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Interaction of Resveratrol and Its Trimethyl and Triacetyl Derivatives with Biomembrane Models Studied by Differential Scanning Calorimetry

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The interaction of resveratrol (*trans*-3,5,4′-trihydroxystilbene) and two of its derivatives (3,5,4′-tri-*O*-methylresveratrol and 3,5,4′-tri-*O*-triacetylresveratrol) with biomembrane models, represented by dimyristoylphosphatidylcholine (DMPC) multilamellar vesicles (MLV), has been studied by differential scanning calorimetry (DSC). The analysis of MLV prepared in the presence of increasing molar fraction of such compounds has been carried out to reveal their maximum interaction with biomembrane models. The results from these studies have been compared with kinetic experiments results, in order to detect the entity and rate of compound absorption by the biomembrane models. The findings indicate that the compounds affected the thermotropic properties of DMPC MLV by suppressing the pretransition peak and broadening the DMPC main phase transition calorimetric peak and shifting it to lower temperatures. The order of effectiveness found was resveratrol ≫ trimethylresveratrol > triacetylresveratrol. The kinetic experiments reveal that in an aqueous medium the absorption of resveratrol by the biomembranes models is allowed, whereas the absorption of its derivatives is hindered; in contrast when a lipophilic medium is employed, all three compounds are easily absorbed.

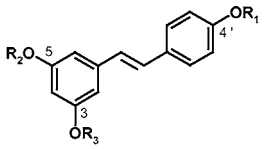
KEYWORDS: Resveratrol; trimethylresveratrol; triacetylresveratrol; differential scanning calorimetry; biomembrane model

INTRODUCTION

Phytoalexins are a group of low molecular weight compounds produced in grape vines and in a large number of plants as a defense response to situations of stress, such as microbial infections and UV irradiation (*1*). Resveratrol (*trans*-3,5,4′-trihydroxystilbene, **1**, **Figure 1**) is a phytoalexin found in *Vitis* spp. and in many other plants and food products and has received much attention due to its possible positive health benefits (*2*). In fact, it has been reported to have potential cancer chemopreventive activities based on its striking inhibitory effects on cellular events associated with cancer initiation, promotion, and progression (*3*−*5*) and it may exert, through its antioxidant properties, a protective effect against atherogenesis (*6*) and against cardiovascular diseases (*7*).

It has also shown a variety of other activities (*8*), among them antimicrobial, antifungal, antileukaemic, and hepatoprotective (*9*−*12*). It is, also, an inhibitor of protein tyrosine kinase (*13*) and DNA polymerase (*14*) and possesses anti-inflammatory activity (*15*) and effect on the COX-2 enzyme (*16*).

Resveratrol may be obtained by chemical synthesis or from grape and other vegetable sources. This, associated with its interesting benefic activity, offers promise for the rational design of new therapeutic agents, and in this context, efforts have recently been devoted to studying the structure−activity relationships of new stilbene-based analogues.



	R_1	R_2	R_3
1	H	H	H
2	CH_3	CH_3	CH_3
3	$COCH_3$	$COCH_3$	$COCH_3$

Figure 1. Resveratrol (**1**), trimethylresveratrol (**2**), and triacetylresveratrol (**3**) structures.

Some studies have been carried out to compare the activity of resveratrol with that of some of its analogues in order to establish the influence of the different portions of its structure on the activity.

It has been reported that the radical-scavenging activity and the antioxidant activity of resveratrol and its analogues are

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significantly dependent on the hydroxyl groups' positions (*17*−*19*). Studying the ability of resveratrol and seven other hydroxylated stilbenes to induce apoptosis in human leukemia cells, it has been found that the activities of the analogues containing 3,4-hydroxyl groups were higher than those of resveratrol and the other analogues (*20*). Other studies have demonstrated the importance of a 3,5-dimethoxy motif in conferring pro-apoptotic activity to stilbene-based compounds (*21*). In a recent work by some of us (*22*), on resveratrol and its lipophilic analogues, 3,5,4′-tri-*O*-methylresveratrol (**2**, **Figure 1**) showed an antiproliferative activity toward DU-145 human prostate cancer cells more potent than that of **1**, and 3,5,4′-tri-*O*-acetylresveratrol (**3**, **Figure 1**) showed activity comparable to that of **1**.

With the aim of acquiring further information on the relationship between structure and activity, we employed resveratrol and its lipophilic derivatives **2** and **3** to study their interaction with biomembranes and in particular the effect of the replacement of all the hydroxy groups with methoxy and acetoxy functions on such interaction. Substantial work at various levels is required to establish a direct connection between molecular mechanisms and activity, owing to the complexity of cellular systems; useful information can be obtained by investigating the interaction of bioactive compounds with model membranes. In this context, multilamellar vesicles are well-established model systems to represent biological membranes (*23*). By using DSC technique, we have studied the interactions of resveratrol and its derivatives, trimethylresveratrol and triacetylresveratrol, with dimyristoylphosphatidylcholine (DMPC) multilamellar vesicles (MLV). Differential scanning calorimetry (DSC) is a nonperturbative technique that allows one to monitor the interaction of bioactive compounds with biomembrane models and then to withdraw consideration about the dissolution and diffusion in and between the lipid phase (*24*−*27*). In fact, if the compounds interact with the phospholipid bilayers, a modification of the lipid transition temperature (T_m, that is, the temperature of the gel to liquid crystal phase transition characteristic of the phospholipid bilayer) should be observed. Usually, molecules interacting with the phospholipids behave as impurities destabilizing the phospholipid ordered structure and shifting the T_m toward lower values with respect to the phospholipid alone (*25*, *28*−*32*). As the decrease of the model membrane T_m depends on the compound amount present in the aqueous−lipid dispersion as well as the kind of interaction, we have determined the maximum interaction occurring between the DMPC and the examined compounds by preparing DMPC MLV in the presence of increasing molar fractions of each compound and submitting them to DSC analysis. Then, to better define the extent and rate of compound interaction and absorption by the biomembrane models, we have performed kinetic experiments and have compared the results with those of the maximum interaction. Significant differences in the interaction as well in its rate and extent between the examined compounds and biomembrane models have been revealed among the compounds related to structural differences.

