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SYNTHESIS AND BIOLOGICAL ACTIVITY OF YUCCAGENIN BISRHAMNOSIDE

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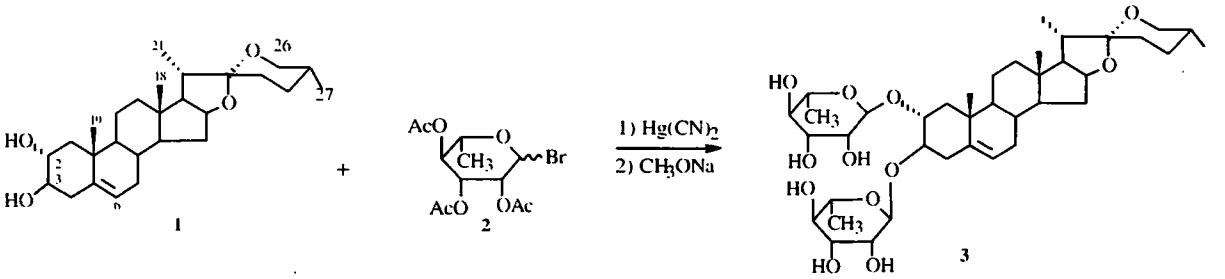
The two hydroxyls of yuccagenin can be glycosylated by Koenigs—Knorr condensation with acetobromorhamnose in dichloroethane in the presence of mercuric cyanide. The bisrhamnoside of yuccagenin markedly lowers the cholesterol and triglyceride content in blood serum of healthy animals and animals with experimental hyperlipidemia.

Key words: synthesis, 2,3-di-O-α-L-rhamnopyranoside, 25(S)-spirost-5-en-2α,3β-diol.

Spirostan steroidal genins and glycosides exhibit various functional activity: antitumor, fungicidal, antimicrobial, and antiviral in addition to membranotropic and antioxidant activities [1]. The present article reports results from a study of synthesis conditions and biological activity of the bisrhamnoside of yuccagenin, a spirostan steroidal genin. Our research demonstrates that the two hydroxyls of yuccagenin can be glycosylated.

Yuccagenin **1** and acetobromorhamnose **2** were condensed by the Koenigs—Knorr method [2] in dichloroethane in the presence of mercuric cyanide [3]. Treatment of the reaction mixture with sodium methoxide in absolute methanol and subsequent chromatography on a silica-gel column gave glycoside **3** in 45% yield.



The structure of the product was established using IR and PMR spectroscopies. The IR spectrum contains absorption bands at 820, 840, 870, 905-925, and 985 cm^{-1}, characteristic of the spiroketal chain, in addition to an absorption band for the hydroxyls at 3550-3520 cm^{-1}. The PMR spectrum of **3** exhibits signals assigned to the aglycone: 3H doublets at 0.58 and 1.01 ppm for the methyls on C-27 and C-21, respectively, and 3H singlets at 0.70 and 0.82 ppm for the C-18 and C-19 methyls. The appearance of the signals for the rhamnose methyls as doublets at 1.49 and 1.53 ppm and the signal of their anomeric protons at 3.47 ppm as a 2H broad singlet indicate that glycosylation occurs at the C-2 and C-3 hydroxyls of yuccagenin. Therefore, glycoside **3** is the 2,3-di-O-α-L-rhamnopyranoside of 25(S)-spirost-5-en-2α,3β-diol.

Considering the beneficial effect of spirostan saponins on impaired lipid exchange [4], we studied the effect of yuccagenin bisrhamnoside on the level of cholsterol and triglycerides in blood serum of healthy animals and animals with experimental hyperlipidemia. A single administration of the studied preparations in doses of 10, 25, 50, and 75 mg/kg to healthy animals decreased the cholesterol and triglyceride levels.

