Studies on Chemical Constituents of Radix Angelicae pubescentis

Yan Zhu[1a], Jiao Feng[1b], QingYang Liu[2*c], Songsong Yang[3*]

[1]College of commerce Liaoning economic management cadre Institute Shenyang, China
[2]Endocrinology department Affiliated Hospital of Liaoning University of Traditional Chinese Medicine Shenyang, China
[3]College of pharmacy Liaoning University of Traditional Chinese Medicine Dalian, China

Abstract—Backgound:Angelicae pubescentis is the root of Angelica pubescens Maxim. f. biserrata Shan et Yuan. The main active ingredients are coumarin and volatile oil, and it has a variety of pharmacological activities such as antibacterial, antioxidant and anti-inflammatory. In this paper, the active components of the CO_2 supercritical extract of Angelicae pubescentis were studied. The results of this study will provide a scientific basis for the study of the chemical composition of Angelicae pubescentis. Purpose: To study the chemical constituents of supercritical extract of Radix Angelicae pubescentis. Method: The supercritical extract of Radix Angelicae pubescentis was separated by silica gel column chromatography to obtain monomers. The structures were identified based on physicochemical properties and spectroscopic data. Result: Eight compounds were separated from the supercritical extract of Radix Angelicae pubescentis, six of which were identified.They are Osthol, Columbianedin, Columbianetin, Columbianetin Acetate, Xanthotoxin and Bergapten. Conclusion: Xanthotoxin and Bergapten were isolated, which structure were similar. The standard substance of Osthol and Columbianedin were producted in order to offer the good material base for quality evaluation and pharmacodynamic study of Radix Angelicae pubescentis. Their purification were more than 99%. The results of this study are of great significance for exploring the effective material basis and medicinal value of the supercritical extract of Radix Angelicae pubescentis.

1. INTRODUCTION

There are many chemical components of Radix Angelicae pubescentis, domestic and foreign scholars have made a lot of research on it. So far, it has been found to contain coumarin, volatile oil, terpenes, sterols, organic acids, sugars and other components.

The main component of Radix Angelicae pubescentis is coumarin. So far, more than 70 kinds of coumarins have been isolated from Ducuo. They include simple coumarins, barking coumarins, picrancoumarins, dicumarins, and some coumarin glycosides. The various coumarin structures are shown in Figure1.

In addition, there are also differences in the main chemical components in different varieties of Radix Angelicae pubescentis. The root of A. pubescens Maxim contains Angelol, Angelicone, Glabralactone, Bergapten, Osthol[1], Umbelliferone, Scoppoletin, Angelic acid, Tiglic acid, Palmitic acid, stearic acid, oleic acid, linolenic acid, phytonol, glucose and a small amount of volatile oil[2]. The root of H. lanatum Michx contains Angelicin, Pimpinellin, Bergapten and other various barking coumarins. In addition to the above components, the leaves also contain psoralen and so on. Among them, the main barking coumarins are bovine parsnips[3]. It also contains 0.26-0.57% volatile oil. So far, the chemical components isolated from Radix Angelicae pubescentis have been far more than these. With the progress of the times and the gradual improvement of scientific and technological means, the chemical components in Radix Angelicae pubescentis will be studied more and more thoroughly.

In this experiment, we utilized column chromatography for the separation of coumarins on the basis of CO_2 supercritical extract. We prepared to product the relevant standard substance, The results of this study are of great significance for exploring the effective material basis and medicinal value of the supercritical extract of Radix Angelicae pubescentis.

2. MATERIALS AND METHODS

2.1 Materials

1) Instruments and equipments:

a) XR4 micro melting point apparatus (Shanghai optical Instrument Factory) The thermometer is not corrected.

b) Burker-ARX-300 Nuclear magnetic resonance spectrometer and Burker-ARX-600 Nuclear magnetic

[a]46581081@qq.com, [b]820331@qq.com, [*c] Corresponding author: qingyang-tcm@163.com

resonance spectromete (Analysis and Testing Center of Shenyang Pharmaceutical University)

c) Shimadzu UV-256-FW self-recording spectrophotometer (Central Laboratory of Liaoning University of Traditional Chinese Medicine)

*d) LC-10A HPLC, SPD-10A De*tector, *LC-10A Pump (Department of Plant Chemistry of Liaoning University of Traditional Chinese Medicine)*

