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Research Article

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Monolayers of three amphiphilic esters of rutin at the air-water interface

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ABSTRACT

The π-A isotherms for monolayers of three amphiphilic rutin esters, rutin-4"'-O-stearate (RS), rutin-4"'-O-laurate (RL) and rutin-4"'-O-caproate (RC), were measured at the air-water interface and an air-aqueous aluminum sulfate solution interface. Although RS and RL showed scant water solubility, they formed monolayers with the liquid-expanded phase when spread on water. An extraordinary value of the limiting molecular area was observed for the RC monolayer, which may be due to the larger water solubility of RC. A model was proposed for the molecular conformations of RS and RL monolayers at the surface pressure at collapse (π_{coll}). In this model, two saccharide groups were under the water and perpendicular to the surface, the aglucone portion was spread out on the surface and the alkyl chain was orientationally disordered in air. When spread on an aqueous aluminum sulfate solution, the formation of a complex between the aluminum ions and the rutin ester resulted in higher values of a_{coll} and lower values of π_{coll} for RS and RL monolayers compared with their values on water, and RC could spread as liquid-expanded phase monolayer. The results described in this paper provide valuable information for investigating the interaction of flavonoids and biomembranes and the anti-oxidant mechanism of flavonoids.

Key words: Rutin ester; Monolayer; *π-A* isotherm; Air-water interface; aluminum ion

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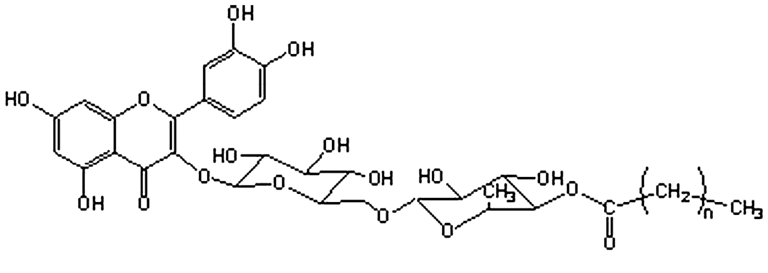
INTRODUCTION

Flavonoids exist widely in various types of plants and exhibit a wide range of biological activities [1-12]. According to their different biological properties, flavonoids have been used in food, cosmetic and pharmaceutical preparations and this subject currently attracts great interest [13, 14]. Unfortunately, most natural flavonoids (mainly flavonoid glucosides) exhibit hydrophilic properties, which results from their polyhydroxyl and saccharide groups. Therefore, flavonoid glucosides show low solubility and stability in lipophilic media [15] and are not effective in stabilizing fats and oils [16]. These properties limit the development of nutritional foods, commodities and drugs containing flavonoids. Esterification of hydroxyl groups by fatty acids is one approach to improving the hydrophobic properties of flavonoid glucosides. Rutin-4"'-O-stearate (RS), rutin-4"'-O-laurate (RL) and rutin-4"'-O-caproate (RC) were obtained by acylation of rutin with stearic acids, lauric acids and *n*-caproic acid, respectively, using immobilized *Candida antarctica* lipase B (Novozym 435) as a catalyst in our laboratory. RS, RL and RC are amphiphilic molecules with a rutin portion as polar groups and alkyl chains as hydrophobic groups (Fig.1). This amphiphilic structure will enable an appropriate hydrophile-lipophile balance to maintain improved solubility and stability of rutin esters in lipophilic media and probably impart a more effective anti-oxidant activity [17].

The absorption and metabolism of flavonoids is involved in the interaction between flavonoids and biomembranes. It is reported that there is a close relationship between anti-oxidant activity, an appropriate hydrophile-lipophile balance of flavonoids and the ability to migrate through the aqueous environment and to cross biomembranes [18, 19]. Knowledge of the properties of flavonoid monolayers at the air-water interface are a prerequisite for investigating the interaction of flavonoids with biomembrane and the anti-oxidant mechanisms of flavonoids. However, little attention has been paid to this subject. The most likely reason is that flavonoids cannot be spread at the air-water interface in the same way that other conjugated aromatic molecules can. Fortunately, amphiphilic flavonoid esters obtained by esterification can be spread at the air-water interface to form a monolayer [20-22]. In the current paper, we studied the properties of Monolayers of amphiphilic RS, RL and RC at the air-water interface and the influence of aluminum ions in the substrate to *π-A* isotherms of RS, RL and RC monolayers to provide valuable information for investigating the interaction between flavonoids and biomembranes and the anti-oxidant mechanism of flavonoids.



n = 16, rutin-4'''-O-stearate (RS)
n = 10, rutin-4'''-O-laurate (RL)
n = 4, rutin-4'''-O-caproate (RC)

Fig.1 Chemical structures of rutin esters

EXPERIMENTAL SECTION

Materials and Methods

The rutin esters, RS, RL and RC were synthesized according to a previously described method [23]. Chloroform and methanol were all analytical reagents from Chengdu Kelong Chemical Plant, Chengdu, PR China.

