The increasing tightness of fully associated states as a function of their increasing stability. The dimerisation of carboxylic acids

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Literature data describing the dilution of carboxylic acids in cyclohexane are reinterpreted according to a monomer–dimer equilibrium (higher order oligomers had previously been postulated in order to explain the data). Apparent dimerisation constants for each of the acids were calculated by incorporating a factor to take into account the varying relative permittivity of the solution during the titration. Dilution titrations of propionic acid using diluents of varying relative permittivities were also carried out and apparent dimerisation constants calculated in a similar manner. The dimerisation constants were found to vary according to the relative permittivity of the diluent, and the tightness of the dimers formed (as evidenced by the chemical shift of the carboxy proton) also varied according to the diluent relative permittivity. The results demonstrate that complex stability is directly related to the strength of the hydrogen bonds which go to form that complex. They also clarify a long-standing debate regarding the origin of an anomalous downfield shift of the carboxylic acid proton seen on dilution of simple aliphatic carboxylic acids with cyclohexane.

Introduction

In a recent paper,[1] for a reversible association $\mathbf{A} + \mathbf{B} \longrightarrow \mathbf{A{\cdot}B}$, we have demonstrated that as the equilibrium constant (K) for the association increases, then the structure of **A·B** cannot be taken as constant. Rather, in a strain-free system, as K increases (for example by the addition to **A** and **B** of structural units which will interact favourably with each other) the fully bound state will typically become tighter (shorter bonds at the **A·B** interface), as demonstrated using the criterion of chemical shift. The work is a further illustration of the enthalpic chelate effect and serves to emphasise that the binding energy which is available from any particular weak interaction is context dependent.[1–3] These results should be of general applicability, and we have therefore sought to demonstrate their relevance to the solution structures of aliphatic carboxylic acids, which are known to form dimers both in the liquid and gas phases (Fig. 1).[4–10]

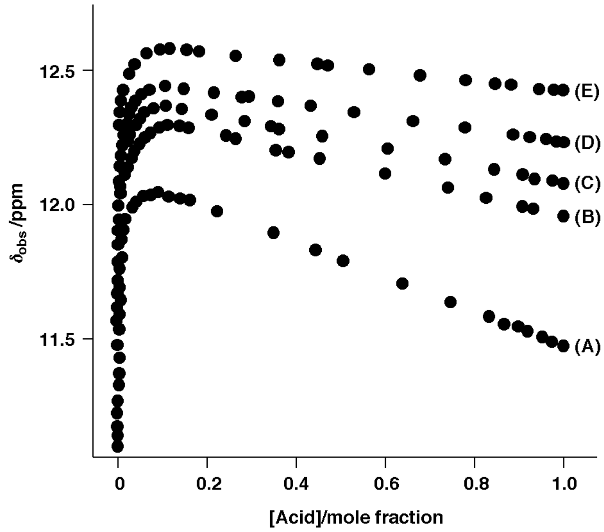


Fig. 2 Chemical shift of the carboxylic acid proton (RCO$_2$$H$) *vs.* mole fraction of acid upon dilution of the neat acid with cyclohexane at 323 K. The different plots represent dilution of the following carboxylic acids: (A) acetic acid, (B) propionic acid, (C) butyric acid, (D) isobutyric acid, (E) pivalic acid. Diagram adapted from *Advances in Molecular Relaxation and Interaction Processes*, Vol. 15, L. L. Kimtys and V. J. Balevicius, "Self-association of carboxylic acids as studied by ^{1}H NMR spectroscopy", pp. 151–161, Copyright 1979, and reprinted with permission from Elsevier Science.

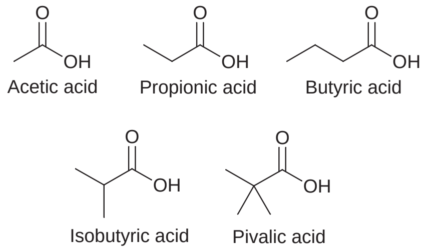
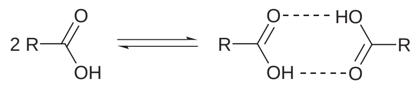


Fig. 1 Structures of carboxylic acids studied in this paper.



