Chemistry of Natural Compounds, Vol. 41, No. 3, 2005

ARABINOGALACTAN POLY- AND OLIGOSACCHARIDES MODIFIED WITH 5-AMINOSALICYLIC ACID

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UDC 615.36:547.454.012

*Arabinogalactan poly- and oligosaccharides from Siberian larch (*Larix sibirica *L.) were modified with 5-aminosalicylic acid. The chemical composition and certain physicochemical properties were studied. The optimal conditions for preparing the modified compounds were found. These compounds were demonstrated to possess anti-ulcer and anti-inflammatory activity in tests on experimental animals.*

Key words: arabinogalactan, polysaccharides, oligosaccharides, chemical modification, anti-ulcer and anti-inflammatory activity.

One of the methods for creating improved forms of medicinal compounds is their modification with natural polysaccharides. Among the polysaccharides that can be used for this purpose, arabinogalactan (AG) isolated by aqueous extraction from wood of the Siberian larch is of great interest because it is very soluble in water and has biological activity [1-4]. The use of water-soluble oxidized macromolecules and oligomers of AG as a polymeric matrix for formulating medicinal preparations is currently of great interest.

We investigated the reaction of AG and its oxidized low-moleclar-weight fractions with an anti-ulcer preparation of 5-aminosalicylic acid (5-ASA). The low-molecular-weight fractions were prepared by oxidative destruction of AG using H_2O_2 and atmospheric oxygen in aqueous medium. The structures of the oxidized AG fractions were previously reported [5].

The modified compounds were obtained by reacting 5-ASA with AG and its oxidized fractions in water at pH 7. The synthesized compounds are light-violet to black powders that are very soluble in water but insoluble in acetone, alcohols, and ether. The natural arabinogalactan (AG_{na}) and polymeric (AG_{pol}) and oligomeric (AG_{ol}) fractions of oxidized polysaccharide were modified by reaction with 5-ASA, which has carboxylic, hydroxyl, and amino groups. It can be assumed that 5-ASA, like salicylic acid, is a relatively weak acid that will be partially dissociated in aqueous medium. Therefore, the form (molecular or ionized) of 5-ASA that reacts with AG must be determined. Thus, the dissociation constants of the carboxylic and hydroxyl groups of salicylic acid are $K_a = 1 \cdot 10^{-3}$ ($pK_a = 3.00$) and $K_a = 1.5 \cdot 10^{-14}$ ($pK_a = 13.82$), respectively [6]. Apparently these functional groups in 5-ASA are weakly dissociated. The presence of amino groups in 5-ASA typifies it also as an organic base that can accept its own protons formed by dissociation. As a result, it is obvious that the degree of dissociation of the carboxylic groups of 5-ASA should increase. Therefore, aqueous solutions of 5-ASA will contain not only acid molecules but also bipolar $^+H_3NC_6H_3(OH)COO^-$ ions. Dissociation of sodium salts of the acid (prepared by neutralization of an aqueous solution of the acid with base) in neutral medium will also form $H_2NC_6H_3(OH)COO^-$ ions. In acid medium, $^+H_3NC_6H_3(OH)COOH$ ions will most likely dominate; in basic medium, $H_2NC_6H_3(OH)COO^-$. Changing the pH of a solution is known to change the degree of dissociation of weak acids and the ratio between the concentrations of 5-ASA molecules and ions and, therefore, the UV spectra. The electronic spectrum of 5-ASA in aqueous solution at pH 7 and [5-ASA] = 10^{-4} M has a single absorption maximum at 330 nm. In acid medium at pH < 7, the absorption maximum in the UV spectrum is observed at λ_{max} 304 nm. Adding solutions of AG_{na}, AG_{pol}, and AG_{ol} to a solution of 5-ASA at pH 7 causes a strong band to appear at λ_{max} 330 nm. A strong band appeared in neutral and basic media if the pH was varied in an aqueous "AG + 5-ASA" system.

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 0009-3130/05/4103-0272

TABLE 1. Effect of Reaction Conditions for Modifying AG and Its Fractions with 5-ASA on Product Composition

Time, h	Complex yield, %	5-ASA in complex, %
	Arabinogalactan + 5-ASA	
24	62.2*a	0.89
24	34.1*b	1.35
24	18.4*c	0.89
0.5	46.2*b	0.9
2	31.9*b	1.1
4	45.4*b	1.3
6	39.8*b	2.0
72	38.4*b	5.5
3	31.8*b	0.94
3	44.8**b	1.46
3	42.2**b*	1.79
	Polymer fraction + 5-ASA	
24	48.1*a	1.07
24	31.5*b	4.33
24	23.0*c	4.66
1	31.95*b	2.93
6	32.55*b	3.4
48	39.75*b	7.5
3	30.20**b	2.81
3	28.8***b	4.1
	Oligomer fraction + 5-ASA	
24	61.6***a	10.9
24	53.5***b	28.9

Reaction temperature, °C: *20, **40, ***60. Ratio of reaction components [poly- or oligosaccharide]:[5-ASA] (mmol/mmol): 1:0.1 (a), 1:1 (b), 1:2 (c).

