JOURNAL OF NATURAL PRODUCTS

ARTICLE

pubs.acs.org/jnp

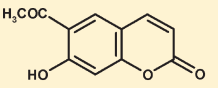
Evaluation of the Interaction of Coumarins with Biomembrane Models Studied by Differential Scanning Calorimetry and Langmuir−Blodgett Techniques

Maria Grazia Sarpietro, Maria Chiara Giuffrida, Sara Ottimo, Dorotea Micieli, and Francesco Castelli*

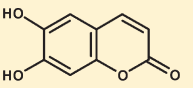
Dipartimento di Scienze del Farmaco, Università di Catania, Viale Andrea Doria 6, 95125 Catania, Italy

Supporting Information

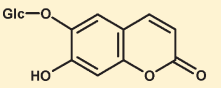
ABSTRACT: Three coumarins, scopoletin (**1**), esculetin (**2**), and esculin (**3**), were investigated by differential scanning calorimetry and Langmuir−Blodgett techniques to gain information about the interaction of these compounds with cellular membranes. Phospholipids assembled as multilamellar vesicles or monolayers (at the air−water interface) were used as biomembrane models. Differential scanning calorimetry was employed to study the interaction of these coumarins with multilamellar vesicles and to evaluate their absorption by multilamellar vesicles. These experiments indicated that **1**−**3** interact in this manner to different extents. The Langmuir−Blodgett technique was used to study the effect of these coumarins on the organization of phospholipids assembled as a monolayer. The data obtained were in agreement with those obtained in the calorimetric experiments.



Scopoletin (**1**)



Esculetin (**2**)



Esculin (**3**)

Coumarins comprise a very large class of compounds found throughout the plant kingdom,[1−3] especially in the families Rutaceae and Umbelliferae.[4] The fact that they are in such a wide range of plants appears to be related to their ability to act as phytoalexins, which are antimicrobial compounds representing an important mechanism of plants to defend themselves against fungal pathogens.[5] Coumarins belong to a group of compounds known as benzopyrans, which consist of a benzene ring joined to a pyrone.[6] Naturally occurring coumarins, derived from 1-benzopyran-2-pyrone, may be present in the free or glycoside form.[7]

Following oral administration, the parent compound, coumarin, is rapidly adsorbed from the gastrointestinal tract and is distributed throughout the body. Pharmacokinetic studies in humans have demonstrated that coumarin is completely adsorbed after oral administration and extensively metabolized by the liver in the first pass, with only between 2% and 6% reaching the systemic circulation intact.[4] Coumarins have a variety of biological effects including analgesic,[8] anti-inflammatory,[9] antimicrobial, antioxidant,[10] antitumor-promoting,[11] and vasodilatatory activities.

In the present study, interactions between the coumarins scopoletin (7-hydroxy-5-methoxycoumarin, **1**), esculetin (6,7-dihydroxycoumarin, **2**), and esculin (6,7-dihydroxycoumarin 6-*O*-β-D-glucopyranoside, **3**) with dimyristoylphosphatidylcholine (DMPC) were investigated by differential scanning calorimetry and Langmuir−Blodgett techniques to gain information about the interaction of these coumarins with cell membranes. DMPC assembled as multilamellar vesicles (MLVs) or monolayers (at the air−water interface) was used as biomembrane models.

Differential scanning calorimetry is a nonperturbative technique largely employed to detect the effects exerted by biomolecules on the lipid bilayers of a cell-like membrane in the processes of entrapment and release inside lipid vesicles.[12−14] When the temperature is increased, MLVs undergo a sharp phase transition from an ordered gel-like structure (L_β) to a disordered fluid-like structure (L_α).[15−18] The differential scanning calorimetry technique can detect such a phase change. The presence of molecules dissolved in the ordered lipid bilayer can cause, depending on their structural features, significant variations in the thermodynamic parameters associated with the lipid phase transition, such as the transition temperature (T_m) and enthalpy changes (ΔH).[19−21] The amplitude of the effect depends on the amount of the chemical dissolved in the lipid structure and on the structural differences of the compounds able to modify the lipophilic/hydrophilic balance.[18,20,22−25]

Langmuir−Blodgett techniques are used to study the interactions between bioactive compounds and phospholipids. Monolayers are an excellent model for studying two-dimensional ordering, with two thermodynamical variables, temperature and pressure, being readily controlled.[26−29] The film-balance method enables phase diagrams of phospholipids to be obtained; these are generally in the form of surface pressure/mean molecular area isotherm curves. The phospholipids are spread over an aqueous subphase, providing monomolecular distribution and the subsequent variation of the available area per molecule. Applying compression on the monolayer, the surface distribution of the molecules changes, and they are forced to go from a "gaseous" or "liquid-expanded" phase at low density to a "liquid-condensed" phase at a higher density and, successively, to a "solid-condensed" phase.[30−33] The results obtained can give indications of the

