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Review

Coagulant Compounds from Rhizomes of *Polygonum bistorta* (Linn.)

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The flour of rhizomes of *Polygonum bistorta* L. was analyzed for its coagulant compounds. These compounds were separated from the flour and identified chemically for the first time. Several spectral methods such as ¹³C NMR, ¹H NMR and FTIR were used to elucidate the structures of coagulant compounds found chemically. Two of these were identified as flavonol of flavonoids. They were 2,3',4',4,6-pentahydroxy flavone and 2,5',6-trihydroxy-4,2'-dimethoxy flavone, which had not been previously reported in these species.

Keywords: Coagulant compound, Rhizomes, Polygonum Bistorta

INTRODUCTION

Polygonum bistorta L. (Polygonaceae) is broadly used in Hebei, Liaoning and Inner Mongolia in China (Editorial Board of China Herbal, 1999). The raw state of this plant has been employed as the drug having numerous effective pharmacological activities such as anti-inflammatory, antibacterial and anti-mutation activity (Duwiejua et al., 1999; Niikawa et al., 1995; Duwiejua et al., 1999). Dysentery with bloody stools, chronic respiratory infection with cough; diarrhoea in severe gastroenteritis, aphthous ulcer, haematemesis, epistaxis, carbuncles, scrofula, haemorrhoidal bleeding have been

treated by using the rhizomes of *Polygonum bistorta* L. in Chinese folk medicine (The State Pharmacopoeia Commission of P.R. of China, 2000). Because of some Particular medicinal and remediable effects of its rhizomes, it can be concluded that in the rhizomes of *Polygonum bistorta* L., there are some triterpenoids, flavones and phenolic acids (Liu et al., 2004; Xiao et al., 2003). Cycloartane type triterpenoids from its rhizomes were isolated and NMR spectroscopy was used to elucidate their structures (Monoharan et al., 2005). Investigation of the roots of this plant afforded seven compounds including triterpenoids, coumarin and a steroid (Sun et al., 2007). So far, there is no detailed chemical study on the rhizomes of this plant to identify the coagulant compounds. This paper describes herein the structure determination of these coagulant compounds on the basis of chemical experiments and spectroscopic methods.

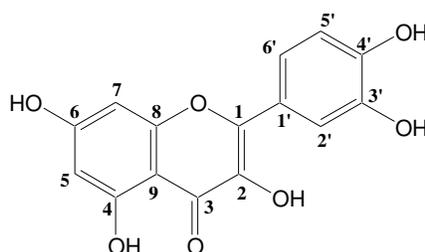


Figure 1.. Structure of coagulant compound B.

Table 1. The ^1H NMR and ^{13}C NMR data of B

Position	$\delta_c(\text{DMSO})$	$\delta_H(\text{DMSO})$
1	133.50	
2	122.38	
3	176.27	
4	149.59	
5	98.61	7.67 (1H, br. s)
6	164.31	
7	93.78	6.40 (1H, br. s)
8	161.15	
9	103.44	
2-OH		12.49 (1H, s)
4-OH		10.8 (1H, br. s)
6-OH		9.61 (1H, br. s)
1'	136.17	
2'	115.48	6.81 (1H, br. s)
3'	148.13	
4'	147.22	
5'	116.03	7.52 (1H, d, J=8.4 Hz)
6'	120.40	6.86 (1H, d, J=8.5 Hz)
3'-OH		9.32 (1H, br. s)
4'-OH		9.38 (1H, br. s)

Experimental

Plant material

The rhizomes of *Polygonum bistorta* L. were brought together directly from the fields of irrigated areas of Ghazvin, Iran.

The rhizomes were cleaned with purified water to take away sludge, cut into slices with a sharp knife and dried in air. The dried samples were ground with an electric grinder to achieve the flour. The flour was maintained in its original state in dry containers with closures tightened so that the flour remained contact less with air for chemical analysis.

Extraction and isolation

The separation and identification processes for finding the coagulant compounds being in the rhizomes of this plant, were be done according to the traditional use of them for blood coagulation. To do this customary

application of the rhizomes, they should be drenched in an organic aqueous and put on an injury until the blood goes to be coagulated. Thus, the extract of the rhizomes flour (100 g) was separated with aqueous ethanol (800 ml) and the flavonols content was identified by the following method. The mixture of the flour and ethanol was stood for 36 h at about zero temperature in a refrigerator. The extraction with ethanol solvent was carried for 6 h followed by evaporation and filtration of the solvent to dryness. 0.5M ethanolic sodium hydroxide was mixed with the ethanolic extract (5 g) so that the mixture property would be led to an alkaline state. The unsolvable part of

the ethanolic extract was filtered, evaporated and crystallized with ethanol. The melting point of this crystallized part, named A, was 135°C.

Aqueous HCl (20%) was added to the alkaline solution without extract so that the pH of the mixture would be reached to 2.5. A little white phenolic precipitate was separated from the mixture and washed with water. The white phenolic precipitate was solved in (10 ml) tetra-hydro furan (THF), the mixture was

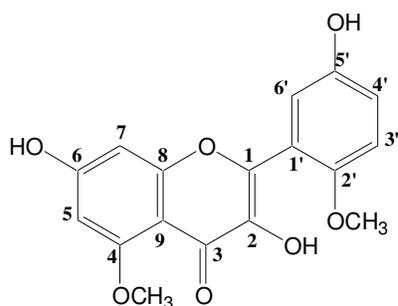


Figure 2. Structure of coagulant compound C.

