

IONOPHORIC AND COMPLEXANT PROPERTIES OF LAGOCHILIN DERIVATIVES

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UDC 577.352.465

The ionophoric and complexant properties of synthetic derivatives of lagochilin, 3,18-O-isopropylidenelagochilin and 3,18-O-ethylidenelagochilin, were studied by BLM methods, conductometry, and IR spectroscopy. The ionophoric activity was found to be highly selective for divalent cations. Studies of the electrical conductivity of alkaline-earth metal salts in the presence of the lagochilin derivatives and analysis of their IR spectra have shown that they can form complexes with various ratios of metal ions.

Key words: diterpenoids, lagochilin, ionophoric activity, bilayer lipid membrane, complexation, alkaline-earth metals, conductometry, IR spectroscopy.

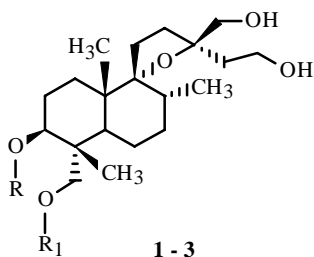
One of the components of the total extracted substances (from 2-5%) of certain *Lagochilus* species growing in Central Asia is the diterpenoid alcohol lagochilin (**1**) [1-3].

Lagochilin and its water-soluble medicinal form lagoden constrict peripheral blood vessels. Therefore, they are strong hemostatic agents [4, 5].

The modulating action of diterpenoids from plants of the *Lagochilus* genus on the blood coagulation system may be due to their ionophoric and membranotropic activities.

Our goal was to study the ionophoric activity of labdane diterpenoids by the bilayer lipid membrane (BLM) method and their complexant properties by conductometry and IR spectroscopy.

For this we studied the diterpenoids lagochilin (**1**), 3,18-O-isopropylidenelagochilin (**2**), 3,18-O-ethylidenelagochilin (**3**), 3,18;15,16-di-O-isopropylidenelagochilin (**4**), lagochilin tetra-O-benzoate (**5**), lagochirzin (**6**), lagochirzidin (**7**), 3,18-O-isopropylidenelagochirzin (**8**), and lagoden (**9**) (sodium salt of lagochirzine).



- 1:** R = R₁ = H;
2: R + R₁ = C(CH₃)₂;
3: R + R₁ = CHCH₃

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TABLE 1. Zero-Current Potentials in Asymmetric Experiments Containing Various Divalent Metals in the *trans*-Chamber (Always Ca²⁺ in the *cis*-Chamber)

Modifier	Me ²⁺			
	Zn ²⁺	Mg ²⁺	Ba ²⁺	Sr ²⁺
2	+5.3±0.1	-4.8±0.5	-5.6±0.5	-11.0±0.7
3	+8.8±0.1	-7.4±0.1	-8.6±0.3	-14.3±0.7

Studies of the ionophoric properties of **1** and its derivatives on planar lipid membranes found that addition of **1** and **4-9** at concentrations up to 200 μM had no effect on the conductivity of the bilayers regardless of the ionic composition of the solutions rinsing the BLM. However, the conductivity of the BLM in the presence of **2** and **3** increased beginning at 5 μM independently of dose in media containing Ca²⁺ at pH 7.4. The maximum specific conductivity appeared at concentrations of 55 μM for **2** and 60 μM for **3**. The optimal concentration of Ca²⁺ was 8 mM for **2** and 10 mM for **3**. This indicates that high Ca²⁺ concentrations saturate and block the conductivity. The final conductivity of the bilayer depended on whether the lagochilin derivatives were added from one or both sides. It was significantly higher in the experiment with lagochilin.

The voltammetric properties of the BLM modified by **2** and **3** were linear and symmetric relative to the zero potential from -100 to +100 mV. This indicates that the limiting factor of ion transport by these compounds is diffusion across the membrane.

The conductivity of BLM modified by lagochilin derivatives depended on the pH of the solution rinsing the bilayer. The maximum specific conductivity was achieved at pH 7.5. This result is probably due to the optimal ionization of the functional groups that participate in the binding of metal ions.