Received: November 22, 2010
Published: March 18, 2011

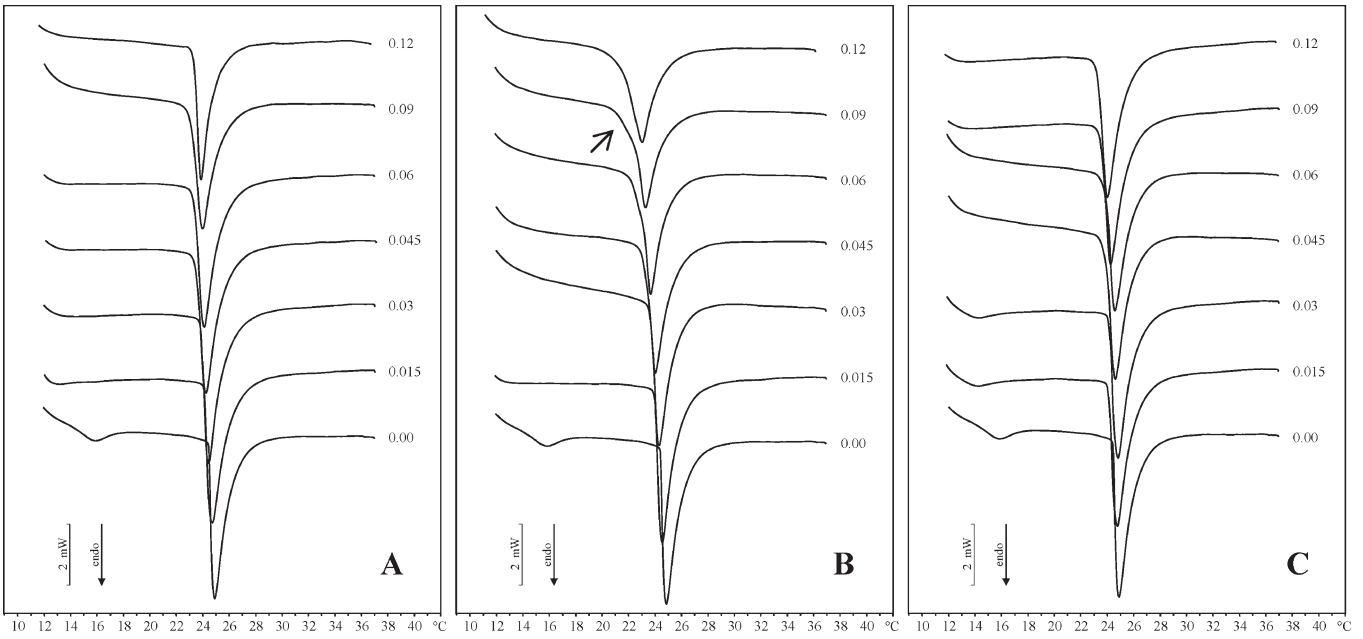
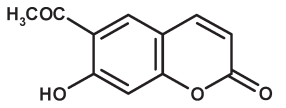
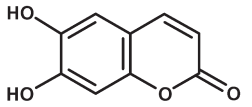


Figure 1. Calorimetric curves, in heating mode, of MLVs prepared in the presence of increasing molar fractions of (A) scopoletin (**1**), (B) esculetin (**2**), and (C) esculin (**3**).

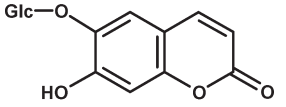
ability of a compound to dissolve in the phospholipid layers used as biomembrane models.



1



2



3

RESULTS AND DISCUSSION

Differential Scanning Calorimetry Analysis. MLVs were prepared in the presence of increasing molar fractions of the studied coumarins, and the related calorimetric curves were compared with that of MLVs prepared in the absence of the compounds evaluated (Figure 1). The calorimetric curve of unloaded MLVs is characterized by two endothermic peaks: a pretransition peak, at about 16.5 °C, associated with the hydrophobic chain tilt, and a main peak, at 24.4 °C, related to the transition from an ordered gel state to a disordered liquid crystalline state.[34]