Film balance measurements were performed using a KSV trough (KSV Instruments Ltd., Helsinki, Finland) with a Wilhelmy type microbalance using a platinum plate. RS, RL and RC were dissolved at a concentration of 1.02, 1.08 and 1.00 mg/mL, respectively, in a mixture of chloroform and methanol with a volume ratio of 9:1. Each solution was spread on the substrate, the temperature of which was controlled to 25.0 ± 0.5 °C. The compression rate was fully controlled in the trough system using a computer.

RESULTS AND DISCUSSION

Monolayers of RS, RL and RC at the air-water interface

The *π-A* isotherms recorded for RS, RL and RC are shown in Fig.2. Following the evaporation of the solvent (20 min), the monolayers were compressed at a rate of 5 mm/min. The isotherms indicated that RS and RL were capable of forming monolayers with the liquid-phase at the air-water interface.

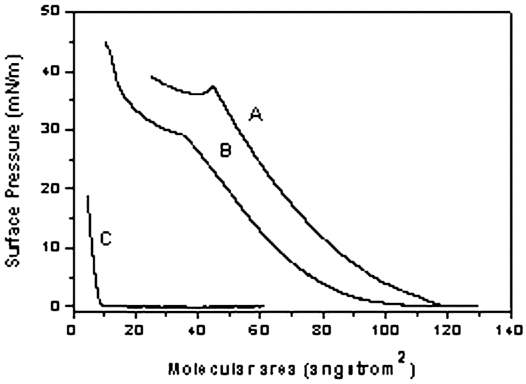


Fig.2 *π-A* Isotherms of rutin esters spread on water
(A) RS (B) RL (C) RC

The intermolecular hydrogen bonds between water and aglucone groups of rutin esters would lead flavones nuclear parent to form a large, flat layer on the air-water interface. The packing of the molecules in the monolayer are affected by two contributions. One is the hydrophobic interaction between the alkyl chains; the other is the interaction between the lateral surfaces of the polar groups. The packing of the molecules in the RS and RL monolayers seemed to be mainly determined by the latter contribution. Compared with the large polar groups, the alkyl chains were relatively short. Near to the collapse of the monolayers, the polar groups were arranged tightly at the air-water interface, while the alkyl chains remained outside the critical interaction distance and remained orientationally disordered in air. Thus, isotherms for RS and RL monolayers with a liquid-expanded phase were observed.

The value of the molecular area at the collapse (a_{coll}) of the RS monolayer was 45 Å^2, which corresponds to the limiting area per molecule (extrapolation of the linear parts on the isotherms to the abscissa) of quercetin palmitate molecules (QP) [18, 19]. This indicates that immediately prior to the RS monolayer collapse, only the flavones nuclear parent contributed to the a_{coll} value. Accordingly, a molecular model of the RS monolayer near to the surface pressure at the collapse (π_{coll}) can be proposed that involves the two saccharide groups being under the water and perpendicular to the interface, with the aglucone portion spreading on the interface and the alkyl chain orientationally disordered in air (Fig.3).

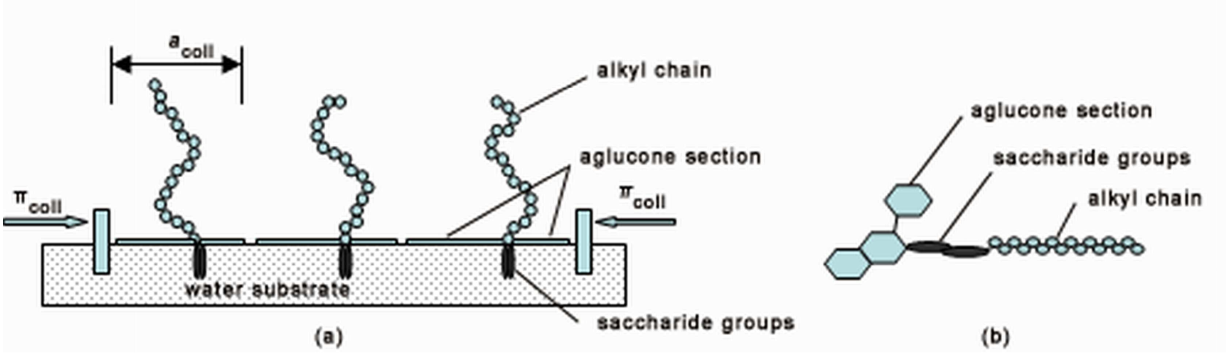


Fig.3 Schemes illustrating the molecular arrangement of RS monolayers near to π_{coll} (a) and RS (b)

