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Spontaneous oscillatory *in vitro* chiral conversion of simple carboxylic acids and its possible mechanism†

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In earlier studies, we have collected experimental evidence (mostly from thin-layer chromatography and polarimetry) on the spontaneous oscillatory *in vitro* chiral conversion of simple carboxylic acids dissolved in 70% aqueous ethanol. To elucidate this phenomenon, we developed a simple theoretical model of two linked Templators. Recently, we have obtained additional experimental evidence of the spontaneous condensation of chiral carboxylic acids, based on the biuret test (amino acids), high performance liquid chromatography, and ^{13}C NMR spectroscopy (profens and hydroxy acids). We briefly describe our experimental results in the context of the existing literature and outline an improved theoretical model for these phenomena. Our system resembles in some respects the reported oscillatory condensation of organic silanols. Here, the key reaction is the formation of carboxylic acid-derived enols. Finally, we discuss the importance of the oscillatory chiral conversion of simple carboxylic acids for biochemistry, pharmacology, and related life sciences. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: chiral carboxylic acids; condensation; enolization; oscillatory chiral conversion; Templator model

INTRODUCTION

The remarkable phenomenon of spontaneous *in vitro* oscillatory chiral conversion of selected profens, amino acids, and hydroxy acids has been the focus of our attention for some time. In papers,[1–3] we reported on the oscillatory chiral conversion of *S*(+)-ibuprofen, *S*(+)-naproxen, *S,R*(±)-2-phenylpropionic acid, *S*(+)-flurbiprofen, *R*(−)-flurbiprofen, and *S,R*(±)-ketoprofen. In papers,[4–6] we described analogous behavior in *L*-alanine, *L*-α-phenylalanine, and *L*-tyrosine.[4–6] In papers,[7,8] the oscillatory chiral conversion of the hydroxy acids *L*-lactic acid, *R*-α-hydroxybutyric acid, *S*-α-hydroxybutyric acid, *R*-mandelic acid, and *S*-mandelic acid was characterized. Most of these oscillatory chiral conversion reactions took place in acidic samples dissolved in 70% aqueous ethanol. Oscillatory chiral conversion has also been reported in the crystallization of the (+) and (−) enantiomers of 2-azabicyclo [2.2.1]hept-5-en-3-one[9].

Much of the experimental evidence for this phenomenon originates from thin-layer chromatography (TLC) and polarimetry. The TLC evidence is mostly in the form of changing concentration profiles and unstable retardation coefficient (R_F) values of the investigated analytes. In Fig. 1, we present an example of TLC results for *S,R*(±)-2-phenylpropionic acid dissolved in 70% aqueous ethanol and then stored for 72 h at 22 °C, with intermittent chromatographic measurements on the stored sample.[1]

Polarimetric evidence was collected in the non-continuous and continuous registration modes. In Fig. 2(a, b), we show examples of the long-term chiral conversion of *S*(+)- and *R*(-)-flurbiprofen dissolved in 70% aqueous ethanol and then stored at 22 °C for several days.[3] These data were collected in the non-continuous registration mode.

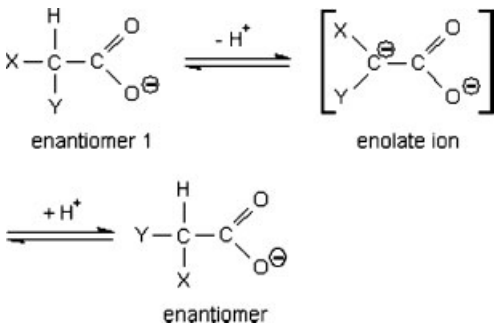
In Fig. 3, we present the continuously registered oscillatory changes of the specific rotation ($[\alpha]_D$) for a freshly prepared 70% aqueous ethanol solution of *R*(-)-flurbiprofen, with monitoring carried out for 540 min.

The general scheme of these chiral conversions can be summarized as

$$\text{enantiomer}(+) \rightleftarrows \text{enol} \rightleftarrows \text{enantiomer}(-) \qquad (1)$$

If we consider chiral conversion of selected carboxylic acids in an aqueous solution in more detail, then Eqn (1) can be replaced by Eqn (1a)[10]:

(1a)



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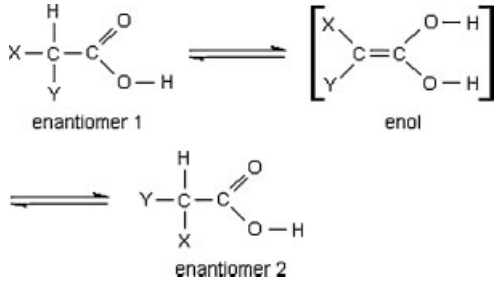
In anhydrous media and in the presence of trace amounts of water, the probable mechanism of chiral conversion is given by Eqn (1b):[11]

(1b)



where X: -NH_2, -OH, -Ar, etc., and Y: -R, etc.

