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Derivatives of Methyllambertianate as Promising Correctors of Cytostatics with Hepatoprotective and Hemostimulating Activity

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Abstract

Results of the development of three original labdanum antioxidants based on the methyl ester of lambertianic acid, an available plant metabolite of Siberian pine *Pinus sibirica* R. Mayr. are presented. As a result of screening with the model of toxic CCl_4-hepatitis induced in mice, the agents surpassing the known flavonoid dihydroquercetin in the anticytolytic and anticholestase action. It is shown that under the conditions of hemodepression caused by the introduction of cytostatic preparation cyclophosphane to rats, the compounds significantly decrease leukopenia by increasing the number of granulocytes and monocytes in blood. At the background of cyclophosphane, the derivatives of methyllambertianate exhibit higher antioxidant activity than dihydroquercetin does. The azlactone demonstrates pronounced anticholestase properties. The pharmacological properties discovered in the new derivatives of lambertianic acid allow considering them as potential correctors of chemotherapeutical preparations.

Key words: methyllambertianate, hepatoprotective, antioxidant, hemostimulating activity

INTRODUCTION

Secondary plant metabolites regulating the vitally important functions of a plant in general and its separate cells are a valuable source of practically important synthones for the development of original medical preparations. As a rule, these compounds are tolerable for animal organisms and possess significant biological activity (antioxidant, anti-inflammatory, hepatoprotective, antiviral, immunomodulating *etc.*). Using them as initial products in synthetic transformation, one may obtain agents with additional useful properties. Promising but relatively poorly investigated class of plant metabolites is labdanoids. It is known that terpenoids of labdanum series possess cytotoxic activity [1], they inhibit the biosynthesis of cholesterol [2] and aggregation of thrombocytes [3], exhibit antilipoxygenase activity [4]. A preparation to treat glaucoma was developed on the basis of plant labdanoid forskolin [5]. One of the representatives of this class of compounds is lambertianic acid produced in *Pinus sibirica* R. Mayr.; it is easily extracted from cedar turpentine and cedar needles with a yield of 3.0 and 0.92 % of the mass of the initial raw material, respectively.

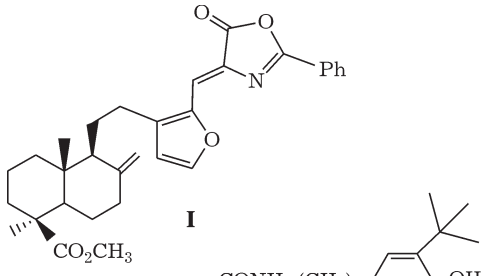
Previously the agents active for the central nervous system and exhibiting nootropic, antidepressant and antipsychotic action were obtained on the basis of lambertianic acid and its methyl ester [6–8]. It was interesting to determine the possibility to obtain from lambertianate some compounds with antioxidant, hepatoprotective and hemostimulating properties that could be used to correct disorders caused by the hazardous action of toxic and medicamental factors. The need for these correcting agents is especially high in oncological practice where they are used as the means of additional therapy to decrease the side effects of highly toxic anti-tumour preparations [9].

Recently new derivatives were synthesized from methyllambertianate: labdane-type azlactone and its derivatives containing screened

phenolic substituents in the side chains with different length of the methylene bridge. The aim of the work was to study their antioxidant, hepatoprotective and hemostimulating activity in animal models.

EXPERIMENTAL

The following compounds have been investigated: of labdane-type azlactone – *Z*-methyl-16-(5-oxo-2-phenyl-oxazole-4-ylidenemethyl)-15,16-epoxy-8(17),13(16),14-labdatriene-18-oat **I** and two its carbamoylvinylbenzamide derivatives – compounds **Ia** and **Ib**.



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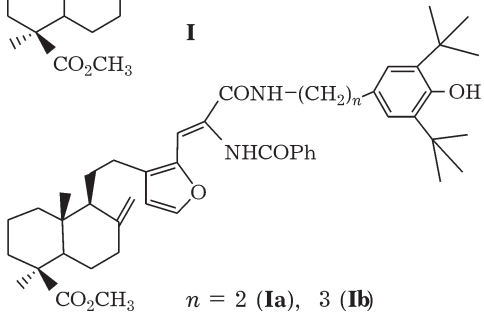
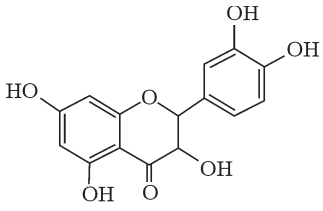


n = 2 (**Ia**), 3 (**Ib**)

Synthesis and physicochemical properties of the compounds are discussed in [10].

