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# The effect of merulinic acid on biomembranes

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**Abstract**

Merulinic acid (heptadecenylresorcinolic acid, resorcinolic acid) is one of the members of resorcinolic lipids, the natural amphiphilic long-chain homologues of orcinol (1,3-dihydroksy-5-methylbenzene). In the present study, membrane properties of merulinic acid were investigated. Merulinic acid exhibits strong haemolytic activity against sheep erythrocytes ($EH_{50}$ of $5 \pm 2$ μM) regardless of the form of its application—direct injection into the erythrocyte suspension or injection as merulinic acid-enriched liposomes. The lysis of erythrocytes induced by merulinic acid was inhibited by the presence of divalent cations. The effectivity of the protection of erythrocytes was highest for $Zn^{2+}$ and weakest for $Mn^{2+}$.

Merulinic acid at low concentrations also exhibits the ability for protection of cells against their lysis in hypoosmotic solutions. This protective effect is significant as, at 10 μM concentration of merulinic acid, the extent of osmotically induced cell lysis is reduced by approximately 40%. Merulinic acid induces increased permeability of liposomal vesicles. This effect was shown to be dependent on the composition of liposomal bilayer and it was stronger when lipid bilayer contained glycolipids (MGDG and DGDG) and sphingomyelin.

Changes of TMA-DPH and NBD-PE fluorescence polarization show that the degree of merulinic acid incorporation into liposomal membrane is not very high. The polar "heads" of the molecules of investigated compounds are localized on the level of fatty acid's ester bonds in phospholipid molecules. Merulinic acid caused the increased fluorescence of the membrane potential fragile probe. This indicated an alteration of the surface charge and a decrease of the local pH at the membrane surface. This effect was visible in both low- and high-ionic strength environment. Merulinic acid causes also a decrease in activity of the membrane-bound enzyme acetylcholinesterase.


*Keywords:* Merulinic acid; Haemolysis; Antihaemolysis; Divalent cation; Acetycholinesterase activity; Fluorescence polarization

## 1. Introduction

Merulinic acid (heptadecenylresorcinolic acid, resorcinolic acid) is one of the members of resorcinolic lipids. Resorcinolic lipids, the natural amphiphilic long-chain homologues of orcinol (1,3-dihydroksy-5-methylbenzene), are demonstrated in numerous plant [1–3], microbial [4–6] and fungal organisms [7]. They exhibit strong amphiphilic character with the values of octanol/water partition coefficient ($logP_{o/w}$) over 7.4 [8]. The resorcinolic lipids exhibit high affinity for lipid bilayer as well as for biological membranes. The incorporation of homologues into liposomal and biological membranes induces an increase of their permeability for small nonelectrolytes and cations [9,10]. This increase of the permeability of membranes may result from formation within the bilayer of the non-bilayer structures, such as reversed micelles or hexagonal phase ($H_{II}$) [11], and often results in haemolysis of cells.

Previous studies on 2,4-dihydroxy-6-(heptadec-8*Z*-enyl)benzoic acid (merulinic acid A) (Fig. 1) showed that it inhibited completely the DNA, RNA and protein synthesis in *Bacillus brevis* cells [7]. In the present study, we focused on the characterization of the biological properties of merulinic acid A as an amphiphilic molecule and its effect on the biological membranes.

## 2. Materials and methods

### 2.1. Materials

Merulinic acid A was isolated chromatographically from fruiting bodies of *Merulius tremellosus* (syn. *Phlebia*

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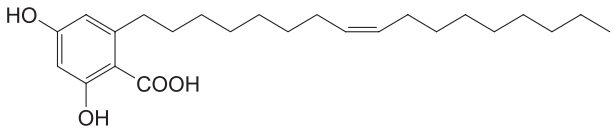


Fig. 1. The structure of merulinic acid A.

*tremellosa* (Schrad.), collected in the forests near Wroclaw, according to the procedure described earlier [7]. For experiments a 5 mM stock methanolic solution was used.

Erythrocytes were isolated from sheep blood collected from randomly chosen individuals at the Department of Epizootiology, Agricultural Academy Wroclaw. Blood was collected into the buffered dextrose (ACD solution) and erythrocytes were separated by centrifugation at 650×*g* for 10 min and subsequently washed three times with 0.9% NaCl buffered with 10 mM Tris–HCl, pH 7.4. Washed erythrocytes were resuspended in the same buffer and the stock suspension of cells (50% hematocrit) was used for experiments.