MODELING STUDIES

Based on the reaction scheme (Eqn (1)), in paper[5] we proposed a simple model of chiral conversion, adapting an earlier oscillatory mechanism designated as the Templator.[12,13] Here, we propose two models which may explain the observed oscillation in these experimental systems. The first model is based on autocatalytic dimerization. Oscillations appear in this model in both enantiomer subsystems. Racemization is a linear reversible reaction coupling the two oscillating subsystems. In such a system, in addition to simple oscillations, quasiperiodic (Fig. 4) and mixed mode oscillations consisting of mixtures of large and small amplitude oscillations are possible as well. Instability of the stationary state leading to pattern formation in a spatially extended system is also possible, because such instability requires that the diffusion coefficient of dimers be smaller than that of monomers, which is physically reasonable.

In our model we consider the concentrations of enantiomers S, R and their homodimers S_2, R_2. We assume that the dimerization reaction may be uncatalyzed

$$2R \leftrightarrow R_2 \quad (1a)$$

$$2S \leftrightarrow S_2 \quad (1b)$$

or catalyzed by the dimers themselves (autocatalysis):

$$2R + R_2 \rightarrow 2R_2 \quad (2a)$$

$$2S + S_2 \rightarrow 2S_2 \quad (2b)$$

For simplicity, we ignore the formation of heterodimers, though it is only necessary to assume that this process is much slower than homodimer formation.

We also consider a reservoir of oligomers, which acts as a sort of buffer, able to remove dimers and introduce monomers into the system. Dimers may be removed by the creation of oligomers R_5, S_5, Q, and P.

$$R_3 + R_2 \leftrightarrow R_5 \quad (3a)$$

$$S_3 + S_2 \leftrightarrow S_5 \quad (3b)$$

$$Q + R_5 \rightarrow P \quad (4a)$$

$$Q + S_5 \rightarrow P \quad (4b)$$

If we make a quasi-steady state approximation for $[R_5]$ and assume that the total concentrations $[R_3]+[R_5]$, $[S_3]+[S_5]$, and [Q] are roughly constant, the rate of removal of dimers is not linear but is instead proportional to $k_2b_2/(1+b_2)$ where b_2 denotes $[R_2]$.

Monomers are introduced to the system from the reservoir by reactions like

$$P \rightarrow R \quad (5a)$$

$$P \rightarrow S \quad (5b)$$

The rates of steps (5a) and (5b) are constant and small but differ because the concentrations of S and R in the reservoir are different. We assume that racemization is a simple linear reaction

$$R \leftrightarrow S \quad (6)$$

The behavior of the system defined above is described by four dimensionless kinetic equations

$$da_1/dt = \epsilon(k_{01} + k_1(b_1 - a_1^2) - 2a_1^2b_1 + k_3(a_2 - a_1)) \quad (7a)$$

$$db_1/dt = a_1^2b_1 - k_1(b_1 - a_1^2) - \frac{k_2b_1}{1+b_1} \quad (7b)$$

$$da_2/dt = \epsilon(k_{02} + k_1(b_2 - a_2^2) - 2a_2^2b_2 - k_3(a_2 - a_1)) \quad (8a)$$

$$db_2/dt = a_1^2b_1 - k_1(b_2 - a_2^2) - \frac{k_2b_2}{1+b_2} \quad (8b)$$

where a_1, b_1, a_2, and b_2 are the dimensionless concentrations of S, S_2, R and R_2, respectively, and k_{01}, k_{02}, k_1, k_2, and k_3 are dimensionless constants which are functions of the rate constants of reactions (1)–(6).

At least two observations support the use of the model described above. First, autocatalytic dimerization has been found in chemical reactions in which the breaking of the mirror symmetry of enantiomers occurs (Soai reaction[14]). Second, oscillations based on autocatalytic dimerization should be more robust at lower temperature, because the dimerization equilibrium (1) should then be shifted toward the formation of dimers. More robust oscillations are indeed seen at lower temperatures in our experiments[1,4,6].

The second approach is based on the model of Plasson *et al.*[15]. In this model, oscillations appear as the result of coupling between enantiomers. This model appears particularly relevant for systems involving condensation or peptidization in which the condensate (dipeptide) SR has different chemical properties than RS, e.g., where only the residue on the left undergoes epimerization.

$$R \leftrightarrow R^* \quad (9a)$$

$$S \leftrightarrow S^* \quad (9b)$$

$$R* + R \rightarrow R_2 \quad (10a)$$

$$S* + S \rightarrow S_2 \quad (10b)$$

$$R_2 \rightarrow 2R \quad (11a)$$

$$S_2 \rightarrow 2S \quad (11b)$$

$$R* + S \rightarrow RS \quad (12a)$$

$$S* + R \rightarrow SR \quad (12b)$$

$$RS \rightarrow S_2 \quad (13a)$$

$$SR \rightarrow R_2 \quad (13b)$$

Oscillations appear in the system (9)–(13) only if the rate constant for the formation of heterodimers (12) is greater than that for homodimers (10) (Fig. 5).

Our model of two linked Templators accounts for the experimentally observed chiral conversion of simple carboxylic acids.