The experiments were performed on noninbred male mice weighing 20–25 g and female Wistar rats weighing 180–200 g, provided by the Laboratory of Animal Breeding of the Institute of Cytology and Genetics, SB RAS (Novosibirsk). During the experiments, the animals were kept in plastic cages on standard pellet diet. All manipulations were carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986). The reference agent in the work was dihydroquercetin [(2*R*,3*R*)-3,5,7,3′,4′-pentahydroxyflavone] (99 % purity) **II**, which has an antioxidant's, the hepatoprotective and capillary-strengthening action [10].



II

The standard model of toxic CCl_4-hepatitis in mice was used to investigate the antioxidant and hepatoprotective activity of the synthesized compounds. The model was reproduced according to the procedure described in [12]. All mice were injected intragastrically with the solution of CCl_4 in vegetable oil (25 %). The compounds under investigation **I**, **Ia**, **Ib** and the reference agent were injected in the same way with a dose of 100 mg/kg as a water-Tween suspension, 1 h before hepatotoxins. Animals in the control group were treated with water-Tween suspension. Each group consisted of 10 animals. After 24 h, the activity of alanine aminotransferase (ALT), aspartate aminotransferase (ACT), alkaline phosphatase (ALP) was determined in mouse serum using the standard kits of reagents (Olvex Co., Russia), and the concentration of malonic dialdehyde (MDA) according to a standard method [13].

The study of the protective action of methyllambertianate derivatives **I**, **Ia**, **Ib** against (CP) cyclophosphane affection was performed with female rats. Cyclophosphane-Lance (medication for injection, Lance-Farm Ltd., Russia) was injected to all animals once intraperitoneally, 125 mg/kg in 0.9 % NaCl solution. The investigated compounds in water-Tween suspension were injected intraperitoneally to the group of rats at a dose of 50 mg/kg for 3 days after the introduction of CP. The animals of the reference group were injected by dihydroquercetin (DHQ) at the same dose in a similar way, and those of control group – only by CP. Each group consisted of 10–12 animals. At the end of the experiment the composition of peripheral blood was examined with the help of hematology analyzer (Medonic Oden, Sweeden). Leukogram was counted under a light microscope in blood smears stained with hematoxylin and eosin. The activity of ALT, ACT, ALP, the total concentration of protein, glucose, and

MDA in the blood serum were determined using standard kits of reagents according to the method described in [13]. The results were statistically treated using the software package Statistica 6.0.

RESULTS AND DISCUSSION

Investigation of the antioxidant and hepatoprotective action in the model of toxic hepatitis showed that azlactone **I** causes anticytolytic effect and reliably decreases the activity of transaminases in blood by a factor of 1.3–1.5 in comparison with the reference (Table 1).

Agent **I** does not give the pas to DHQ in anticytolytic action, which is confirmed by the absence of statistically significant differences between the indices of the corresponding groups. Its derivatives with sterically hindered phenyl substituent **Ia** and **Ib** also exhibit reliable anticytolytic effect. Agent **Ia** causes a decrease in the activity of transaminases in blood by a factor of 1.3, agent **Ib** by a factor of 1.7–1.9 in comparison with the reference. Both agents exhibit not smaller effect on ALT level than DHQ does (the differences between the groups are uncertain), while agent Ib exceeds the reference compound by a factor of 1.7 in the effect on the activity of AST. All the methyllambertianate derivatives under test reliably decrease the activity of ALP, which is the evidence of their anti-cholestasic action. Azlactone **I** decreases the level of ALP 1.9 times, while its derivatives decrease it by a factor of 1.5 and 2 with respect to the reference. As far as the manifestation of the anti-cholestasic effect is concerned, agents **I** and **Ib** exceed the reference by a factor of 1.4–1.5, while compound **Ia** affects not weaker than DHQ does.

All the derivatives of lambertianate, similarly to DHQ, did not exhibit the antioxidant effect under the conditions of the present experiment. because the concentration of MDA in the corresponding goups had no reliable differences with the reference group (see Table 1).

