$C_{35}H_{54}O_{15}$, mp 196-200°C $[\alpha]_D^{20}$ − 3.9° (c 0.5; methanol). From its R_f values in various solvent systems and a mixed melting point, the reduced substance was identical with the glycoside (I) that had been isolated.

SUMMARY

A new cardenolidic bioside has been isolated from the seeds of Coronilla scorpioides; it has been called coronillobiosidol and is 3β-O-[O-β-D-glucopyranosyl)-(1 → 4)-β-D-glucopyranosyl]-14β, 19-dihydroxy-5α-card-20(22)-enolide.

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IMMOBILIZATION OF Oospora lactis LIPASE

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A number of new sorbents for the immobilization of lipases have been synthesized from Silokhrom and Porokhrom, and also from microcrystalline cellulose. The conditions for the immobilization of lipases have been selected and some of their properties have been studied.

At the present time, immobilized lipases are being widely used in scientific investigations [1, 2] and various biotechnological processes [3, 4]. At the same time, the search for new approaches to immobilization and the selection of a suitable support continues to remain an urgent one.

The aim of our investigations was to obtain an immobilized lipase with the aid of traditional approaches to the immobilization of enzymes and to evaluate its stability and some catalytic properties.

As the support, we used Silokhrom and Porokhrom, and the biospecific sorbent Liposorb-4 which we had synthesized previously [5].

To obtain a sorbent with a greater sorption capacity, partial optimization of the conditions of immobilization was carried out by the mathematical planning of experiment work. The factors varied were the concentrations of 3-aminopropyl-3-ethoxysilane and of 2,4-toluylene diisocyanate, and the temperature and time of the reaction; as a parameter for evaluating the sorbent we used the amount of adsorbed protein and the lipase activity determined before and after contact with the sorbent. Under the conditions of a batch reactor, the following optimum conditions for the modification of the sorbents were established: for the first sorbent — temperature 90 to 95°C; reaction time 5 h; ratio of 3-aminopropyl-3-ethoxysilane to Silokhrom 1:4; and for the second sorbent, respectively, 78-80°C, 6 h, and ratio of 2,4-toluylene diisocyanate to Porokhrom 1:5.

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TABLE 1. Influence of the Method of Immobilization on the Yield of Enzyme

Support	Amt. of immobilized protein, mg/g of Support	Degree of binding,%	
		as protein	as activity
Silokhrom	157,3	70,7	27,6
Porokhrom	95 7	43 0	11,8
Liposorb-4	175,6	78,9	43,5

TABLE 2. Stability of Immobilized Lipases on Storage

Time of storage, days	Lipase activity, % of initial		
	Silokhrom	Porokhrom	Liposorb
10	95	95	97
30	80	78	80
60	63	60	75
90	57	55	53
120	50	42	17
150	45	40	0
180	40	38	0

TABLE 3. Stability of Immobilized Lipase on Repeated (1-10 times) Use

Method of immobilization	Residual activity, %									
	1	2	3	4	5	6	7	8	9	10
Activated Silokhrom	100	61,3	52,3	44,7	36,5	23,1	12,1	7,6		
Porokhrom	100	45,3	32,6	20,1	5,7	0		0		
Liposorb-4	100	71,8	64,1	51,7	43,8	34,1	23,4	16,1	16,0	12,1

As follows from Table 1, the specific amount of enzyme per 1 g of support depended on the method of immobilization, and it amounted to 95.7 mg for the modified Silokhrom.

When the lipase preparations were stored at 5°C for six months, the activity of the enzyme changed differently according to the support used (Table 2).

The most stable preparations were those obtained with the use of the modified Silokhrom (which retained 40% of the initial activity) and with the modified Porokhrom (30% of the initial activity). The lipase immobilized on Liposorb lost its activity completely in aqueous suspension in the course of five months (Table 2). Nevertheless, in a study of the possibility of repeated use, an advantage of the lipase immobilized on Liposorb clearly appeared (Table 3).

In the majority of cases, the immobilization of the enzymes led to an increase in their stability and to a shift in the temperature optimum in the direction of higher temperatures as compared with the native enzymes. And, in fact, as a result of immobilization we succeeded in stabilizing the enzyme. Thus, for example, the native lipase lost 50% of its activity in 90 min at 40°C (Fig. 1, a), while the immobilized enzyme could work under these conditions with no change in its activity for 3 h (Fig. 1, b). It was impossible to change the optimum temperature of the action of the enzyme as a result of immobilization (the optimum temperature of the immobilized lipase was 37-39°C, just as for the native enzyme), although a broadening of the temperature optimum was achieved (Fig. 1, c). It was also established that the pH maximum of the activity both of the soluble and of the immobilized lipase was located at pH 7.2-7.5. As a result of immobilization, the pH optimum shifted in the acid direction by only 0.3-0.4 pH units (Fig. 1, d).

EXPERIMENTAL

The source of lipase was the fungus Oospora lactis Uz LM-2. The conditions for the growth of the fungus and for the isolation of the total lipase preparation were as described previously [6].

Lipase activity was determined by the method of Ota and Yamada [7]. As the unit of lipase activity we took that amount of enzyme that split out 1 μmole of fatty acid from a 40% emulsion of olive oil in a 2% solution of polyvinyl alcohol at 37°C in 1 h.

The Porokhrom and Silokhrom were activated with the aid of 3-aminopropyl-3-ethoxysilane or 2,4-toluene diisocyanate followed by treatment with fatty acid residues having alkyl radicals of different lengths.

