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SYNTHESIS OF GLYCOPEPTIDE DERIVATIVES OF GLYCYRRHIZINIC ACID AND THEIR IMMUNOMODULATORY PROPERTIES

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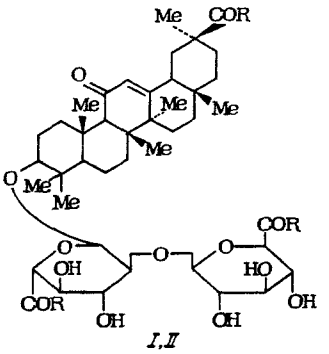
The anti-inflammatory activity, low toxicity and absence of serious side effects provide a rationale for examining new synthetic derivatives of glycyrrhizinic acid as medicinal compounds [1, 3-5]. Among glucosidic derivatives there is special interest in a new class – the triterpene glycopeptides, which are modelled on the amide type of bond present in natural glycoproteins [1].



I, II

I: R=OH (I); L-Met(OMe) (IIa); L-Leu(OMe) (IIb); L-Ala(OMe) (IIc); D-Glu(OMe)$_2$ (IId); L-Ile (Otert-Bu) (IIe); β-Ala-Gly(OMe) (IIf); Gly-L-Leu(OMe) (IIg); Gly-L-Asp(OMe)$_2$ (IIh).

In developmental work on the synthesis of glycopeptide derivatives of glycyrrhizinic acid we have obtained the new derivatives (IIa-h) and studied their immunomodulatory properties.

The syntheses of the glycopeptides IIa-g were carried out by condensing glycoside I without prior protection with the hydrochlorides of the methyl and tert-butyl esters of optically pure aminoacids and dipeptides using the method of activated (N-hydroxysuccinimide) esters in the presence of N,N'-dicyclohexylcarbodiimide (DCC) and triethylamine (TEA) in tetrahydrofuran (THF) and dioxane. The yields of the target glycopeptides II were in the range of 49-86%.

EXPERIMENTAL (CHEMISTRY)

To obtain analytically pure samples the products were crystallized from methanol/ether or acetone/hexane. All compounds II obtained were homogeneous according to TLC data, and were characterized by IR and UV analysis as well as by elemental analysis. In the IR spectra of glycopeptides II absorption bands for OH and NH groups were observed in the 3600-3200 cm^{-1} region, the mixed ester groups (COOMe, COO-tert-butyl) at 1750-1720 cm^{-1}, the conjugated carbonyl (-C(11)=O) at 1650-1630 cm^{-1}, and amide group at 1560-1520 cm^{-1} (amide II) and

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TABLE 1

Compound	Yield, % (experimental condition)	mp, °C	$[\alpha]_D^{20}$	Rf Silufol	Elemental composition
IIa	78 THF	Amorphous	+29° (c 0,27)	0,54 [b]	$C_{60}H_{95}N_3O_{19}S_3$
IIb	75 (THF)	162—165	+30° (c 0,15)	0,30 [a]	$C_{63}H_{101}N_3O_{19}$
IIc	52 (THF)	148—151	+29° (c 0,06)	0,72 [c]	$C_{54}H_{83}N_3O_{19}$
IId	49 dioxane	119—115	+50° (c 0,02)	0,76 [b]	$C_{63}H_{95}N_3O_{25}$
IIe	71 dioxane	88—90	+33° (c 0,09)	0,36 [a]	$C_{72}H_{119}N_3O_{19}$
IIf	46 dioxane	139—142	+54°	0,64 [c]	$C_{60}H_{92}N_6O_{22}$
IIg	86 (THF)	160—163	+32° (c 0,1)	0,56 [b]	$C_{69}H_{110}N_6O_{22}$
IIh	43 dioxane	Amorphous	+26° (c 0,035)	0,50 [b]	$C_{73}H_{92}N_9O_{25}$

*a Chloroform—alcohol 10:1, b) Chloroform—alcohol 5:1, c) Chloroform—alcohol 4:1.

TABLE 2. The Effect of Glycopeptides of Glycyrrhizinic Acid on the Production of Antibody-Producing Cells (APC) in Noninbred White Mice

Compound	Dose mg/kg	Number of animals	Number of APC per 10^6 splenocytes
IIa *	2	7	2086±249
IIb *	2	8	2022±576
IIc *	2	7	2999±913
IId	2	5	507±736
IIe	2	5	280±27
IIf *	2	6	3436±1025
IIh *	2	6	2049±376
Control	Physiological solution	7	1290±351

*Compounds IIa-c, IIf, IIh showed a stimulatory effect on antibody genesis in comparison with the control. Glycopeptides IId and IIe reduced the initial immune response.

1260-1220 cm^{-1} (amide III). In the UV spectra of all glycopeptides an intense absorption maximum at 249-251 nm (log ε = 3.90-4.10) was observed, characteristic for 18-β-glycyrrhizinic acid 12-ene-11-one system [1]. Physicochemical constants for the synetic compounds are presented in Table 1.