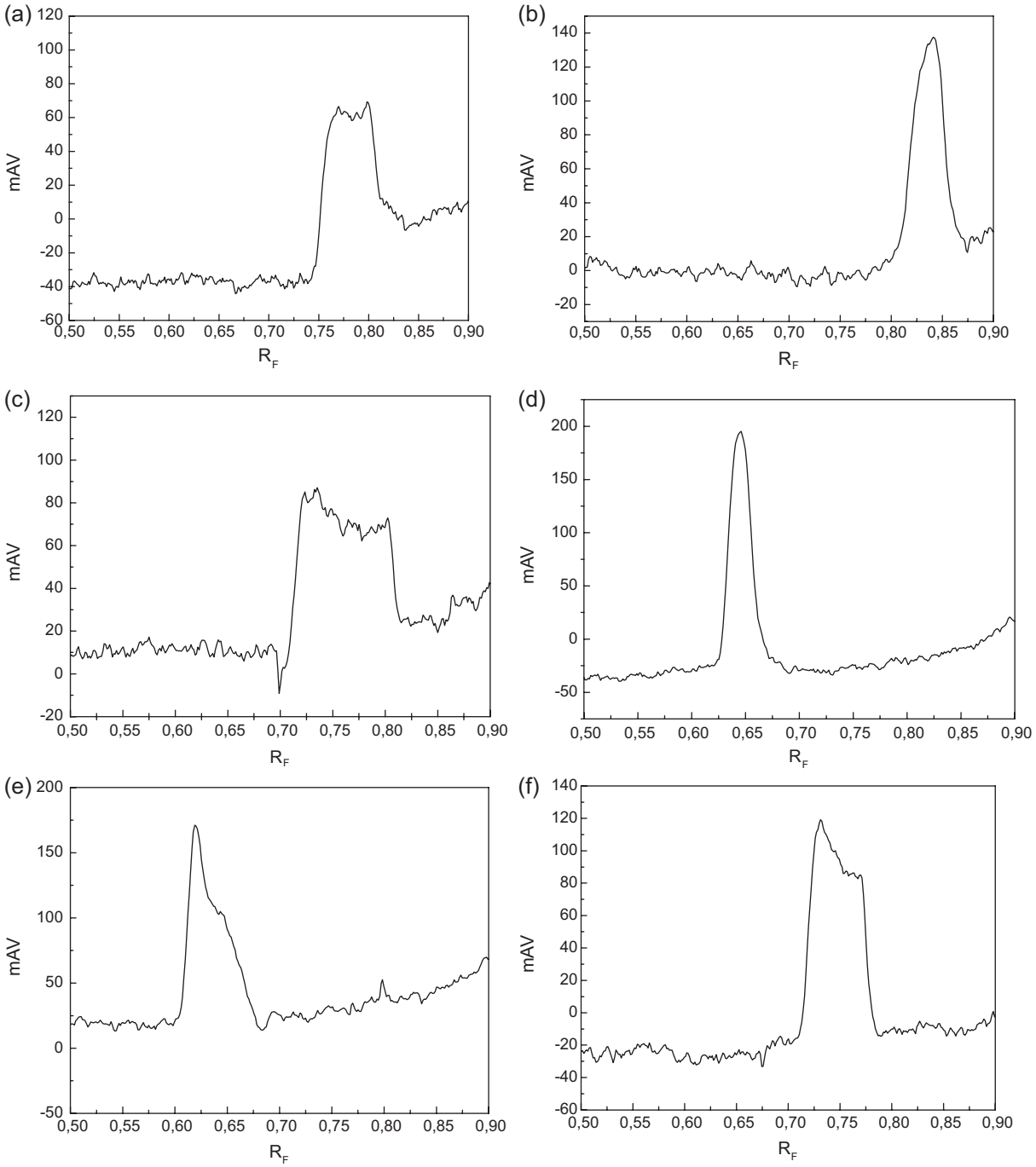


Figure 1. Sequence of densitometric concentration profiles of *S,R*(±)-2-phenylpropionic acid after: (a) 0 h (racemic mixture); (b) 22.5 h (*S*(+) form); (c) 27.5 h (racemic mixture); (d) 46.5 h (*R*(−) form); (e) 51.5 h (shift from *R*(-) form to racemic mixture); and (f) 70.5 h (racemic mixture); storage time at 22°C. Changes of the peaks' concentration profiles are accompanied by changing R_F values. Stationary phase: silica gel 60 F_{254} (precoated TLC plates; Merck, Darmstadt, Germany; cat. # 1.05715) impregnated with *L*-arginine. Mobile phase: acetonitrile–methanol–water, 5:1:0.75 (v/v), containing several drops of acetic acid[1]

However, it does not propose any realistic chemical reaction (or physical process) responsible for the oscillatory removal of the reaction template (assumed to be an H-bonded cyclic homodimer of the starting carboxylic acid). The extreme simplicity of the process summarized in Eqn (1) draws attention to the intermediate reaction product (enolate ion or enol) as the possible origin of a nonlinear process. So far, no oscillatory process involving this intermediate reaction product has been discovered.