In theory, when the polar group of a monolayer molecule is large and the alkyl chain is relatively short, the value of a_{coll} is determined by the structure and the number of polar groups retained at the air-water interface. Although the initial numbers of RS and RL molecules spread on water were equal and RS and RL have the same polar group and rutin section, the a_{coll} value of RL monolayer was 35 Å^2 , which was smaller than that of the RS monolayer. This was probably due to partial dissolution of RL in water.

A longer alkyl chain favors an array of molecules in the monolayers, thus the value of π_{coll} of the RS monolayer was 37.3 mN/m, which was larger than that of the RL monolayer, 29.0 mN/m.

For the π-A isotherm of RC, a rapid increase in the slope of the curve indicates the formation of a Langmuir monolayer with a liquid-condensed phase. By extrapolating the linear portion of the curve to zero pressure, a limiting area of about 8 Å^2 was obtained. This average molecular area was very small compared with the large polar group of RC. Furthermore, the value of π_{coll} could not be obtained for the same spreading conditions as RS and RL. The Langmuir trough experiments are useless for RC due to its larger water solubility.

We examined the relaxation of the RS and RL monolayers after compression and the result is shown in Fig.4. After compression, the barrier was fixed once the surface pressure reached 25 mN/m. The surface pressure was then observed continuously as a function of time. The RL monolayer relaxed to a surface pressure of about 6 mN/m in 250 min. In the case of the RS monolayer, the surface pressure was stable at about 15 mN/m after a slight relaxation. This fact means that RS has strong enough hydrophobicity to maintain the surface pressure for a long period, while RL partially dissolved in water dues to the shorter alkyl chain. This fact was also confirmed by the recompression π-A isotherms for RS and RL shown in Fig.5. After the surface was compressed to a pressure of 25 mN/m, the monolayers were expanded and then recompressed until collapse. Recompression π-A isotherms were observed, although some decrease of the area per molecule was observed. If the monolayer molecules dissolved into the water substrate, the π-A isotherm would shift to a smaller area per molecule. Once injected onto the air-water surface, RS and RL molecules might spread as pseudo-stable molecular assemblies due to the quick evaporation of the solvent. During the relaxation process, RS showed a few amount of dissolution of the molecules and could maintain a stable monolayer. RL, with shorter alkyl chains, showed a more pronounced tendency for dissolution into the water substrate due to its lower hydrophobicity.