Variations of the calorimetric curves (i.e., peak shape and/or transition temperature) of MLVs prepared in the presence of increasing molar fractions of the compounds examined can indicate that an interaction between the DMPC and these substances occurred. Scopoletin (**1**) (Figure 1A) caused the disappearance of the pretransition peak already at the lowest molar fraction (0.015). From an examination of the calorimetric curves obtained, it was evident that with increasing compound amount the main peak gradually shifted toward the lower temperature and broadened. In the presence of increasing molar fractions of esculetin (**2**) (Figure 1B), the pretransition peak disappeared, while the main peak shifted toward lower temperature and broadened. At 0.09 molar fraction, a phase separation appeared (as indicated by an arrow in the figure); such a phenomenon indicates a loss of homogeneity of the bilayers, i.e., an inconsistent distribution of the compound within the DMPC bilayers, with the formation of "compound-rich" and "compound-poor" domains in the vesicles. Esculin (**3**) (Figure 1C) caused slight shifts of the main peak toward lower temperatures and the gradual disappearance of the pretransition peak.

The transition temperature changes observed in these calorimetric curves are reported in Figure 2 as $\Delta T/T^0_m$ ($\Delta T = T_m - T^0_m$, where T^0_m is the transition peak temperature of pure DMPC MLVs and T_m is the transition peak temperature of MLVs prepared in the presence of each coumarin) against the molar fraction of the coumarin present in the MLVs aqueous dispersion.

All of the coumarins examined caused the decrease of the transition temperature with increased molar fraction. The results show that compound **2** caused the greatest variation of thermotropic parameters, while compound **3** destabilized the phospholipidic bilayer least. Scopoletin (**1**) showed an intermediate behavior. The observed transition temperature decrease indicates a membrane destabilization due to the insertion of these test compounds between the phospholipid. This destabilization is greater for scopoletin (**1**) and esculetin (**2**) probably because of their greater lipophilicity than esculin (**3**).

In Figure 3, the ΔH variations are reported as $\Delta\Delta H/\Delta H^0$ ($\Delta\Delta H = \Delta H - \Delta H^0$, where ΔH^0 is the enthalpy variation of pure DMPC MLVs and ΔH is the enthalpy variation of MLVs

dx.doi.org/10.1021/np100850u |J. Nat. Prod. 2011, 74, 790–795

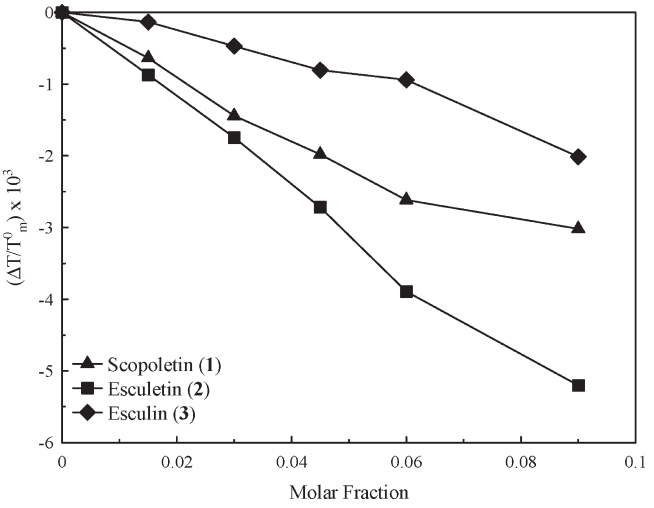


Figure 2. Transition temperature variations, as $\Delta T/T^0_m$ ($\Delta T = T_m - T^0_m$ where T^0_m is the transition temperature of MLVs and T_m is the transition temperature of MLVs prepared in presence of compound), as a function of compound molar fraction in the MLVs aqueous dispersion.

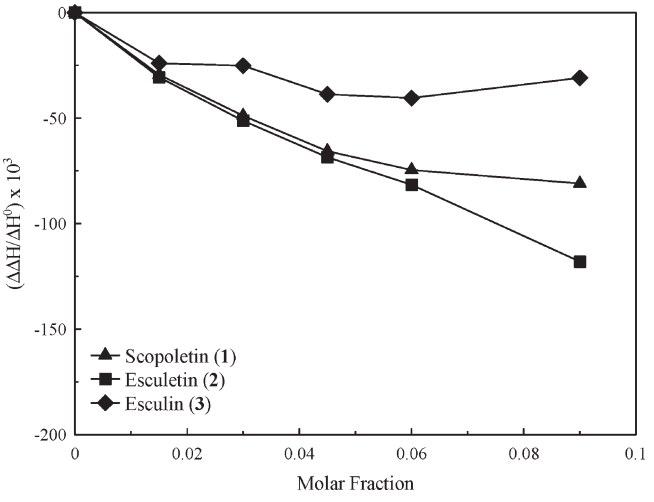


Figure 3. ΔH variations, as $\Delta\Delta H/\Delta H^0$ ($\Delta\Delta H = \Delta H - \Delta H^0$ where ΔH^0 is the enthalpy variation of MLVs and ΔH is the enthalpy variation of MLVs prepared in the presence of each compound), as a function of compound molar fraction present in the MLVs aqueous dispersion.