EXPERIMENTAL RESULTS

We have recently demonstrated that the profens, amino acids, and hydroxy acids we have investigated undergo not only chiral conversion but also spontaneous condensation[16,17]. In Reference [16], the ability of *R*-phenylglycine and *S*-phenylglycine (dissolved in 70% aqueous ethanol) to undergo rapid, nearly instantaneous, peptidization was demonstrated by TLC and the biuret test. An analogous, though less rapid, outcome

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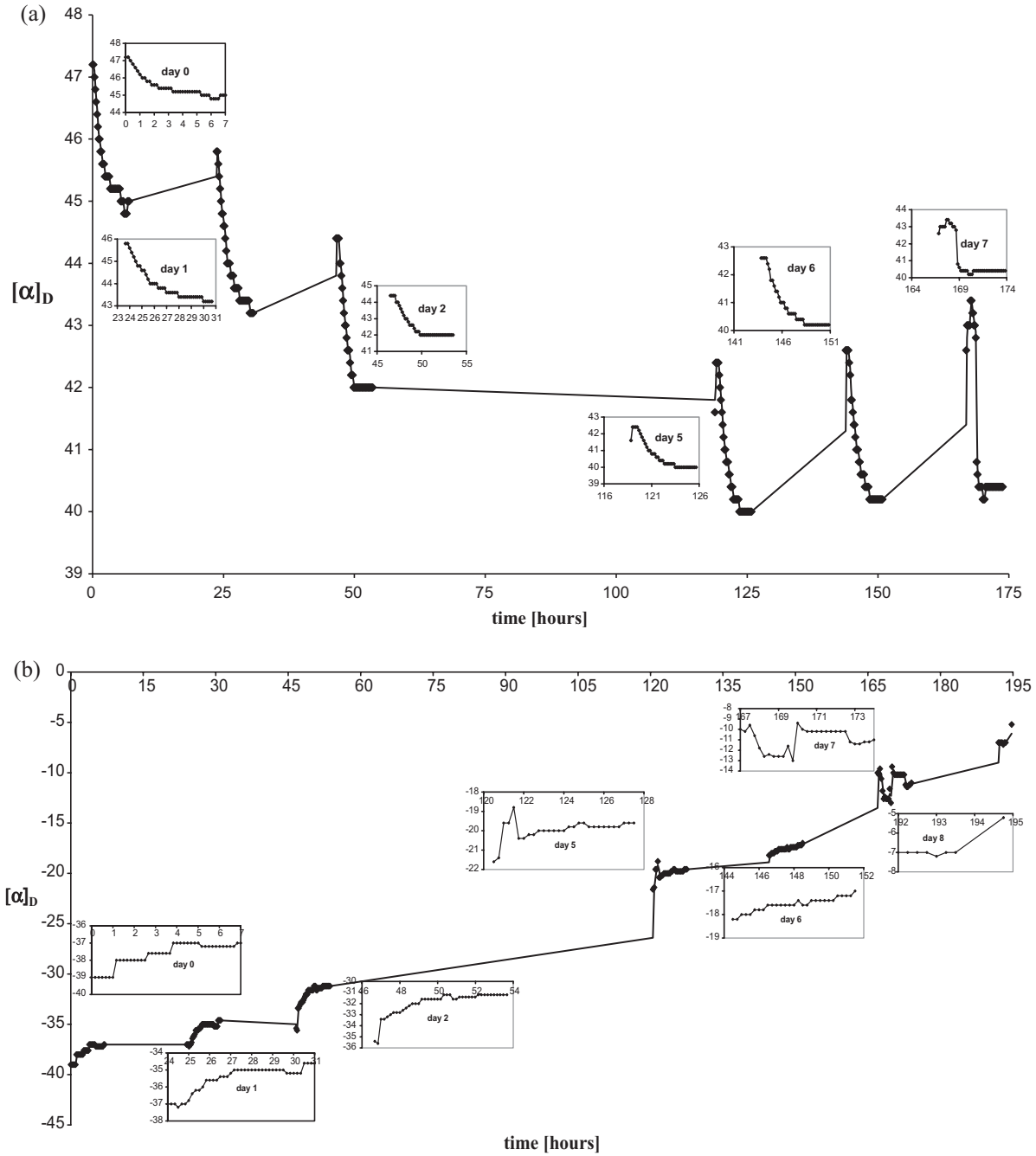