Studies of cation—anion selective conductivity of lagochilin derivatives showed that membranes modified by them are highly selective for divalent cations but not for monovalent ones. In fact, BLM modified by **2** and **3** were nonconductive in three-fold concentration gradients of NaCl and KCl (50 mM/150 mM; *cis/trans*). However, cation—anion selectivity of modified lipid bilayers was observed when the bilayer separated solutions of a 2:1 electrolyte. Thus, the zero-current potential (V^*) in CaCl₂ [5 mM (*cis*)/15 mM (*trans*)] was 9.6 ± 0.5 mV if the BLM was modified by **2**. Under analogous conditions for membranes modified by **3**, V^* was slightly higher, 12.0 ± 0.5 mV. The theoretical potential calculated for this CaCl₂ gradient was 12.5 mV. Therefore, membranes modified by **2** are highly selective for Ca²⁺ whereas those modified by **3** are ideally selective.

Addition of NaCl up to a concentration of 150 mM to both chambers of an experimental cell containing CaCl₂ decreased insignificantly V^* to 8.87 ± 0.1 and 10.1 ± 0.2 mV for membranes modified by **2** and **3**, respectively. The decrease of V^* is apparently due to a small shunting of the Ca²⁺ current by Na and Cl ions although the modified membranes are impermeable to monovalent cations in the absence of divalent metal ions. A calculation of the relative permeability coefficients for Ca²⁺ and Na⁺ ions in this system confirmed this hypothesis. Moreover, it was observed that the relative permeability for Na⁺ ions was less with Ca²⁺ on both sides of the bilayer than in a simple double-ion system. This again confirmed the high permeability of lipid bilayers modified by lagochilin derivatives for divalent cations.

Measurement of double-ion potentials is very informative for a comparative analysis of the selectivity of modified membranes for various ions. This type of experiment was performed in two steps. In the first step, permeabilities of Ca²⁺ and monovalent cations were compared; in the second, those of Ca²⁺ and other divalent cations.

It was found that double-ion potentials using CaCl₂ (*cis*, 10 mM)/NaCl (*trans*, 20 mM) were -8.87 ± 0.4 mV and -10.47 ± 0.3 mV; using CaCl₂ (*cis*, 10 mM)/KCl (*trans*, 20 mM), -9.1 ± 0.5 mV and -9.9 ± 0.1 mV for **2** and **3**, respectively.

The relative permeabilities of various divalent cations were estimated for BLM modified by lagochilin derivatives that separated solutions (10 mM) of 2:1 electrolytes, one of which (in the *cis*-chamber of the experimental cell) was always CaCl₂ solution. Table 1 gives the double-ion potentials.

It was found that Zn²⁺ ions most easily permeate through bilayers modified with membrane-active lagochilin derivatives. Zero-current was attained by setting positive potentials in the CaCl₂ chamber. Zero-current was achieved with the other studied divalent cations by setting negative potentials in the CaCl₂ chamber (Table 1). For BLM modified by either of the two membrane-active lagochilin derivatives, the tested cations could be placed in the following order of capability to permeate through the bilayer: Zn²⁺ > Ca²⁺ > Mg²⁺ > Ba²⁺ > Sr²⁺ [6]. The permeability coefficients depended on the actual lagochilin derivative and were for this order 0.66:1.0:1.45:1.55:2.36 and 0.51:1.0:1.78:1.95:3.05 for **2** and **3**, respectively.