The products of modifying AG and its oxidized fractions that contain 5-ASA absorb in the range 315-317 nm. Solutions of the polysaccharides do not absorb in this range. Therefore, the appearance of a band at λ_{max} 315-317 nm can be explained by the reaction of 5-ASA and the investigated biopolymers. A similar phenomenon is observed in acid medium. Therefore, the reaction of AG and 5-ASA was studied further at pH 7.0-7.1.

Model experiments in which D-galactose and galacturonic acid replaced the biopolymers were performed in order to establish the nature of the functional group of 5-ASA that reacts with AG at pH 7. The UV spectrum of the product from reaction of D-galactose and 5-ASA taken in a 1:1 mole ratio has an absorption maximum at λ_{max} 333 nm, which coincides with the absorption maximum of 5-ASA in neutral medium. Therefore, the hydroxyls and ether bond of D-galactose (meaning AG also) do not react with the functional groups of 5-ASA. It should be considered that sodium salts of AG uronic acids are formed in neutral medium. Then these dissociate to carboxylate ions. These functional groups are probably involved when the biopolymer is modified. In fact, 5-ASA reacts with galacturonic acid to form a product with λ_{max} 317 nm. In summary, the model experiments lead to the conclusion that the reaction of the protonated amino group of 5-ASA with carboxylate ions (uronic acids) of AG and (or) its oxidized fractions is most likely. The structures of the resulting compounds were confirmed by elemental analysis and IR and UV spectroscopy (Table 1). The IR spectra of the compounds show shifts to low frequency by 20-50 cm^{-1} for the absorption maximum at 3600-3100 cm^{-1}, corresponding to hydroxyl stretching vibrations, and by 10-15 cm^{-1} for the C–O ether bond of pyranose and furanose rings at 1200-1100 cm^{-1}. This also indicates that intermolecular H-bonds form between 5-ASA and the polysaccharide fractions. Furthermore, in the 1500-1750 cm^{-1} range, the absorption band for carbonyl vibrations at 1750 cm^{-1} weakens and a moderate absorption band for 5-ASA benzene-ring vibrations appears at 1580 cm^{-1}.

TABLE 2. Anti-inflammatory, Analgesic, and Anti-ulcer Activity of Products from Reaction of AG and Its Oxidized Fractions with 5-ASA

Compound	Amount of "acetic twitching"	"Hot plate," s	Average % mass increase of paw	Amount of stomach lining destruction
Arabinogalactan	7.0±1.5	17.0±1.2*	65.0±5.1	7.5±1.0, P<0.05
Polymer fraction	6.0±1.0	14.0±1.3	65.5±3.4*	10.5±1.4, P<0.05
Oligomer fraction	4.0±0.7	12.0±1.0	53.0±2.5*	5.5±0.9, P<0.05
Analgin	3.0±1.0*	17.0±0.7*	-	-
Voltaren	-	-	55.0±1.5*	-
Control	6.2±0.9	13.0±1.1	64.0±4.3	-
Arabinogalactan + 5-ASA	4.7±1.1	11.0±1.2	62.0±2.6	2.2±1.3, P<0.02
Polymer fraction + 5-ASA	5.8±0.7	14.5±1.1	60.0±1.7*	1.5±1.0, P<0.01
Oligomer fraction + 5-ASA	4.3±0.6	13.0±1.1	63.0±2.4	2.7±1.4, P<0.02

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*$P < 0.05$ is the reliability relative to the control.

The electronic spectra of the compounds show a hypsochromic shift of the absorption maximum for the aromatic component of modified AG and its fractions at λ_{max} 317 nm, in contrast with λ_{max} 330 nm for 5-ASA.

Table 1 shows the effect of the reaction conditions on the composition of the resulting products. The results were optimal using equimolar amounts of the starting compounds. Increasing the AG/5-ASA molar ratio from 0.1 to 1.0 regularly increases the amount of bonded 5-ASA. Further increasing the amount of added 5-ASA does not increase the amount of 5-ASA in the reaction products. Increasing the reaction temperature does not practically increase the content of 5-ASA. Increasing the reaction time increases substantially the amount of 5-ASA in the products. However, changing the nature of polysaccharide fraction has a significant effect on the composition of the reaction products. The highest content of 5-ASA in the modified compounds is observed for the oligomer fraction. This is due to the high content of functional groups in this fraction.