Scheme 1 Dimerisation of carboxylic acids.

For over 40 years, it has been accepted that the proton chemical shift changes of RCO$_2$$H$ (where R lies in the range CH$_3$ to C$_4$H$_9$) upon dilution of the neat carboxylic acid with an inert solvent (*e.g.*, cyclohexane) cannot be interpreted in terms of a simple monomer–dimer equilibrium (Scheme 1) alone. The reason for this conclusion is clearly illustrated by the data of Kimtys and Balevicius[10] for acetic, propionic, butyric, isobutyric, and pivalic acids [Fig. 2, R = H$_3$C, H$_5$C$_2$, n-H$_7$C$_3$, i-H$_7$C$_3$, (CH$_3$)$_3$C]. On dilution of the neat acids with cyclohexane, the chemical shifts of the carboxylic acid protons (which are well-established to have much greater δ values in the dimer than in the monomer) first *increase* (until *ca.* 0.1 mole fraction of acid is reached) before decreasing (due to increasing population of the monomer). It has been argued that dilution of the neat acid should lead to a gradual reduction in the fractional population of dimer relative to monomer, resulting in a decrease in the observed value of δ (δ_{obs}). Therefore, it was proposed by Reeves and Schneider,[4] and has been accepted by subsequent investigators, that the initial increase in δ_{obs} upon

Table 1 Carboxylic acid relative permittivities[13] (and the temperatures at which those values were determined) and dimerisation parameters obtained from curve-fitting to a plot of δ_{obs} *vs.* carboxylic acid concentration for titrations of cyclohexane ($\varepsilon_r = 2.02$) into carboxylic acid solutions at 323 K. The original data were obtained by Kimtys and Balevicius[10]

Acid	Relative permittivity (ε_r)	K_{dim}^{obs}/M^{-1}	$\delta_{dim}^{cyclohex}$ /ppm[b]	Δ/ppm dm^3 mol^{-1}[c]
Acetic	6.20 (293 K)	1200 ± 150	12.21 ± 0.02	−0.041 ± 0.002
Propionic	3.44 (298 K)	1100 ± 150	12.45 ± 0.02	−0.034 ± 0.002
Butyric	2.98 (287 K)	2000 ± 200	12.50 ± 0.02	−0.036 ± 0.002
Isobutyric	2.58 (293 K)	2900 ± 200	12.54 ± 0.02	−0.026 ± 0.002
Pivalic	2.62[a] (298 K)	9600 ± 2000	12.63 ± 0.03	−0.022 ± 0.004

[a] Indicates static relative permittivity.[14] Pivalic acid is solid at 298 K and the static relative permittivity is anticipated to have a higher value than would the corresponding dynamic relative permittivity due to the probable greater ordering of molecules in the solid compared to the liquid. [b] Theoretical limiting chemical shift of dimeric carboxylic acid in a medium of 100% cyclohexane. [c] Δ = Rate of change of δ_{dim} with concentration of acid. Negative values indicate that δ_{dim} increases as the proportion of cyclohexane increases, whilst positive values indicate a decrease in δ_{dim} as the proportion of cyclohexane increases.

dilution is due to the presence of other aggregated species (possibly polymers[4,5,9,10] or trimers[6] or tetramers[7]) which are particularly present at high concentrations of the carboxylic acid. Here, we point out that in the light of our earlier work,[1] the dilution curves can be understood without invoking the presence of any species other than the two represented in Scheme 1.