Figure 2. Oscillatory changes of the specific rotation, $[\alpha]_D$, for (a) *S*(+)-flurbiprofen and (b) *R*(−)-flurbiprofen dissolved in 70% aqueous ethanol and stored at 22°C. The general trend of the changes is indicated by the solid line, and insets show the changes on selected days of the experiment[3]

was obtained with *L*-alanine and *L*-phenylalanine. In reference[17], we presented experimental evidence for the spontaneous condensation of selected hydroxy acids (i.e., *L*-lactic acid and *S*(+)-mandelic acid). We have also amassed (unpublished) experimental evidence of spontaneous condensation of profens[18]. The evidence for hydroxy acid and profen condensation originates from ^{13}C NMR spectroscopy. In Fig. 6, we present an example of ^{13}C NMR spectroscopic evidence of the spontaneous condensation of *L*-lactic acid and *S*(+)mandelic acid, and in Fig. 7, of spontaneous condensation of *S*(+)-ketoprofen. High performance liquid chromatography (HPLC) experiments[19] provide further support for the spontaneous condensation of *S*(+)-ketoprofen. In Fig. 8, we present a sequence of chromatographic 'snapshots' showing formation and then disappearance of aging products derived from ketoprofen (most probably, ketoprofen condensates), and oscillations of the peak heights corresponding to the starting material, *S*(+)-ketoprofen, and the first condensation product. The HPLC peak heights are roughly proportional to the respective concentrations.

Summing up, storage of profens, amino acids, and hydroxy acids in aqueous organic solution results in two parallel spontaneous processes: (i) oscillatory chiral conversion and (ii) condensation. A simple scheme for such a process in profens is shown below.

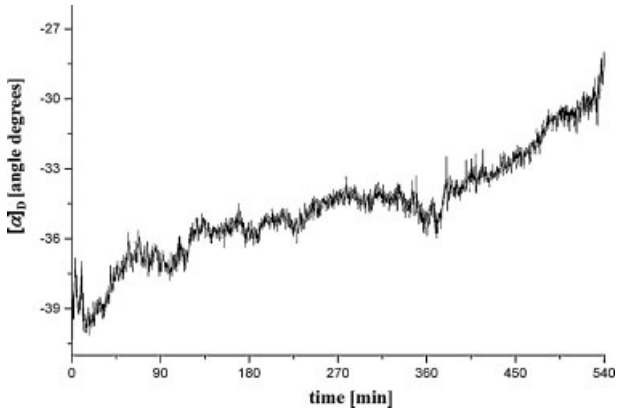
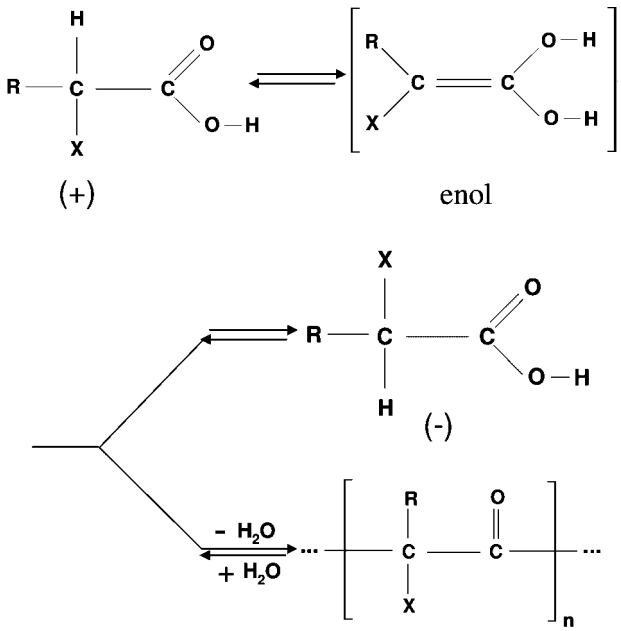


Figure 3. Oscillatory changes of the specific rotation, $[\alpha]_D$, for *R*(-)-flurbiprofen dissolved in 70% aqueous ethanol and stored at 22°C for 540 min



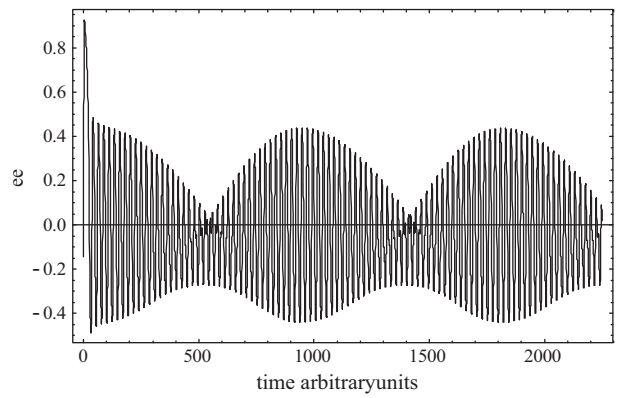


Figure 4. Complex behavior of the enatiomeric excess ee = $(a_1 - a_2 + 2b_1 - 2b_2)/(a_1 + a_2 + 2b_1 + 2b_2)$ simulated with the coupled Templator model