MATERIALS AND METHODS

Materials. *trans*-Resveratrol (*trans*-3,5,4′-trihydroxystilbene) was purchased from Sigma (purity ≥ 99%) and used as received.

3,5,4′-Tri-*O*-methylresveratrol was prepared from resveratrol according to a standard methylation procedure. Resveratrol (100 mg, 0.44 mmol) was placed into a boiling flask and dispersed with 40 mL of acetone and 65 mg of potassium carbonate; 70 μL of dimethyl sulfate was added to this suspension and then heated for 24 h under a reflux condenser. Acetone was removed from the mixture by a rotary evaporator. The resulting mixture was purified by LC (silica gel, CH_2Cl_2 in *n*-hexane from 20 to 100%), thus affording 85 mg of **2**. MS-FAB and ^{1}H NMR data of **2** are in agreement with those reported in the literature (*11*). 3,5,4′-Tri-*O*-acetylresveratrol was prepared from resveratrol in standard conditions using acetic anhydride in pyridine (1:1, v/v). MS-FAB and ^{1}H NMR spectra of the product **3** were in perfect agreement with those reported in the literature (*33*). The ^{1}H NMR spectra were recorded on a Varian Unity Inova spectrometer at 500 MHz and performed at constant temperature (27 °C). Electron impact (EI MS) mass spectra were recorded on a ZAB 2-SE instrument. Analytical thin-layer chromatography was performed on silica gel (Merck 60 F_{254}) plates using cerium sulfate as developing reagents.

1,2-Dimyristoyl-*sn*-glycero-3-phosphatidylcholine was supplied by Genzyme Pharmaceuticals (Liestal, Switzerland). Lipids were chromatographically pure as assessed by two-dimensional thin-layer chromatography. Lipid concentration was determined by the phosphorus analysis (*34*); a 50 mM Tris buffer solution, adjusted to pH 7.4, was employed.

Reagents were of commercial quality and were used as received (Merck, Sigma-Aldrich, and Carlo Erba); only solvents were distilled and dried using standard techniques.

Liposome Preparation. Multilamellar vesicles were prepared as follows: stock solutions of DMPC, resveratrol (**1**), 3,5,4′-tri-*O*-methylresveratrol (**2**), and 3,5,4′-tri-*O*-acetylresveratrol (**3**) were prepared using chloroform/methanol (1:1, v/v) for DMPC, chloroform/methanol (9.5:0.5; v/v) for **1**, and chloroform for **2** and **3** as solvents. Aliquots of DMPC solution were distributed in glass tubes to have 0.01032 mmol of DMPC in all of the tubes; then aliquots of solutions of compounds **1**−**3** were added to have a defined molar fraction of the examined compounds with respect to the phospholipid. The solvents were removed under nitrogen flow, and the resulting films were dried under vacuum to eliminate eventual solvents residues. One hundred and sixty-eight microliters of a 50 mM Tris buffer (pH 7.4) was added to the films, and the samples were heated at 37 °C for 1 min and successively shaken for 1 min three times and kept at 37 °C for 1 h to permit the MLV to homogenize and allow the compounds to partition between the lipid and aqueous phases. One hundred and twenty microliters (0.007375 mmol) was transferred to a 160 μL aluminum DSC pan, which was hermetically sealed, and submitted to calorimetric analysis.

DSC Analysis. A Mettler Toledo STAR^e system equipped with a DSC-822^e calorimetric cell and Mettler TA-STAR^e software was used. The samples were submitted, at least four times to check the results reproducibility, to the following procedure: (i) a heating scan between 5 and 37 °C at 2 °C/min; (ii) a cooling scan between 37 and 5 °C at 4 °C/min.

The sensitivity was automatically chosen as the maximum possible by the calorimetric system, and the reference pan was filled with Tris buffer solution. The calorimetric system was calibrated, in transition temperature and enthalpy changes, by using indium, stearic acid, and cyclohexane by following the procedure of the DSC 822 Mettler TA STAR^e instrument.

After the DSC analysis, aliquots of all samples were extracted from the calorimetric aluminum pans and used to determine, by the phosphorus assay (*34*), the exact amount of phospholipids present in each sample.

Compound Partition between Phospholipid and Aqueous Phases. To know the real amount of compounds present in the lipid phase, forming MLV, samples of MLV prepared in the presence of 0.06 and 0.12 molar fractions of **1**−**3** were submitted to DSC analysis to check the complete interaction (partition equilibrium reached) with the liposomes, by comparing the calorimetric results with those obtained following the experiments reported in the previous section. The compound partition studies between aqueous and lipid phases were performed at 0.06 and 0.12 molar fractions to be sure that the partition found was the same at lower and high concentrations without saturation in the liquid phase, which can cause errors in the determination of the compounds inside the vesicles.