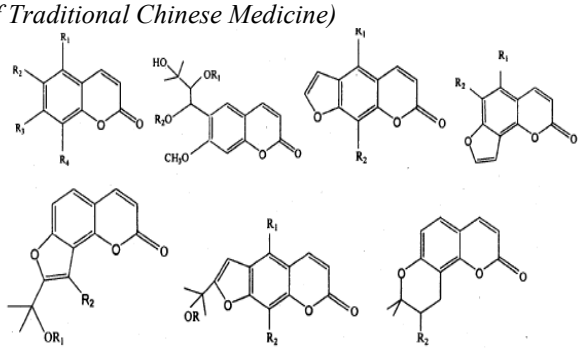

Figure 1. Structural classification of coumarin in Radix Angelicae pubescentis

2) *Reagents:* Silica gel（TLC, produced by Qingdao Marine Chemical Plant), Silica gel(Column, 100-200mesh, produced by Qingdao Marine Chemical Plant).

All chemicals for chromatography were of analytical grade. All chemicals for HPLC were of chromatographic grade.

2.2 Methods

In this experiment, we utilized column chromatography for the separation of coumarins on the basis of CO_2 supercritical extract.

1) Extract: We crushed the herbs of Radix Angelicae pubescentis. We take 1kg of crushed herbs between the 40-mesh screen and the 60-mesh screen, and then put them into the extraction tank. According to the CO_2 flow rate of 1.5 ml/min, extraction time of 20 minutes, pressure of 20 Mpa, temperature of 40℃, the operation was carried out to obtain the independent active supercritical extract.

2) Separation: Add 87g of supercritical extract of Radix Angelicae pubescentis into 100~200 mesh silica gel and stir them evenly. It was used for elution of mixture of petroleum ether and ethyl acetate. One stream fraction is collected every 500ml, and then solvent was recovered. After thin layer detection, flow fractions of similar composition were merged.

a) 25 to 30 flow fractions were placed stationary after they were combined. After solvent was dried, the mixture of petroleum ether and ethyl acetate was recrystallized to give a white needle-like crystal. It was defined as compound II.

b) 35 to 38 flow fractions were placed stationary after they were combined. After solvent was dried, the mixture of petroleum ether and ethyl acetate was recrystallized to give a white needle-like crystal. It was defined as compound I.

c) 46 to 48 flow fractions were placed stationary after they were combined. After solvent was dried, the mixture of petroleum ether and ethyl acetate was recrystallized to give a white needle-like crystal. It was defined as compound VI.

d) 52 to 54 flow fractions were placed stationary after they were combined. After solvent was dried, the mixture of petroleum ether and ethyl acetate was recrystallized to give a white needle-like crystal. It was defined as compound IV.

e) 60 to 62 flow fractions were placed stationary after they were combined. After solvent was dried, the mixture of petroleum ether and ethyl acetate was recrystallized to give a white needle-like crystal. It was defined as compound V.

f) 68 to 71 flow fractions were placed stationary after they were combined. After solvent was dried, the mixture of petroleum ether and acetone was recrystallized to give a white needle-like crystal. It was defined as compound III.

The process of separation is showed in Figure 2.

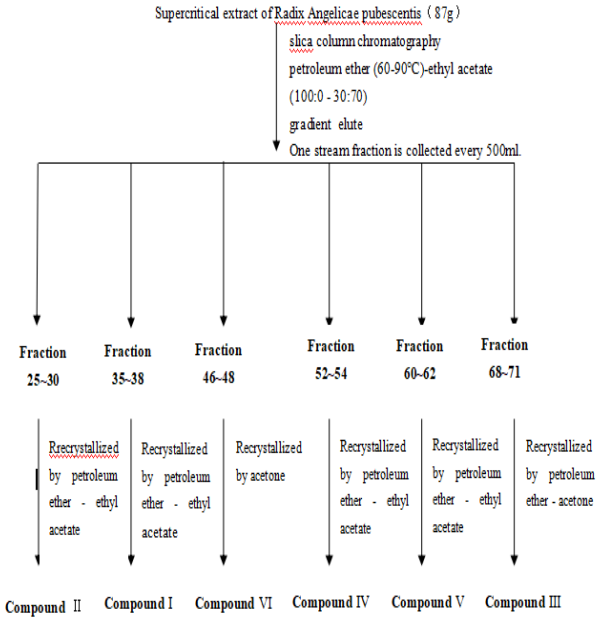

Figure 2. Structural classification of coumarin in Radix Angelicae pubescentis

3. Results & Discussion

In this experiment, we used CO_2 supercritical extraction technology to get the extract of monosacrum. We systematically separated the extracts and thus obtained eight compounds. We identified six of them by physicochemical properties and spectral data analysis. They includs five furan coumarins and one simple coumarins. They are Osthol, Columbianetin, Columbianetin Acetate, Xanthotoxin, Bergapten. The structures of the compounds were showed in Figure 3.