prepared in the presence of each compound) against the molar fraction of the compounds present in the MLVs dispersion. All three coumarins caused a decrease of the enthalpy variation, in the order **2** > **1** > **3**. This behavior is similar to that observed in the transition temperature variation.

The decrease of the T_m and ΔH values leads to the conclusion that all three coumarins interact with phospholipid bilayers, causing their fluidization. Esculin (**3**) stays close to the polar head of the phospholipids, whereas scopoletin (**1**) and esculetin (**2**) remain in proximity to the polar head but span toward the hydrophobic chains of the phospholipids.

Further permeation experiments were carried out leaving the MLVs aqueous suspension in contact with a fixed amount (0.09 molar fraction with respect to DMPC) of each coumarin, for increasing incubation times. A 0.09 molar fraction was used in the experiments, and when MLVs were prepared in the presence of

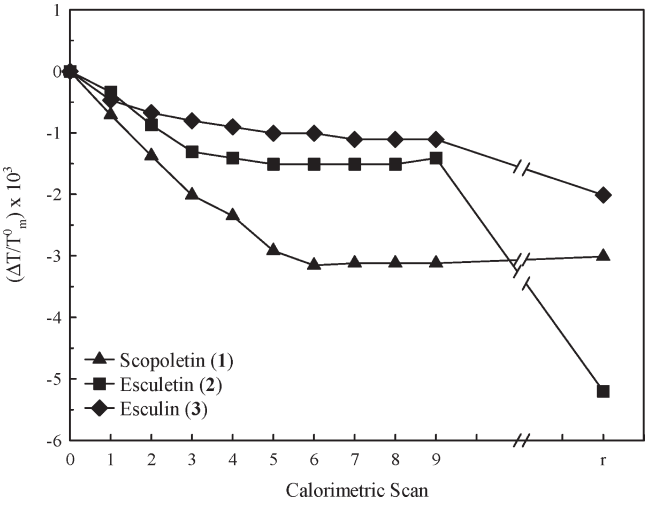


Figure 4. Transition temperature variations, as $\Delta T/T^0_m$, of MLVs left in contact with coumarins at 0.09 molar fraction as a function of the calorimetric scans. The value *r* belongs to MLVs prepared in the presence of coumarins at 0.09 molar fraction.

different molar fractions of coumarins, they gave well-defined transitions. On the other hand, lower molar fractions did not really give appreciable shifts in the T_m to follow the permeation processes. The calorimetric curves obtained were compared with that of MLVs as well as of MLVs prepared in the presence of a 0.09 molar fraction of each compound. Each scan (calorimetric curves) was recorded at intervals of 1 h (Figure S1, Supporting Information). These experiments permit an assessment of the ability of the compounds to dissolve in the aqueous medium and reach and cross the lipid bilayers of MLVs. The eventual changes of the thermotropic parameters of pure MLVs can be related to the amount of each coumarin that penetrated into the bilayers over time. If the complete transfer and miscibility of the compounds with MLVs occurred, a calorimetric curve would be measured similar to that obtained when the vesicles were formed in the presence of a 0.09 molar fraction of the compound (curve *r*).

Scopoletin (**1**) caused the disappearance of the pretransition peak after the first scan, and with the increase of the incubation time, the main peak gradually shifted toward lower temperatures, reaching curve *r*. Also, coumarins **2** and **3** caused the disappearance of the pretransition peak after the first scan but provoked only a small shift of the main peak to lower temperature, and curve *r* was not reached.

Variations of the transition temperature as a function of the calorimetric scans are shown in Figure 4. In this figure, the *r* values are used as reference and represent the value that should be obtained if the compound is completely taken up by MLVs. All three coumarins caused decreases of the transition temperature. However, some differences were clearly visible since **2** and **3** provoked only small decreases of T_m, whereas **1** provoked a strong and complete decrease of T_m, which reached the value *r*. These results indicate that esculin (**3**) and esculetin (**2**), due to their more hydrophilic (or less hydrophobic) character than scopoletin, can dissolve in the aqueous medium and come in contact with the MLV surface, but they are slowly and incompletely taken up by MLVs. They could remain anchored to the MLVs surface through hydrogen bonding with the polar head of the phospholipid. Scopoletin (**1**), in being less hydrophilic and in

having more affinity for the hydrophobic environment of the bilayer, was completely taken up by the MLVs.