The samples were transferred to a centrifuge tube and centrifuged at $(60 \times 10^3)g$ for 1 h using a Beckman L8-60M centrifuge, at

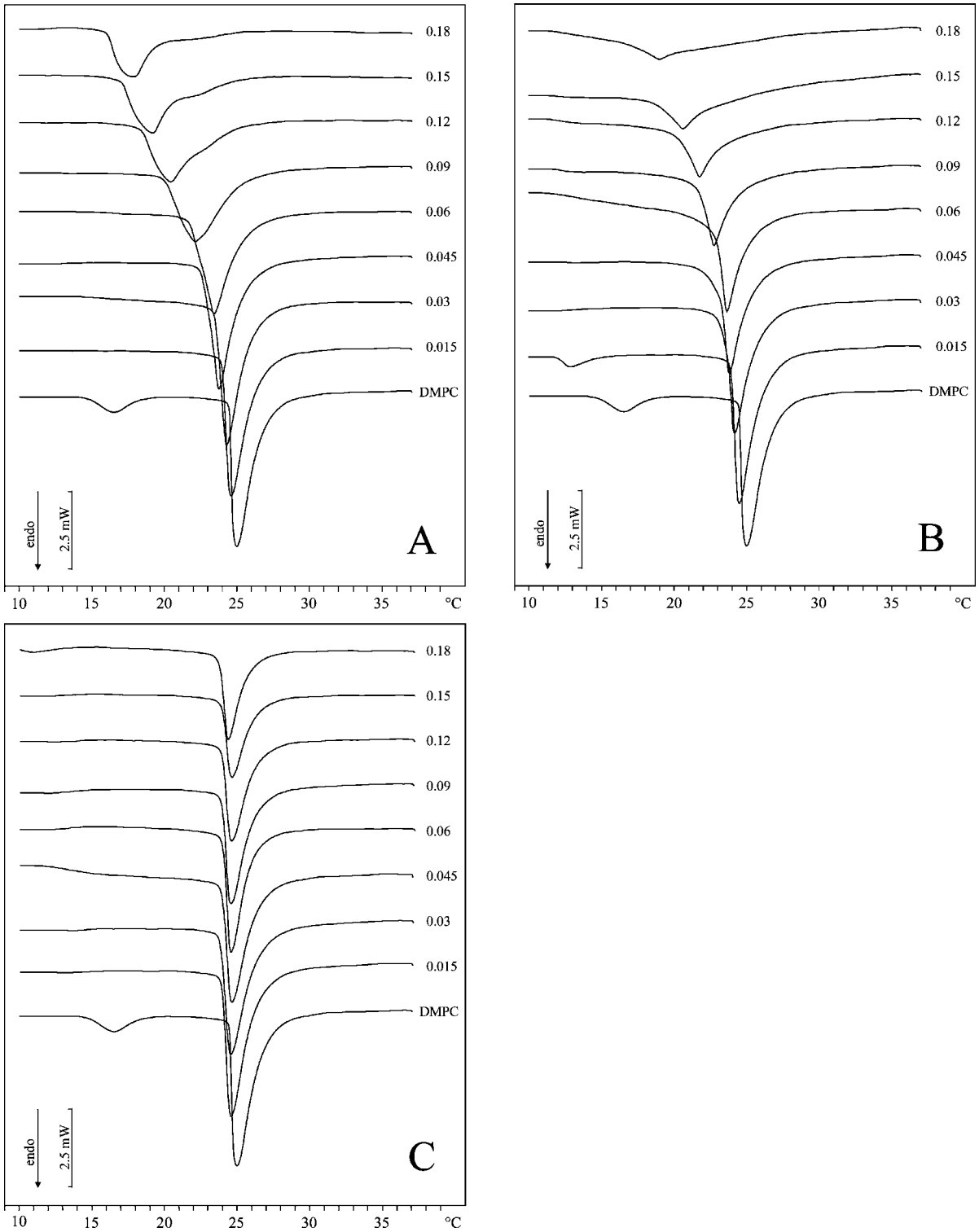

Figure 2. Calorimetric curves, in heating mode, of DMPC MLV prepared in the presence of increasing molar fractions of (**A**) resveratrol, (**B**) trimethylresveratrol, and (**C**) triacetylresveratrol.

controlled temperature. The supernatant was separated by the phospholipid pellet and then both were dried and lyophilized. The lyophilized samples were dissolved in chloroform/methanol (9.5:0.5; v/v) for **1** and only chloroform for **2** and **3**. The amount of compound present in the two fractions (aqueous and lipid) was determined by UV−vis spectroscopy at the maximum absorbance wavelength (309 nm for **1** and **2**, 301 nm for **3**) using a Varian Cary 500 Scan UV−vis NIR spectrophotometer. Each determination was made in triplicate.

Permeation Kinetics Experiments. An exact amount (to have a 0.12 molar fraction) of the powdered examined compounds was weighed in the bottom of the DSC aluminum pan, and 120 μL (0.007375 mmol) of the DMPC MLV aqueous dispersion was added. The aluminum pan was hermetically sealed and the sample submitted to the following calorimetric analysis: (i) a heating scan between 5 and 37 °C, at the rate of 2 °C/min, to detect any interaction between compound and MLV; (ii) an isotherm scan at 37 °C, to permit the compound to dissolve in the aqueous medium, reach the MLV surface, penetrate the phospholipid bilayers, and interact with them; (iii) a cooling scan between 37 and 5 °C, at the rate of 4 °C, to bring the phospholipid system back to the ordered state.

