Chemistry of Natural Compounds, Vol. 44, No. 3, 2008

REACTION OF ARABINOGALACTAN WITH ISONICOTINIC ACID HYDRAZIDE

L. A. Badykova,[1*] R. Kh. Mudarisova,[1] G. S. Khamidullina,[2] Kh. K. Aminev,[3] and Yu. B. Monakov[1]

UDC 615.36:547.454.012

The reaction of an arabinogalactan and its oxidized forms with the antituberculosis preparation isonicotinic acid hydrazide was investigated. The chemical composition and certain physicochemical properties of the modified compounds were studied. A correlation was found between the concentration of carboxylic acids in the polysaccharides and the drug content in the modified compounds.

Key words: arabinogalactan, polysaccharides, chemical modification, antituberculosis activity.

Drug discovery is one of the thrust areas of modern medicinal chemistry. The search for and development of new antituberculosis agents have recently become of interest because of the drug resistance of mycobacteria to existing drugs. One promising direction for creating such drugs is the addition of common tuberculostatics to polysaccharides [1-3]. It is known that the polysacccharide arabinogalactan (AG) has a broad spectrum of biological activity [4-5]. However, its tuberculostatic activity has not been reported. Herein the modification of AG and its oxidized forms by the antituberculosis drug isonicotinic acid hydrazide (INAH) and the antituberculosis activity of the resulting compounds are studied.

Modified compounds were prepared by the reaction of INAH with AG and its oxidized high-molecular-weight (AG_{HMW}) and low-molecular-weight (AG_{LMW}) fractions. The preparation and structure of the oxidized AG fractions have been reported previously [6, 7].

The synthesized compounds are light-brown to white powders that are very soluble in water and insoluble in acetone, alcohols, and ether.

The angles of rotation of the products were $[\alpha]_D^{25}$ +11.9° (AG), +8.4° (AG_{HMW}), +5.3° (AG_{LMW}), +14.0° (AG + INAH), +20.0° (AG_{HMW} + INAH), and +39.0° (AG_{LMW} + INAH).

The modified AG compounds (AG + INAH, AG_{HMW} + INAH, and AG_{LMW} + INAH) were studied by spectral methods.

Electronic spectra of the products had an absorption band with λ_{max} (H_2O) 250 nm in contrast with the electronic spectrum of INAH with an absorption band at 262 nm. Solutions of the polysaccharides do not absorb in this region. The hypsochromic shift in the electronic spectra is probably related to steric hindrance from the polysaccharide matrix that disrupts the coplanarity of the conjugated chromophore and is indicative of donor—acceptor interaction between the polymer and the low-molecular-weight compound.

IR spectra exhibited low-frequency shifts of absorption maxima in the range 3600-3100 cm^{-1} that correspond to hydroxyl stretching vibrations by 130-140 cm^{-1} and of absorption maxima of C–O ether stretching vibrations in the pyranose and furanose rings at 1200-1100 cm^{-1} by 19-20 cm^{-1}. This may indicate formation of intermolecular H-bonds between INAH and the polysaccharides. Furthermore, the absorption band at 1750 cm^{-1} that is typical of carbonyl vibrations in the range 1500-1750 cm^{-1} weakened. An absorption band of medium strength appeared at 1550 cm^{-1} and was attributed to vibrations of the pyridine ring of INAH.

1) Institute of Organic Chemistry, Ufa Scientific Center, Russian Academy of Sciences, 450054, Ufa, prosp. Oktyabrya, 71, Russia, fax (3472) 35 60 66, e-mail: badykova@mail.ru; 2) GUZ Respublikanskii Protivotuberkuleznyi Dispanser, 450075, Ufa, prosp. Oktyabrya, 155, Russia, tel: (3472) 31 29 50; 3) Bashkir State Medical University, 450000, Ufa, ul. Lenina, 3, Russia. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 221-223, May-June, 2008. Original article submitted July 4, 2007.

0009-3130/08/4403-0279

TABLE 1. Effect of Modification Conditions of AG, AG_{HMW}, and AG_{LMW} by INAH on Drug Content in the Modified Form*

AG:INAH mole ratio	τ, h	T, °C	INAH content, mol per mol polymer
		AG	
1:1	3 (6, 10)	20	0.01
1:1	24 (48)	20	0.02
1:0.1 (1:0.5)	24	20	0.01
1:2 (1:3)	24	20	0.02
1:1	3	0 (40, 60)	0.01
1:1	3	90	0.02
		AG_{HMW}	
1:1 (1:2) (1:3)	4	20	0.12
		AG_{LMW}	
1:1 (1:3)	4	20	0.40

*Changes of parameters not affecting INAH yield are shown in parentheses.

NMR spectroscopy confirmed that modified compounds (polysaccharides—INAH) were formed. The PMR spectrum of mixed AG and INAH contained resonances for aromatic protons that were broadened and transformed. The resonances for C-6 and C-2 appeared as a broad singlet (8.75 ppm) instead of a doublet (8.74 ppm, 6.1 Hz). The resonances for C-3 and C-5 (7.75 ppm, 6.2 Hz) also changed from a doublet to a broad singlet at 7.77 ppm.