Surface Tension Measurements. Molecular interactions between coumarins **1**−**3** and DMPC were also investigated by applying the Langmuir−Blodgett technique, which uses the lipid monolayer at the air−water interface. The advantage of such a simple model membrane is that measurement of the pressure as a function of the surface area isotherms can be carried out conveniently and analyzed in various environmental conditions such as pH and temperature and then referred to the corresponding properties of bilayer vesicles. The molecular area/surface pressure isotherms (Figure S2, Supporting Information) were recorded at pH 4 and 37 °C (above the transition temperature of the phospholipid) in order to affect a DMPC monolayer behavior as a fluid membrane. DMPC shows a gaseous state from 120 to 115 Å^2 and a liquid expanded state from molecular areas lower than 115 Å^2.

Compounds **1**−**3** do not form monolayers. For all three coumarins, the isotherms of the mixed monolayers at molar fractions between 0.024 and 0.09 of coumarins are very similar to that of DMPC. At higher molar fractions, especially 0.5 and 0.75, the isotherms shifted toward smaller areas per molecule.

Interesting information can be found by reporting, at different surface pressures, the molecular area as a function of the molar fraction of the compound present in the monolayer (Figure 5). The mean molecular area of a two-component monolayer can be calculated by $A = A_1X_1 + (1 - X_1)A_2$, where A is the mean molecular area, X_1 is the molar fraction of the component 1, and A_1 and A_2 are the areas of the two pure components (phospholipids and drug in this case) at the same surface pressure. Reporting in a graph A/X_1, a straight line is obtained if the monolayer components are completely immiscible or possess an ideal miscibility.[35] Any deviation from the straight line indicates an interaction between the molecules. In particular, a positive deviation indicates that repulsive interactions occur, whereas a negative deviation is related to the occurrence of attractive forces between the molecules. The isotherms were measured at specific values of surface pressure, namely, 10, 20, and 30 mN/m. Scopoletin (**1**) (Figure 5A), at all molar fractions and all surface pressure values considered, caused a positive deviation, indicating repulsive interactions taking place among the molecules of the monolayer. Esculetin (**2**) (Figure 5B) also showed positive deviations for all the surface pressure values taken into account. Esculin (**3**) (Figure 5C) at 10, 20, and 30 mN/m, produced positive deviations from the 0.048 molar fraction, whereas the experimental values coincided with the ideal values at 0.024 molar fraction. This means that, at a low molar fraction of **3**, the monolayer behaved in an ideal way, but above a 0.048 molar fraction, the molecules repelled one another.

In conclusion, the present studies indicate that the three coumarins, scopoletin (**1**), esculetin (**2**), and esculin (**3**), interact with a biomembrane model constituted by the multilamellar vesicles of dimyristoylphosphatidylcholine. They caused decreases of transition temperature as well as of the enthalpy variation and then destabilization and fluidization of the phospholipid bilayer. The same fluidization was observed when the monolayer was employed as a biomembrane model. The results of the permeation experiments provide evidence that the absorptions of coumarins **1**−**3** are strictly dependent upon their structure. These results give information on the interaction and absorption of coumarins **1**−**3** by the biological membranes and on their mechanism of action.