Results and discussion

Since the hydrogen bonds which hold the two molecules of carboxylic acids together in a dimer are essentially electrostatic in nature,[8] it is to be expected that these hydrogen bonds will become stronger as the relative permittivity of the medium is reduced. Associated with this increase in hydrogen bond strength, there should be a shortening of the hydrogen bonds and a resultant increased downfield shift of the acidic proton in the fully bound state of the dimer.[1,2] The relative permittivity of the five carboxylic acids studied by Kimtys and Balevicius are given in Table 1. They give rise to the expectation that, in the neat acids, the loosest dimer [with the smallest δ value for the carboxylic acid proton in the dimer state (δ_{dim})] will be that for acetic acid, with a gradual tightening of the dimer structure, and an associated increase in δ_{dim} along the series R = $H_3C \rightarrow H_5C_2 \rightarrow n\text{-}H_7C_3 \rightarrow i\text{-}H_7C_3 \rightarrow (CH_3)_3C$. Additionally, since cyclohexane has a relative permittivity ($\varepsilon_r = 2.02$) smaller than that of any of the carboxylic acids studied, the structure of any given dimer in this solvent should be the tightest, with the largest δ_{dim} value for the carboxylic acid proton. These expectations are exactly those evident from the data of Fig. 2; as the proportion of cyclohexane increases, the relative permittivity of the medium decreases, resulting in a tightening of the dimer and a corresponding increase in δ_{dim} leading to a greater value of δ_{obs}. As the concentration of cyclohexane increases beyond a mole fraction of acid of 0.1, greater dissociation of dimers into monomers occurs leading to a corresponding reduction in the value of δ_{obs}. To understand the increase in δ_{dim} along the series of R groups, it is not necessary to postulate a role for the inductive effect of these groups (although this cannot be precluded). It is sufficient to note that as the bulk of the hydrocarbon R group is increased, the effective relative permittivity of the medium surrounding the dimer interface will decrease with a resulting increase in δ_{dim}. This effect is observed for the neat acids; it is also observed in the theoretical data for the dimers in very dilute solution in cyclohexane (see later, Table 1). Evidently, although cyclohexane has a relative permittivity smaller than any of the studied carboxylic acids, the polarity of the medium surrounding any one dimer molecule (Scheme 1) is less in the case of the pivalic acid dimer surrounded by cyclohexane molecules than in the case of the acetic acid dimer surrounded by cyclohexane molecules.

Support for the above proposals was obtained by curve-fitting the data of Kimtys and Balevicius according to the equations for a dimeric association. In order to account for the expected variation of δ_{dim} with the variation in relative permittivity of the medium at different points during the titration, an extra variable was incorporated into the curve-fitting regime (see Experimental). This variable allowed δ_{dim} to change linearly with the concentration of acid. This is a reasonable assumption given that δ_{dim} was predicted to reflect the average relative permittivity of the medium, and this varies approximately linearly with acid concentration. Three variables are therefore optimised by this curve-fitting regime: (i) the theoretical limiting chemical shift of the carboxylic acid proton in a medium of 100% cyclohexane ($\delta_{dim}^{cyclohex}$); (ii) the rate of change of δ_{dim} with concentration of acid (Δ; positive values of Δ indicate an increase in δ_{dim} as the proportion of acid relative to solvent increases, whereas negative values indicate decreasing δ_{dim} with increasing concentration of acid); (iii) the observed dimerisation constant of the acid (K_{dim}^{obs}).

An important qualification must be made at this point. It is our hypothesis that as the relative proportions of carboxylic acid and solvent vary during the dilution titration, the limiting chemical shift of the carboxylic acid proton also varies due to a change in the tightness of the carboxylic acid dimers formed. A corollary of this hypothesis is that the dimerisation constant should also vary as a function of the acid concentration. We therefore propose that there is no unique dimerisation constant for each carboxylic acid in the titrations performed by Kimtys and Balevicius. Rather, the dimerisation constant varies between a value when the medium is 100% cyclohexane and a different value when the mole fraction of acid is 1 (*i.e.*, undiluted acid). The *apparent* dimerisation constants calculated by our curve-fitting regime (K_{dim}^{obs}) are therefore derived as some (unknown) combination of these limiting values. Thus, although the observed dimerisation constants (K_{dim}^{obs}) are dependent on the dimerisation propensity of each acid, we can draw only qualitative conclusions regarding the stability of dimer formation for the different acids.

