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BIOMIMETIC AGGREGATES FORMED BY HETEROCYCLIC AMPHIPHILES

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Abstract – This paper describes interesting self-assembling properties of heterocyclic bolaamphiphiles derived from *(E)*-urocanic acid (3-[1*H*-imidazolyl-(4)-yl]propenoic acid). The structures of the aggregates obtained in water were studied by transmission electron microscopy. The formation of an elegant biomimetic superstructure was obtained from a bolaamphiphile having two different head-groups: a urocanic moiety and a methyl carboxylate.

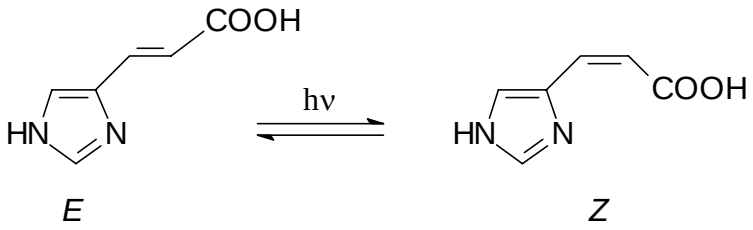
Beyond molecular chemistry, interactive advances in conceptualization in chemistry and biology have led to the development of supramolecular chemistry.[1] While molecular chemistry is based on the covalent bond, supramolecular chemistry deals with more complex systems, supermolecules and organized polymolecular systems controlled by intermolecular bonds. This term includes ion pairing (electrostatic), hydrophobic and hydrophilic interactions, hydrogen bonding, host-guest interactions, pi stacking and Van der Walls interactions. These interactions form the basis of highly specific biological processes such as the folding of polypeptide chains into functional proteins,[2] the formation of the DNA double helix,[3] and the aggregation of phospholipids to form cell membranes.[4, 5] Thus, supramolecular chemistry is a biomimetic approach of the chemical objects considered henceforth as "cooperative systems".

In this perspective, the effect of a molecule's structure on its reactivity is not only sought in the molecule as an isolated object but also in the primary aggregates formed by the self-association of several molecules[6] (micelles, vesicles,[7] fibres, monolayers, liquid crystal, etc.). These organized systems have potential applications in molecular recognition,[8] catalysis [9,10] and transport processes.[11, 12, 13]

The research undertaken in our laboratory is in keeping with this mode of thinking and concerns the self-organized systems formed by amphiphiles in water. The idea is to connect the amphiphile structures with

the shapes and the features of the aggregates in order to adapt the amphiphile to the chosen application usually related to the nature of the polar head(s). [14]

This has led us to develop the study of amphiphilic derivatives of urocanic acid (scheme 1), an important natural heterocyclic chromophore present in the skin.[15-18]



Scheme 1: *E* and *Z* isomers of urocanic acid

These molecules have potential applications in:

- cosmetology, because urocanic acid has a large absorption band and acts as a photoprotective agent.[19, 20]
- the treatment of transplant rejection because the Z isomer has an immunosuppressive activity; [21, 22]
- chemical decontamination because the imidazole ring has a catalytic effect on ester hydrolysis.

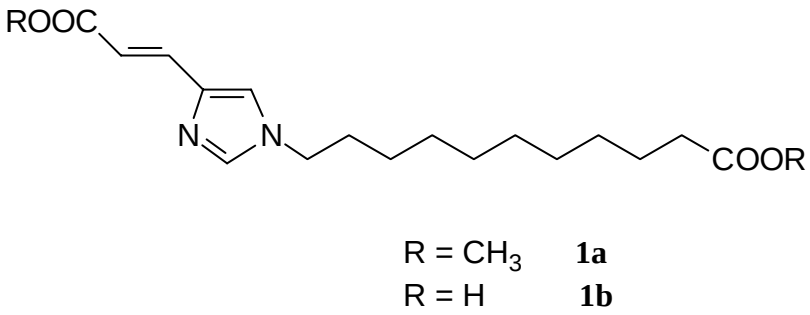
Also, from the galenic point of view, urocanic amphiphiles can be introduced more easily into formulations, whereas urocanic acid is practically insoluble in organic and aqueous media and difficult to formulate.

It was interesting to study the effect of a structural change on the aggregation behavior of the amphiphiles in relation with their photoprotective, immunosuppressive and catalytic activities.