For the model of cyclophosphane affection, it was established that azlactone **I** reliably decreases the activity of ALP with respect to the

TABLE 1

Effect of the derivatives of methyllambertianate on the biochemical parameters of blood serum of mice with hepatitis induced by CCl_4

Groups	ALT, units/L	AST, units/L	ALP units/L	MDA, μmol/L
Reference	880.80±37.12	554.00±48.34	174.6±15.6	3.56±0.35
I	701.50±61.29*	373.16± 49.00*	94.2±12.6**#	4.55±0.46#
Ia	655.80±55.46**	411.07±43.58*	119.4±7.2*	3.75±0.12#
Ib	515.58± 60.41***	290.14±46.17**#	84.6±9.6**#	3.07±0.24
Dihydroquercetin	577.24±25.81***	480.47±41.14	130.8±8.4*	3.28±0.11

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ with respect to the reference group.

#$p < 0.05$ with respect to dihydroquercetin.

TABLE 2

Effect of the derivatives of methyllambertianate on the average values of biochemical parameters of blood serum of mice at the background of intoxication with cyclophosphane

Groups	ALT, units/L	AST, units/L	ALP, units/L	MDA, μmol/L	Total protein, g/L	Glucose, mmol/L
Reference	54.3±6.8	73.9±7.9#	129.0±9.5	1.93±0.09#	63.1±2.34	16.43±1.02
I	62.6±2.9	81.4±7.1#	87.1±14.2*#	1.92±0.07#	60.26±4.17	14.93±0.64
Ia	67.3±3.8	69.5±4.7#	122.8±15.1	1.98±0.05	59.89±2.32	3.53±0.55*
Ib	65.3±4.3	86.4±9.2#	146.2±16.7	1.86±0.17	58.73±2.92	14.64±0.36
Dihydroquercetin	55.2±5.2	53.7±3.9*	146.3±13.6	2.57±0.09*	63.14±1.52	14.19±0.70

*$p < 0.05$ with respect to the reference group.

#$p < 0.05$ with respect to dihydroquercetin.

TABLE 3

Effect of the derivatives of methyllambertianate on the average values of the parameters of peripheral blood of rats

Groups	RBC	HCT	WBC	HGB	PLT	MCV	RDW%	MPV
Reference	5.06±0.21	28.0±1.2	0.9±0.1	15.5±0.8	93.9±8.4	55.3±1.0	10.2±0.4	8.8±0.01
I	5.34±0.20	30.2±1.0	1.5±0.1*	16.6±0.7	107.9±9.6	55.0±0.5	10.8±0.6	8.7±0.04
Ia	5.25±0.31	28.4±1.2	1.1±0.2	16.3±0.3	97.4±8.8	53.9±0.8	10.7±0.6	8.6±0.05*
Ib	4.33±0.24*	23.7±1.4*	1.7±0.3*	14.2±0.6	101.1±17.2	58.5±1.9	10.6±0.3	8.5±0.1*
DHQ	4.19±0.20*	22.6±0.9*	1.4±0.2	14.2±0.6	88.3±8.9	55.3±0.8	10.2±0.6	9.3±0.1*
Norm	6.87±0.14	38.7±0.7	17.0±0.9	19.7±0.6	257.0±21.2	57.1±0.9	13.3±0.7	9.0±0.1

Note. DHQ – dihydroquercetin, RBC – number of erythrocytes, HCT – hematocrit, WBC – number of leucocytes, mean volume of erythrocyte, RDW% – percentage of the distribution of red blood over the absolute mass, content in erythrocyte, MCHC – mean concentration of hemoglobin.

*$p < 0.05$ with respect to the reference group.

reference compound and the reference group. The recorded anti-cholestasic effect was 1.7 times with respect to DHQ. Quite contrary, its derivatives **Ia** and **Ib** do not cause anti-cholestasic action (Table 2). All the synthesized compounds (**I**, **Ia** and **Ib**) did not cause substantial changes in the concentration of MDA in blood in comparison with the reference group. At the same time, they decreased the level of MDA by a factor of 1.3–1.4 in comparison with DHQ; the latter compound under the conditions of this experiment caused an increase in the intensity of peroxide oxidation. At the background of CP, the derivatives of methyllambertianate did not affect the activity of transaminases, while DHQ reliably caused a decrease in the activity of one of them, AST. No significant differences between the agents and reference compounds in their effect on general metabolism parameters (total protein, glucose) were detected.

As a result of the investigation of the effect of methyllambertianates on the parameters of peripheral blood, it was established that azlactone **I** exhibits hemostimulating effect as it reliably causes an increase in the number of leucocytes in peripheral blood by a factor of 1.7 with respect to the reference group (Table 3). The corresponding effect of DHQ turned out to the less clearly pronounced (by a factor of 1.5).