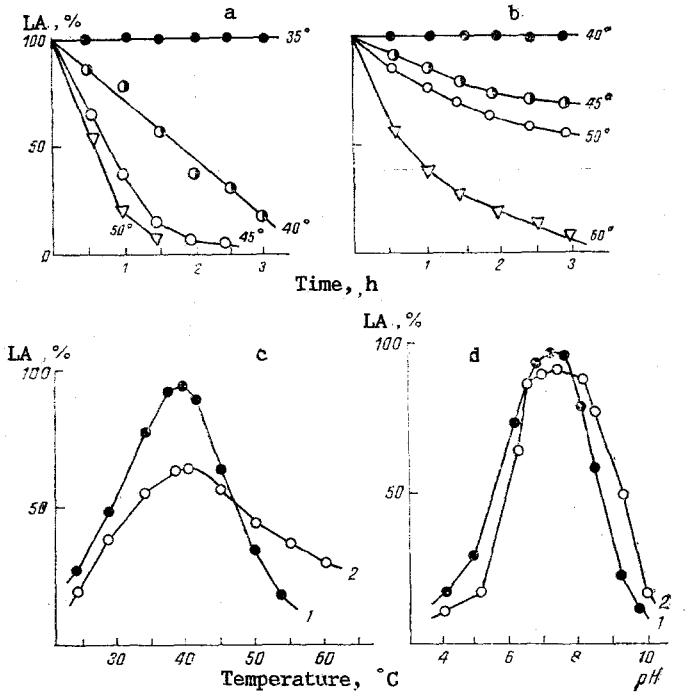

Fig. 1. Thermal stability of the native (a) and immobilized (b) lipase from Oospora lactis; temperature (c) and pH (d) dependences of lipase activity: 1) native; 2) immobilized lipase.

Preparation of the Immobilized Lipase. The support (5 g) was added to 1.0 g of a technical lipase preparation dissolved in 10 ml of 0.005 M phosphate buffer pH 7.4, containing 0.01 N $CaCl_2$. The mixture was incubated for 6 h in the case of activated Silokhrom and 5 h in the cases of Porokhrom and Liposorb at 22-25°C with constant stirring, and was then washed with the same buffer containing 1 M NaCl on a Büchner funnel until the washing solutions showed no absorption at 280 nm. The preparations of immobilized enzyme obtained were stored in the form of suspensions in the refrigerator at 5°C.

Determination of Lipase Activity on Storage. Samples of immobilized lipase were kept in the refrigerator at 5°C in distilled water for six months. The residual activity of the enzyme was determined every ten days. Microbiological contamination was prevented by the use of 1-2 drops of toluene.

Determination of the Thermal Stability of the Immobilized Lipase. The immobilized lipase in 0.01 M phosphate buffer, pH 7.5, was thermostated at 35-60°C, and the residual lipase activity was determined after definite intervals of time. The apparent inactivation rate constants K_{in} of the native and immobilized enzymes were calculated from the formula

$$K_{in} = \frac{23}{t} \log \frac{A}{A_0},$$

where A_0 is the initial activity of the enzyme; and

A is the activity at the given moment of time.

Determination of the Temperature Optimum. The activities of a suspension of immobilized lipase and of a solution of the native enzyme were determined when the reaction mixtures were incubated at 30-60°C.

Determination of the pH Optimum. The activity of a suspension of immobilized enzyme or of a solution of the enzyme in distilled water with a 40% emulsion of olive oil in a 2% solution of polyvinyl alcohol in 0.1 M phosphate buffer was determined at various pH values.

Change in the Lipase Activity on Repeated Use. Immobilized lipase was thermostated at 37°C, and an emulsion of olive oil was added. After the mixture had been stirred for 60 minutes, the immobilized enzyme was filtered off and was washed with buffer solution, the lipase activity in the filtrate was determined, and the part of the enzyme remaining on the filter was resuspended and added to the rection vessel for reuse.

SUMMARY

Sorbents for the immobilization of lipases have been synthesized from Silokhrom and Porokhrom and also from the biospecific sorbent Liposorb, based on microcrystalline cellulose. Immobilized forms of the lipase from the fungus Oospora lactis have been obtained, and some of their properties have been studied.

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SYNTHESIS OF C-TERMINAL PEPTIDE FRAGMENTS OF THE HEAVY CHAIN OF HEMAGGLUTININ OF INFLUENZA VIRUS OF SUBTYPES H1 AND H3

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It is proposed to use the chromogenic 2-[4-phenylazo)benzylsulfonyl]ethyl group, which can be eliminated by organic bases in aprotic solvents, for the protection of a C-terminal carboxy group in the synthesis of peptides. The synthesis of a number of 10–16-membered peptides corresponding to C-terminal fragments of the heavy chain of the hemagglutination of influenza virus of subtypes H1 and H3 has been performed with the use of this group.

The main protective antigen of the influenza virus, hemagglutinin (HA), is a highly variable membrane protein consisting of two polypeptide chains – heavy (HA_1) and light (HA_2) – linked by disulfide bonds. According to their antigenic affinities, HAs are divided into several serosubtypes of which subtypes H1 and H3 are of the greatest importance for man. Within the serosubtypes, also, an extremely high variability of the amino acid sequence is observed, but the HA of each subtype has several extended conservative regions having undergone practically no amino acid substitutions in the process of the drift evolution of the subtype. One of the most interesting regions is the C-end of HA_1. It has been shown in a number of papers [1-3] that antibodies obtained against synthetic C-terminal fragments of HA_1 of subtype H3 also interact specifically with the HAs and viruses of different strains of subtype H3 immobilized on a solid support, but the biological properties of such antibodies have not been studied.

With the aim of a detailed investigation of their immunogenic properties, we have synthetized several peptides, (Ia-c) and (IIa-c), consisting of C-terminal fragments of HA_1 of subtypes H1 and H3 having different lengths – from 10 to 16 amino acid residues (Table 1).

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