Erythrocytes ghosts were prepared from sheep erythrocytes according to the procedure described previously [12].

The fluorescent probes, fluorescein-PE, 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatrien, *p*-toluenesulfonate (TMA-DPH) and *N*-(-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoetanolamine, triethylammonium salt (NBD-PE) were from Molecular Probes (Eugene, OR, USA). Egg yolk phosphatidylcholine (PC), monogalactosyldiglyceride (MGDG), digalactosyldiglyceride (DGDG) and sphingomyelin (Sph) were from Sigma-Aldrich (Poznan, Poland), dipalmitoylphosphatidylcholine (DPPC) from Lipid Products (Nutfield, Surrey, GB). Remaining chemicals were of the best available purity from POCh (Gliwice, Poland).

### 2.2. Haemolytic activity of merulinic acid

The haemolytic effect of merulinic acid A was assayed at 37 °C. Into 4 ml of 0.9% NaCl and 1 mM EDTA buffered with 10 mM Tris–HCl, pH 7.4, erythrocyte suspension (20 μl) was added and after 5-min preincubation the microliter amounts of merulinic acid A were injected. The samples were incubated for 30 min and the amount of liberated haemoglobin was determined colorimetrically (570 nm) in the supernatant after centrifugation of the sample at 650×*g* for 10 min. The absorbance of supernatants obtained after the identical amounts of erythrocytes were injected into 4 ml of distilled water was used as the measure of 100% haemolysis.

For the studies of the effect of divalent cations on merulinic acid A-induced haemolysis, the experiments were performed similarly but the merulinic acid A concentration that induced 50% haemolysis was used. This concentration was determined from the merulinic acid A concentration dependence of the extent of haemolysis. The cations ($Cd^{2+}$, $Co^{2+}$, $Mn^{2+}$ and $Zn^{2+}$) were injected in microliter amounts of their chloride salts into the sample prior to addition of erythrocytes and merulinic acid A.

### 2.3. Haemolytic activity of liposomal form of merulinic acid

In this experiment merulinic acid A was added into erythrocyte suspension not as a methanolic solution, but as a form incorporated into liposomal membrane. Liposomes containing merulinic acid A were prepared as follows. Phosphatidylcholine and merulinic acid (74:26 w/w) were dissolved in chloroform and mixed. The chloroform was removed under the stream of nitrogen, an experimental buffer (1 ml 140 mM NaCl buffered with 10 mM Tris–HCl, pH 7.4) was added and the sample was vortexed to obtain suspension of multilamellar vesicles. This suspension was subjected to eight freezing (at −70 °C) and thawing (at +50 °C) cycles and was sonicated for 30 s with a sonicator (Microson/Micronix).

The haemolytic activity of liposomal form of merulinic acid was assayed at room temperature. Into 4 ml of 140 mM NaCl buffered with 10 mM Tris–HCl, pH 7.4, erythrocyte suspension (20 μl) was added and after preincubation the small volume of liposomes suspension was injected under continuous stirring. Total concentration of merulinic acid in liposomal form in the incubation mixture was equal to $EH_{50}$ determined for its free form. The samples were incubated for 30 min and the amount of liberated haemoglobin was determined colorimetrically (570 nm) in the supernatant after centrifugation of the sample at 650×*g* for 10 min. In the control, erythrocytes were incubated with PC liposomes without incorporated merulinic acid. For determination of the 100% of haemolysis, the absorbance of supernatants obtained after the identical amounts of erythrocytes were injected into 4 ml of distilled water was used.

To determine the effect of protein on haemolytic activity of liposomal form of merulinic acid, the experiments were performed similarly to those described above but in the presence of bovine serum albumin (BSA; 32 g/l) in the incubation buffer.

### 2.4. The effect of merulinic acid on hypoosmotically induced haemolysis

The effect of merulinic acid A was assayed at 37 °C. Into 0.8 ml 0.9% NaCl buffered with 10 mM Tris–HCl, pH 7.4, erythrocyte suspension (200 μl) and microliter amounts of merulinic acid were added. After 5-min preincubation, 200 μl of this samples was added to 4-ml 0.55% NaCl buffered with 10 mM Tris–HCl (pH 7.4) under continuos stirring. Samples were incubated for 30 min and the amount of liberated haemoglobin was determined colorimetrically (570 nm) in the supernatant after centrifugation of the sample at 650×*g* for 10 min. The absorbance of supernatant obtained after the identical procedure without added studied lipids into samples was the measure of 100% haemolysis.