The characteristic viscosities of the prepared compounds are practically the same as those of AG and the starting oxidized fractions. This indicates that the chains of the polysaccharide fractions were not destroyed during the modification. The compounds had $[\eta] = 0.03$-0.036.

The acute toxicity of the studied compounds was >5000 mg/kg. These compounds are moderately toxic or slightly toxic substances. All compounds exhibited high anti-ulcer activity. Any AG fraction acts as a synergist for 5-ASA anti-ulcer activity. The anti-ulcer activities of the modified compounds were greater than those of the starting compounds by an average of 2-7 times (depending on the structure) (Table 2). Screening found that the oligomer fraction and the modified compound based on the polymer fraction inhibited the development of inflammation analogously to voltaren. It can be seen that the oligomer fraction exhibited analgesic activity analogous to analgin for the "acetic twitching" model. AG showed an analgesic effect analogous to analgin for the "hot plate" model (Table 2). The remaining compounds had weak analgesic activity.

An analysis of the results indicates that the higher anti-ulcer and anti-inflammatory activities of the oligomer fraction and the modified compound based on the polymer fraction are due to their lower molecular weights.

EXPERIMENTAL

We used AG of molecular weight 40,000 that was isolated by aqueous extraction of Siberian larch wood [7]. AG was isolated and purified of water-soluble phenolic compounds, including dihydroquercetin, using polyamide sorbent. This produced an aqueous solution of polysaccharide containing traces of flavonoids. The ratio of galactose and arabinose units in the polymer was 5.6:1 [8].

IR absorption spectra were recorded on a UR-20 spectrophotometer in mineral oil. Optical density was determined on a Specord M-40 specctrophotometer in water. The pH values of solutions were measured with an ANION 4100 pH-meter and adjusted by adding NaOH (0.1 M). The specific rotation was measured on a Perkin—Elmer (model 141) polarimeter.

Characteristic viscosities of aqueous solutions of AG and its oxidized fractions and modification products were measured at 30 ± 1°C in a Ubbelohde viscosimeter with a hanging level [9].

Synthesized products were purified of unreacted 5-ASA by precipitation from water into ethanol, which did not cause 5-ASA unbound to the polymer to precipitate. The purity of the preparation was monitored by TLC on Silufol plates using *n*-butanol:pyridine:water (6:4:5) and ninhydrin developer.

General Method for Preparing Modified Products. Polysacchride (1 g) or oligosaccharide (5.55 mmol base) was dissolved in water (20 mL) at pH 7-7.1. 5-ASA (0.85 g, 5.55 mmol) was suspended in water (20 mL). The pH was adjusted to 7-7.1. The polysaccharide solution was vigorously stirred and treated dropwise with the 5-ASA solution at 25°C. The reaction was carried out for 3 h. When the reaction was complete, the product was isolated by precipitation using ethanol and reprecipitated in alcohol. The solid was separated, washed three times with alcohol and then diethylether, and dried in vacuo.

The pharmacological activities were studied and the acute toxicities were determined for AG, its oxidized fractions, and the modification products using white mongrel mice and rats of mass 18-20 and 150-200 g kept under standard conditions in a vivarium.

The acute toxicity was determined in mice using the Kerber method and a single intraventricular injection.

The anti-inflammatory activities of the compounds were studied in mice using a model of adema caused by aponeurosis administration of a 1% carragheenin solution. The effect was evaluated using the percent decrease of paw adema after 6 h. The standard was an intraventricular administration of voltaren (8 mg/kg). Each compound was tested in six animals.

The anti-ulcer activities were tested in rats using an experimental ulcer model caused by intraventricular administration of indomethacin (20 mg/kg) one hour before reproduction of the model. The effect was evaluated after one day using the decrease in the amount of stomach ulceration.

The analgesic activities of the compounds were studied in mice using the "acetic twitching" (0.75% solution of acetic acid i.p.) and "hot plate" (54°C) models. The standard was an intraventricular administration of analgin (50 mg/kg). Compounds were administered once intraventricularly (50 mg/kg) one hour before reproduction of the models. This was more than 1/100 of the LD_{50}. Each compound was tested in six animals.

ACKNOWLEDGMENT

The work was supported financially by the Foundation "Leading Science Schools" (grant NSh-728.2003.3).

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