^{13}C NMR spectra of the reaction products of arabinogalactan and INAH exhibited shifts of the resonances for C-2/C-6 and C-3/C-5 by 0.1-0.15 ppm and a shift of the signal for C-4 by 0.2 ppm. The greatest shift to weak field by 0.3 ppm was seen for C-7. Therefore, the biopolymers were modified at the INAH amino group and the carboxylic acid of AG and its oxidized fractions. Thus, the most probable reaction is coordination of the polysaccharide and INAH as follows:

$$AG–COOH + H_2N–C_6H_5N_2OO \rightarrow AG–COOH\cdots H_2N–C_6H_5N_2O \rightarrow AG–COO^{-}\cdots{}^{+}H_3N–C_6H_5N_2O,$$

where AG–COOH = arabinogalactan, $H_2N–C_6H_5N_2O$ = INAH.

We studied the effect of the modification conditions of AG, AG_{HMW}, and AG_{LMW} by INAH on the drug content of the modified form (Table 1). The best results were obtained using equimolar amounts of the starting compounds. Table 1 shows that increasing the molar ratio by 10 times (from 0.1 to 1.0) increased slightly the amount of bound drug. Then, increasing the amount of added INAH did not increase the INAH content in the products. The reaction temperature and time had no effect on the drug content in the complex. Judging from the results, the reaction was practically complete after 1 h.

Changing the polysaccharide had a large effect on the composition of the products. The INAH content in them increased on going from AG to its oxidized forms. The greatest INAH content was observed in modified compounds of AG_{LMW}. This was due to the increased concentration of carboxylic acids in the oxidized AG. Initial AG contained 0.047 mol of COOH groups per mole of polymer; AG_{HMW}, 0.12; AG_{LMW}, 0.76.

Tuberculostatic tests found that AG and modified compounds of AG, AG_{HMW}, and AG_{LMW} possess tuberculostatic activity *in vitro* against pathogenic mycobacteria of the same level as free INAH. Partial growth inhibition of tuberculosis mycobacteria was noted for AG_{HMW} and AG_{LMW}.

Thus, it can be concluded that the INAH amino group and AG carboxylic acids react during modification of AG and its oxidized fractions by INAH. A correlation was found between the concentration of carboxylic acids in the polysaccharides and the drug content in the modified compounds. Modified compounds containing from 1 to 18% of the drug and possessing good tuberculostatic activity were obtained.

EXPERIMENTAL

We used AG of molecular weight (M) 40,000 and oxidized forms with high molecular weight (AG_{HMW}, M = 22,000) and low molecular weight (AG_{LMW}, M = 4,000). Modified compounds were synthesized using INAH (analytically pure). INAH and polysaccharides were reacted in water. PMR spectra in D_2O were recorded on a Bruker AM-300 spectrometer (operating frequency 300 MHz); ^{13}C NMR spectra, with wide-field proton decoupling on the same spectrometer (operating frequency 75.47 MHz). We used 3-5% solutions of polysaccharides and INAH in D_2O with DSS internal standard. ^{13}C NMR spectra were recorded at 25 ± 0.5°C with a 15 s delay between pulses. IR spectra in mineral oil were recorded on a Shimadzu spectrophotometer. Optical density was determined on a Specord M-40 instrument. The pH values of solutions were measured using a Anion 4100 pH-meter and were adjusted by adding NaOH solution (0.1 M). Specific rotation was measured using a Perkin—Elmer Model 141 polarimeter.

General Method for Preparing Modified Compounds. Polysaccharide (1 g, 5.55 mmol) and INAH (0.85 g, 5.55 mmol) were dissolved separately in distilled water (20 mL each). The polysaccharide solution was stirred vigorously and treated dropwise with the INAH solution at 25°C. The reaction was performed for 3 h. The product was isolated by precipitation by ethanol and reprecipitated from water in ethanol. The solid was separated, washed with alcohol three times and with diethylether, and dried in vacuo.

Tuberculostatic activity was studied by serial dilutions using Lowenstein—Jensen medium to which (before testing) the studied compounds were added (1 μg/mL). The test cultures were human *Mycobacterium tuberculosis*. The culture suspensions were prepared from a bacterial standard (500 million microbes per mL, 5 units). The resulting suspension (0.2 mL) was innoculated into tubes with nutrient medium containing the tested compounds of each dilution and without them (controls). The tubes were incubated for 21 d at 37°C.

REFERENCES

1. L. A. Kayukova and K. D. Praliev, *Khim.-farm. Zh.*, **34**, 1, 12 (2000).
2. K. P. Khomyakov, A. D. Virnik, S. N. Ushakov, and Z. A. Rogovin, *Vysokomol. Soedin.*, **7**, 6, 1035 (1965).
3. E. B. Dol′berg, B. G. Yasnitskii, L. N. Shuteeva, and I. P. Kovalev, *Zh. Prikl. Khim.*, **46**, 9, 2121 (1973).
4. Yu. S. Ovodov, *Bioorg. Khim.*, **24**, No. 7, 483 (1998).
5. A. O. Arifkhodzhaev, *Khim. Prir. Soedin.*, 185 (2000).
6. I. M. Borisov, E. N. Shirokova, R. Kh. Mudarisova, R. R. Muslukhov, Yu. S. Zimin, S. A. Medvedeva, G. A. Tolstikov, and Yu. B. Monakov, *Izv. Ross. Akad. Nauk, Ser. Khim.*, **2**, 305 (2004).
7. I. M. Borisov, E. N. Shirokova, V. A. Babkin, G. A. Tolstikov, and Yu. B. Monakov, *Dokl. Ross. Akad. Nauk*, **383**, 774 (2002).