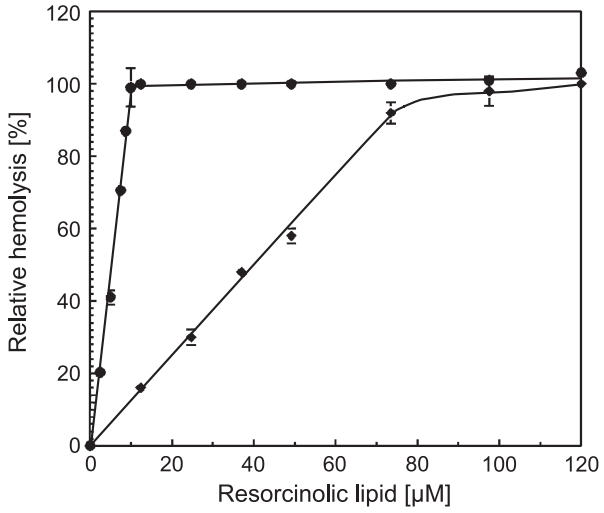


Fig. 2. Dependence of the extent of lysis of sheep erythrocytes on the concentration of free form of merulinic acid (●) and heptadecenylresorcinol (♦). Merulinic acid and resorcinolic lipids were added into erythrocyte suspension as a methanolic solution. After 30 min of incubation, the amount of liberated haemoglobin was determined colometrically (570 nm) in the supernatant after centrifugation of the sample at 650×*g* for 10 min.

### *2.5. The effect of external merulinic acid on liposomal membrane permeability*

Liposomes containing calcein were prepared by the extrusion technique using 5–7 mg of lipid mixture hydrated with 0.5-ml 20 mM calcein buffered in 20 mM Tris–HCl, pH 7.4. Untrapped calcein was removed from liposomal suspension by gel filtration on Sepharose 4 B column (1×20 cm) eluted with 20 mM Tris–HCl, pH 7.4. Ten microliters of liposomes was incubated for 15 min with microliter amounts of studied lipids in the elution buffer at room temperature in the dark, and then the leakage of the calcein was determined fluorimetrically (excitation 490 nm, emission 520 nm). The content of calcein in liposomes was assessed by determination of its fluorescence after lysis of liposomes with 0.1% Triton X-100 (final concentration).

Relative fluorescence of sample ($F$) was calculated as follows:

$$F = \frac{100(F_t - F_0)}{(F_\infty - F_0)}\,[\%],$$

where $F_t$—fluorescence of samples after incubation with phenolic lipid; $F_0$—fluorescence of samples in the beginning (before incubation with phenolic lipid); $F_\infty$—maximal fluorescence after lysis of liposomes.

### *2.6. The effect of merulinic acid on liposomal surface charge and mobility of lipids*

Experiments were assayed in 14 and 140 mM NaCl solutions buffered with 10 mM Tris–HCl, pH 7.4 at room temperature. The final concentration of fluorescent probe (fluorescein-PE) in liposomal membrane was 0.5 mol%; the final concentration of merulinic acid in incubation medium varied between 1 and 10 mol% with respect to the liposomal lipids.

The small unilamellar vesicles (SUV) were prepared from egg PC shortly before measurements at room temperature. Lipids together with fluorescent probes (fluorescein-PE, NBD-PE or TMA-DPH to the final concentration in liposomal membrane 0.5 mol% versus total lipid concentration) were dissolved in chloroform and mixed in the appropriate ratios. The chloroform was removed under the stream of nitrogen, an experimental buffer was added and the sample was vortexed to obtain suspension of multilamellar vesicles. Suspension was sonicated for 15 min with a sonicator (Microson/Micronix) to obtain transparent SUV vesicles suspension.

Merulinic acid was added to the appropriate liposome suspensions containing 0.5 mg of lipid mixtures in the microliter amounts, necessary to reach required molar ratios with respect to the liposomal lipids. The samples were incubated for 2 min and the value of fluorescence polarization was determined using the excitation and emission wavelengths appropriate to the probes used (SFM 25, Kontron).

### *2.7. The effect of merulinic acid on acetylcholinesterase activity*

The effect of merulinic acid on the apparent acetylcholinesterase activity was assayed at 37 °C. Into 3 ml of 0.1 M $Na_2HPO_4/NaH_2PO_4$ (pH 8.0), 50-μl suspension of erythrocyte ghosts and 100-ml 0.4% DTNB buffered 0.1 M $Na_2HPO_4/NaH_2PO_4$ (pH 7.0) with 0.15% $Na_2CO_3$ were added. Subsequently, microliter amounts of merulinic acid A were added. After 5-min preincubation, 20 μl of 0.075 M acetylcholine iodide was added. The changes of absorbance of the samples were recorded continuously for 10 min.