As far as other parameters of blood are concerned, a trend to their normalization under the action of compound **I** was detected; the positive effect of this compound exceeds the effect of DHQ. Thus, under the action of azlactone **I** the number of erythrocytes and thrombocytes increases by a factor of 1.3 and 1.2, respectively; the level of hematocrit and hemoglobin increases by a factor of 1.3–1.2 in comparison with that caused by the introduction of DHQ. (It was noted that in the group with the introduction of DHQ the average content

TABLE 4

Effect of the derivatives of methyllambertianate on average values of the parameters of leucogram of blood of rats at the background of intoxication with cyclophosphane

Groups	Eosinophils	Neutrophils		Monocytes	Lymphocytes
		Stab cell	Segmented cell		
Reference	0	0	1.43±0.48	2.43±0.81	96.14±0.51
I	0	0	1.14±0.51	3.71±0.42	95.14±0.40
Ia	0.29±0.18	0	3.43±0.84	4.57±0.57	91.86±0.99
Ib	0.43±0.20	0	1.29±0.68	5.71±1.04	92.57±1.64
DHQ	0	0	0.71±0.29	4.71±0.47	94.57±0.48
Norm	0.86±0.46	0	19.29±1.44	7.14 ±1.06	72.61±1.44

MCH	MCHC
30.6±0.6	55.5±0.6
31.??±0.3	56.4±0.6
31.1±0.6	57.7±0.5*
33.0±0.8*	57.4±0.3*
33.9±0.3*	61.3±0.8*
29.7±0.5	52.0±0.4

HGB – hemoglobin, PLT – thrombocytes, MCV – MPV – volume of thrombocytes, MCH – mean hemoglobin

and the concentration of hemoglobin in an erythrocyte are reliably higher than in the reference group). At the background of hemodepression caused by CP, one of the derivatives of azlactone **Ib** caused the shifts to the same direction as those caused in the parameters of peripheral blood by DHQ.

The same agent (**Ib**) reliably decreases leukopenia increasing the number of leucocytes in blood by a factor of 1.9 in comparison with the reference group, which exceeds the effect of DHQ (1.6 times). In addition, for agent **Ib**, we revealed a small (by a factor of 1.2) decrease in the number of erythrocytes in comparison with the reference group, which correlates with the same decrease of hematocrit index. Relative decrease in the erythrocyte mass in the animals of this group is compensated by a small reliable increase of the quantitative index of hemoglobin in erythrocytes (MSN, MSNS). Compound **Ia** did not cause reliable changes in the parameters of peripheral blood, thus it did not cause hemostimulating action.

At the background of neutropenia caused by CP, we observed a trend to an increase in the number of granulocyte cells under the action of all the compounds under test **I**, **Ia** and **Ib**. In the same groups, in the blood of animals we revealed relative increase in the number of monocytes. The observed trend to stimulation of the cells of leukocyte series turned out to be more clearly expressed for compound **Ib** in comparison with DHQ (Table 4).

So, it was demonstrated that the derivatives of methyllambertianate **I** and **Ib** after intragastric introduction in the dose of 50 mg/kg at the background of hemodepression caused by the injection of CP decrease leukopenia and exceed DHQ in the stimulating action on the leukocyte part of blood. Agent **Ia** under the same conditions does not exhibit significant hemostimulating effect.

CONCLUSIONS

Derivatives of lambertianate **I**, **Ia** and **Ib** introduced intragastrically cause hepatoprotective action decreasing the manifestation of cytolysis and cholestasis processes at the background of toxic CCl_4-hepatitis. Under the conditions of affection with cyclophosphane, azlactone **I** exceeds DHQ in anti-cholestasic and antioxidant effect, for its derivatives **Ia** and **Ib** anticytolytic effect is lower and the antioxidant effect is higher in comparison with DHQ.

At the background of hemodepression caused by cyclophosphane, azlactone exhibits hemostimulating effect which is expressed as an increase in the number of leucocytes with a trend to normalization of other parameters of the peripheral blood. Compound **Ib** decreases leukopenia and exceeds DHQ in the stimulating effect on the leukocyte part of blood. Agent **Ia** under the same conditions does not exhibit significant hemostimulating effect.

It was concluded that the introduction of di-*tert*-butylphenoxycarbomoylvinyl substituent into the molecule of methyl ester of lambertianic acid causes a decrease in protecting properties; the length of the methylene bridge before the screened phenyl substituent is important. With an increase in the length of the linker between the labdan and phenol substituent, the activity of the compounds increases. The results provide evidence of the promising character of methyllambertianate as a synthone for obtaining potential correctors of cytostatic preparations [14, 15].

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