Table 2. The ^1H NMR and ^{13}C NMR data of C

Position	$\delta_c(\text{DMSO})$	$\delta_H(\text{DMSO})$
1	129.30	
2	125.05	
3	194.28	
4	174.64	
5	95.75	6.01 (1H, br. s)
6	172.48	
7	96.47	5.95 (1H, br. s)
8	151.60	
9	105.89	
2-OH		10.20 (1H, br. s)
4-OCH ₃		3.74 (3H, br. s)
6-OH		9.56 (1H, s)
1'	122.41	
2'	149.30	
3'	115.33	7.07 (1H, d, J=7.6 Hz)
4'	115.53	6.92 (1H, d, J=8.3 Hz)
5'	137.07	
6'	114.92	7.52 (1H, br. s)
2'-OCH ₃		4.58 (3H, s)
5'-OH		9.09 (1H, s)

filtered, evaporated and crystallized with ethanol to obtain the second product of extract named B. The unsolvable part of the white phenolic precipitate in THF was crystallized with (5 ml) ethanol and (2 ml) n-hexane to create the third product of extract named C. The melting point of compounds B and C were 321°C and 349°C, respectively

General experimental procedures

All NMR spectra were recorded on a Bruker ARX-300 instrument with TMS as internal reference. IR spectra were taken on a Shimadzu FT-500 infrared spectrophotometer. Melting points were measured on a Yanaco MP-S3 micro-melting point meter.

RESULTS AND DISCUSSION

According to some identification analyses (Shriner et al., 2004) and the measured melting points, it was proved

that in compounds B and C, there were some phenolic structures such as phenolic OH, but in compound A, they could not be recognized. Thus, compounds B and C would be flavonols with coagulant properties.

To confirm the above identification and structural elucidation of coagulant compounds B and C, three spectral methods such as ^{13}C NMR, ^1H NMR and FTIR were used.

The molecular formula for compound B, $\text{C}_{15}\text{H}_{10}\text{O}_7$, was determined on the basis of chemical and spectral analyses (Figure 1).

The IR spectrum of B indicated the presence of phenolic and alcoholic OH ($\nu_{\text{max}} > 3100 \text{ cm}^{-1}$), ketonic C=O ($\nu_{\text{max}} 1610 \text{ cm}^{-1}$), C=C of alkenes ($\nu_{\text{max}} 1680, 1570 \text{ cm}^{-1}$), C=C of aromatics ($\nu_{\text{max}} 1570, 1500 \text{ cm}^{-1}$), etheric C-O ($\nu_{\text{max}} 1250, 1170 \text{ cm}^{-1}$), alcoholic C-O ($\nu_{\text{max}} 1020 \text{ cm}^{-1}$), aromatic C-H ($\nu_{\text{max}} 750, 630 \text{ cm}^{-1}$). The ^1H NMR spectrum in DMSO gave three aromatic proton signals at δ 6.81, 6.40, 7.67, 6.86 and 7.52, four phenolic proton signals at δ 9.32, 9.38, 9.61 and 10.80, and one hydroxylic proton signal at δ 12.49. The ^{13}C NMR

spectra showed 15 carbon signals of the twelve aromatic carbons (δ 93.78, 98.61, 103.44, 115.48, 116.03, 120.4, 122.38, 136.17, 145.49, 147.22, 148.13, and 149.59), two carbons linked to oxygen and a double bond (δ 161.15, 164.31) and one carbonyl carbon (δ 176.27). The ^1H NMR and ^{13}C NMR signals of B were assigned in detailed according to Table 1. It could be concluded that the structure of compound B was designated as 2, 3', 4', 4, 6-pentahydroxy flavone.

The molecular formula for compound C, $\text{C}_{17}\text{H}_{14}\text{O}_7$, was determined on the basis of chemical and spectral analyses (Figure 2).

The IR spectrum of C indicated the presence of phenolic and alcoholic OH ($\nu_{\text{max}} >3100 \text{ cm}^{-1}$), ketonic C=O ($\nu_{\text{max}} 1615 \text{ cm}^{-1}$), C=C of alkenes ($\nu_{\text{max}} 1680, 1575 \text{ cm}^{-1}$), C=C of aromatics ($\nu_{\text{max}} 1575, 1500 \text{ cm}^{-1}$), CH_3 ($\nu_{\text{max}} 1450, 1370 \text{ cm}^{-1}$), etheric C-O ($\nu_{\text{max}} 1280, 1180 \text{ cm}^{-1}$), alcoholic C-O ($\nu_{\text{max}} 1010 \text{ cm}^{-1}$), aromatic C-H ($\nu_{\text{max}} 820, 700 \text{ cm}^{-1}$). The ^1H NMR spectrum in DMSO gave two methoxy proton signals at δ 3.74, 4.58, five aromatic proton signals at δ 5.95, 6.01, 6.92, 7.07, and 7.52, two phenolic proton signals at δ 9.09, and 9.56 and one hydroxylic proton signal at δ 10.49. The ^{13}C NMR spectra showed 17 carbon signals of the two methoxy carbons (δ 64.23, 65.33), twelve aromatic carbons (δ 95.75, 96.47, 105.89, 114.92, 115.33, 115.53, 122.41, 122.05, 129.30, 137.07, 149.30, and 151.60), two carbons linked to oxygen and a double bond (δ 172.48, 174.64) and one carbonyl carbon (δ 194.28). The ^1H NMR and ^{13}C

NMR signals of C were assigned in detailed according to Table 2. It could be concluded that the structure of compound C was designated as 2, 5', 6-trihydroxy-4, 2'-dimethoxy flavone.

Finally, it should be said that from the first ethanolic extract (5 g), (0.02 g) compound B and (0.03 g) compound C were obtained.

CONCLUSIONS

The rhizomes of *Polygonum bistorta* L. can be used as a natural non-toxic source of coagulant compounds in medicine and pharmacy. Also, these compounds are obtained from the rhizomes in a non-complicated chemical manner.

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