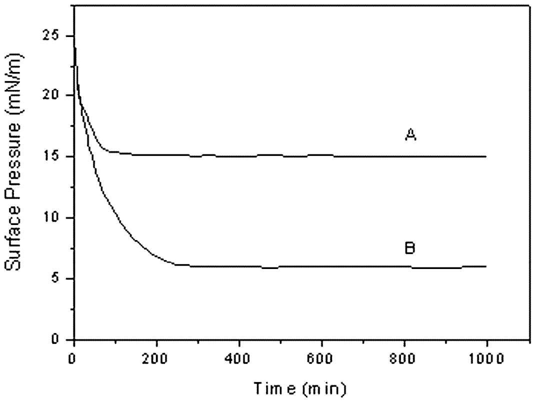


Fig.4 Relaxation of RS and RL spread on water

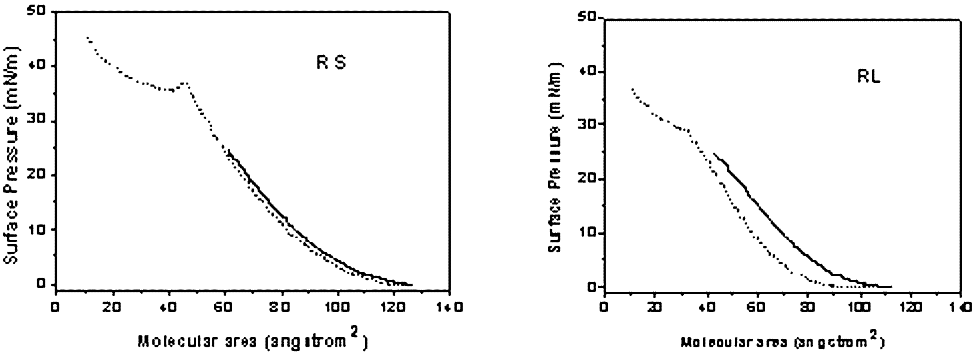


Fig.5 π-A Isotherms for the recompression of RS and RL spread on water

In general, reasons for the smaller area per molecule in the isotherms could be (1) incomplete dissolution of the sample into the spreading solvent and (2) loss of monolayer molecules due to partial dissolution into the substrate. No precipitates could be detected when preparing the RS and RL solutions so explanation (1) was excluded. Thus, the reason for the smaller area per molecule during recompression, especially in case of RL, would be the partial dissolution of monolayer molecules in water.

The solubility of fatty acids in water decreases as the length of the alkyl chain is increased. To obtain an insoluble monolayer of a non-ionized fatty acid, the molecule must contain at least twelve carbon atoms [24]. The behavior of RS and RL at the air-water surface could be interpreted similarly to the case of fatty acids. That is, RS and RL dissolved in water like fatty acids and RL was more water-soluble than RS. Because the polar groups of RS and RL were larger than that of fatty acids, longer alkyl chains (at least eighteen carbon atoms) than for a non-ionized fatty acid were required for RS and RL to form a stable monolayer at the air-water interface.

The influence of the compression rate on the RS and RL isotherms is shown in Fig.6 and Fig.7, respectively. Following the evaporation of the solvent (20 min), the π-A isotherms of RS were measured at compression rates of 1, 5 and 8 mm/min and the π-A RL isotherms were measured at compression rates of 3, 5 and 8 mm/min. Almost identical isotherms were observed for the RS monolayers when compression rates were at 5 and 8 mm/min i.e. at compression rates greater than 5 mm/min, the isotherms of RS monolayers were independent of the compression rate. The smallest value of a_{coll} of the RS monolayer was observed at a compression rate of 1mm/min. This was because a few RS dissolved in water when molecules were kept at the interface for a longer time. For the same reason, a pronounced decline in the value of a_{coll} of the RL monolayer was observed as the compression rate was reduced.

The largest π_{coll} value for the RS monolayer, 40.0 mN/m, was obtained at a compression rate of 1 mm/min. Also, the largest π_{coll} value of RL monolayer, 30.0 mN/m, was obtained at the lowest compression rate, 3 mm/min. In theory, the value of π_{coll} is determined by the structure of the alkyl chains. The large polar groups of RS and RL required sufficient time for molecules to form a thermodynamically stable structure. A slower compression rate allowed a longer time for molecules to be at the interface, so polar groups as well as alkyl chains became thermodynamically stable. Thus, the π_{coll} values for RS and RL monolayers increased as the compression rate was reduced.

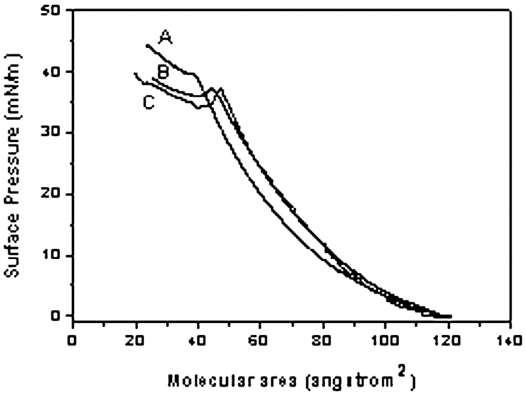


Fig.6 *π-A* Isotherms for RS spread on water at various compression rates
(A) 1 mm/min (B) 5 mm/min (C) 8 mm/min

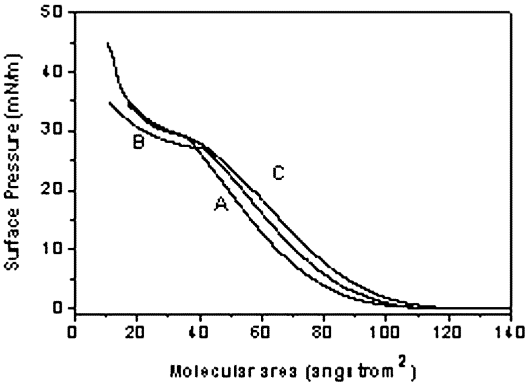


Fig.7 *π-A* Isotherms for RL spread on water at various compression rates
(A) 3 mm/min (B) 5 mm/min (C) 8 mm/min

π-A Isotherms for RS, RL and RC monolayers at an air-aqueous aluminum sulfate solution interface

The *π-A* isotherms of RS, RL and RC monolayers at an air-aqueous aluminum sulfate solution interface are shown in Fig.8. RS, RL and RC solutions were spread on a 1×10^{-4} mol/L aluminum sulfate aqueous solution. Following evaporation of the solvent, the *π-A* isotherms were measured at a compression rate of 5 mm/min.

