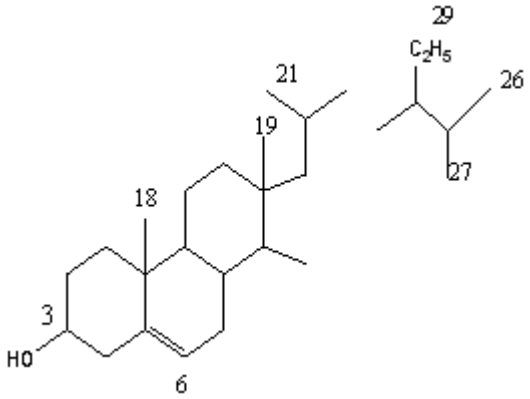

Fig. 2. Arbitrary atom numbering

Table 1. 'H-NMR Data of I At 400 MHz in $CDC1_3$ δ in ppm , J in Hz.Arbitrary atom

Atom	Compound I
H-C(6)	5. 28(t ,J= 5. 2 Hz)
H-C(3)	3. 46(t)
H-C(19)	1. 04(s)
H-C(21)	0. 94(d, J=4. 4 H z)
H-C(29)	0.84(t, J=6. 8 Hz)
H-C(27)	0.78(dÿJ=7. 2 Hz)
H-C(26)	0.73(dÿJ=7. 2Hz)
H-C(18)	0.61(s)

s),2. 94(1H, dd),along with the corresponding ^{13}C-NMR (Table3) signals at δ(C) 129.24,118.61, 118.53, 136.02, 137.71 (C5,6,7,8), 153.50 (C4), 40.46 (C9), 21.69 (C1,3), 67.72(C2).The DEPT shows that Compound I has three quaternary carbon atoms, benzene ring and obvious isopropyl structure. Based on the figures above, the molecular formula of compound I is given as $C_{11}H_{15}N0_3$. The Compound I is identified as isopropyl(4-hydroxymethyl) phenyl)carbamate (Fig.3)

Data of I corresponding arbitrary atom numbering see **Fig.4.**

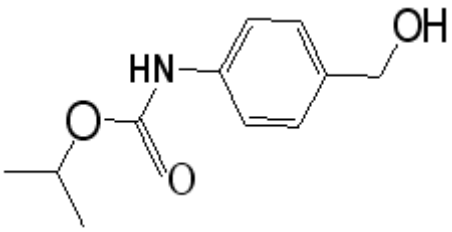

Fig. 3. Compound I structural formula

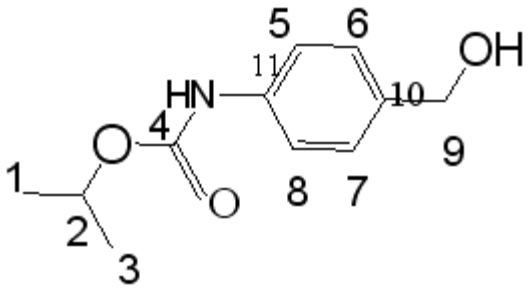

Fig. 4. Arbitrary atom numbering

Table 2. 'H-NMR Data of Compound I At 400 MHz in $CDC1_3$ δ in ppm

Atom	Compound I
H-C(5)	7. 61(1H, dd)
H-C(8)	7.59 (1H,dd)
H-C(6)	7.26 (1H, dd, H-2)
H-C(7)	7.25 (1H,dd, H-2)
H-C(1,3)	1.34(6H, m)
H-N	8. 52 (1H, s)
H-C(2)	5. 06 (1H, m)
H-O	3. 99(1H, s)
H-C(9)	2. 94(1H, dd)

Table 3. ^{13}C-NMR Data of Compound I At 400 MHz in $CDC1_3$ δ in ppm

Atom	Compound I
C(6)	129.24
C(5)	118.61
C(7,8)	118.53
C(10)	136.02
C(11)	137.71
C(4)	153. 50
C(9)	40. 46
C(1,3)	21.69

Compound I, white needle crystal(acetone), The EI-MS fragments at m/z 339,and the EI-MS also shows that there is a characteristic absorption peak of the long chain fatty hydrocarbon. Difference value of 57, 71, 85, 97, 111,125 each other is 14.The 'H-NMR($CDCl_3$) (Table 4) spectrum of I showed the signals of four aromatic resonances at δ(H) 4.67(m,2H),1.84(brs,1H),1.17(m,3H),1.02(m,3H), along with the corresponding 13C-NMR (Table 5) signals at δ(C) 34.7(CH_2), 31.9 (CH_2), 29.66~29.08 (many CH_2),25.04(CH_2),22.66(CH_2),18.75(CH_3).