Rate of the reaction ($V$) was calculated as follows [12].

$$V = \frac{A/\mathrm{min}}{1.36 \times 10,000}\,[\mathrm{mol/l/min}]$$

where $A$—absorbance of the sample.

## 3. Results and discussion

For the initial characterization of biological properties of merulinic acid, the studies on its haemolytic properties, both in its free form as well as in the form of merulinic acid-enriched liposomes, were conducted. Additionally, we have tested the effect of merulinic acid on the resistance of erythrocytes upon the hypotonically induced lysis. Further characterization of the merulinic acid properties deals with the determination of the localization of its molecules within

the membrane and its effect on the membrane surface pH. Finally, the effect of merulinic acid on the membrane-bound enzymes was determined using, as an example, erythrocyte membrane acetylcholinesterase.

Merulinic acid, despite its previously described biological properties [7], exhibits strong haemolytic activity against sheep erythrocytes ($EH_{50}$ of 5±2 μM), which is stronger than that observed for other resorcinolic lipids (e.g., 5-*n*-heptadecenylresorcinol) used as a reference (Fig. 2). This haemolytic activity is similar regardless of the form of the application of merulinic acid—direct injection into the erythrocyte suspension (Fig. 2) or injection as merulinic acid-enriched liposomes (Fig. 3A), containing the same amount of merulinic acid as in the case of its free form (methanolic solution). The strong activity in the latter case, although slightly less effective in the term of $EH_{50}$ (about 40% increase), indicates that the compound can be easily exchanged between liposomal and erythrocyte membranes, thus resulting in their haemolysis. These experiments indicate that lytic agents can act on cells not only from their monomeric/micellar forms from the external solution but they may be also exchanged from supramolecular carriers (e.g., liposomes) to the cellular membranes resulting a similar to the "free" form biological effects. Numerous groups have demonstrated earlier that the presence of proteins, e.g., serum albumin, in the external media protects erythrocytes against heamolysis induced by various lytic agents [13,14]. Haemolytic activity of the liposomal merulinic acid, as well as of its free form (data not shown), was practically abolished in the presence of serum albumin (BSA) at the concentration similar to the physiological one (Fig. 3B). This effect may be related to the direct interaction of albumin with the outer surface of the bilayer (stabilization) and the protection from the transfer of merulinic acid from the environment or of liposomes to the erythrocyte membrane. This has potential meaning in pharmacological applications of merulinic acid as (similarly to resorcinolic lipids) modifier of liposomal bioactive substance carriers. Strong haemolytic properties of merulinic acid, the compound structurally similar to 5-*n*-heptadecenylresorcinol, whose haemolytic properties are moderate, its $EH_{50}$ is at approximately eight times higher concentration (Fig. 2), are attributed to the presence of the carboxylic ring substituent. This substituent is responsible for the increase of the solubility of merulinic acid in aqueous solutions and, therefore, the availability of larger number of molecules for the interaction with the membrane lipid bilayer.

Lysis of the cells, induced by various compounds, can be modulated by the presence of divalent cations or protons in the medium. This effect is dependent on the lytic agent, cell type and origin as well as on the cation and its concentration [15,16]. This protective effect of the cells against lysis by divalent cations has been demonstrated for many different lytic agents [13,15–19] including natural resorcinolic lipids isolated from rye grains [20,21]. It was interesting to check if, similar to the already demonstrated, divalent cations

