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EFFECTS OF 1-[β-ALKYLSULFONYLETHYL]SILATRANES ON THE REPAIR PROCESSES OF CONNECTIVE TISSUE

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Many 1-substituted silatranes – $RSi(OCH_2CH_2)_3N$ – possess high specific physiological activity [1, 2, 16, 17]. They are stimulators of protein and nuclei acid biosynthesis in the regeneration of connective tissue [6, 9], pilotropic agents [4], stabilizers of erythrocyte membranes [6], inhibitors of platelet aggregation [7] and lipid peroxidation [11, 14], etc. Among the 1-substituted silatranes, 1-(organylthioalkyl)silatranes of the type of $RS(CH_2)_nSi(OCH_2CH_2)_3N$ and their derivatives containing a tricoordinated sulfur atom are of definite interest. Earlier we showed that 1-(propylthiomethyl)silatrane (R = Pr, n = 1) possesses pilotropic activity [4], while sulfonium salts of the type of $RR'S^+CH_2Si(OCH_2CH_2)_3N\ I^-$ have a pronounced antiulcerogenic activity [7, 8].

Developing these investigations, we synthesized 1-(β-alkylsulfinylethyl)silatranes $RSOCH_2CH_2Si(OCH_2CH_2)_3N$ with R = Me(I) and R = Et(II) and investigated their influence on the proliferative-repair reaction of connective tissue.

Compounds I and II were produced with yields up to 90% by oxidation of the corresponding 1-(β-alkylthioethyl)silatranes $RSCH_2CH_2Si(OCH_2CH_2)_3N$ with 30% H_2O_2. The reaction is carried out in $CHCl_3$ medium (i.e., in a two-phase system) at 0-10°C. The silatranes I and II are white fine crysetalline powders with the specific odor of sulfur compounds. They are readily soluble in water (whereupon they slowly hydrolyze), lower alcohols, and polar organic solvents. Their structure has been demonstrated by methods of PMR and IR spectroscopy. Compounds I and II are virtually nontoxic (LD_{50} for noninbred white mice in the case of intraperitoneal injection is more than 3000 mg/kg).

EXPERIMENTAL CHEMICAL

The PMR spectra (10-15% solutions in $CDCl_3$ were investigated, internal standard TMS) were obtained on a Tesla BS-487C spectrometer (80 MHz, Czechoslovakia). The IR spectra were recordeed on a UR-20 spectrophotometer (German Democratic Republic) in tablets with KBr.

The initial 1-(β-methylthioethyl)silatrane was produced according to the procedure of [3] by transesterification of (β-methylthioethyl)trimethoxysilane with triethanolamine. Yield 95%. Colorless crystals with mp 125-126°C form $CHCl_3$). Found %: C 43.21; H 7.54; S 12.56; Si 11.05. $C_9H_{19}O_3NSSi$. Calculated %: C 43.35; H 7.68; S 12.83; Si 11.26. PMR spectrum, δ, ppm: 2.07 (s, CH_3), 2.64 (m, SCH_2), 0.76 (m, CH_2Si), 3.76 (t, OCH_2), 2.81 (t, CH_2N).

1-(β-Methylsulfinylethyl)silatrane (I). To a solution of 2.5 g 1-(o-methylthioethyl)-silatrans in 5 ml of $CHCl_3$, cooled to 0°C, 1.3 g of 30% H_2O_2 was added with mixing and cooling, maintaining the temperature of the reaction mixture around 0°C. Then the reaction mixture was mixed for another 10 min at 10°C and for 10 min at 20-25°C. After removal of the amorphous precipitate of polymeric hydrolysis products, the two-layered filtrate was separated and the aqueous layer extracted with three 2-ml portions of $CHCl_3$. The combined

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chloroform solutions were dried over freshly calcined $MgSO_4$ and evaporated under vacuum to a volume of 3 ml. The silatrane I formed was precipitated with absolute ether, suction filtered, and washed twice with the same solvent. Yield 2.4 g (~90%), mp 155-158°C. Found %: C 41.20; H 7.43; S 11.50; Si 0.12. $C_9H_{19}O_4NSSi$. Calculated %: C 40.75; H 7.21; S 12.06; Si 10.58. IR spectrum, ν_{max}, cm^{-1}: 1042 (S=O). PMR spectrum, δ, ppm: 2.48 (s, CH_3), 0.62 (m, CH_2Si), 3.77 (t, OCH_2), 2.84 (t, CH_2N).

1-(β-Ethylsulfinylethyl)silatrane (II) was synthesized analogously to I. Yield 90%; mp 139-141°C. Found %: C 42.20; H 7.30; S 11.54; Si 9.89. $C_{10}H_{21}O_4NSSi$. Calculated %: C 43.01; H 7.59; S 11.45; Si 10.05. IR spectrum, ν_{max}, cm^{-1}: 1045 (S=O). PMR spectrum, δ, ppm: 1.30 (s, CH_3–C), 0.66 (m, CH_2Si), 3.77 (t, OCH_2), 2.84 (t, CH_2N) (the protons of the CH_2SOCH_2 fragment form an extremely complex overlapping multiplet).