We first investigated the organization in aqueous media of monopolar long-chain urocanic (N- or O-alkylated) derivatives.[23] These compounds gave micelles only in strongly basic conditions (pH ≥ 13)[24] and co-micelles with cetyltrimethylammonium bromide. These systems show a high catalytic effect on ester hydrolysis.[25] In order to obtain aggregates at neutral pH, we then developed the study of bipolar amphiphiles having two urocanic head-groups.[24, 26] The morphologies of the aggregates formed by these bolaamphiphiles (micelles, vesicles or fibres) have been found to be strongly dependent on three parameters: pH, structure of the urocanic moiety and position of the connecting link.[27] This work revealed that the position of the functions generating hydrogen bonding plays a key role in the structure of the aggregates. Hydrogen bonding seems to be a driving force for the formation of fibres.[28]

In order to increase the possibilities of structural modulations, we have been interested by unsymmetrical bolaamphiphiles bearing head groups of different sizes. In the literature, few examples of unsymmetrical bolaamphiphiles have been given.[29-31] We first decided to synthesize the bolaamphiphile (**1b**) having a large (urocanic moiety) and a smaller group (carboxylic acid or methyl carboxylate) (Scheme 2). It was

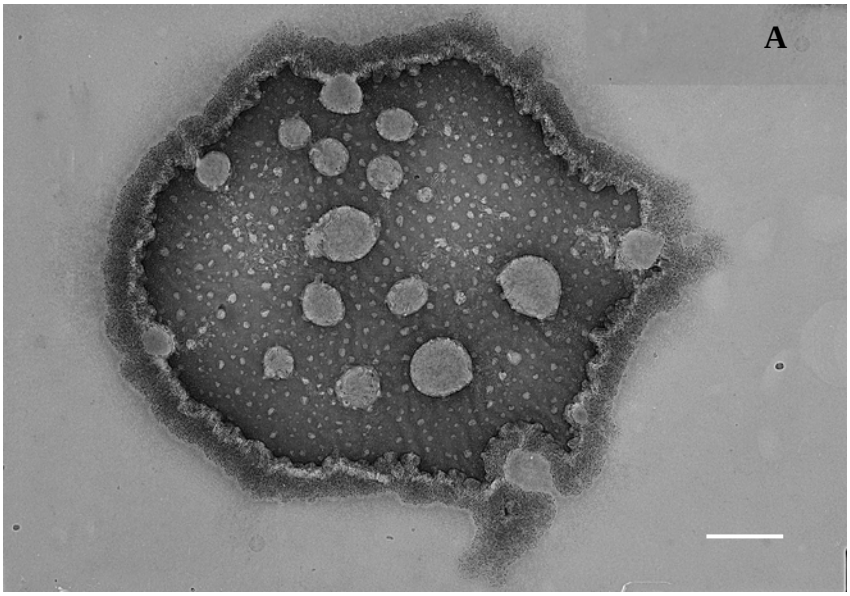
synthesized by N-alkylation of urocanic methyl ester using a previously described method under solid-liquid phase-transfer catalysis.[32] In order to confirm the effect of functions generating hydrogen bonding on the aggregate structure, we compared the aggregation mode of **1b** and of the synthesis intermediate (**1a**)**.**



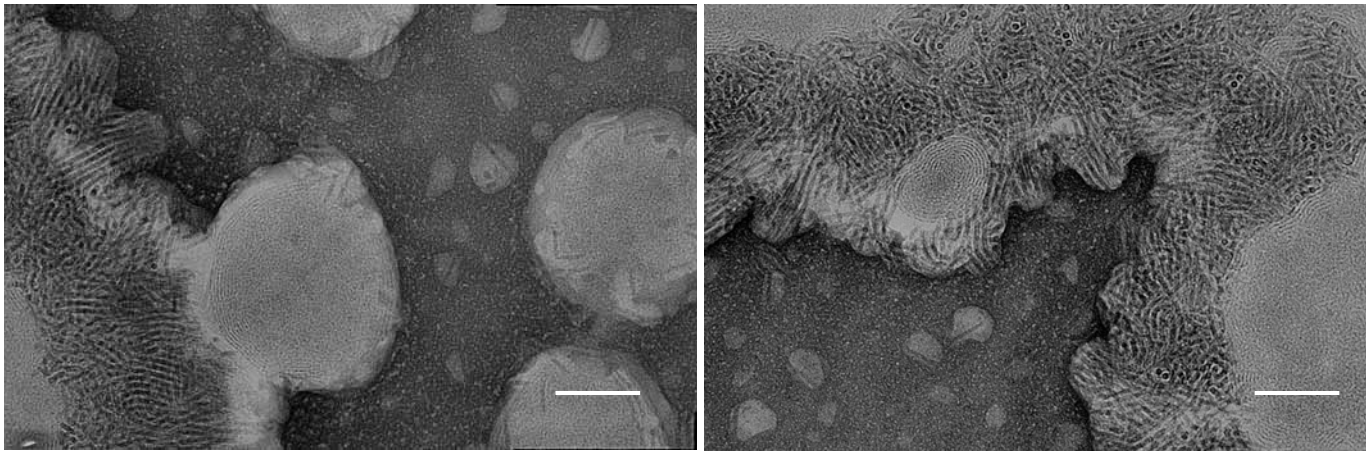
Scheme 2: Unsymmetrical bolaamphiphilic derivatives of urocanic acid

Elegant organized structure was observed by transmission electronic microscopy with the amphiphile diester (**1a**), which resembles a biological cell (Figure 1). The TEM pictures of this structure show two aggregation forms: plurilamellar vesicles (diameter ~ 150–500 nm) in which the thickness of the lamellae (~ 3 nm) corresponds to the length of the amphiphilic molecule, and thicker (~ 8 nm) lamellar structures.