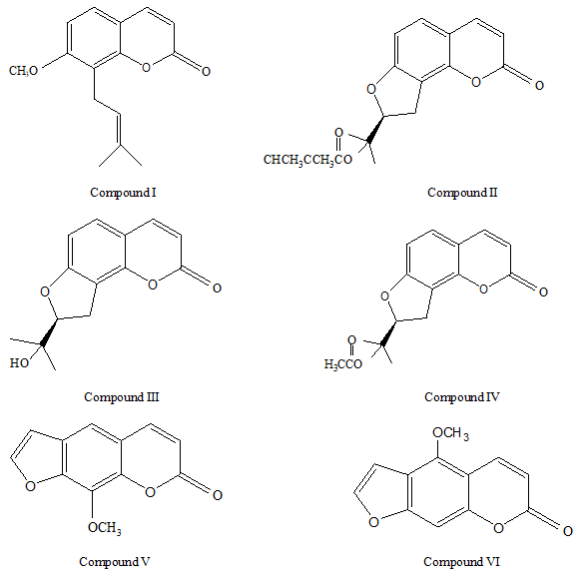

Figure 3. The structures of the compounds we separated in Radix Angelicae pubescentis

3.1Chemical composition and structure identification of effective parts

1) Compound I: It is a white needle crystal (Petroleum ether - ethyl acetate), which m.p. is 82.2-82.5 ℃. It showed blue fluorescence under ultraviolet light. It's physical constant and spectroscopic data is showed as follows.

a) UVλMeOH maxnm: 257, 322.

b) ^{1}HNMR ($CDCl_3$)(ppm): ξ 1.67 (3H, s), 1.84 (3H ,s), 3.53 (2H, d, J=7.3Hz), 3.92 (3H, s), 5.22 (1H, t, J=7.3Hz), 6.23 (1H, d, J=9.5Hz), 6.84 (1H, d, J=8.6Hz), 7.29 (1H, d, J=8.6Hz), 7.60 (1H, d, J=9.5Hz).

c) ^{13}CNMR ($CDCl_3$)(ppm): ξ 161.4, 160.1, 152.7, 143.7, 132.6, 126.2, 121.0, 117.8, 112.9, 107.3, 56.0, 25.7, 21.8, 17.9.

The physical constants and spectral data were consistent with those reported Osthol [4]. ^{1}HNMR and ^{13}CNMR spectral data of Osthol were showed in Table 1.

2) Compound II: It is a white needle crystal (Petroleum ether - ethyl acetate), which m.p. is115-117 ℃. It showed bluish violet fluorescence under ultraviolet light. It's physical constant and spectroscopic data is showed as follows.

a) UVλMeOH maxnm: 326, 260, 265.

b) ^{1}HNMR ($CDCl_3$)(ppm): ξ 1.60 (3H, s), 1.64 (3H, s), 1.67 (3H, s), 1.89 (3H, d, J=1.5Hz), 3.38 (2H, d, J=8.5Hz), 5.12 (1H, t, J=8.5z), 5.98 (1H, q, J=1.5z), 6.21 (1H, d, J=9Hz), 6.74 (1H, d, J=8Hz), 7.26 (1H, d, J=8Hz), 7.62 (1H, d, J=9Hz).

c) ^{13}CNMR ($CDCl_3$)(ppm): ξ 167.0, 163.9, 161.0, 151.2, 144.0, 137.7, 128.8,128.6,113.5,112.9, 112.1, 106.6, 89.2, 82.0, 27.6, 22.2, 21.2, 20.5, 15.6.

The physical constants and spectral data were consistent with those reported Columbianedin[5]. ^{1}HNMR and ^{13}CNMR spectral data of Columbianedin were showed in Table 2.