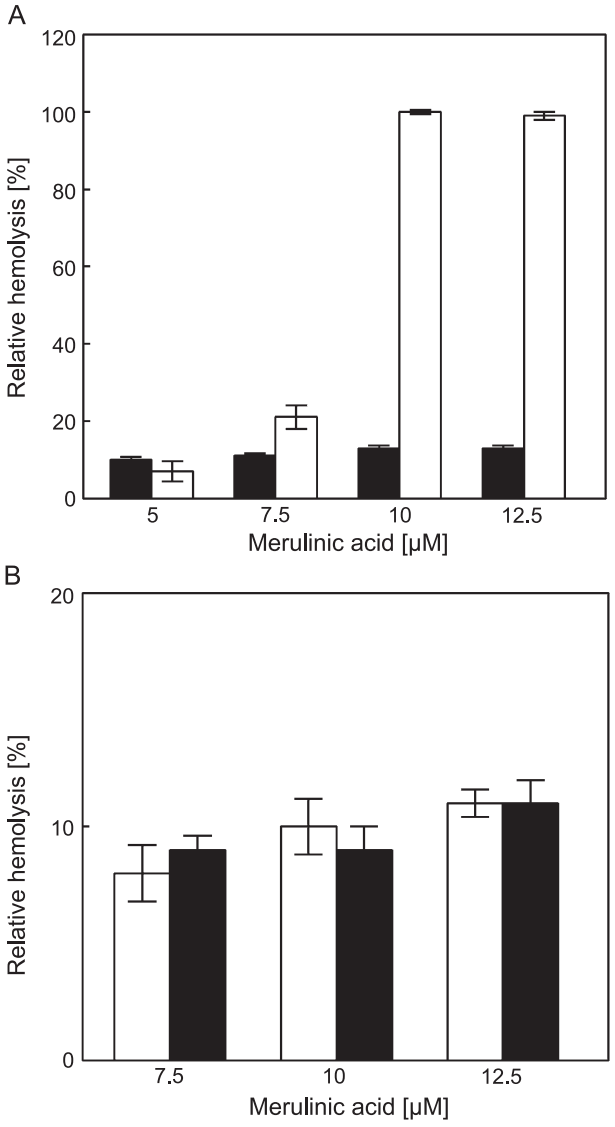


Fig. 3. Dependence of the extent of lysis of sheep erythrocytes on the concentration of liposomal form of merulinic acid in the absence (A) and presence (B) of BSA. Please note differences between scale of *Y* axes. Open bars—liposomes from egg PC with incorporated merulinic acid; black bars—liposomes from egg PC without incorporated merulinic acid. Merulinic acid was added into erythrocyte suspension as a liposomal form. After 30 min of incubation, the amount of liberated haemoglobin was determined colorimetrically (570 nm) in the supernatant after centrifugation of the sample at 650×*g* for 10 min. In the control, erythrocytes were incubated with PC liposomes without incorporated merulinic acid.

would also have protective effect also on the lysis induced by merulinic acid. It was demonstrated that the lysis of erythrocytes induced by merulinic acid was also inhibited by the presence of divalent cations (Fig. 4), similarly as the lysis induced by 5-*n*-alkylresorcinols [20,21]. The effectivity of the protection of erythrocytes was highest for $Zn^{2+}$ and weakest for $Mn^{2+}$, which is similar to the effect observed for long-chain 5-*n*-alkylresorcinols [20,21].

Merulinic acid at low concentrations, well below those inducing lysis of erythrocytes, also exhibits the ability for protection of cells against their lysis in hypoosmotic

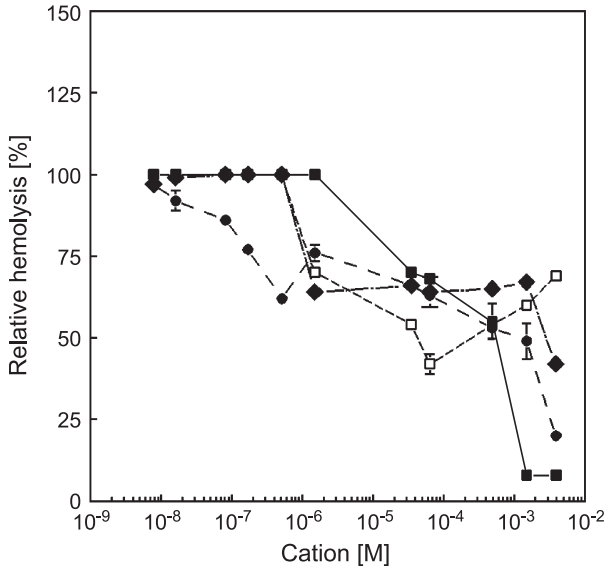


Fig. 4. Effect of $Cd^{2+}$, $Co^{2+}$, $Mn^{2+}$ and $Zn^{2+}$ ions on relative haemolysis of sheep erythrocytes induced by merulinic acid at the concentration of 50% absolute lysis (5±2 μM) (■) $Zn^{2+}$, (□) $Mn^{2+}$, (●) $Co^{2+}$, (♦) $Cd^{2+}$. Cations were injected in microliter amounts of their chloride salts into the samples prior to addition of erythrocytes and merulinic acid A. Merulinic acid was added into erythrocyte suspension as a methanolic solution. The merulinic acid A concentration that induced 50% haemolysis was used. After 30 min of incubation, the amount of liberated haemoglobin was determined colorimetrically (570 nm) in the supernatant after centrifugation of the sample at 650×*g* for 10 min.