EXPERIMENTAL BIOLOGICAL

Methods of Investigation. The influence of silatranes I and II on the wound process in the case of local application, including an assessment of the effect on the period of healing and a quantitative biochemical characterization of the granulation-fibrous tissue (GFT) developing in the wound defect, was studied on 112 laboratory white rats weighing about 200 g. To obtain a flat healing wound, a square piece of skin was excised with the subcutaneous tissue, on an area of 400 mm^2. To standardize the shape and size of the wound and prevent its rapid contraction, a metallic frame with internal area equal to the original area of the wound was sutured to the edges of the wound defect. After the operation the animals were kept in individual cages. On the fifth day after the operation, the frame was removed, after which contraction and epithelization of the wound occurred unhindered. Dropoff of the scab with exposure of the epidermized surface served as the criterion of wound healing.

To obtain GFT we sued an experimental model – an open skin defect. The GFT was investigated after seven days by a system of quantitative biochemical analysis [12]. Hydroxylysine, which is a more specific amino acid for collagen than hydroxyproline, was determined according to the method of [13]. The results of the biochemical analyses were calculated as concentrations in percent (in grams per 100 g of dry defatted tissue) and as the absolute content of the components to be determined (in milligrams) in the entire weight of newly formed GFT. The method of [5, 15] was used for the statistical treatment of the results. Silatranes I and II were applied in the form of 0.5% and 5% liniments based on a mixture of lanolein and castor oil (LC) in a 1:2 ratio. The experiment was accompanied by two controls: in the first the wound defect was not subjected to any influences ("pure" control); in the other a liniment base – placebo (LC) was applied on the surface. Silatranes I and II were compared with the recommended pharmacopoiea agent for local treatment of wounds and burns – acemin, which was used in the prepared drug form (ointment).

DISCUSSION OF THE RESULTS

The acemin ointment used for comparison shortened the period of healing of the experimental wounds by approximately 10% (19.5 ± 0.5 and 20.8 ± 0.3 days). This result corresponds to the indices of the biochemical investigation of the GFT (Tables 1 and 2). An increase in the RNA/DNA coefficient, an increase in the concentration and absolute accumulation of collagen, and a small increase in the concentrations of glycoconjugates – proteoglycans and dialoglycoproteins – were observed.

The liniment basis (LC) itself had no stimulating effect on the development of the GFT. It induces (in the placebo series) an increase in the content of nucleic acids and specific components of collagen – hydroxyproline and hydroxlysine. Under the influence of LC, the healing period was shortened to practically the same degree as under the influence of the acemin ointment (19.2 ± 0.1 and 19.0 ± 0.5 days).

Silatrane I in an LC base significantly increases the concentration and absolute content of lipids in the tissue. This increase is especially pronounced when the 5% liniment is used (180% relative to the placebo).

The same liniment intensifies the accumulation of collagen to a substantial degree and increases the RNA/DNA coefficient. Such biochemical changes have a favorable effect on the morphogenetic potentials of the GFT, and healing of the experimental wounds was 18%

TABLE 1. Influence of 1-(β-Methylsulfinylethyl)silatrane (I) and 1-(β-Ethylsulfinylethyl)silatrane (II) (0.5% and 5% liniments based on LC) on Individual Values of the Biochemical Indices of the GFT (M ± m)

Indices of GFT	Liniment I		Liniment II		Control	Placebo	Acemin (ointment)
	0.5 %	5 %	0.5 %	5 %			
Weight of tissue, g	1.17±0.38	1.18±0.32	1.18±0.42	0.90±0.22	1.15±0.25	1.06±0.22	0.99±0.23
Lipids*	5.20±0.25	6.30±0.35	3.60±0.20	3.60±0.30	3.70±0.20	3.50±0.10	—
DNA	4.60±0.02	3.40±0.22	4.05±0.01	4.20±0.18	3.20±0.04	3.76±0.01	3.12±0.06
RNA	2.96±0.10	2.83±0.05	2.60±0.10	2.43±0.01	2.33±0.04	2.66±0.15	2.58±0.11
Hydroxyproline	2.14±0.05	2.59±0.13	1.98±0.04	2.08±0.13	1.69±0.11	2.09±0.14	2.25±0.06
Hydroxylysine	0.40±0.01	0.41±0.02	0.32±0.02	0.36±0.01	0.30±0.01	0.36±0.02	0.32±0.02
Tyrosine	2.82±0.09	2.92±0.16	3.14±0.35	2.85±0.04	3.21±0.02	2.92±0.08	2.34±0.15
Arginine	4.26±0.52	4.66±0.52	4.35±0.69	4.12±0.10	4.65±0.48	4.24±0.62	4.36±0.19
Hexosamines	0.74±0.03	0.70±0.03	1.00±0.13	0.85±0.02	0.86±0.03	0.75±0.03	1.00±0.11
Hexuronic acids	0.53±0.01	0.70±0.03	0.71±0.12	0.67±0.14	0.62±0.05	0.62±0.01	0.73±0.11
Hexoses	1.92±0.14	1.85±0.38	2.00±0.40	1.85±0.06	2.33±0.10	2.63±0.20	1.97±0.33
Sialic acids	0.66±0.02	0.62±0.04	0.75±0.08	0.73±0.01	0.73±0.01	0.65±0.04	0.99±0.03