For TLC, Silufol plates (Czech SSR) were used in solvent systems (a) chloroform—alcohol 10:1, (b) 5:1, and (c) 4:1. For visualization, phosphotungstic acid (20% in ethanol) was used, followed by heating to 110-120°C (5 min). Column chromatography was conducted with silica gel L (40/110 Czech SSR).

IR spectra were recorded on an IR-20 spectrophotometer as mulls in Vaseline oil. Electronic spectral absorptions were measured on a Specord M-40 in methanol. The specific rotations were determined on a Perkin-Elmer 141M polarimeter with a 1 dm path length. DCC from the firm "Ferak" (West Berlin) was used in the work.

General Method for Obtaining Glycopeptides IIa-h: To a solution of 2 mmoles glycyrrhinic acid of mp 209-211 °C (lit. 210 °C [10]) in 50 ml dry THF or dioxane kept at 0-5 °C was added 10-10.4 mmoles HOSu (N-hydroxysuccinimide) plus 6-6.4 mmoles DCC, and the mixture

TABLE 3. The Effect of Glycopeptides of Glycyrrhizinic Acid on HDT with Sheep Erythrocytes in Noninbred Mice (in doses of 10 μg/kg)

Compound	No. of animals	Increase in paw weight %
Control (physiological solution)	8	16,3±1,5
IIa	6	20,8±4,3
IIb	5	18,2±4,4
IIc	7	19,1±1,8
IId	6	25,0±4,0
IIe	6	30,1±4,5
IIf	5	34,8±1,7
IIg	6	15,5±3,2
IIh	8	20,5±1,5
MDP	5	20,8±8,5

TABLE 4. Effects of Glycopeptides of Glycyrrhizinic Acid on HDT with Bovine Serum Albumin (BSA) in Noninbred Mice (in doses of 2 mg/kg)

Compound	No. of animals	Increase in paw weight %
Control (physiological solution)	7	17,4±1,8
IIa	5	19,7±3,0
IIb	6	23,0±3,9
IIc	5	24,4±1,9
IId	7	20,4±1,5
IIe	6	25,0±3,0
IIf	6	33,1±1,2
IIg	6	28,0±5,0
IIh	5	14,4±1,4

maintained at this temperature for 3 h, followed by 6 h at room temperature, then the mixture was stored overnight in a refrigerator. The precipitated dicyclohexylurea was filtered off, and the cold solution was stirred as 6-7 mmoles of the corresponding hydrochloride ester of the amino acid or dipeptide, plus 9.8-10.8 mmoles of distilled triethylamine (TEA) were added. The mixture was stirred in the cold for 1 h, then at room temperature for 24 h. The solvent was removed in a vacuum, the residue dissolved in methylene chloride (200 ml) and the solution washed with a 5% solution of hydrochloric acid, then water, then 5% $NaHCO_3$, then the organic phase was dried with $MgSO_4$. After removal of the solvent at a temperature of less than 50 °C, the target compounds II were obtained. These were shown to be homogeneous by TLC. To obtain analytically pure samples the products were recrystallized from acetone/hexane or methanol/ether (see Table 1).

EXPERIMENTAL (PHARMACOLOGY)

The immunomodulatory properties of glycopeptides II were studied using the models of humoral immune response according to the method of Jerne and Nordin with the modification of Cunningham [6], and the cellular immune response.

The primary immune response was judged by the number of antibody producing cells (APC) in the spleens of mice immunized with sheep erythrocytes. The influence of the test compounds on cellular immunity was determined using the model of hypersensitivity of the delayed type (HDT) with sheep erythrocytes and bovine serum albumin (BSA) as test agents.

The set of preparations included for comparison a known immunomodulator (a fragment of bacterial peptidoglycans) N-acetyl-muramoyl-L-alanyl-D-isoglutamine (MDP)* [8, 9, 11], the

*MDP was kindly provided by Candidate in Chemical Sciences T. M. Andronov (Institute of Biological Chemistry M. M. Shemyakin, Moscow).

clinical use of which is restricted due to its pyrogenicity, high toxicity and other side effects [2, 7]. The compounds under study were administered to the animals intraperitoneally in a single dose a day after immunization.

The effects of glycopeptides II on the primary immune response was studied using white mice of undefined lineage with mass 18-20 g. The animals were immunized intraperitoneally with a 5% suspension of sheep erythrocytes in 0.5 ml volume. After a day, the test compounds were administered intraperintoneally in doses of 2 mg/kg. On the fifth day the number of antibody producing cells (APC) in the spleen was determined (see Table 2).

The action of glycopeptides (II) on cellular immunity was studied using the model HDT on white mice of unknown lineage sensitized with a suspension of sheep erythrocytes ($5 \cdot 10^4$ intraperitoneally). The test compounds were administered a day after immunization in doses of 10 μg/kg. Resolving doses of sheep erythrocytes were administered sub-plantarally (to the sole of the paw) in 0.05 ml solution 6 days after administration of the test agents. On the seventh day the increase in the mass of paw was measured (see Table 3). MDP was administered for comparison in the same dose.