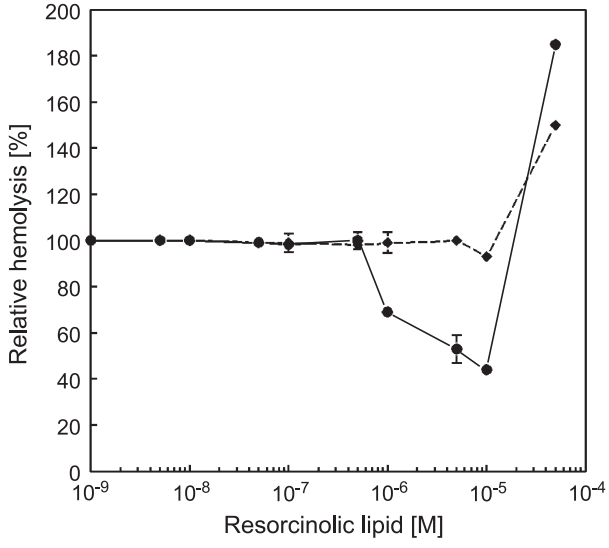


Fig. 5. Dependence of the extent of hypoosmotically induced lysis of sheep erythrocytes on sublytic concentration of free form of merulinic acid (●) and heptadecenylresorcinol (♦). Samples contained erythrocyte suspension and microliter amounts of merulinic acid in 0.9% NaCl buffered with 10 mM Tris–HCl, pH 7.4. After 5-min preincubation, 200 μl this sample was removed and added to 4-ml 0.55% NaCl buffered with 10 mM Tris–HCl (pH 7.4). Samples were incubated for 30 min and the amount of liberated haemoglobin was determined colorimetrically (570 nm) in the supernatant after centrifugation of the sample at 650×*g* for 10 min. The absorbance of supernatant obtained after the identical procedure without added studied lipids into samples was the measure of 100% haemolysis.

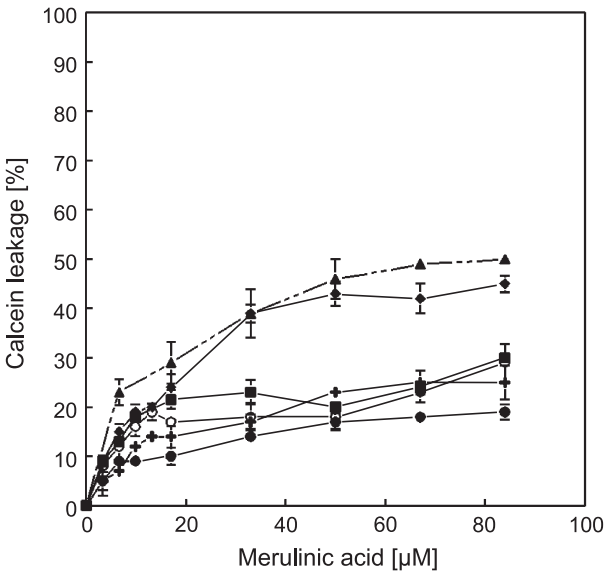


Fig. 6. Leakage of calcein from phospholipid vesicles induced by merulinic acid. Liposomes were prepared from PC (●), PC/PS (80:20 w/w) (■), PC/PE (80:20 w/w) (○), PC/SM (70:30 w/w) (▲), PC/MGDG (67:33 w/w) (+), PC/DGDG (67:33 w/w) (♦). Liposomes containing calcein were prepared by the extrusion technique. Untrapped calcein was removed from liposomal suspension by gel filtration on Sepharose 4B column. Samples contained 10 μl of liposomes in 20 mM Tris–HCl, pH 7.4, and microliter amounts of studied lipids were incubated for 15 min at room temperature in the dark, and then the leakage of the calcein was determined fluorimetrically (excitation 490 nm, emission 520 nm). The content of calcein in liposomes was assessed by determination of its fluorescence after lysis of liposomes with 0.1% Triton X-100 (final concentration).