*In grams per 100 g of fresh tissue; indices cited below in grams per 100 g of dry defatted tissue.

TABLE 2. Influence of Compounds I and II (0.5% and 5% liniments based on LC) on the Absolute Values (in mg) of the Biological Indices of the GFT

Index of GFT	Liniment I		Liniment II		Control	Placebo	Acemin (ointment)
	0.5 %	5 %	0.5 %	5 %			
Weight of tissue	246	248	241	180	230	181	208
Lipids	60,8	75,3	41,4	32,4	42,55	36,75	—0
DNA	11,4	8,43	9,76	7,56	7,36	6,77	6,57
RNA	7,28	7,02	6,27	4,37	5,36	4,79	5,38
Hydroxyproline	5,26	6,42	4,71	3,74	3,89	3,76	4,67
Hydroxylysine	0,98	1,02	0,77	0,65	0,68	0,65	0,67
Tyrosine	6,94	7,24	7,57	5,13	7,38	5,26	4,8
Hexosamines	1,82	1,81	2,41	1,53	1,97	1,35	2,08
Hexuronic acid	1,30	1,74	1,71	1,21	1,43	1,12	1,52
Hexoses	4,72	4,59	4,82	3,33	5,36	4,73	4,10
Sialic acids	1,63	1,54	1,81	1,32	1,68	1,17	2,06

accelerated in comparison with LC and 28% in comparison with the control (15.6 ± 0.2 days). Thus, 5% silatrane I liniment significantly surpasses acemin ointment in its influence on the biochemical indices of the GFT and in wound-healing activity. The wound-healing activity of a 0.5% liniment of I approximately corresponds to the activity of acemin ointment (19.0 ± 0.3 days).

Despite the great chemical similarity of silatranes I and II, the latter in an LC-liniment base is less effective. When it is used the biochemical indices of the GFT differ little from the placebo series, and there is also no wound-healing effect (20.2 ± 0.2 days).

The data obtained serve as a new confirmation of the fact that the biolgoical activity of silatranes is determined not only by the presence of the silatrane group but also by the structure of the substituent at the silicon atom. This is evidenced by the difference in the wound-healing effect of silatranes I and II.

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HYPOLIPIDEMIC PROPERTIES OF BETULIN GLYCOSIDES

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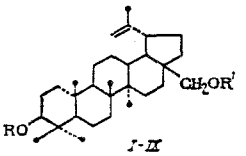
In recent years interest in triterpene compounds, which possess varied biological activity and are widespread in the plant world, has increased. Reports have appeared about hypocholesterolemic and antiatherosclerotic effects of certain triterpene saponins and triterpenic acids [1, 3, 5].

Betulin (I) is a pentacyclic triterpenoid of the lupane series, one of the major components of the bark of various species of the birch genus [14, 17, 20, 23]. The availability [2, 17, 22] and biological activity [6, 15] of betulin make it a valuable natural source for use both in the native state and in the form of various conversion products [7-9, 12, 13].

Earlier, at the laboratory of organic synthesis of the Pacific Ocean Institute of Bioorganic Chemistry of the Far Eastern Science Center, Academy of Sciences of the USSR, a number of glycosides were synthesized on the basis of betulin, its derivatives and other pentracyclic triterpenoids [4, 21, 22]. The low toxicity of betulin [16, 19] and the absence of any data in the literature on hypocholesterolemic properties of its glycosides, prompted us to invesetigate the indicated compounds.



I-III

R = H (I, II, V, VIII), Ac (III, Va), Glcp (IV, VI), Glcp $(Ac)_4$ (IVa), α-Glcp (1→4)-α-Glcp (VII), IX); R^1 = H (I, III, IV, VII), Ac (II, IVa), Glcp (V, VI), Glcp $(Ac)_4$ (Va), α-Glcp (1 → 4)-α-Glcp (VIII, IX).

We selected six betulin glycosides for a study of the biological activity: the glucoside at C-3 (IV), the glucoside at C-28 (V), the bis-glucoside (VI), the maltoside at C-3 (VII), the maltoside at C-28 (VIII), and the bis-maltoside (IX) [21].

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