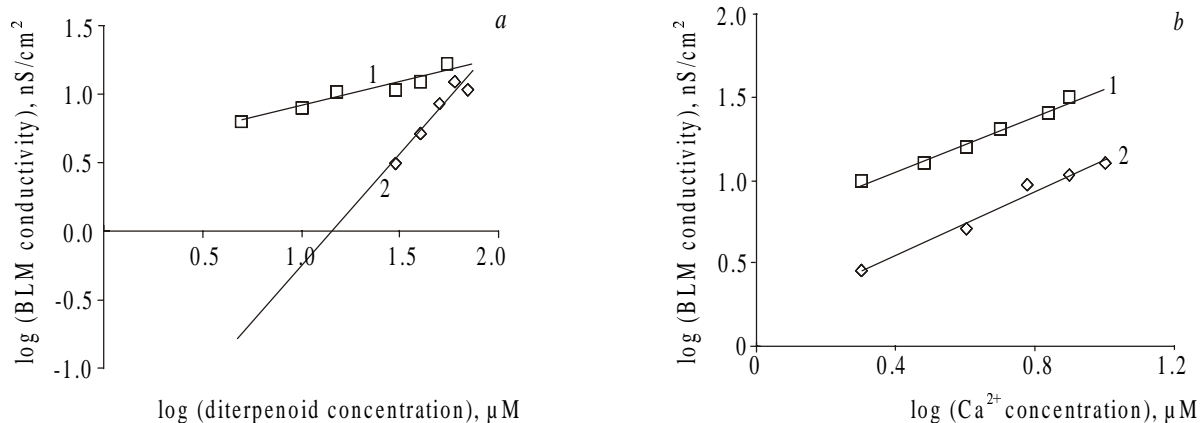


Fig. 1. BLM conductivity as a function of concentration of **2** (1) and **3** (2) (a) and of Ca²⁺ concentration in the presence of **2** (1) and **3** (2) (b) in log—log coordinates.