The procedure was run at least eight times, to reveal any variation in the calorimetric curves during the entire incubation time.

Transmembrane Transfer Experiments. Sixty microliters (0.003687 mmol) of DMPC MLV dispersion prepared in the presence of 0.12 molar fraction of compound (loaded MLV) were delivered in a 160 μL DSC aluminum pan, and 60 μL of an equimolar DMPC MLV dispersion (empty MLV) was added. The pan was hermetically sealed, and the sample was submitted to the calorimetric analysis as reported under Permeation Kinetics Experiments.

RESULTS AND DISCUSSION

We used a DSC technique to study the effect of resveratrol (**1**) and two lipophilic resveratrol derivatives (**2** and **3**) on the thermotropic behavior of DMPC MLV employed as biomembrane models and to evaluate whether different substituents on the resveratrol backbone are related to eventual effects on the lipid phase as well as the solubility in the lipid phase, as previously done using other bioactive compounds (*31*, *35*−*39*). We prepared DMPC MLV in the presence of increasing molar fractions of the examined compounds, and the related calorimetric curves were compared with that of pure DMPC MLV and reported in **Figure 2**. A pretransition peak that is related to the tilt of the phospholipid acyl chains and a main calorimetric peak, associated with the phospholipid gel−liquid crystalline phase transition, related to the passage from an ordered to a disorded state of the lipid packing caused by the temperature increase, characterize the pure DMPC curve. Variations of calorimetric curves (i.e., shape and/or transition temperature) of liposomes prepared in the presence of increasing molar fractions of the examined compounds, with respect to the DMPC MLV one, indicate that an interaction between the DMPC and the compounds occurs. From an examination of the calorimetric curves, it appears to be evident that **1** (**Figure 2A**) causes the disappearance of the DMPC pretransition peak for all of the molar fractions used, indicating that it localizes in the polar region of the lipid bilayers. In addition, with increasing compound amount, the main peak gradually shifts toward lower temperature and broadens. The peak broadening indicates the decrease of the cooperativity of the main transition and the induction of disorder in the structured lipids (*40*). Starting from 0.09 molar fraction of resveratrol, a phase separation is also visible. The permethylated derivative **2** (**Figure 2B**) abolishes the pretransition peak (at molar fractions > 0.015) and causes the shift toward lower temperature and the broadening (for molar fraction ≥ 0.12) of the main peak, as its amount in the MLV aqueous dispersion increases. The peracetylated derivative **3** (**Figure 2C**) causes the disappearance of the pretransition peak but, differently from the other two compounds, it causes a very small shift of the main peak and the peak shape remains almost unchanged for all of the tested concentrations. The transition temperature variations obtained from these curves are reported in **Figure 3** (solid symbols) as $\Delta T/T°_m$ ($\Delta T = T_m - T°_m$, where $T°_m$ is the transition peak temperature of pure DMPC MLV and T_m is the transition peak temperature of DMPC MLV prepared in the presence of each compound) against the compounds' molar fractions present in the MLV aqueous dispersion. As stated, MLV were prepared in the presence of each compound at different molar fractions in the lipid aqueous dispersion with the possibility that they entirely localize in the lipid bilayers of the MLV or in part remain in the aqueous medium.

Nevertheless, the observed effects have been attributed to the compound present in the aqueous lipid dispersion and not really dissolved in the phospholipid membranes, and then it is worthwhile to determine the partition of each compound between aqueous and lipid phases to know the exact amount of compound present in the lipid phase of MLV. From our partition studies, 90.00% of **1**, 99.16% of **2**, and 99.00% of **3** were found

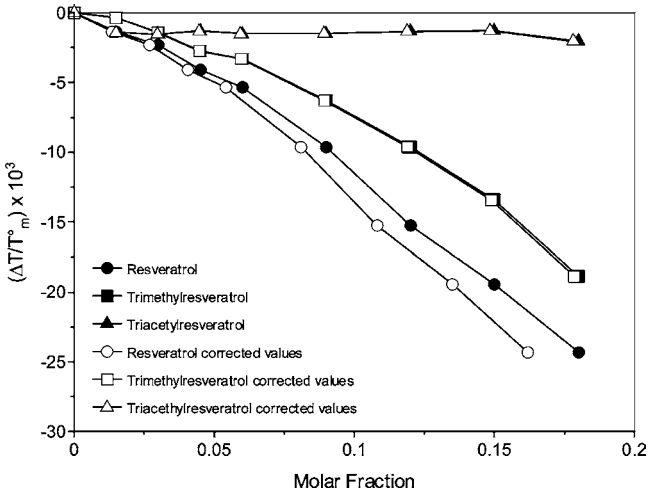

Figure 3. Transition temperature variations, as $\Delta T/T°_m$ ($\Delta T = T_m - T°_m$, where $T°_m$ is the transition peak temperature of pure DMPC MLV and T_m is the transition peak temperature of DMPC MLV prepared in the presence of compound), as a function of compound molar fractions in the MLV dispersion. Solid symbols represent the effect of the compound dispersed in the lipid aqueous dispersion; open symbols represent the effect of the real compound amounts present in the lipid ordered structure obtained after the partition coefficient correction of the solid symbols.