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TABLE 1. Effect of Yuccagenin and Polysponin Bisrhamnosides on Development of Hyperlipidemia (HP) Caused by Injecting Rats with Triton WR-1339 (M ± m, n = 6-8)

Experimental conditions	Cholesterol		P	Triglycerides		P
	mmol/l	% of control		mmol/l	% of control	
Helathy animals	1.97±0.067	-	-	0.683±0.05	-	-
Control (Triton HP)	6.82±0.28	-	-	4.25±0.19	-	-
HP + yuccagenin bisrhamnoside	4.94±0.27	-27.6	<0.01	3.28±0.24	-22.8	<0.01
HP + polysponin	5.56±0.17	-18.5	<0.01	3.76±0.28	-11.5	>0.05

Administration of yuccagenin bisrhamnoside at a dose of 10 mg/kg (4.5-1.7%) gave the greatest lowering of the indicators although it was statistically insignificant. The greatest lowering of the cholesterol and triglyceride levels was observed for administration of the preparation at a dose of 50 mg/kg. The lowering of the cholesterol level in blood serum by 20.2% and the tryglyceride level by 14.5% was statistically significant ($P < 0.05$). Administration under analogous conditions of the known hypolipidemic agent polysponin at a therapeutic dose lowered the content of cholesterol by 13.5% and of triglycerides by 4.7%.

Therapeutic administration of yuccagenin bisrhamnonside and polysponin to rats with Triton-induced hyperlipidemia prevented the acute disruption of lipid exchange. Whereas in the control group the cholesterol level increased by almost 3.5 times and the triglyceride level by 6.2 times, administration of yuccagenin bisrhamnoside lowered the cholesterol content by 27.6%; triglycerides, by 22.8% (Table 1).

The standard polysponin lowered the cholesterol (18.5%) and triglycerides (11.5%) less.

Thus, the results indicate that yuccagenin bisrhamnoside markedly lowers the cholesterol and triglyceride levels in blood serum of healthy animals and animals with experimental hyperlipidemia. Yuccagenin bisrhamnoside in experiments with healthy animals and those with Triton-induced hyperlipidemia has a greater therapeutic effect than an analogous type of agent, polysponin, for inducing hypocholesteremia and hypotriglyceridemia.

EXPERIMENTAL

KSK silica gel containing 5% gypsum was used for TLC. Crystalline 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl bromide was obtained by the literature method [5]. Dichloroethane was distilled twice over $CaCO_3$ and twice over $CaCl_2$ before use. Mercuric cyanide was recrystallized from alcohol and dried under vacuum. Molecular sieve (4 Å) was heated for 3 h at 400 C before use. IR spectra were recorded on a UR-20 instrument; PMR spectra, on a BS-567A instrument (Tesla) (C_5D_5N, 100 MHz, HMDS, δ, ppm, 0 = TMS).

Synthesis of Yuccagenin 2,3-Di-O-α-L-rhamnopyranoside (3). A mixture of yuccagenin (**1**, 6.0 g), acetobromorhamnose (**2**, 2.0 g, 5.7 mmole), mercuric cyanide (32 g), and molecular sieve (4 Å, 17 g) in anhydrous dichloroethane (150 ml) was stirred for 6 h at room temperature with exclusion of atmospheric moisture by a stream of nitrogen. The course of the reaction was monitored by TLC using $CHCl_3$—CH_3OH (100:1).

The reaction mixture was filtered. The filtrate was diluted with $CHCl_3$ (200 ml); washed with aqueous KI (20%, 2×20 ml), $NaHCO_3$ solution, and water; dried over anhydrous Na_2SO_4, and evaporated under vacuum. The dry solid was dissolved in absolute methanol (100 ml) and treated with sodium methoxide (20 ml, 0.1 N) in CH_3OH. A few drops of acetic acid were added after 1 h. The mixture was evaporated to dryness. The solid was chromatographed on a grade L silica-gel (75 g) column and eluted with $CHCl_3$—CH_3OH—H_2O (85:14:1).

Yield, 4.5 g of crystalline product (45%), $C_{39}H_{62}O_{12}$, mp 205-207 C (alcohol), $[\alpha]_D^{22}$ = -113.2 ± 2 (c = 0.98, pyridine). IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3550-3250 (OH), 820, 840, 870, 905-925, 985 (spiroketal chain). PMR spectrum (C_5D_5N, δ, ppm): 0.58 (3H, d, CH_3-27), 0.70 (3H, s, CH_3-18), 0.82 (3H, s, CH_3-19), 1.01 (3H, d, CH_3-21), 1.49 (3H, d, CH_3 rhamnose), 1.53 (3H, d, CH_3 rhamnose), 3.47 (2H, br. s, H-1′ and H-1″ rhamnose), 3.42 (2H, m, H-26), 5.18 (1H, m, H-6).

Experimental hyperlipidemia in rats was induced by intraperitoneal injection of Triton WR-1339 according to the literature [6]. The total cholesterol and triglyceride contents were determined by the literature method [6].

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