solutions (Fig. 5). This protective effect is significant as, at 10 μM concentration of merulinic acid, the extent of osmotically induced cell lysis is reduced by approximately 40%. In this respect, merulinic acid is more active in comparison to other phenolic lipids. For instance, 5-*n*-heptadecenylresorcinol (a compound lacking the carboxylic attachment to the ring) reduces hypoosmotically induced haemolysis by only 7%. On the other hand, anacardic acid (pentadecylphenolic acid, pentadecylsalicylic acid), lacking the second hydroxyl group, also protects erythrocytes against hypoosmotic lysis only by 10%, however, at lower concentration (1 μM). This activity of merulinic acid, called "antihaemolytic", is similar to that demonstrated for cardol (5-*n*-pentadec(en)ylresorcinol), which at the concentration of 10 μM inhibits 50% of hypoosmotically induced lysis of erythrocytes (data not shown).

Besides the ability for the concentration-dependent alteration of barrier properties of natural membranes, merulinic acid also induces increased permeability of liposomal vesicles (Fig. 6). This effect was shown to be dependent on the composition of liposomal bilayer and it was stronger when lipid bilayer contained glycolipids (MGDG and DGDG) and sphingomyelin. This observation suggests that cells in which the natural membrane lipid asymmetry is disturbed would be more susceptible to the action of lytic agents that would be present in the outside

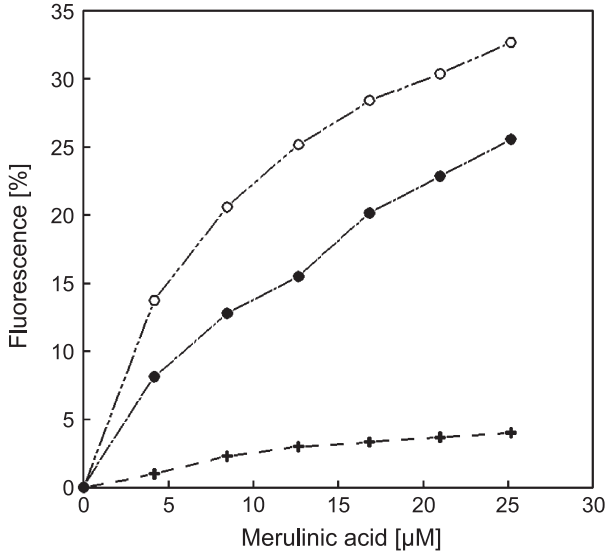


Fig. 7. Dependence of the extent of fluorescence of fluorescein-PE on the concentration of merulinic acid. (+) Methanol; (○) merulinic acid in 14 mM NaCl buffered with 20 mM Tris–HCl, pH 7.4; (●) merulinic acid in 140 mM NaCl buffered with 20 mM Tris–HCl, pH 7.4. The final concentration of fluorescein-PE in liposomal membrane was 0.5 mol%. Merulinic acid was added to the appropriate SUV liposome suspensions in microliter amounts and after 2-min incubations, the value of fluorescence intensity was determined.

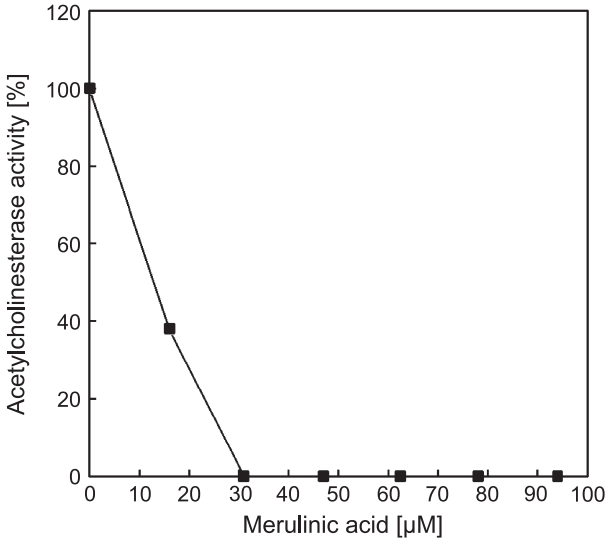


Fig. 9. Effect of merulinic acid on acetylcholinesterase activity. Samples contained suspension of erythrocyte ghosts and DTNB buffered with 0.1 M $Na_2HPO_4/NaH_2PO_4$ (pH 7.0), 0.15% $Na_2CO_3$ and microliter amounts of merulinic acid A. After 5-min preincubation, acetylcholine iodide (substrate) was added. The changes of absorbance of the samples were recorded continuously for 10 min.

medium (e.g., serum). On the other hand, this observation also suggests the possible stronger effect of resorcinolic lipids on the glycolipid/sphingolipid-rich biomembranes, e.g., chloroplast or mitochondria.