in the lipid phase. The consequence is that for the resveratrol the observed effect is exerted by an amount lower than that considered to be in the membrane. The partition coefficient results were used to modify the data previously obtained (solid symbols) to get the curves representing the effect of the real molar fraction of each compound present in the MLV (open symbols of **Figure 3**). Just for resveratrol the curve was deeply modified, indicating that the perturbing effect was exerted by a fraction of compound really present in the lipid bilayer, whereas the other two lipophilic compounds are well dissolved in the lipid matrix. These partition results suggest also the high affinity of **2** and **3** for the lipids, but the thermotropic behavior is due not only to the amount of the compound but also to its localization.

All of the tested compounds interact with DMPC MLV but in different ways. They cause the decrease of the transition temperature, with **1** exerting the highest effect and **3** the lowest. Moreover, the temperature decreases in a concentration-dependent way in the cases of **1** and **2**, whereas it decreases up to 0.015 molar fraction and remains constant for the remaining molar fractions when **3** is present. Resveratrol and trimethylresveratrol exert a strong interaction, decreasing the transition temperature and then increasing the phospholipid bilayer fluidization. Molar fractions being equal, **2** acts to a lesser extent than **1**. In the calorimetric curves we can see a unique main peak up to 0.06 molar fraction for **1** and 0.09 molar fraction for **2**, meaning that, up to these molar fractions, they uniformly localize in the bilayers. Then, a phase separation is seen at higher molar fraction, indicating, in terms of homogeneity, a nonuniform distribution of the compounds in the DMPC bilayers with the formation of "resveratrol-rich" and "resveratrol-poor" domains in the phospholipid bilayers for compound **1**, whereas **2** shows a not well-defined phase separation represented by an evident broadening of the calorimetric peak.

To reveal the ability of the studied compounds to be absorbed by the phospholipid membranes through an aqueous medium, we carried out a series of experiments leaving a fixed amount of DMPC MLV dispersion in contact with an exact amount (to have a 0.12 molar fraction) of each powdered compound for