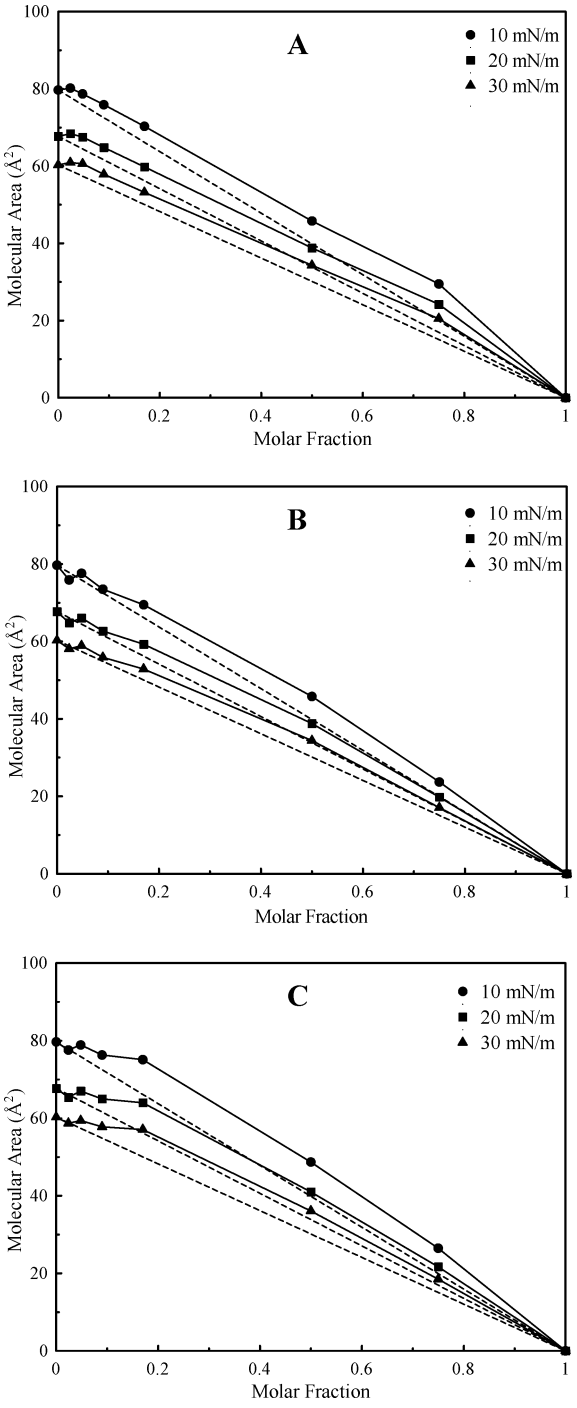


Figure 5. Molecular area of the mixed monolayers of DMPC and (A) scopoletin (**1**), (B) esculetin (**2**), and (C) esculin (**3**) at the air−water interface plotted as a function of the compound molar fraction, at 10, 20, and 30 mN/m.

EXPERIMENTAL SECTION

Materials. Scopoletin (**1**) (purity ≥ 99%), esculetin (**2**) (purity = 98%), and esculin (**3**) (purity ≥ 98%) were purchased from Sigma Aldrich. Synthetic L-α-dimyristoylphosphatidylcholine was obtained from Genzyme Pharmaceuticals (Liestal, Switzerland) and was chromatographically pure as assessed by two-dimensional thin-layer chromatography.[36] A 50 mM Tris buffer solution, adjusted to pH 4, was used for liposome production. A 5 mM Tris (pH 4) buffer solution in ultrapure Millipore

dx.doi.org/10.1021/np100850u |J. Nat. Prod. 2011, 74, 790–795

water with resistivity of 18.2 MΩ cm was used as subphase for the Langmuir−Blodgett experiments conducted. An acidic pH (pH 4) was chosen considering that pharmacokinetic studies in humans have demonstrated that coumarin is completely absorbed from the gastrointestinal tract after oral administration.[4] Although the gastric pH is ≤3, pH 4.0 was used, as this is the lowest pH at which the DMPC MLVs packing is unmodified.[37]

Differential Scanning Calorimetry Analysis. A Mettler Toledo STAR^e system equipped with a DSC-822^e calorimetric cell and Mettler TA-STAR^e software was used. 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, using indium, stearic acid, and cyclohexane, by following the procedure of the DSC 822 Mettler TA STAR^e instrument.

Multilamellar Vesicle Preparation. Stock solutions of DMPC and coumarins **1**−**3** were prepared in $CHCl_3$−MeOH (1:1). Aliquots of DMPC solution were distributed in glass tubes in order to afford 0.01032 mmol of DMPC in all tubes; aliquots of solutions of coumarins **1**−**3** were added to have a defined molar fraction of the examined compounds with respect to the phospholipid (0.00; 0.015; 0.03; 0.045; 0.06; 0.09). Solvent was removed under nitrogen flow, and the resulting films were lyophilized to eliminate solvent residues. A 168 μL amount of a 50 mM Tris buffer (pH 4.0) was added to the films, and the samples were heated at 37 °C (above the gel-to-liquid crystalline phase transition of the phospholipid) for 1 min and successively shaken for 1 min; this procedure was repeated three times, and samples were then kept at 37 °C for 1 h to homogenize the liposomes.

Coumarin/MLV Interaction. A 120 μL aliquot of each MLV suspension (corresponding to 0.007375 mmol of DMPC) was transferred to a 160 μL aluminum pan, hermetically sealed, and submitted, at least four times to check reproducibility, to the following calorimetric procedure: (1) a heating scan between 5 and 37 °C at 2 °C/min; (2) a cooling scan between 37 and 5 °C at 4 °C/min.