The effect of merulinic acid on bilayer properties was also demonstrated when alteration of the surface charge and local pH was studied (Fig. 7). Merulinic acid, as the molecule containing ionised carboxylic moiety, caused the increased fluorescence of the membrane probe. This indicated an alteration of the surface charge and a decrease of the local pH at the membrane surface. This effect was visible in both low- and high-ionic strength environment.

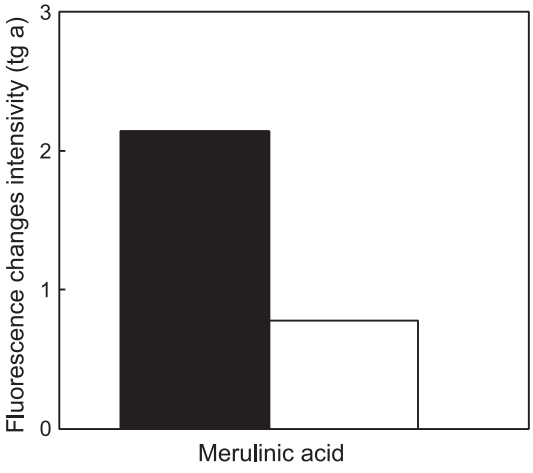


Fig. 8. Effect of merulinic acid on fluorescence intensity of incorporated fluorescence dye (TMA-DPH and NBD-PE) as illustrated by the values of a tangent of the slope of fluorescence changes. Open bars—NBD-PE; black bars—TMA-DPH. The final concentration of fluorescent probes in liposomal membrane was 0.5 mol%. Merulinic acid was added to the appropriate SUV liposome suspensions in microliter amounts and after 2-min incubations, the value of fluorescence polarization was determined.

On the other hand, experiments concerning the effect of studied compound on the properties of the bilayer across its thickness, monitored by the alteration of fluorescence behaviour of probes localizing at different depths of the bilayer (NBD-PE in the hydrophilic region and TMA-DPH in the deeper region, below C4 of the acyl chains of phospholipids), indicate that merulinic acid rather affects the properties of the hydrophobic region of the bilayer than the hydrophilic one (Fig. 8). This, rather unexpected, behaviour may be related to the presence of unsaturation in the merulinic acid side chain. This may cause stronger effect in the properties of, otherwise saturated, phospholipid bilayer than the effect of more polar headgroup on the behaviour of the polar region of the bilayer.

The decrease of the apparent activity of membrane-bound enzyme, acetylcholinesterase, upon incorporation of merulinic acid (Fig. 9) may result from the alteration of the distribution and/or the alteration of the mobility of membrane phospholipids (fluidity of the bilayer). Another reason for the observed decrease of the enzyme's activity may be related the state of, as called, boundary lipids, i.e., lipids that are closely bound/related to the membrane enzyme. In case of acetylcholinesterase, hitherto studied resorcinolic lipids also induced marked decrease of its apparent activity [22]. The similarity of the net effect of these compounds, noted previously, and the effect of more polar compounds (such as merulinic acid) may suggest an

existence of a more complex interactions. These interactions may involve not only the effect of the polar (hydrophilic) region of the bilayer modifier but also the effect of the hydrophobic alk(en)yl side chain. Both regions seem to participate in the alteration of the native enzyme environment by, e.g., inducing the lipid environment-dependent conformational changes of the enzyme molecule and the decrease of the spatial adjustment of the enzyme molecule within the actual lipid environment. These changes, in consequence, will lead to the decrease of its apparent activity. Similar effects have been also observed for other amphiphilic molecules [23–25] and for modulation the activity of phospholipase $A_2$ by selected resorcinolic lipids [26]. As the biological activities of the anacardic acids, being the homologues of alkylsalicylic acid, are of the general interest [27–30], the recognition of the metabolic and physiological processes affected by merulinic acid is also important. These compounds, as metabolic precursors or resorcinolic lipids [31], may play a role in the biological and biomedical effects of the products they are present in, especially the fungal and cereal ones.

Further studies concerning the biological activities of alkylphenolic (anacardic) and alkylresorcinolic (merulinic) acids, as well as the most abundant in cereal materials, alkylresorcinols, are under way.

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