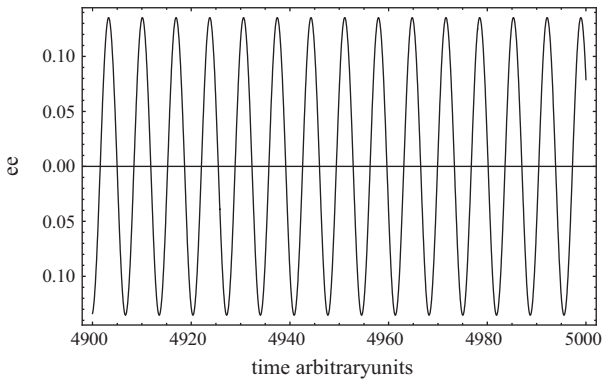


Figure 5. Enantiomeric excess ee = $([R] - [S] + 2[R_2] - 2[S_2])/c$ for $c = 1$, $k_9 = 1$, $k_{-9} = 0.1$, $k_{10} = 1$, $k_{11} = 1$, $k_{12} = 100$, $k_{13} = 1$, simulated with Eqns (9)–(13)

DISCUSSION

With amino acids and hydroxy acids, the analogous condensation pathways are also possible, resulting, respectively, in peptidization and esterification. The kinetics of spontaneous condensation of profens, amino acids, and hydroxy acids in aqueous ethanol has not yet been studied in detail, and hence it cannot be concluded whether it is nonlinear or linear in nature. In our forthcoming studies, we will focus on the investigation of these dynamics.

In this context, it is noteworthy that oscillatory condensation has been reported in another system[20,21]. Interestingly, the experimental evidence presented in those studies also originates

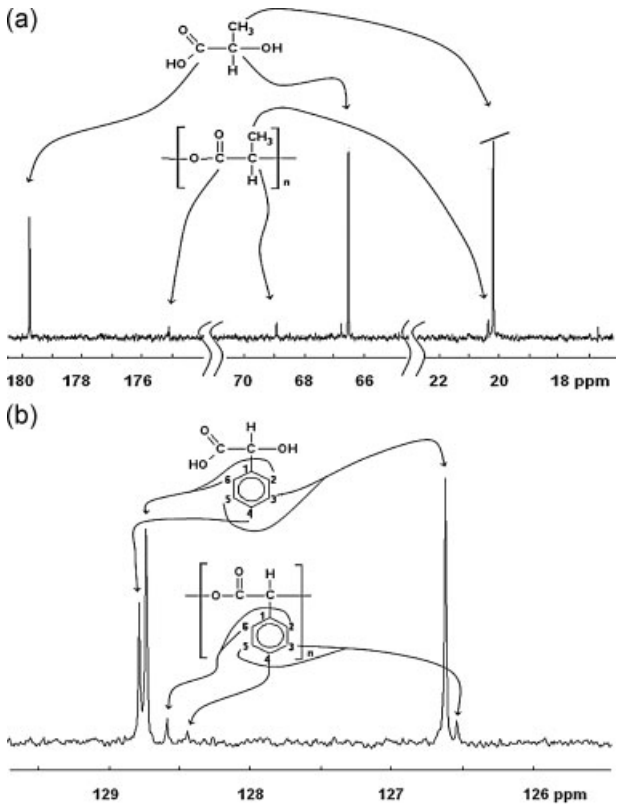


Figure 6. 100 MHz ^{13}C NMR spectra of (a) *L*(+)-lactic acid and (b) *S*(+) mandelic acid, first dissolved and stored for 10 days in pure ethanol, and then recorded in $CDCl_3$ at 25 °C[16]