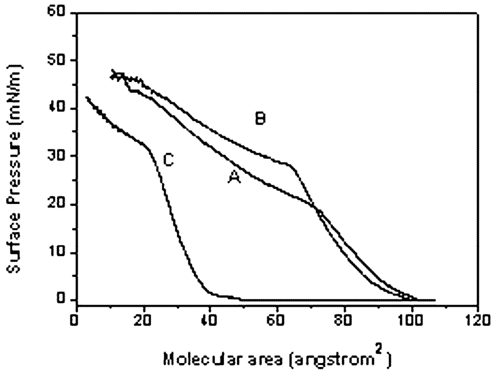


Fig.8 *π-A* Isotherms of rutin esters spread on an aqueous aluminum sulfate solution
(A) RS (B) R (C) RC

During the compression of RS and RL monolayers, the fluorandiol complex of rutin ester and the aluminum ion-rich phase was observed at the surface within the two barriers, which suggested the formation of a multilayer. For the compression of an RC monolayer, the substrate became visibly yellow, which suggests the dissolution of some complex between RC and aluminum ions. Isotherms of RS, RL and RC monolayers with the liquid-expanded phase were also observed on the aqueous aluminum sulfate solution and the values of a_{coll}, 74, 65 and 22 $Å^2$ respectively, were larger than those on water. Reasons for the larger values of a_{coll} could be (1) more molecules remaining at the surface and (2) structural change of molecules at the surface. From the results of research on complexes of quercetin with aluminum ions by Cornard, et al [25], it can be concluded that the *ortho*-diphenolic hydroxyl group of the rutin ester was involved in formation of a complex at the surface with stoichiometry rutin ester:aluminum ions 2:1 (Fig.9). Once the complex formed, the polar group became larger and more molecules were constrained at the surface. These two factors led to larger values of a_{coll}. The conformation of the complex also brought molecules of RS, RL and RC monolayers into contact each other at a lower pressure. Smaller values of π_{coll} for RS and RL monolayers were observed compared with their values on water and thus the value of π_{coll} for the RC monolayer, 32.0 mN/m, was obtained.

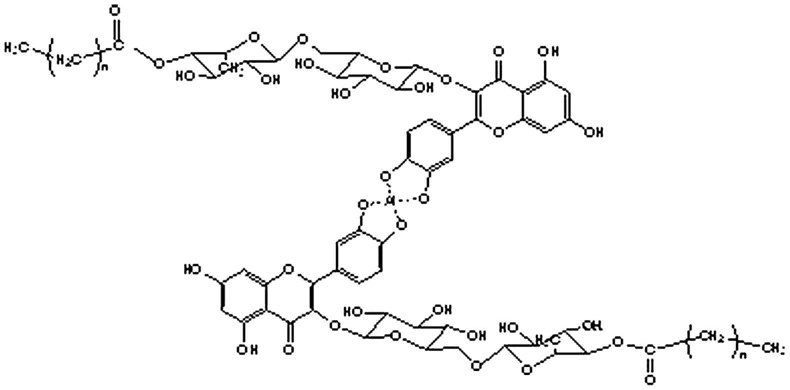


Fig.9 The complex between rutin esters and aluminum ions

CONCLUSION

The properties of monolayers of RS, RL and RC depended on the length of the alkyl chain and the substrate properties. When spread on water, RS and RL both presented monolayers with the liquid-expanded phase, and RS could maintain a stable monolayer, while RL showed a tendency for dissolution in water due to its shorter alkyl chain. In the case of RC with an alkyl chain of 4, a definitely well solubility in water was occurred, and the Langmuir trough experiment was useless. When spread on an aqueous aluminum sulfate solution, RS and RL presented monolayers with the liquid-expanded phase as their layers on water, but showed the larger values of a_{coll} and π_{coll}. RC also could spread as liquid-expanded phase monolayer due to the formation of a complex between aluminum ions and RC.

Acknowledgments
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