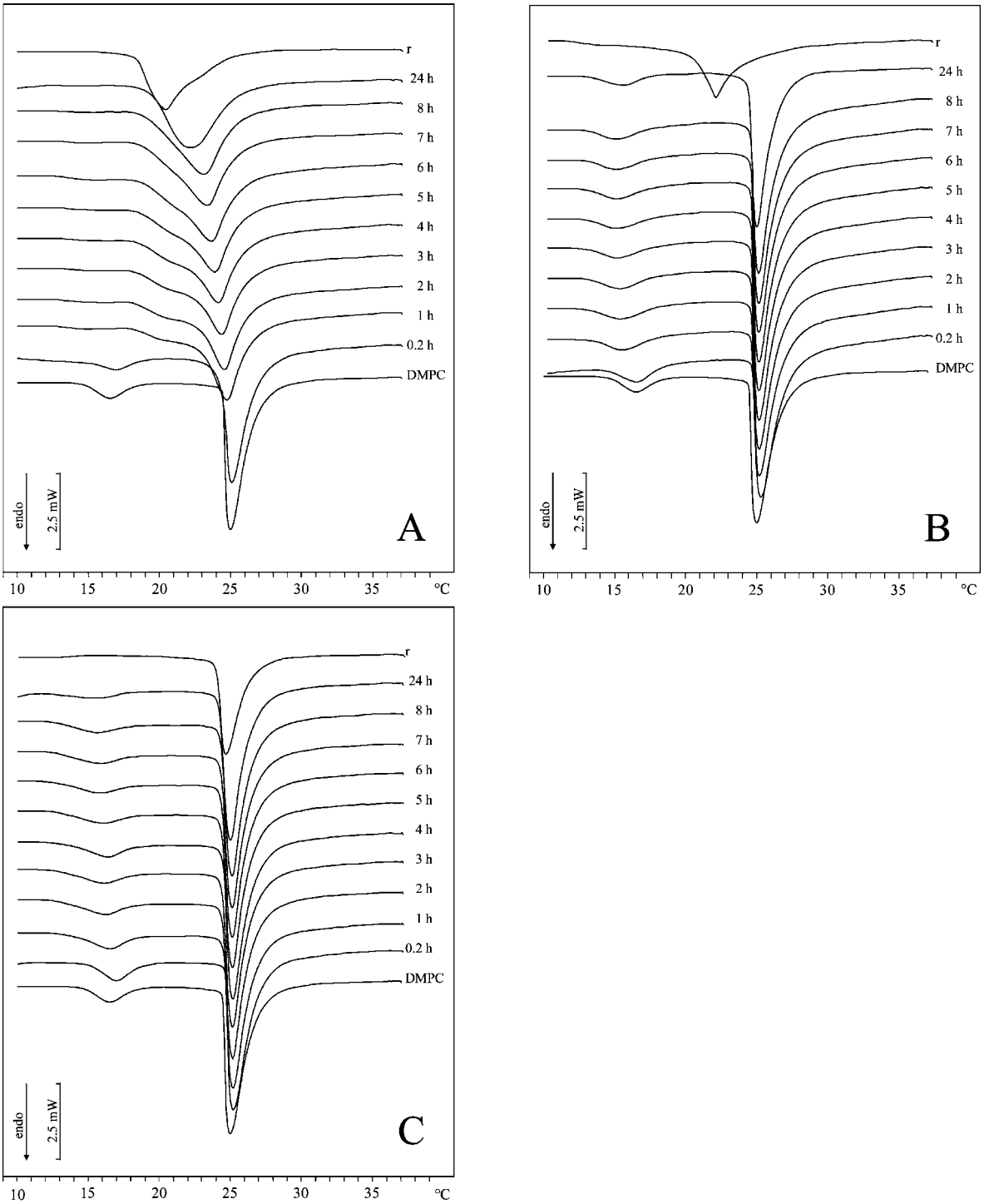

Figure 4. Calorimetric curves, in heating mode, of DMPC MLV left in contact with a 0.12 molar fraction of (**A**) resveratrol, (**B**) trimethylresveratrol, and (**C**) triacetylresveratrol at increasing incubation time. Curves *r* belong to DMPC MLV prepared in the presence of a 0.12 molar fraction of compound prepared as described under Liposome Preparation.

increasing incubation periods and recording at 1 h intervals the calorimetric curves, which are shown in **Figure 4**. This compound amount was chosen to have the highest calorimetric temperature shift associated with a well-defined DSC peak, which will permit the transfer of the compounds to the MLV to be followed. The curves are compared with that of pure DMPC MLV and that of DMPC MLV prepared in the presence of a 0.12 molar fraction of compound (curves *r*) prepared as described under Liposome Preparation. If the compound dissolves and migrates through the aqueous medium, reaching the MLV surface, and is able to interact with the phospholipid bilayers, we should observe a gradual variation of the calorimetric curve shape and position with respect to that of DMPC MLV and, at the end of the process, a similarity with the curve *r*. This means that a complete partition of the compound between lipid and aqueous phases has happened. Looking at the curves related to MLV/resveratrol permeation kinetics (**Figure 4A**), after 1 h of incubation, the complete disappearance of the pretransition peak is visible. Interesting features are seen in the main peak recorded after 1 h of incubation. It is constituted by two parts: a shoulder, at lower temperature, and a well-defined peak, at higher temperature. As the incubation period increases,

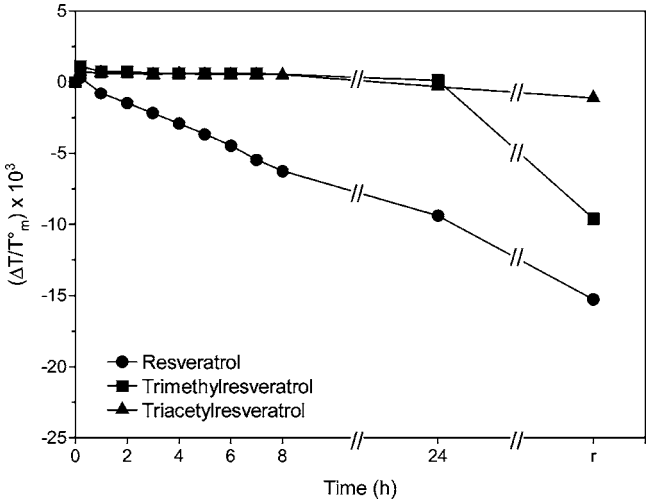

Figure 5. Transition temperature variations, as $\Delta T/T°_m$, of DMPC MLV left in contact with a 0.12 molar fraction of compound, as a function of incubation time. The *r* values represent the transition temperature variation of DMPC MLV prepared in the presence of a 0.12 molar fraction of compound as described under Liposome Preparation and can be considered as the maximum interaction between compound and MLV.

a reduction of the shoulder is caused by the gradual merging to the main peak that simultaneously shifts toward lower temperature. After 24 h, they merge in a unique, broad peak. On the contrary, curves from MLV/trimethylresveratrol (**Figure 4B**) and MLV/triacetylresveratrol (**Figure 4C**) permeation kinetics do not show any appreciable variation in the shape or in the transition temperature even after 24 h. The transition temperatures of all permeation kinetics calorimetric curves are reported in **Figure 5** as $\Delta T/T°_m$ against the incubation times. The *r* values represent the transition temperature variation of MLV prepared in the presence of 0.12 molar fraction of each compound (obtained by curves *r* in **Figure 4**) and are considered as the maximum obtainable interaction between MLV and the examined compounds. The lipophilic analogues **2** and **3** do not cause transition temperature variation, whereas resveratrol causes an evident decrease of the transition temperature variation. None of the lines reaches the value *r*, but that of resveratrol tends to it. Taking into account **Figure 5** together with the calorimetric curves in **Figure 4**, we can draw the following conclusions. Resveratrol dissolves in the aqueous medium (see the partition coefficient of resveratrol with respect to the other two compounds), reaches the MLV, and interacts with the bilayers. Initially, the compound is localized in the outer bilayer rather than in the inner ones as testified by the contemporaneous presence of the shoulder (that indicates bilayers containing a high molar fraction of compound) and of the main peak (that indicates bilayers containing a low amount of compound). Then it, gradually, transfers to the inner bilayers, as testified by the approaching and merging of shoulder and main peak. Compounds **2** and **3** do not seem to transfer to and interact with phospholipid bilayers, probably due to their highly hydrophobic nature, as it appears from the partition experiments, that do not allow them to dissolve in the aqueous medium and then to reach the MLV surface.