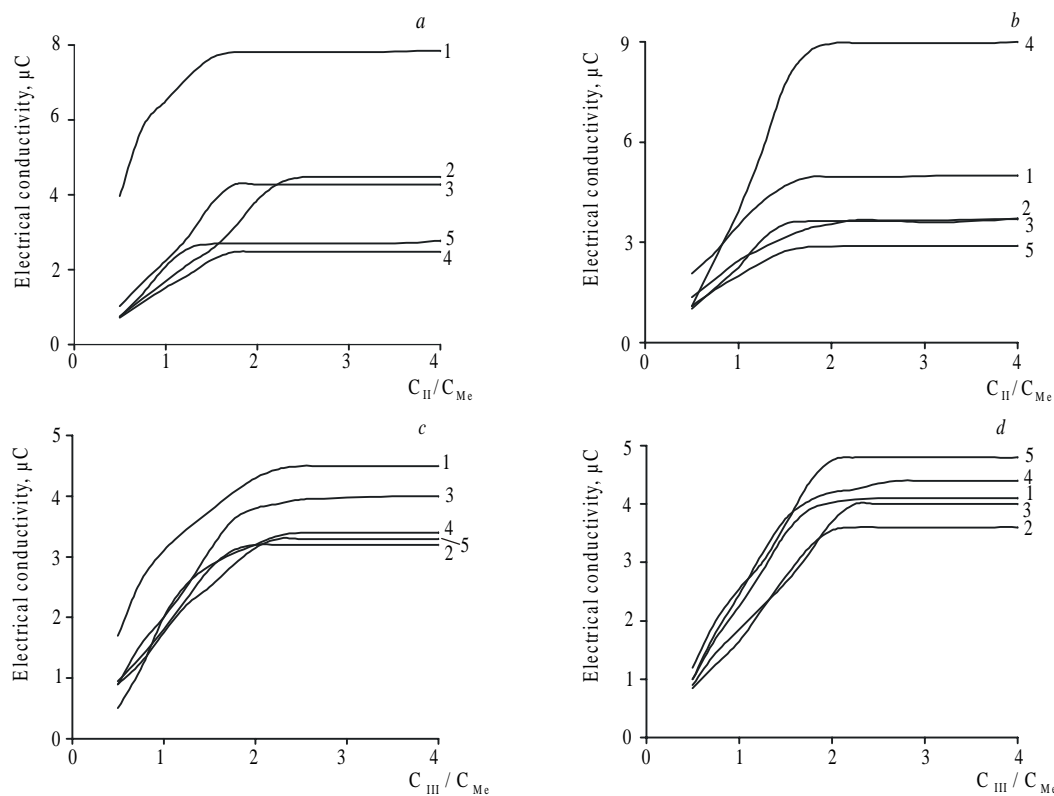


Fig. 2. Conductometric titration curves of **2** in ethanol (a) and acetone (b) and **3** in ethanol (c) and acetone (d) by divalent metal ions: Ca²⁺ (1), Zn²⁺ (2), Mg²⁺ (3), Sr²⁺ (4), and Ba²⁺ (5). Differences in the electrical conductivities of solutions of lagochilin derivatives with metal and of metal without lagochilin derivatives are shown, μC. C_{II}/C_{Me} and C_{III}/C_{Me} are the ratios of concentration of **2** and **3**, respectively, to metal concentration.

The steady-state conductivity of the BLM was studied as a function of diterpenoid concentration in order to estimate the minimal number of lagochilin derivatives forming the ionophore—cation complex. Under conditions where the lagochilin derivatives were added to both chambers, it was found that the BLM conductivity depended linearly on the concentrations of these compounds (5–60 μM) in log—log coordinates (Fig. 1a). The slope was 1.1 ± 0.2 for **2** and 2.03 ± 0.4 for **3**. The amount of ions transferred by one current-carrying structure must be known in order to determine the possible stoichiometry of the complex [7]. Analysis of the BLM steady-state conductivity as a function of Ca²⁺ concentration showed (Fig. 1b) that these plots are also linear in log—log coordinates with slopes of 1.2 ± 0.3 for **2** and **3**.

TABLE 2. Quantitative Properties of Complexation of **2** and **3** by Divalent Cations in Ethanol and Acetone

Cations (10^{-3} M)	$K \cdot 10^4$		$r, \text{Å}$		$\Delta G, \text{kJ}$	
	Ethanol	Acetone	Ethanol	Acetone	Ethanol	Acetone
2 (0.01 M)						
Ca ²⁺	0.98	0.59	5.59	6.47	-22.77	-21.51
Mg ²⁺	0.10	0.33	4.95	6.24	-17.11	-20.07
Zn ²⁺	0.08	0.16	5.33	5.99	-16.56	-18.28
Sr ²⁺	0.14	0.06	5.28	8.16	-17.95	-15.85
Ba ²⁺	1.43	2.14	5.36	5.53	-23.71	-24.70
3 (0.01 M)						
Ca ²⁺	0.16	0.16	4.90	6.19	-18.28	-18.28
Mg ²⁺	0.06	0.09	5.27	6.07	-15.85	-16.85
Zn ²⁺	0.15	0.07	4.75	6.16	-18.12	-16.23
Sr ²⁺	0.14	0.11	5.45	6.31	-17.95	-17.35
Ba ²⁺	0.11	0.08	5.47	6.25	-17.35	-16.56

These investigations indicate that the current-carrying complex of **2** contains one diterpenoid molecule, of **3**, two molecules and one Ca²⁺.

Thus, it has been found that certain labdane diterpenoids (**2** and **3**) have ionophoric activity, are highly selective for divalent cations, and form current-carrying complexes with Zn and alkaline-earth cations.

It is known that alkaline-earth cations can under certain conditions form coordination bonds to oxygen-containing molecules [8]. We studied the conductivity of solutions of **2** and **3** with various metal ions by conductometry in order to obtain accurate data on their complexant properties. Furthermore, their IR spectra were studied in order to show the presence of coordination bonds between the metal ion and lagochilin derivatives. The solvents were ethanol and acetone, the molecules of which can coordinate to metal ions [9].

The investigation of the electrical conductivity of Zn and alkaline-earth salts in alcohol and acetone solutions in the presence of **2** and **3** has confirmed the hypothesis that these diterpenoids can form complex compounds. The experimental data showed that the solution electrical conductivity increased with increasing concentration of lagochilin derivatives and then reached a plateau (Fig. 2a, b, c, and d). At these concentrations, the binding of the Zn cation and alkaline-earth metals to the lagochilin derivatives is maximal [10].