The results from the curve-fitting are illustrated in Fig. 3 and summarised in Table 1. It can be seen that using this modified curve-fitting regime, a good fit was obtained between the theoretical equations for dimerisation and the data (unlike the case if the extra factor was not included). A number of the predictions outlined earlier are borne out by the data. First, the expected variation in apparent dimerisation constants for the different acids is followed approximately, with acids of lower relative permittivities exhibiting higher apparent dimerisation constants. Second, the relationship identified previously[2] between dimer stability (as manifested by K_{dim}) and dimer strength (as manifested by δ_{dim}) can be seen from the relative values of $\delta_{dim}^{cyclohex}$ and K_{dim}^{obs}. As the dimerisation constants (K_{dim}^{obs}) of the acids increase, the $\delta_{dim}^{cyclohex}$ values also increase. This indicates that the dimers which are more stable are also formed from stronger, tighter hydrogen bonds leading to more downfield δ_{dim} values. Finally, as the difference in relative permittivity between the

Table 2 Solvent relative permittivities[13] and dimerisation parameters for propionic acid obtained from curve-fitting to plots of δ_{obs} *vs.* propionic acid concentration for titrations of a variety of solvents into propionic acid at 300 K

Solvent	Relative permittivity (ε_r)	K_{dim}^{obs}/M^{-1}	$\delta_{dim}^{solvent}$/ppm	Δ/ppm dm^3 mol^{-1}
Hexane	1.89 (293 K)	3300 ± 400	13.02 ± 0.05	−0.036 ± 0.005
Cyclohexane	2.02 (293 K)	1500 ± 300	12.98 ± 0.04	−0.029 ± 0.003
Toluene	2.38 (296 K)	330 ± 70	12.43 ± 0.04	0.016 ± 0.003
2,6-dichlorotoluene	3.36 (301 K)	270 ± 40	12.33 ± 0.03	0.022 ± 0.003
Dichloromethane	8.93 (298 K)	80 ± 10	12.25 ± 0.03	0.035 ± 0.003

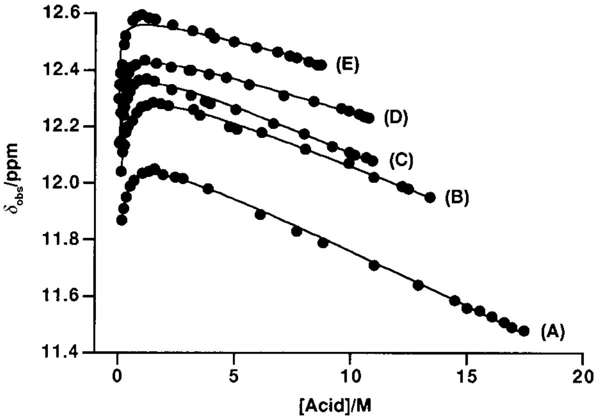


Fig. 3 Dimerisation curve-fits to the data of Kimtys and Balevicius[10] using a least-squares curve-fitting regime. The different plots represent dilution of the following carboxylic acids: (A) acetic acid (K_{dim}^{obs} = 1200 M^{-1}), (B) propionic acid (K_{dim}^{obs} = 1100 M^{-1}), (C) butyric acid (K_{dim}^{obs} = 2000 M^{-1}), (D) isobutyric acid (K_{dim}^{obs} = 2900 M^{-1}), (E) pivalic acid (K_{dim}^{obs} = 9600 M^{-1}).

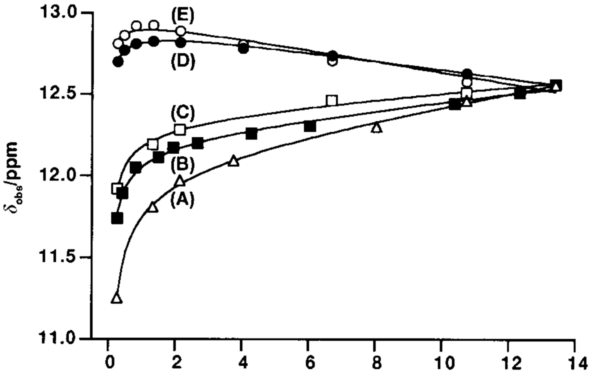


Fig. 4 Dilution titrations of propionic acid (ε_r = 3.44) in solvents of differing relative permittivity at 300 K. The curves associated with each set of points were obtained by curve-fitting according to the equations for a dimeric association. The different plots represent titrations using the following solvents: (A) dichloromethane [ε_r = 8.93 (298 K)], (B) 2,6-dichlorotoluene [ε_r = 3.36 (301 K)], (C) toluene [ε_r = 2.38 (296 K)], (D) cyclohexane [ε_r = 2.02 (293 K)], (E) hexane [ε_r = 1.89 (293 K)]. Relative permittivities at the indicated temperatures were obtained from reference 13.