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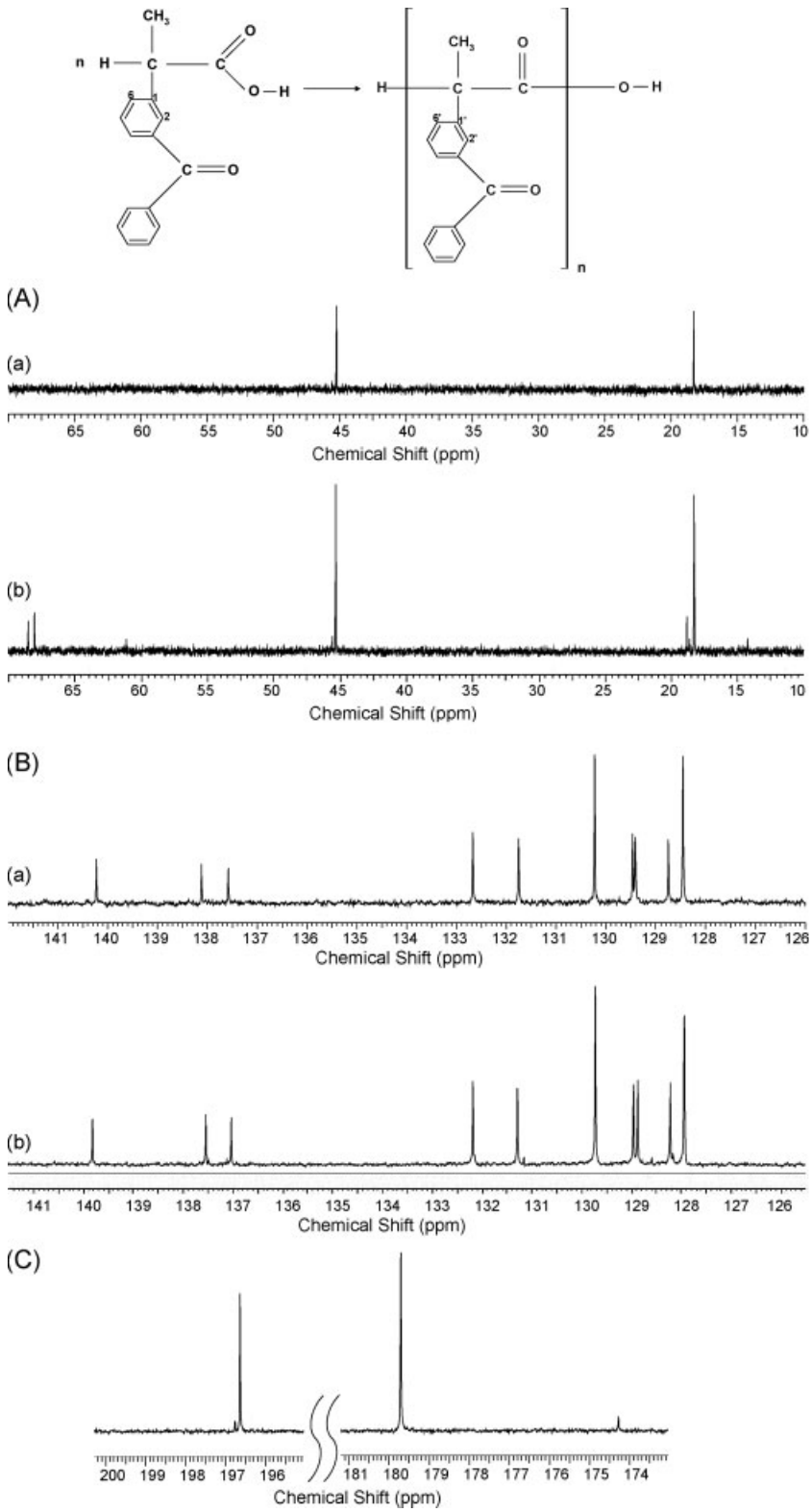

Figure 7. 100 MHz ^{13}C NMR spectra of (a) *S*(+)-ketoprofen monomer and (b) *S*(+)-ketoprofen condensate. *S*(+)-Ketoprofen sample was first dissolved in pure ethanol and then stored in solution for 10 days. Spectra were recorded at 25 °C in $CDCl_3$. (A) Aliphatic range. Methyl line at 18.26 ppm in (a) is accompanied by two additional lines at 18.61 and 18.81 ppm in (b), which originate from the methyl groups in the repeating units of the condensate. The signal of the methine carbon at 45.31 ppm in (a) is flanked by a new small line at 45.55 in (b), coming from the end groups. The new lines at 68.03 and 68.51 ppm in (b) originate from new quaternary carbons formed in the main chain of the polymer. (B) Aromatic range. In spectrum (b) two small new peaks appear at 129.09 and 131.65 ppm, respectively, which can be attributed to C-2′ and C-6′, respectively, in the new molecular environment of ketoprofen condensate (as compared with spectrum (a)). (C) Carbonyl range. After 10 days storage, the carbonyl signal at 179.68 ppm in (a) is flanked by two additional lines, at 174.29 and 196.76 ppm, respectively, in (b)