TABLE 1 ^{1}HNMR AND ^{13}CNMR SPECTRAL DATA OF OSTHOL ($CDCL_3$)

No. H-Proton	ξ (ppm)	No.C-Proton	ξ (ppm)
		2	161.4
H-3	6.23	3	112.9
H-4	7.60	4	143.7
H-5	7.29	5	126.2
H-6	6.84	6	107.3
		7	160.1
		8	112.9
		9	152.7
		10	117.8
H-1'	3.53	1'	21.8
H-2'	5.22	2'	121.0
		3'	132.6
H-4'	1.84	4'	25.7
H-5'	1.67	5'	17.9
OCH_3	3.92	OCH_3	56.0

TABLE 2 ^{1}HNMR AND ^{13}CNMR SPECTRAL DATA OF COLUMBIANEDIN ($CDCL_3$)

No. H-Proton	ξ (ppm)	No.C-Proton	ξ (ppm)
		2	163.9
H-3	6.21	3	112.1
H-4	7.62	4	144.0
H-5	7.26	5	128.6
H-6	6.74	6	106.6
		7	160.5
		8	113.5
		9	151.2
		10	112.9
H-1'	3.38	1'	21.8
H-2'	5.12	2'	88.7
		3'	85.3
H-4'	1.64	4'	20.5
H-5'	1.60	5'	21.2
		1"	167.0
		2"	128.8
H-3"	5.98	3"	138.6
H-4"	1.67	4"	15.6
H-5"	1.89	5"	27.6

3) Compound III: It is a colorless column crystal (Petroleum ether - ethyl acetate), which m.p. is158.2-158.4 ℃. It showed bluish violet fluorescence under ultraviolet light. It's physical constant and spectroscopic data is showed as follows.

a) UVλMeOH maxnm: 328, 261.

b) ^{1}HNMR ($CDCl_3$)(ppm): ξ 1.25 (3H, s), 1.37 (3H, s), 3.32 (2H, d, J=8.3Hz), 4.80 (1H, t, J=8.3Hz), 6.20 (1H, d, J=9.5Hz), 6.75 (1H, d, J=8Hz), 7.26 (1H, d, J=8Hz), 7.63 (1H, d, J=9.5Hz).

c) ^{13}CNMR ($CDCl_3$)(ppm): ξ 163.6, 161.0, 151.2, 143.9, 128.7, 114.0, 113.0, 112.2, 106.6, 91.3, 71.7, 27.5, 25.9, 23.9.

The physical constants and spectral data were consistent with those reported Columbianetin[6]. ^{1}HNMR and ^{13}CNMR spectral data of Columbianetin were showed in Table 3.

4) Compound IV: It is a colorless prismati crystal (Petroleum ether - ethyl acetate), which m.p. is133.2-134.1 ℃. It showed blue-purple fluorescence under ultraviolet light. It's physical constant and spectroscopic data is showed as follows.

a) UVλMeOH maxnm: 250, 260, 325.

b) ^{1}HNMR ($CDCl_3$)(ppm): ξ 1.52 (3H, s), 1.58 (3H, s), 1.99 (3H, s), 3.28 (2H, m), 5.16 (1H, t), 6.21 (1H, d, J=9.5Hz), 6.75 (1H, d, J=8Hz), 7.28 (1H, d, J=8Hz), 7.65 (1H, d, J=9.5Hz).

c) ^{13}CNMR ($CDCl_3$)(ppm): ξ 170.1, 163.8, 160.9, 151.1, 143.9, 128.8, 113.3, 112.9, 112.1, 106.6, 88.6, 82.0, 27.5, 22.2, 21.8, 20.8.

The physical constants and spectral data were consistent with those reported Columbianetin Acetate[7]. ^{1}HNMR and ^{13}CNMR spectral data of Columbianetin Acetate were showed in Table 4.

TABLE 3 ^{1}HNMR AND ^{13}CNMR SPECTRAL DATA OF COLUMBIANETIN ($CDCL_3$)

No. H-Proton	ξ (ppm)	No.C-Proton	ξ (ppm)
		2	163.6
H-3	6.20	3	112.2
H-4	7.63	4	143.9
H-5	7.26	5	128.7
H-6	6.75	6	106.6
		7	161.0
		8	114.3
		9	151.2
		10	113.7
H-1’	3.32	1’	23.9
H-2’	4.80	2’	91.9
		3’	73.3
H-4’	1.37	4’	27.5
H-5’	1.25	5’	25.9

TABLE 4 ^{1}HNMR AND ^{13}CNMR SPECTRAL DATA OF COLUMBIANETIN ACETATE ($CDCL_3$)