Figure 1: Transmission electron micrograph (negative stain, 2% uranyl acetate) of aggregates formed from bolaamphiphiles (**1a**) in aqueous medium at pH 6.3. Micrographs **B** are enlargements of part of **A**. Bar represents: **A**: 550 nm and **B**: 100 nm



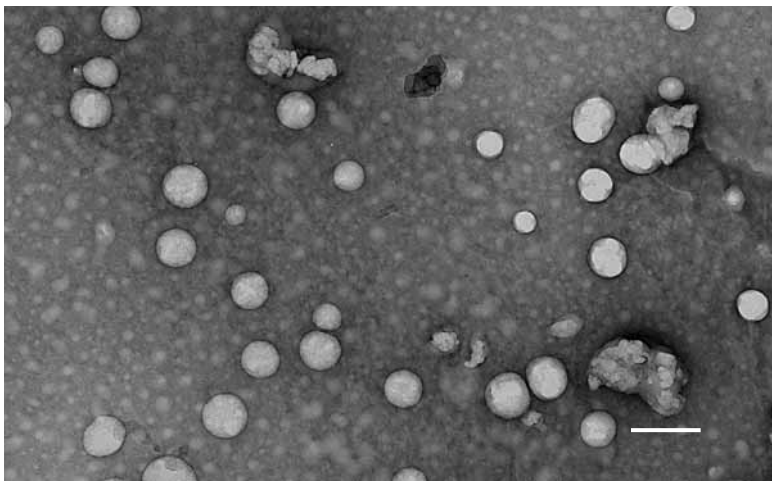
B



These aggregates were observed from suspensions (pH of the bulk phase: 6.3) made in double-distilled water in order to avoid salt effects. In acidic medium (pH of the bulk phase: 2), this compound gave only vesicles (diameters ~ 120 and 1200 nm). Due to the hydrolysis of compound (**1a**), the study in basic medium was not possible. The diacid bolaamphiphile (**1b**) gave only vesicles at all pH values (d ~ 40 – 250 nm) (Figure 2). These observations give additional importance to the interesting aggregation properties of bolaamphiphilic structures, which are the basic constituents of the cell membrane in certain archeobacteria.[33]

This work emphasizes the importance of possibility of hydrogen bonds on the aggregation behavior and shows that slight changes induce important modifications. By analogy, compounds having similar aromatic head-groups form thermotropic mesophases via carboxylic groups whereas no mesogenic phase was obtained by methyl ester analogs.[34]

Figure 2: Transmission electron micrograph (negative stain, 2% uranyl acetate) of vesicles formed from bolaamphiphiles (**1b**) in water (pH 6.3). Scale bar represents 380 nm.



EXPERIMENTAL

SYNTHESIS

Materials. Commercial products (Aldrich Chemical Co.) were used without further purification. Solvents were freshly distilled and dried before using.

Methods. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC 200, 250 spectrometers at nominal frequencies of 80 or 200 MHz for ^{1}H and 50 MHz for ^{13}C, δ given in ppm and *J* in Hertz. Mass spectra were recorded on a Nermag R10-10DCI instrument for DCI (NH_3) and a ZAB–HS instrument (WG–Analytical, Manchester, UK) using the Electro-Spray mode. Melting points were determined on an Electrothermal apparatus (capillary tubes). Microanalysis was carried out on a Carlo Erba 1106 at the ENSCT (Toulouse, France).

11-Bromoundecan-1-oic acid esterification was performed using the method described for methyl urocanate.[32] Methyl 11-bromoundecan-1-oate was purified by flash-chromatography on silica gel (eluent: chloroform/heptane: 50/50 v/v) and obtained as a yellow oil in 85% yield. ^{1}H-NMR (80 MHz, $CDCl_3$): δ 1.28 (m, 12H, 6 CH_2); 1.50 (m, 2H, C*H*$_2$CH$_2$COOCH$_3$); 1.76 (m, 2H, C*H*$_2$CH$_2$Br); 2.29 (t, *J = 7.4*, 2H, C*H*$_2$COOCH$_3$); 3.39 (t, *J = 6.7*, 2H, C*H*$_2$Br); 3.65 (s, 3H, C*H*$_3$OOC). ^{13}C-NMR (50 MHz, $CDCl_3$): δ 24.9–34.1; 45.1; 51.4; 174.2; MS-ES positive m/z = 300.9 $(M + Na)^+$, 302.9 $(M + Na)^+$