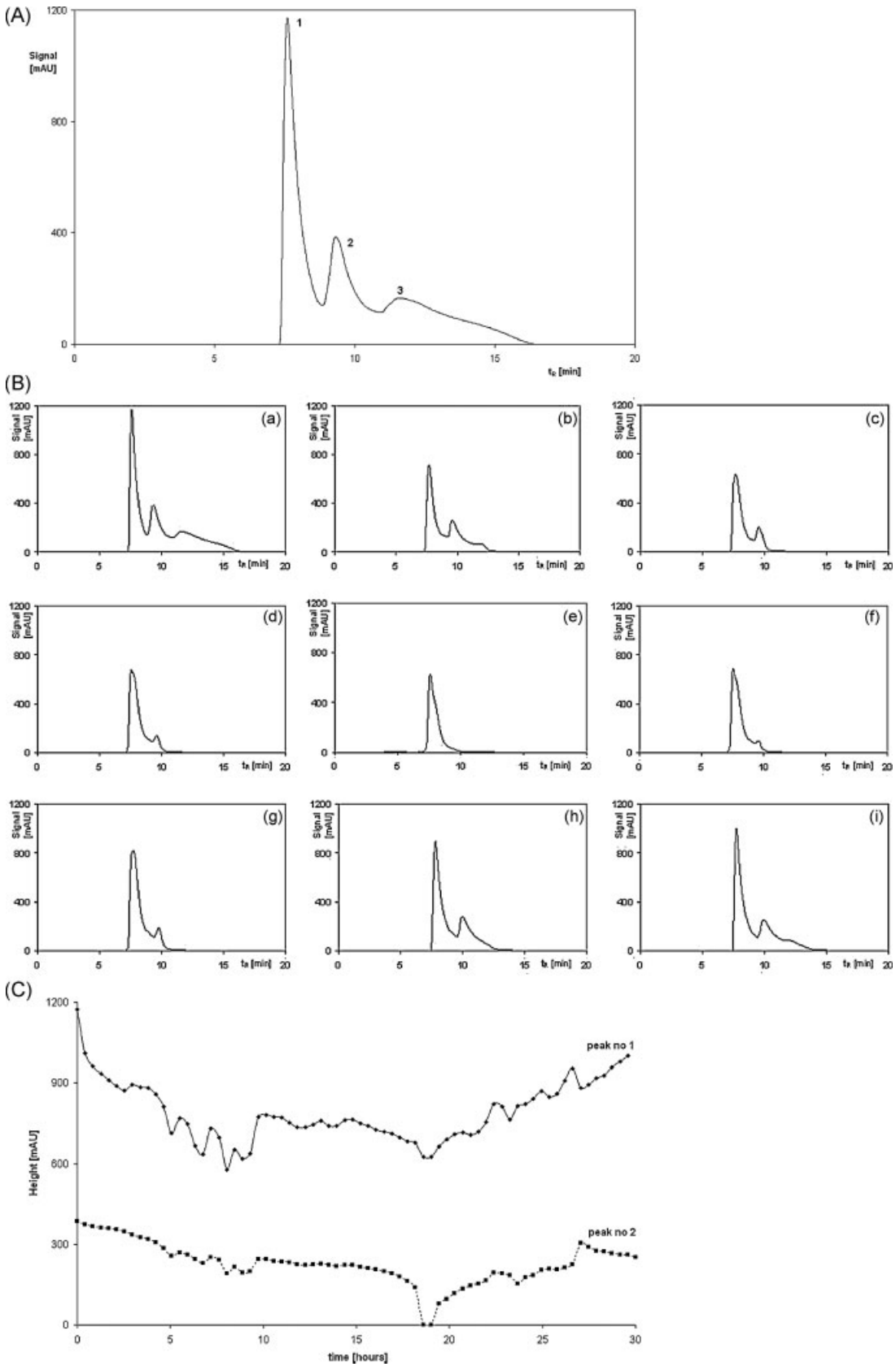

Figure 8. (A) Chromatogram of a freshly prepared solution of *S*(+)-ketoprofen in acrylonitrile recorded at 259 nm. Retention times: peak 1, 7.66 min; peak 2, 9.68 min; peak 3, 11.80 min. (B) Sequence of chromatographic concentration profiles of *S*(+)-ketoprofen dissolved in acetonitrile after (a) 0 h; (b) 5.5 h; (c) 9.5 h; (d) 18 h; (e) 19 h; (f) 20 h; (g) 24.5 h; (h) 28 h; and (i) 30 h storage time at 22 °C. (C) Time course of the chromatographic peak heights of an *S*(+)-ketoprofen solution stored at 22 °C for 30 hours. Peak numbers as in (A)[19]

from TLC, which emphasizes the utility of this simple analytical technique in tracing the structural lability of organic compounds. Even more relevant to our own research is that the oscillatory condensation documented by these authors involves organosilanols (e.g., $R_2Si(OH)_2$ or $RR'Si(OH)_2$). There are several analogies between the results presented in references[20,21] and those originating from our own laboratory, and also between the conclusions of the two research teams:

(i) In selected molecular structures of the relevant organosilanols, the molecular fragment $=Si(OH)_2$ appears[20,21]. In our study, we postulate the presence of an intermediate enol structure with the analogous molecular fragment $=C(OH)_2$ (see Eqn (1b)).
(ii) In both studies, condensation of the respective substrates and the spontaneity of this process is well documented.
(iii) In both cases, condensation takes place in aqueous organic solution.
(iv) In the condensation of organosilanols, association of the monomers and oligomers is postulated as the key effect responsible for the oscillatory nature of the process. In our model of two linked Templators, the H-bonded homodimers of the carboxylic acids are suggested as templates for chiral conversion (further association of enols with these templates results in a sterically oriented structural change).
(v) Finally, chirality seems to be a key feature in both processes. In our study, chiral carboxylic acids are the starting material, while with $RR'Si(OH)_2$ organosilanols, oligomeric condensates have asymmetric Si atoms (and the number of these atoms equals the number of coupled monomer units).

Further investigation of oscillatory processes involving simple carboxylic acids occurring spontaneously *in vitro* in abiotic aqueous organic systems seems challenging for both purely scientific reasons and also for practical ones. It seems evident that insufficient attention has so far been paid to *in vitro* studies of structurally simple compounds of high pharmaceutical and/or biochemical importance (profens, amino acids, and hydroxy acids are certainly among such compounds). As a result, processes that run spontaneously in abiotic *in vitro* systems could erroneously be attributed to *in vivo* systems only (as, e.g., in the case of *in vivo* chiral conversion of profen drugs[22]) and then claimed to be inherent in physiological systems and biochemical processes alone. In that way, misinterpretation of natural processes can easily occur, with serious and potentially negative consequences.

Acknowledgements

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