Chemistry of Natural Compounds, Vol. 34, No. 6, 1998

CARBOHYDRATES OF *Allium*
X. GLUCOFRUCTANS OF *Allium suvorovii* AND THEIR BIOLOGICAL ACTIVITY

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UDC 547.917

Glucofructans have been isolated from the onion Allium suvorovii *Rgl., and their qualitative chemical compositions and the nature of the bonds between the fructofuranose residues have been determined. It has been established that glucofructan-II is a compound with low toxicity and exhibits a pronounced hypolipidemic activity in relation both to intact animals and to animals with experimental hyperlipidemia and atherosclerosis.*

The medicinal properties of *Allium suvorovii* Rgl. have long been known in folk medicine. In view of this, and also the useful food properties of this onion and a continuing investigation of plants of the genus *Allium*, we have studied the isolation of a homogeneous fraction of the glucofructans of *A. suvorovii* (fam. Alliaceae) and their biological activity [1, 2].

Comminuted bulbs of *A. suvorovii* gathered in the dormant stage in the environs of the village of Sina (western Hissar) were treated with chloroform, alcohol, and water. The yield of water-soluble glucofructan was 60% on the absolutely dry raw material. Gel chromatography on Sephadex G-75 showed that the initial glucofructan (GF) was polydisperse; i.e., it consisted of a group of glucofructans of the same structure but with different molecular weights: from 5000 to 50000.

Weight-average molecular masses were determined from a calibration curve of the dependence of molecular mass on elution volume (V_e) [3]. To obtain a homogeneous product, the initial glucofructan was treated with alcohol, and the fractions shown in Table 1 were obtained. The yield of the homogenous fraction GF-II amounted to 33—35% of the initial glucofructan or 15—17% of the raw material. GF-II was a colorless hygroscopic powder possessing no reducing capacity, readily soluble in water to form a clear solution, and giving no coloration with a 0.1 N solution of iodine.

In the product of the complete acid hydrolysis of GF-II, by PC (systems 1 and 2) we detected glucose and fructose, the quantitative level of the latter by Kolthoff's method [4] amounted to 98.1%.

The IR spectrum of GF-II contained absorption bands at 825, 870, 940, and 3320—3600 cm^{-1}. Absorption bands at 855 and 930 cm^{-1} are typical for 2→6-β-bonds [5] linking fructofuranose residues, with a standard deviation of ±15 cm, while absorption bands in the 815, 880, and 945 cm^{-1} regions are typical for the absorption of 2→1-β-bonds linking fructofuranose units. A region of wavelengths near 945 cm^{-1} covers the vibration of the fructofuranose ring [6].

We have investigated some aspects of the biological action of the glucofructans from *A. suvorovii* bulbs. One of their important biological effects is the capacity of these glucofructans for lowering the levels of cholesterol, β-lipoproteins, and triglycerides in the blood serum in intact animals and under conditions of experimental hyperlipidemias.

The most pronounced hypocholesteremic and hypolipidemic effect was possessed by a glucofructan of the mixed type — GF-II. Thus, under the conditions of endogenous hypercholesteremia, GF-II in a dose of 50 mg/kg (five times smaller than for clofibrate) lowered the level of cholesterol in blood serum by 18.8% and that of β-lipoproteins by 42%, while clofibrate in a dose of 250 mg/kg lowered these indices by 3 and 14.5%, respectively. In rats with Triton hyperlipidemia, this effect was similar to that of clofibrate (Table 2).

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 0009-3130/98/3406-0656$20.00

TABLE 1. Results of the Fractionation of the Initial Glucofructan from *A. suvorovii* Rgl.

Glucofructan*	Ethanol added		Yield of GF, %	Mol. mass
	ml	volume ratio with the extract		
GF-I	1400	1:2.3	38.3	40000-50000
	1500	1:2.6	40.0	4000-5600
GF-II	3400	1:5.6	33.0	15000
	3600	1:6.0	35.0	15000
GF-III	Mother solution		28.0	1000-5000

* The initial glucofructan dissolved in water in a ratio of 1:10.

TABLE 2. Influence of the Preparations on the Levels of Cholesterol, β-Lipoproteins, and Triglycerides in the Blood Serum of Experimental Animals ($M \pm m$; $n = 8$—10)

Index	Intact animals, mg/%		Hyperlipidemia, mg/%			
			endogenous		Triton-induced	
	cholesterol	β-lipoproteins	cholesterol	β-lipoproteins	cholesterol	triglycerides
Control	73.8±5	69.9±5	111.8±3.6*	80.2±8.1*	329±49	684±41.18
Glucofructan, 10 mg/kg	63.4±2.2	56.8±2.6	94.6±4.0**	66±3.5**	-	-
Glucofructan, 50 mg/kg	57.6±5.1*	41.1±3.1*	90.8±8.3*	40.6±4.6**	169±25**	553±36.8**
Clofibrate, 250 mg/kg	61.3±2.7*	60.6±2.7	96.4±4.27**	68.6±4.9**	140±11.8**	363±37.2**

* Significant changes in comparison with the intact animals.