No. H-Proton	ξ (ppm)	No.C-Proton	ξ (ppm)
		2	163.8
H-3	6.21	3	112.1
H-4	7.65	4	143.9
H-5	7.28	5	128.8
H-6	6.75	6	106.6
		7	160.9
		8	113.3
		9	151.1
		10	112.9
H-1’	3.28	1’	22.2
H-2’	5.16	2’	88.6
		3’	82.0
H-4’	1.58	4’	27.5
H-5’	1.52	5’	20.8
		1”	170.1
H-2”	1.99	2”	21.8

5) Compound V: It is a white needle crystal (petroleum ether - ethyl acetate), which m.p. is 142-143.2 ℃. It showed yellow fluorescence under ultraviolet light. It’s physical constant and spectroscopic data is showed as follows.

a) UVλMeOH maxnm: 299, 248.

b) ^{1}HNMR ($CDCl_3$)(ppm): ξ 4.27 (3H, s), 6.34 (1H, d, J=9.5Hz), 6.80 (1H, d, J=2Hz), 7.33 (1H, s), 7.67 (1H, d, J=2Hz), 7.75 (1H, d, J=9.5Hz).

c) ^{13}CNMR ($CDCl_3$)(ppm): ξ 160.4, 147.6, 146.6, 144.3, 142.9, 132.7, 126.1, 116.4, 114.6, 112.9, 106.7, 61.2.

The physical constants and spectral data were consistent with those reported Xanthotoxin[7]. ^{1}HNMR and ^{13}CNMR spectral data of Xanthotoxin were showed in Table 5.

TABLE 5 ^{1}HNMR AND ^{13}CNMR SPECTRAL DATA OF XANTHOTOXIN ($CDCL_3$)

No. H-Proton	ξ (ppm)	No.C-Proton	ξ (ppm)
		2	160.4
H-3	6.34	3	112.1
H-4	7.75	4	144.3
H-5	7.33	5	112.9
		6	126.1
		7	146.6
		8	132.7
		9	142.9
		10	116.4
H-11	6.80	11	106.7
H-12	7.67	12	146.0
OCH_3	4.27	OCH_3	61.2

6) Compound VI:It is a white needle crystal (petroleum ether - ethyl acetate), which m.p. is 187-188 ℃. It showed yellowish-green fluorescence under ultraviolet light. It’s physical constant and spectroscopic data is showed as follows.

a)UVλMeOH maxnm: 220, 247, 267, 308.

b) ^{1}HNMR ($CDCl_3$)(ppm): ξ 8.15 (1H, d, J=9.7Hz), 7.59 (1H, d, J=2.3Hz), 7.12 (1H, s), 7.02 (1H, d, J=2.3Hz), 6.27 (1H, d, J=9.7Hz), 4.27(3H,s).

c) ^{13}CNMR ($CDCl_3$)(ppm): ξ 161.3, 158.4, 152.7, 149.6, 144.8， 139.3， 112.7， 112.5， 106.4， 105.0， 93.8， 60.1.

The physical constants and spectral data were consistent with those reported Bergapten[7]. ^{1}HNMR and ^{13}CNMR spectral data of Bergapten were showed in Table 6.

TABLE 6 ^{1}HNMR AND ^{13}CNMR SPECTRAL DATA OF BERGAPTEN ($CDCL_3$)

No. H-Proton	ξ (ppm)	No.C-Proton	ξ (ppm)
		2	161.3
H-3	8.15	3	112.7
H-4	6.27	4	139.3
		5	151.2
		6	112.5
		7	158.4
H-8	7.12	8	93.8
		9	149.6
		10	106.4
H-11	7.02	11	105.0
H-12	7.59	12	144.8
OCH_3	4.27	OCH_3	60.1

4. CONCLUSIONS

A systematic chemical separation of Radix Angelicae pubescentis CO_2 supercritical extracts was carried out, eight compounds were obtained, and their chemical composition was preliminarily studied. Six of them were identified by various chromatographic methods combined with spectral data. They are Osthol, Columbianedin, Columbianetin, Columbianetin Acetate, Xanthotoxin, Bergapten. Xanthotoxin and Bergapten were isolated,which structure were similar. The standard substance of Osthol and Columbianedin were producted in order to offer the good material base for quality evaluation and pharmacodynamic study of Radix Angelicae pubescentis. Their purification were more than 99%. The results of this study are of great significance for exploring the effective material basis and medicinal value of the supercritical extract of Radix Angelicae pubescentis.

ACKNOWLEDGMENT

This work was suppored by Research Planning Project of Liaoning Vocational and Technical Education Association (LZY20630) and Research project of Liaoning Provincial Education Department (LJKQZ2021161).

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