After the calorimetric analysis, aliquots of all samples were withdrawn from the pans and used to determine the exact amount of phospholipids present in each sample by using a phosphorus assay.[36]

Permeation Experiments. An exact amount (corresponding to a 0.09 molar fraction of compound with respect to the phospholipid) of the bulk tested compounds was weighted in the bottom of a calorimetric aluminum pan, and 120 μL (0.007375 mmol) of the MLV aqueous dispersion was added. The aluminum pan was hermetically sealed, and the sample was submitted to the following calorimetric scans: (1) a heating scan between 5 and 37 °C, at a rate of 2 °C/min, to detect any interaction between each compound and MLVs, during a first heating of the sample, bringing the MLVs to a disordered state; (2) an isothermal incubation period of 1 h at 37 °C in the calorimetric cell, to allow the compound to dissolve in the aqueous medium, reach the MLVs surface, penetrate the phospholipid bilayers, and interact with them; (3) a cooling scan between 37 and 5 °C, at a rate of 4 °C, to bring the phospholipid system back to the ordered state. The procedure was run at least eight times.

Surface Tension Measurements. Film balance measurements were performed using a KSV minitrough apparatus that included a 24225 mm^2 (available area) Teflon trough, two mechanically mobile coupled hydrophilic barriers (in Delrin), a platinum surface pressure sensor, and operating software.

A 5 mM Tris (pH 4) solution in ultrapure Millipore water with resistivity of 18.2 MΩ cm was used as subphase. DMPC and coumarins **1**−**3** were dissolved in chloroform in order to obtain equimolar solutions (0.00103 mmol/mL). Mixed DMPC/compound solutions were prepared successively to obtain the following molar fractions with respect to the phospholipid for each compound: 0.024, 0.048, 0.09, 0.17, 0.50, and 0.75. A Hamilton syringe was cleaned three times with chloroform and then with the examined solutions. Aliquots of 30 μL of the mixed solutions as well as of the pure components were spread drop by drop over the aqueous subphase. After 10 min, during which solvent evaporation occurred, the floating films were compressed linearly using the mobile barriers at a rate of 10 mm/min. Surface pressure versus molecular area isotherms were recorded by film balance measurements. Before spreading the sample, subphase purity was checked by closing and opening the barriers and ensuring that surface pressure readings did not differ by more than ±0.1 mN/m so that no impurities were present on its surface. The KSV system was checked using stearic acid.[35] The experiments were performed at a subphase temperature of 37 °C (a temperature above the DMPC phase transition, at which the phospholipid is in a disordered state). The temperature was kept constant by a thermostated circulating water bath. The effect of a foreign compound dissolved in the ordered lipid structure can then be amplified, giving more information on its packing in the lipid matrix and on the consequent loss of lipid cooperativity. Each experiment was repeated at least three times to obtain reproducible results

■ ASSOCIATED CONTENT

Supporting Information. Calorimetric curves of MLV left in contact with scopoletin (**1**), aesculetin (**2**), and esculin (**3**), at increasing incubation times. Surface pressure/molecular area isotherms of DMPC and scopoletin (**1**), DMPC and aesculetin (**2**), and DMPC and esculin (**3**) mixed monolayers at the air−water interface. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +390957385099. Fax: +39095580138. E-mail: fcastelli@dipchi.unict.it.

■ ACKNOWLEDGMENT

The authors thank the MIUR (Fondi di Ateneo) for financial support.

■ REFERENCES

(1) Egan, D.; O'Kennedy, R.; Moran, E.; Cox, D.; Prosser, E.; Thornes, R. D. *Drug Metab. Rev.* **1990**, *22*, 503–529.
(2) Egan, D.; O'Kennedy, R. *J. Chromatogr. B* **1992**, *582*, 137–143.
(3) Finn, G.; Kenealy, E.; Creaven, B.; Egan, D. *Cancer Lett.* **2002**, *183*, 61–68.
(4) Keating, G.; O'Kennedy, R. In *Coumarins. Biology, Applications and Mode of Action*; O'Kennedy, R.; Thornes, R. D., Eds.; John Wiley & Sons: New York, 1997; pp 23−66.
(5) Grayer, R. J.; Kokubun, T. *Phytochemistry* **2001**, *56*, 253–263.
(6) Lake, B. *Food Chem. Toxicol.* **1999**, *37*, 423–453.
(7) Dewick, P. M. In *Medicinal Natural Products*; John Wiley & Sons, Ltd., 2002; pp 121−166.
(8) Soine, T. O. *J. Pharm. Sci.* **1964**, *53*, 231–264.
(9) Chen, Y. F.; Tsai, H. Y.; Wu, T. S. *Planta Med.* **1995**, *61*, 2–8.
(10) Shaw, C. Y.; Chen, C. H.; Hsu, C. C.; Chen, C. C.; Tsai, Y. C. *Phytother. Res.* **2003**, *17*, 823–825.
(11) Fujioka, T.; Furumi, K.; Fujii, H.; Okabe, H.; Mihashi, K.; Naksno, Y.; Matsunaga, H.; Katano, M.; Mori, M. *Chem. Pharm. Bull.* **1999**, *47*, 96–100.
(12) Castelli, F.; Sarpietro, M. G.; Messina, C.; De Lazzari, A.; Di Rosa, D.; Giannetto, A. *Eur. J. Pharm. Sci.* **2003**, *19*, 237–243.
(13) Castelli, F.; Raudino, A.; Fresta, M. *J. Colloid Interface Sci.* **2005**, *285*, 110–117.