To verify if a lipophilic medium is more suitable for the compounds' absorption by the biomembrane model, transmembrane kinetics experiments were carried out leaving DMPC MLV (empty MLV) with DMPC MLV prepared in the presence of 0.12 molar fraction of each compound (loaded MLV) and the compounds' transfer from the loaded to the empty MLV was detected by DSC. The 0.12 molar fraction was chosen to have a well-defined DSC peak, which will change its shape during the compound transfer from loaded to empty MLV. Calorimetric curves are shown in **Figure 6**. Curve *r* belongs to MLV prepared in the presence of 0.06 molar fraction of compound and is used as a reference curve. In fact, if the compound passes from loaded MLV ($X = 0.12$) to empty MLV, loaded MLV will lose half of the compound, whereas empty MLV will enrich in the compound to reach a unique MLV population containing an intermediate molar fraction (0.06) of compound between the starting ones. With regard to the experiment with **1** (**Figure 6A**), the first calorimetric scan (curve 0.2 h) loses the pretransition peak and shows a broad main peak that is shifted to lower temperature, between the empty and loaded MLV peaks. No further significant shifts are seen in the successive curves, whereas the main peak broadening becomes less evident. For **2** (**Figure 6B**), the first calorimetric curve (at 0.2 h) shows three regions, a pretransition peak, a big shoulder at about 22 °C, and a main peak. Within the first hour of incubation, the pretransition peak disappears and the shoulder merges with the main peak that shifts toward lower temperature. As the incubation time increases, the main peak gradually sharpens. With regard to **3** (**Figure 6C**), with respect to the pure DMPC MLV curve, the pretransition peak disappearance at the first scan (0.2 h) and a small shift of the main peak toward lower temperature are seen. **Figure 7** groups the transmembrane kinetic temperature results, expressed as $\Delta T/T°_m$, against the incubation times. The *r* values represent the transition temperature variations of MLV prepared in the presence of a 0.06 molar fraction of compound (curves *r* in **Figure 6**). A decrease in the transition temperature is obtained for the three examined compounds; moreover, the decrease is visible during the first hour of incubation, and the values *r* are always reached, meaning a complete and fast transfer of compounds from loaded to empty MLV until a concentration equilibrium is obtained with all of the MLV containing the same amount of compound (0.06 molar fraction).

On the basis of the results reported above, first evidence is that all of the examined compounds interact with the biomembrane model. From our partition results, which indicate resveratrol as the most hydrophilic compound and trimethyl- and triacetylresveratrol possessing comparable hydrophobicity, one should expect resveratrol to exhibit a weaker interaction with the phospholipid bilayer with respect to trimethyl- and triacetylresveratrol. Nevertheless, the highest interaction is exhibited by **1** and the lowest by **3**. An interaction of all the compounds with the phospholipid headgroups is suggested by the disappearance of the pretransition peak (*40*). It seems reasonable that resveratrol, due to its hydroxy groups, localizes between the phospholipid headgroups, forming hydrogen bonds; as a consequence, the phospholipid molecules are pushed away and the ordered phospholipid structure is strongly destabilized. This hypothesis finds a further justification in the pronounced decrease of the transition temperature. The less polar compound **2** is probably localized next to the headgroups protruding toward the acylic chains and consequently weakening the chain−chain hydrophobic interactions. Compound **3** should localize next to the headgroups at the interface phospholipid/aqueous medium without marked modification on the biomembrane model phospholipidic structure.

Further considerations arise from the results of the kinetic experiments. The first one, where the ability of each compound to pass the aqueous medium to reach the MLV surface being taken up by the phospholipid bilayers, shows that only resveratrol, in a free form, is effectively taken up by the biomembrane