The quantitative characteristics of complexation of lagochilin derivatives to divalent metal cations were calculated from titration curves near the maximal binding. Table 2 gives the complexation constants (K), Gibbs free energies (ΔG), and Stokes radii of the complexes formed (r).

These diterpenoids can form complexes with all studied divalent cations in ethanol and acetone and are complexants for alkaline-earth metals. The stoichiometry of the complexes formed with divalent metal ions differ and depend mainly on the nature of the solvent. The different values of the complexation constants are consistent with selectivity for separate cations. The selectivity for forming complexes of **2** with divalent cations decreases in the order Ca²⁺ > Zn²⁺ > Mg²⁺ > Ba²⁺ > Sr²⁺ in ethanol and Sr²⁺ > Ca²⁺ > Mg²⁺ > Zn²⁺ > Ba²⁺ in acetone. For **3**, the selectivity for complexation decreases in the order Ca²⁺ > Zn²⁺ > Sr²⁺ > Ba²⁺ > Mg²⁺ in ethanol and Ba²⁺ > Sr²⁺ > Ca²⁺ > Mg²⁺ > Zn²⁺ in acetone.

The thermodynamics of complexation, in particular, the free-energy change ΔG , showed that complexation of the studied lagochilin derivatives with Zn cations and alkaline-earth metals is spontaneous without energy losses.

Additional confirmation of the complexing properties of **2** was obtained by analyzing IR spectra of this compound and its complex with Ca²⁺ in a 1:1 ratio (**2**:Ca²⁺ = 1:1). The IR spectrum of starting **2** contains absorption bands at 1250, 1200, 1080, and 860 cm⁻¹. Bending vibrations of methyls are responsible for the bands at 1250 and 1200 cm⁻¹. The bands at 1080 and 860 cm⁻¹ result from vibrations of the carbon skeleton in the isopropylidene radical [11]. Moreover, an absorption band at 3100 cm⁻¹ in the initial spectrum caused by OH stretching vibrations shifted to higher frequency at 3180 cm⁻¹. Intermolecular H-bonds formed by OH groups in **2** are probably decreased and weakened. The reason for this is evidently the presence of two methyls that create steric hindrance upon formation of intermolecular H-bonds.

The IR spectrum of the 1:1 **2**:Ca²⁺ complex has no absorption bands at 3180 and below 1300 cm⁻¹ like that of starting **2** because the Ca²⁺ ions form coordination bonds to the OH oxygen atoms, destroying the intermolecular H-bonds. This is reflected in the changes of the IR spectrum of the complex that were mentioned above.

In general, these lagochilin derivatives satisfy the main requirements for compounds capable of transporting alkaline-earth metals across biological membranes: 1) the amount of ligand oxygen atoms is sufficient to bind cations in the ionophore molecule and 2) the C and H atoms are present in an amount sufficient to form a lipophilic shell.

However, it should be noted that there are differences in the membrane-active properties due to structural features of **2** and **3**. Thus, the small increase of the hydrophobicity (nonpolarity) on going from **3** to **2** (owing to the presence of an additional methyl) was accompanied by a decrease in the selectivity of the modified membranes for Zn²⁺. However, this led to an increase in the permeability of the modified membranes for Sr²⁺. Differences were observed in the stoichiometries of the complexes of these lagochilin derivatives for Ca²⁺. Thus, the value is 1:1 for **2** whereas it is close to 2:1 for **3**.

Thus, the membrane-active properties of the diterpenoids depend on their chemical structures. Only two of the labdane diterpenoids (**2** and **3**) could increase the permeability of lipid bilayers for cations independently of dose. The remaining diterpenoids of this type did not exhibit ionophoric properties. The different membrane activities of the studied lagochilin derivatives is most probably due to their different degrees of affinity for lipids and capabilities to assimilate into the lipid matrix. Apparently the presence of a sufficient number of methyls in these diterpenoids imparts to them the required lipophilicity and thereby optimizes assimilation into membranes.