(14) Sarpietro, M. G.; Caruso, S.; Librando, V.; Castelli, F. *Mol. Nutr. Food Res.* **2005**, *49*, 944–949.
(15) Lee, A. G. *Biochim. Biophys. Acta* **1991**, *57*, 341–362.
(16) Houslay, M. D.; Stanley, K. K. In *Dynamics of Biological Membranes: Influence on Synthesis, Structure and Function*; John Wiley: New York, 1982; pp 40−91.
(17) Tenchov, B. *Chem. Phys. Lipids* **1991**, *57*, 165–177.
(18) Marsh, D. In *Nonmedical Applications of Liposomes*; Barenholz, Y.; Lasic, D. D., Eds.; CRC Press: Boca Raton, FL, 1996; Vol. II, pp 1−16.
(19) Jain, M. K.; Wu, N. M. *J. Membr. Biol.* **1977**, *34*, 151–201.
(20) Jain, M. K. In *Introduction to Biological Membranes*; Jain, M. K., Ed.; John Wiley & Sons: New York, 1988; pp 122−165.
(21) Castelli, F.; Sarpietro, M. G.; Micieli, D.; Stella, B.; Rocco, F.; Cattel, L. *J. Colloid Interface Sci.* **2007**, *316*, 43–52.
(22) Lohner, K. *Chem. Phys. Lipids* **1991**, *57*, 341–362.
(23) Silvius, J. R. *Chem. Phys. Lipids* **1991**, *57*, 241–252.
(24) Bach, D. In *Biomembrane Structure and Function*; Chapman, D., Ed.; Macmillan: London, 1984; pp 1−41.
(25) Huang, C.; Li, S. *Biochim. Biophys. Acta* **1999**, *1422*, 273–307.
(26) Brockman, H. *Curr. Opin. Struct. Biol.* **1999**, *9*, 438–443.
(27) Kaganer, V. M.; Möhwald, H.; Dutta, P. *Rev. Modern Phys.* **1999**, *71*, 779–819.
(28) Brezesinski, G.; Möhwald, H. *Adv. Colloid Interface Sci.* **2003**, *100−102*, 563–584.
(29) Castelli, F.; Sarpietro, M. G.; Rocco, F.; Ceruti, M.; Cattel, L. *J. Colloid Interface Sci.* **2007**, *313*, 363–368.
(30) Möhwald, H. *Annu. Rev. Phys. Chem.* **1990**, *41*, 441–476.
(31) Prieto, I.; Camacho, L.; Martin, M. T.; Mobius, D. *Langmuir* **1998**, *14*, 1853–1860.
(32) Krasteva, N.; Vollhardt, D.; Brezesinki, G. *J. Phys. Chem. B* **2000**, *104*, 8704–8711.
(33) Vollhardt, D.; Fainerman, V. B. *Adv. Colloid Interface Sci.* **2000**, *86*, 103–151.
(34) Walde, P. In *ASP Encyclopedia of Nanoscience and Nanotechnology*; Nalwa, H. S., Ed.; American Scientific Publishers: Stevenson Ranch, CA, 2004; pp 43−79.
(35) Gaines, G. L., Jr. *Insoluble Monolayers at Liquid-Gas Interfaces*; Wiley-Interscience: New York, 1966.
(36) Rouser, G.; Fleischer, J.; Yamamoto, A. *Lipids* **1970**, *5*, 494–496.
(37) Tocanne, J. F.; Teissié, J. *Biochim. Biophys. Acta* **1990**, *1031*, 111–142.