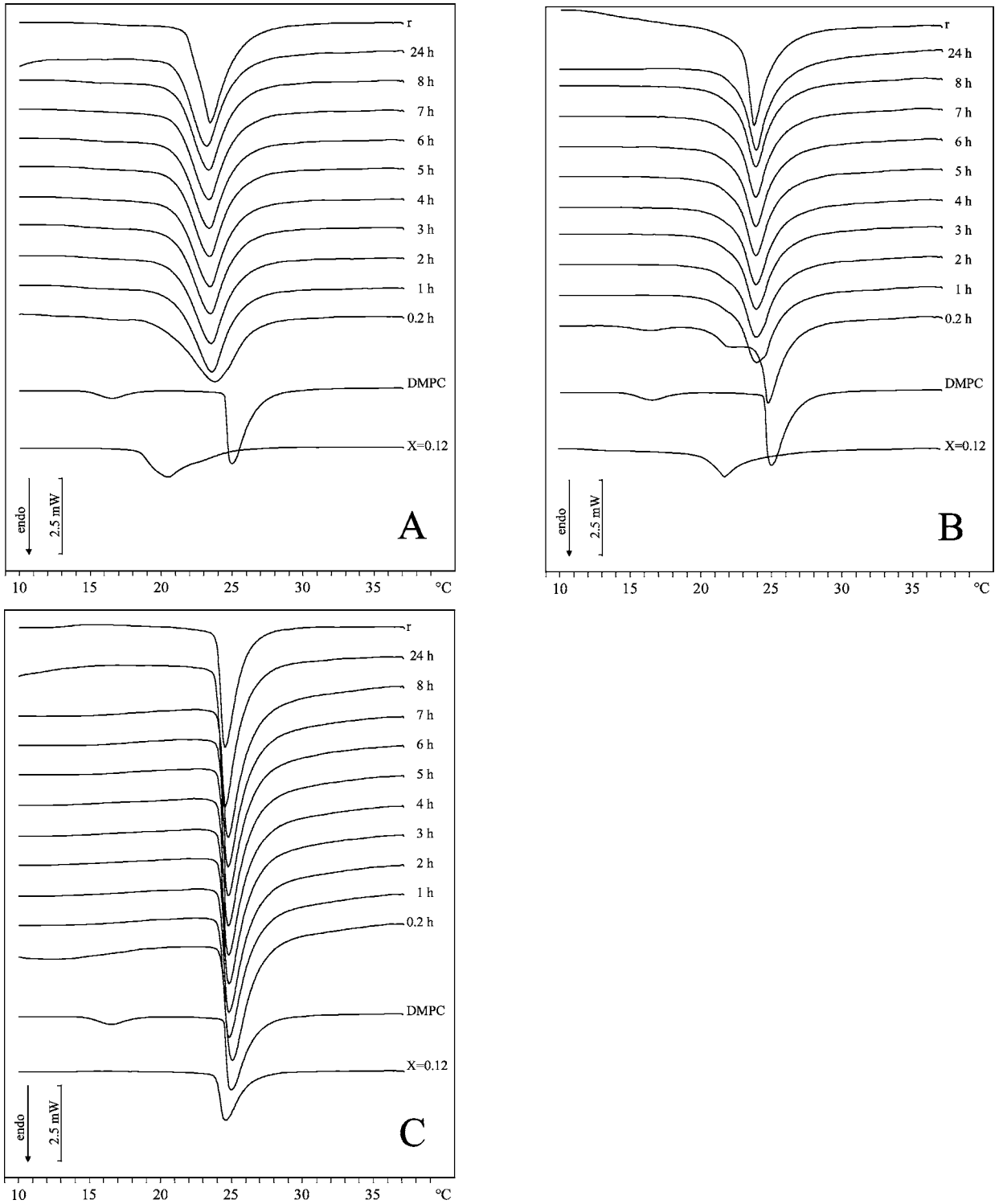

Figure 6. Calorimetric curves, in heating mode, of DMPC MLV left in contact with an equimolar amount of DMPC MLV prepared in the presence of a 0.12 molar fraction of (**A**) resveratrol, (**B**) trimethylresveratrol, and (**C**) triacetylresveratrol at increasing incubation time. Curves *r* belong to DMPC MLV prepared in the presence of a 0.06 molar fraction of compound as described under Liposome Preparation.

model, whereas uptake of **2** and **3** is very poor. The second one was designed to mimic the uptake of the compounds in a medium more similar to the physiological fluids: we evaluated the ability of resveratrol and its lipophilic analogues to move from the phospholipidic bulk to the biomembrane models. In this situation, the three compounds show a more similar behavior: in particular, the profile obtained for **2** strongly resembles that of **1**, thus suggesting that the higher lipophilicity of the tri-*O*-methylresveratrol, although reducing its mobility in an aqueous medium, does not significantly block its uptake by biomembranes.

As a first conclusion, this work corroborates the evidence about the important role of resveratrol as a component of food and beverages, which may impart beneficial effects to human health, indicating that this compound may be effectively taken up by cell membranes from aqueous media, notwithstanding its partly hydrophilic moieties. With regard to its lipophilic analogues **2** and **3**, although further study is required to obtain

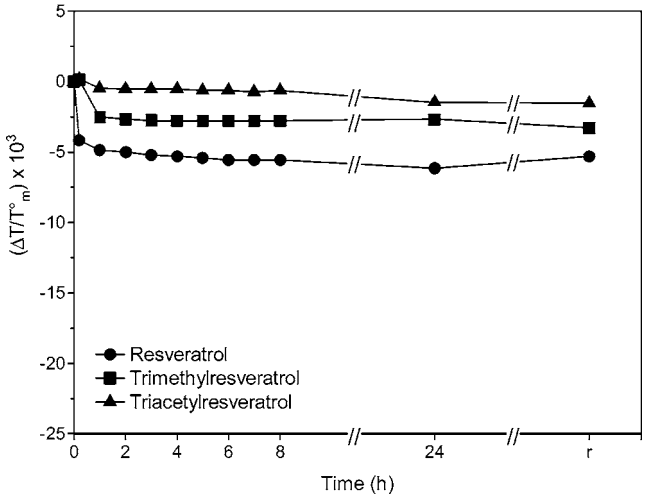

Figure 7. Transition temperature variations, as $\Delta T/T°_m$, of a fixed amount of pure DMPC MLV left in the presence of equimolar DMPC MLV prepared in the presence of a 0.12 molar fraction of compounds, as a function of incubation time. The *r* values represent the transition temperature variation of DMPC MLV prepared in the presence of a 0.06 molar fraction of compound prepared as described under Liposome Preparation and can be considered as the maximum interaction between compound and MLV.

unambiguous conclusion, it is worth noting that also these compounds appear able, in due conditions, to be effectively taken up by biomembranes; due to the more potent antiproliferative activity reported for **2** with respect to resveratrol (*21*, *22*), this may be of particular interest in the future design of resveratrol analogues as cancer chemopreventive agents or adjuvant of current anticancer drugs.

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