solvent and the acid being titrated increases [*i.e.*, as the magnitude of ε_r(acid) − ε_r(cyclohexane) increases], the rate of variation of δ_{dim} with acid concentration (Δ) also increases. In all cases, Δ is negative indicating that δ_{dim} increases with increasing cyclohexane concentration, *i.e.*, the dimers become tighter as the proportion of cyclohexane increases. The values obtained from the data of Kimtys and Balevicius thus demonstrate clearly the effect of the medium relative permittivity on δ_{dim} and K_{dim}^{obs}.

Similar titrations to those of Kimtys and Balevicius were carried out for propionic acid using a variety of diluents with different relative permittivities (Fig. 4). Dimerisation constants were obtained by curve-fitting to the data, as above, and the values so obtained, as well as the shapes of the dilution curves, were found to reflect the relative permittivities of the different solvents used (Table 2). For dilutions with solvents of lower relative permittivity than that of propionic acid there was initially a downfield shift of δ_{obs} upon dilution, indicative of a tightening of the dimer interface. For dilutions in solvents of higher relative permittivity than that of propionic acid, the initial downfield shift of δ_{obs} (hitherto regarded as characteristic of the presence of polymeric species)[4–7,9,10] was not observed (Fig. 4). Indeed, an upfield shift upon dilution was observed which was greater than that which would have been expected on the basis of dissociation of dimers into monomers. When the relative permittivity of the solvent used for dilution closely matched that of the acid (propionic acid ε_r = 3.44, 2,6-dichlorotoluene ε_r = 3.36), the shape of the plot of δ_{obs} *vs.* mole fraction of propionic acid approached that characteristic of a two-state (monomer–dimer) equilibrium. The dimerisation constants and theoretical limiting values of δ_{dim} ($\delta_{dim}^{solvent}$) derived from these titrations were also found to reflect the relative permittivities of the solvents used. As the solvent relative permittivities increase, both K_{dim}^{obs} and $\delta_{dim}^{solvent}$ values decrease. Similar variations in curve-shape to those obtained for titrations of propionic acid with 2,6-dichlorotoluene were obtained for titrations of other carboxylic acids with a solvent of similar relative permittivity to that of the particular acid used (Fig. 5). The curve-shapes obtained from these titrations are characteristic of that expected for a two-state (monomer–dimer) equilibrium where δ_{dim} is approximately invariant at all concentrations of acid.

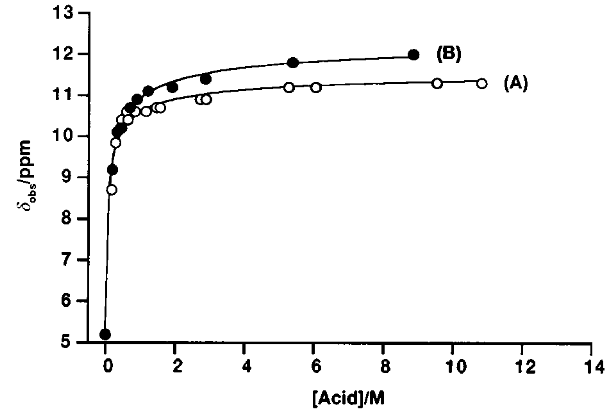


Fig. 5 Plots of δ_{obs} *vs.* concentration of acid for dilution titrations of (A) isobutyric acid (ε_r = 2.58) and (B) pivalic acid (ε_r = 2.62) using a solvent of similar relative permittivity to that of the acids [*o*-xylene; ε_r = 2.56 (293 K)[13]] at 363 K. The curves associated with each set of points were obtained by curve-fitting according to the equations for a dimeric association (*cf.* Fig. 4).