HDT tests with bovine serum albumin in complete Freund's adjuvant (BSA in CFA) were conducted similarly with white mice of undefined lineage according to the following scheme: on the first day the antigen BSA was administered sub-plantarally as an oil-water emulsion, on the eighth day a 2% suspension of BSA was administered to the base of the tail. On the following day the test compounds were administered intraperitoneally in doses of 2 mg/kg. On the 9th day post-immunization the increase in paw weight was measured (see Table 4).

The results are presented in Tables 2-4.

In the reproduction of the HDT test with sheep erythrocytes (see Table 3) the greatest adjuvant activity at doses of 10 μg/kg was shown by compound IIe and IIf, containing fragments L-Ile (O-tert-butyl) and β-Ala-Gly(OMe)respectively. The stimulatory effect of these compounds on the cellular reaction was expressed more than for MDP administered in a single dose. The immunotropic activity of the other compounds in the HDT reaction with sheep erythrocytes was weakly expressed or did not register at all.

In the HDT test with BSA with complete Freund's adjuvant, glycopeptides IIc, IIe, IIf and IIg stimulated the cellular response in comparison with the control. The remaining compounds did not elicit a statistically significant response in increasing the swelling of animals' paws as an indicator of cellular immune response in the given model.

The acute toxicities of the glycopeptides IIa and IIc were determined by intraperitoneal administration. The LD_{50} for compound IIa was 934 ± 61.3 mg/kg, and for IIc was 950 ± 50 mg/kg.

In correspondence with the regulations of the Governmental Committee standards of the Soviet Minister No. 579 (Mar. 10, 1976), the given compound data relates them to Class 3 of slightly dangerous compounds.

Thus, the new synthetic derivatives of glycyrrhizinic acid of the glycopeptide type are of interest for practical medicine as immunomodulators. Among the compounds obtained are those stimulating the primary immune response (APC) in comparison with the control as well as showing effects on cellular immunity (HDT) greater than MDP. In contrast to earlier derivatives of glycyrrhizinic acid these are less toxic [1-5].

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF POLYMERIC DERIVATIVES OF GAMMA-AMINO-BUTYRIC ACID

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In the combined treatment of malignant neoplasms, there has in recent years been much interest in the use of drugs which reduce immunodepression and effectively increase the immune response of the body to tumors [1, 2]. The search for new drugs with high immune stimulatory activity has involved both naturally occurring and synthetic compounds. It has, for instance, been shown [4, 7] that some polymers, particularly polyelectrolytes, display such adjuvant properties when administered in conjuction with antigens.

The aim of the present investigation was to obtain non-toxic, water-soluble polyelectrolytes, and to examine their immune-modifying and antitumor properties.

The polymers used to obtain new polyelectrolytes were copolymers of N-vinylpyrrolidone (I) with maleic anhydride (II) and 2-chloroethyl vinyl ether (III). Copolymerization of (I) and (II) was carried out in a solvent in the presence of azobisisobutyronitrile (ABN) by the method of Nikolaev et al. [6]. Monomers (I) and (III) were block copolymerized in sealed ampuls in the presence of ABN at 60°C. Analysis showed the copolymers obtained had the structures (IV) and (V). γ-Aminobutyric acid (VI) was introduced into each of these copolymers by polymer-analogous reactions.

In this way, there were obtained the maleic acid N-vinylpyrrolidone-mono-N'-(3-carboxy)-propylamide copolymer (VII), which is an anionic polyelectrolyte, and the ethylene glycol N-vinylpyrrolidone-monovinyl ether copolymer esterified with γ-aminobutyric acid (VIII), a cationic polyelectrolyte.

To confirm the ionogenic character of these copolymers, the relationship of the viscosity of aqueous solutions of the compounds to the pH of the solutions was examined. In the case of (VII), plots of viscosity against pH showed a single maximum in the alkaline region (pH 11.2), indicating that this polyelectrolyte is typically anionic. That (VIII) is cationic is shown by the presence in the plot of the viscosity of its aqueous solution against pH of a maximum in the strongly acidic region (pH 1.8), due to protonation of the amino-group.

It was of interest to carry out a joint experimental examination of the antitumor and immunostimulant properties of these polyelectrolytes.

Antitumor activity was assessed in mice with intramuscularly grafted Lewis pulmonary carcinoma and melanoma B-16, metastasizing to the lungs. The tests showed that neither (VII) nor (VIII) had any appreciable effect on the growth of the Lewis pulmonary sarcoma, although (VII) had some antimetastatic activity, reducing the number of metastases by 20.5% ($p > 0.05$) as compared with the controls. Greater antiblastic activity was shown by (VII) against melanoma B-16, primary tumor growth being reduced by 24.4% ($p < 0.05$) and the number of metastases by 88.3% ($p < 0.05$), although (VIII) was not as active, only showing a tendency to inhibit the growth of the primary tumor and reduce the number of metastases as compared with the control (Fig. 1).

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