Combine with HSQC spectrogram ,chemical shift of main carbon may identify: c-15 (122.5) c-16 (18.7), c-3 (24.9), c-16 (18.7); corresponding typical proton chemical shift may identify: H-15(5.42,m), H-16(3.93,brs), H-1(3.64, m), H-16(0.97, t). c-14, (139.7), c-1 (173.3) were satured carbon and corresponding proton chemical shift was not found. To sum up, structure of chemical compound b! was indetified (*E*)-methyl 2-(3-methoxypropyl)hexadec-14-enoate ,and it was a new chemical compound(Fig.5).

Data of III corresponding arbitrary atom numbering see Fig.6.

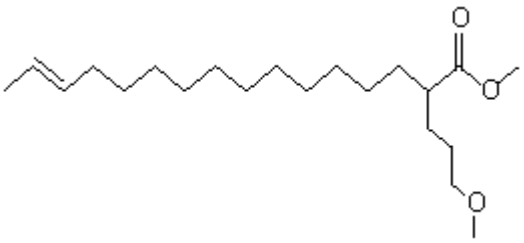

Fig. 5. Compound III structural formula

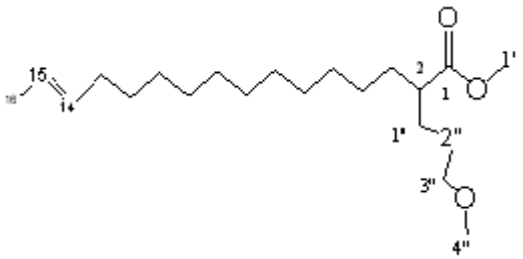

Fig. 6. Arbitrary atom numbering

Table 4. 'H-NMR Data of Compound II At 400 MHz in $CDC1_3$ δ in ppm

Atom	Compound II
H-C(3'')	4. 67(m, 2H)
H-C(2)	1. 84(brs,1H)
H-C(4'')	1.17(m, 3H)
H-C(1')	1.02(m,3H)

Table 5. ^{13}C-NMR Data of Compound II At 400 MHz in $CDC1_3$ δ in ppm

Atom	Compound II
C	34.70(CH_2)
C	31.90(CH_2)
C(2-14)	29.66^,29.08(Y*NCH_2)
C	25.04(CH_2)
C	22.66(CH_2)
C(16)	18.75(CH_3)
C(1)	173.29
C(14)	139.70
C(15)	122.54
C(3'')	73.64
C(4'')	56.67
C(2)	42.29
C(1')	50.02

Experiment result showed that this was separation of crude extraction to effective constituent, mixing element need further separate, until to get single component.Combining solvent method and silica gel column chromatography to separate and extract root of Mirabilis jalapa, using two methods mutual complementation, to make up for deficiency that solvent method got impurity sample and silica gel column chromatography could not treat a lot of sample once. solvent method and silica gel column chromatography combined with thin layer chromatography ,it was very effective Separation and Purification Technology, achieve satisfied effect; extract to get three crystals. So far this experiment only finished preliminary separation of petroleum ether extract of root of Mirabilis jalapa. Experimental studies found (lei *et al*., 2011) that sherwood oil extract , chloroform extract, normal butanol extract had better fuction to mice Morphine dependence withdrawal syndrome. Preliminary experiment (Cai *et al*., 2007) showed sherwood oil extract mainly included Steroid and Volatile oil ; chloroform extract Contained mainly alkaloid, coumarins, anthraquinones, ester and lactone; normal butanol extract mainly had saccharides, glucideÿflavonoid, Organic acids and Phenols. Separate to get three crystalsÿwhether they had pharmacological activity ÿneed test and verify further by animal experimentr. This study provided basis for further development of medicinal value and scientific evidence for chemical classification. But we need further studied if three cr ystals of root of Mirabilis jalapa were effective constituent of having hypnosis , hypnogenesis , hypnotize , mesmerize,Sedation and analgesia.

ACKNOWLEDGEMENTS

This work was financed by the research fund of Department of education of Jilin Province. Accept my thanks for Changchun Institute of Applied Chemistry and test center of Beihua University for testing spectrum data in this pager.

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