EXPERIMENTAL

IR spectra of **2** and the 1:1 **2**:Ca²⁺ complex were recorded on a Specord 71 IR spectrophotometer. Samples were prepared as suspensions in mineral oil.

Electrical conductivity of solutions was measured using a Redelkis OK-102/1 conductometer (Budapest, Hungary) at 25 ± 1 °C with a constant salt concentration and variable lagochilin derivative concentration. A weighed portion of lagochilin derivative was dissolved in ethanol or acetone (20 mL) before measuring the electrical conductivity and subsequently diluting the starting solution of compound in ethanol or acetone (0.01 M) with given volumes of salt solution (10⁻³ M) [12]. We used ethanol and acetone and solutions of Zn and alkaline-metal salts (10⁻³ M) in the experiments.

Planar BLM were formed by the Montal—Mueller method [13] from azolectin [10-15 µL of lipid solution in octane (0.5%)] in an opening (0.2-0.4 mm diameter) in the diaphragm (20 µm thick) of a teflon dual-chamber cell at 25 ± 1 °C. Electrical parameters of membranes were measured by setting the potential. The potential was applied to the *cis*-chamber of the experimental cell. The *trans*-chamber of the experimental cell was virtual ground. The conductivity of the membrane (G) was determined as G = I/V, where I is the current passing through the membrane and V is the potential in the *cis*-chamber [14]. The conductivity of unmodified BLM was <5 pC. Doubly distilled water and tris-HCl buffer (1 mM) at pH 7.5 were used to prepare all solutions.

REFERENCES

1. M. M. Abramov, *Dokl. Akad. Nauk Uzb. SSR*, No. 3, 41 (1958).
2. O. S. Chizhov, A. V. Kessenikh, I. P. Yakovlev, B. M. Zolotarev, and V. S. Pastukhov, *Izv. Akad. Nauk, Ser. Khim.*, 1983 (1970).
3. L. G. Vorontsova, O. S. Chizhov, B. L. Tarnopol'skii, and V. I. Andrianov, *Izv. Akad. Nauk, Ser. Khim.*, 338 (1975).
4. I. E. Akopov, *Farmakol. Toksikol. (Moscow)*, **6**, 40 (1961).
5. T. R. Abdurakhmanov, Doctoral Dissertation in Medical Sciences, Moscow (1988).
6. R. K. Ziyatdinova, P. G. Kosymbetov, S. V. Bessonova, V. Shlyonsky, U. N. Zainutdinov, and B. A. Salakhutdinov, in: *Proceedings of the XIIth International Symposium on Supramolecular Chemistry (ISSC)*, Eilat, Israel (2002), p. 57.

7. A. Y. Abramov, M. V. Zamaraeva, A. I. Hagelgans, R. R. Azimov, and O. V. Krasilnikov, *Biochem. Biophys. Acta*, **1512**, 89 (2001).
8. M. Blaghen, N. Lahlou, F. Z. Dzairi, A. Moutaouakkil, and M. Talbi, *Nat. Toxins*, **7**, No. 5, 179 (1999).
9. Yu. N. Kukushkin, *Sorosovskii Obrazovatel'nyi Zh.*, No. 9, 56 (1997).
10. I. M. Andreev, G. G. Malenkov, A. M. Shkrob, and M. M. Shemyakin, *Mol. Biol.*, **5**, No. 4, 614 (1971).
11. L. A. Kazitsyna and N. B. Kupletskaya, *Application of UV-, IR-, and NMR-Spectroscopy in Organic Chemistry* [in Russian], Vysshaya Shkola, Moscow (1971).
12. V. V. Skorchelletti, *Theoretical Electrochemistry* [in Russian], GNTI, Khim. Lit., Leningrad (1963).
13. M. Montal and P. Mueller, *Proc. Natl. Acad. Sci. USA*, **69**, 3561 (1972).
14. D. E. Goldman, *J. Gen. Physiol.*, **27**, 37 (1943).