** Significant changes in comparison with untreated animals (control) having hyperlipidemias at $P < 0.05$.

Under conditions of experimental atherosclerosis, the administration of GF-II (100 mg/kg) decreased the spreading of the atherosclerotic lesions in the aorta. Thus, in an experimental group the index of atherosclerotic damage of the aorta was considerably lower than in the control (38, compared with 67.4%). A determination of cholesterol and triglycerides in the blood serum, the aorta, and the myocardium of experimental animals showed that in rabbits treated with GF-II these indices were considerably lower than in the control group and in animals that had received polysponin (Fig. 1). In its antiatherosclerotic effect, GF-II is superior to the drug polysponin used at the present time.

Pharmacological investigations showed that GF-II is a nontoxic compound and its administration in doses of 500—200 mg/kg to mice (perorally) caused no changes whatever in the behavior of the experimental animals. In effective doses, the drug had no effect on the arterial pressure and the respiration of the experimental animals.

In view of the fact that GF-II is a compound of low toxicity exhibiting pronounced hypolipidemic activity, further investigations in the direction of experimental therapy may lead to the creation of an original drug normalizing lipid dysmetabolism.

EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at 40±5°C. PC was conducted on Filtrak FN-12 paper by the descending method with the following systems: 1) butanol—pyridine—water (6:4:3 by volume); spots revealed with acid phthalate; and 2) water-saturated phenol, lower layer; spots revealed with the Bonner reagent [7]. Their spectra were taken on a UR-20 instrument in tablets with KBr.

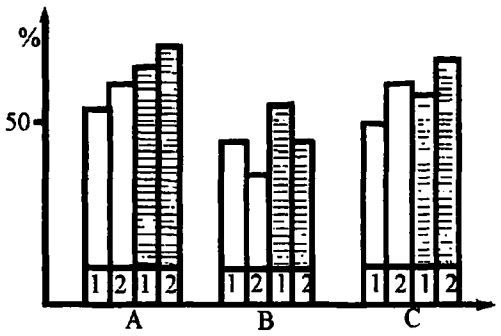

Fig. 1. Influence of glucofructan-II (▭) and polysponin (▤) on the levels of cholesterol (*1*) and triglycerides (*2*) in the blood serum (*A*), the aorta (*B*), and the myocardium (*C*) of rabbits with experimental atherosclerosis (% on the control). The differences from the control are statistically significant.

Isolation of the Glucofructan. The material of finely ground *A. suvorovii* bulbs that was retained a 0.1-mm sieve (100 g) was treated with chloroform in a ratio of 1:20 for 2 h. After elimination of the chloroform by filtration through a paper filter, the raw material was treated with 80% alcohol in a ratio of 1:20 at the boil for 1 h twice. The alcoholic extracts were eliminated by filtration through a paper filter and the raw material was subjected to extraction with water in a ratio of 1:30 at room temperature with constant stirring for three hours. After removal of the raw material by filtration through unbleached calico, the filtrate was evaporated to 1 liter in a rotary evaporator (40±5°C) and was freed from protein by Sevag's method [2]. Then the substances of the alliin type that impart the pungent odor and taste to onions were oxidized with hydrogen peroxide (technical grade, conc. 30%, 10 ml) with constant stirring at room temperature for 5 h. The extracts were decolorized by passage through type OU-V carbon (10 g) and silica gel. The filtrate was evaporated in a rotary evaporator to a viscous syrup and triturated with acetone to form a dry powder. The yield of the glucofructan amounted to 60% of the absolutely dry raw material.

Gel Chromatography. Samples (20 mg in 2 ml of water in each case) of the glucofructans, sucrose (V_e = 73.2 ml), raffinose (V_e= 70.1 ml), inulin (V_e = 57.5 ml), and dextrans D-10000 (V_e = 53.1 ml), D-20000 (V_e = 49.5 ml), and D-40000 (V_e = 43.0 ml) were deposited on a column (1.8 × 61 cm) of Sephadex G-75. The molecular masses were found to be: for GF-I, 40000—50000 (V_e = 43—32 ml); for GF-II, 15000 (V_e = 50.7 ml); and for GF-III, 1000—5000 (V_e = 71.6—56.5 ml).

Acid Hydrolysis. In each case, 0.1 g of material in 10 ml of 0.5% H_2SO_4 was heated in the boiling water bath for 4 h. The hydrolysates were neutralized with calcium carbonate, treated with KU-4 cation-exchanger (H^+), and concentrated in vacuum. The treated hydrolysates GF-I, GF-II, and GF-III were subjected to PC (systems 1 and 2; revealing agents 1 and 2), and fructose and glucose were detected.

Experimental endogenous hyperlipidemia was induced in rats by starving them for a day with unlimited drinking water [8] and the intraperitoneal injection of Triton WR-1339 in a dose of 225 mg/kg [9]. Experimental atherosclerosis was produced in rabbits by the oral administration of cholesterol (0.3 g/kg in cottonseed oil) for 120 days. During the last four weeks the animals were given GF-II in a dose of 100 mg/kg, in addition to the cholesterol. The atherosclerotic damage to the aorta in the experimental animals was evaluated by direct planimetry, and the corresponding index was calculated [10]. The levels of triglycerides [11], cholesterol [12], and β-lipoproteins [13] in the organs and the blood serum were determined.

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