Bolaamphiphile (**1a**): *N*-alkylation of *(E)*-methyl urocanate was carried out according to a previously published method[32] from a mixture of *(E)*-methyl urocanate (886 mg, 5.83 mmol), methyl 11-bromoundecan-1-oate (1.63 g, 5.83 mmol), K_2CO_3 (8 g, 58.2 mmol) and 18-crown-6 (154 mg, 0.583 mmol) in 80 mL of anhydrous THF. The product (**1a**) was purified by flash chromatography (silica gel, chloroform/ethanol, 98/2 v/v) to obtain a white solid (740 mg, 36 %); mp: 78°C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.24 (m, 12H, 6 CH_2); 1.58 (t, *J = 6.7*, 2H, C*H*$_2$CH$_2$COOCH$_3$); 1.75 (t, *J = 6.7*, 2H, C*H*$_2$CH$_2$N); 2.27 (t, *J = 7.3*, 2H, C*H*$_2$COOCH$_3$); 3.64 (s, 3H, C*H*$_3$OOCCH$_2$); 3.74 (s, 3H, C*H*$_3$OOC-CH=); 3.89 (t, *J = 7.1*, 2H, C*H*$_2$N); 6.52 (AB system, J_{AB} *= 15.6*, 1H, CH=C*H*$_A$-COOCH$_3$); 7.07 (s, 1H, H_5im); 7.45 (s, 1H, H_2im); 7.53 (AB system, J_{AB} *= 15.6*, 1H, C*H*$_B$=CH-COOCH$_3$); ^{13}C NMR (50 MHz, $CDCl_3$) : δ 24.9 - 34.4; 47.4; 51.5; 60.2, 115.3; 115.9; 121.6; 138.3; 138.5; 168.2; 174.4. Anal. Calcd for $C_{19}H_{32}N_2O_4$: H, 8.63; C, 64.69; N, 7.99. Found: H, 9.00; C, 65.20; N, 7.86; MS-DCI (NH_3) m/z = 351 MH^+ (100%).

Bolaamphiphile (**1b**). Compound (**1a**) hydrolysed according the previously described method,[35] gave **1b** in 38% yield; mp: 198°C; ^{1}H-NMR (200 MHz, DMSO-d_6): δ 1.24 (m, 12H, 6 CH_2); 1.48 (t, *J = 7.0*, 2H, C*H*$_2$CH$_2$COOH) ; 1.69 (t, *J = 7.0*, 2H, C*H*$_2$CH$_2$N) ; 2.18 (t, *J = 7.3*, 2H, C*H*$_2$COOH) ; 3.95 (t, *J = 7.1*, 2H, CH_2N) ; 6.27 (AB system, J_{AB} *= 15.5*, 1H, CH=C*H*$_A$-COOH) ; 7.41 (AB system, J_{AB} *= 15.5*, 1H, C*H*$_B$=CH-COOH) ; 7.58 (s, 1H, H_5im) ; 7.72 (s, 1H, H_2im) ; 11.92 (COOH). ^{13}C NMR (50 MHz, DMSO-

d_6): δ 24.4-33.6; 46.1; 114.8; 122.7; 136.6; 136.9; 139.1; 166.0; 174.4. Anal. Calcd for $C_{17}H_{26}N_2O_4$ + ½ H_2O: H, 8.21; C, 61.61; N, 8.45. Found: H, 7.95; C, 61.34; N, 8.26; MS-DCI (NH_3) m/z = 323 MH^+ (100%).

BOLAAMPHIPHILE AGGREGATION IN AQUEOUS SOLUTION

8.76 mg of bolaamphiphile (**1a**) or 8.06 mg of bolaamphiphile (**1b**) were poured into 3 ml of double-distilled water or of 10^{-2} mol.L^{-1} HCl solution or of 10^{-1} mol.L^{-1} NaOH solution (5 10^{-3} mol.L^{-1}). The pH of the suspensions so obtained was 6.3 or 2 or 9.9 respectively. At this last pH, compound (**1a**) was hydrolysed. The aqueous suspensions were sonicated with a titanium probe (High-Intensity Ultrasonic Processor 600-Watt Model), at 110 watt and 0°C, for 15 min using an 80 % duty cycle, and observed by transmission electronic microscopy (TEM) using a JEOL JEM 200 CX electron microscope operating at 200 kV. Aliquots were applied to carbon-coated Formvar grids, and negatively stained with 2% uranyl acetate to reveal the aggregates. After one month, the same aggregates were observed from the different suspensions.

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