The results demonstrate the importance of both the solvent relative permittivity and the carboxylic acid relative permittivity on the strength of the dimers formed. The data of Kimtys and Balevicius demonstrate that the different relative permittivities of the acids used result in them forming dimers of varying stability in cyclohexane, whilst the data presented in this paper show that a variation in solvent relative permittivity also results in a variation in dimer stability. Additionally, they

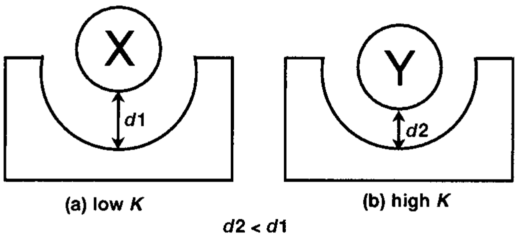


Fig. 6 Schematic representation of the association of (a) an entity X and (b) a similar entity Y binding with different equilibrium constants to a receptor. The complex formed in (b), with a high equilibrium constant, is tighter than that in (a) and relatively few bound states are accessible to the ligand. As a result, the distance *d*2 is typically less than the distance *d*1.

illustrate the validity of the hypothesis that the change in δ_{obs} upon dilution in cyclohexane can be accounted for according to a two-state (monomer–dimer) equilibrium. This hypothesis removes the necessity for the postulation of additional oligomeric states of the acids. Although they do not prove the absence of such additional states, by application of Occam's razor, such states should not be postulated in the absence of clear evidence for their existence in solution.

Finally, the relationship between dimer stability (K_{dim}) and dimer strength (δ_{dim}) is also clear from the data described. This relationship is represented schematically in Fig. 6 where two similar associations (not necessarily carboxylic acid dimerisations) formed with different values of *K* are depicted. Where the value of *K* is higher, the interface is correspondingly tighter and there are reduced bond lengths across the binding interface. This relationship has now been illustrated both for a relatively simple system (dimerisation of carboxylic acid dimers) and for more complex systems (dimerisation of glycopeptide antibiotics and ligands binding to glycopeptide antibiotics),[2,3] indicating that it is likely to be of general relevance in molecular recognition.

Experimental

Materials

Propionic acid, isobutyric acid, pivalic acid, and 2,6-dichlorotoluene were purchased from Aldrich. *o*-Xylene was purchased from Lancaster Synthesis Ltd. All acids and solvents were obtained as reagent grade materials (*ca.* 98% purity) and were fractionally distilled two or three times (under reduced pressure for high boiling point liquids) prior to use. After distillation, all acids and solvents were stored under an argon atmosphere and over molecular sieves (4 Å) in order to prevent contamination with water.

^{1}H NMR spectroscopy

Experimental procedure. Samples were prepared immediately prior to NMR analysis by addition of the appropriate volumes of acid and solvent to NMR tubes. All glassware used was dried *in vacuo* prior to use in order to prevent contamination with water.

^{1}H NMR spectra were recorded on Bruker DRX500 and DRX400 spectrometers. All spectra were one-dimensional and were recorded with 16k data points. Data was processed with XWIN-NMR software and referenced to the residual protiosolvent peaks of an external reference of dimethyl sulfoxide-d_6 (δ 2.52) or 1,1,2,2-tetrachloroethane-d_2 (δ 6.00).

Calculation of dimerisation constants and limiting dimer chemical shift values. ^{1}H NMR titration data were plotted as δ_{obs} [observed chemical shift of the carboxylic acid proton (RCO$_2$*H*)] *vs.* concentration of acid. For all acids, a monomer chemical shift of 5.2 ppm was assumed,[10,11] as was a fast exchange between monomer and dimer species and a linear variation of δ_{dim} with the proportion of acid present. Dimerisation parameters were calculated by least-squares curve-fitting of the plotted data to the theoretical equations (1) and (2) for a

$$K_{dim} = \frac{[\text{dimer}]}{[\text{monomer}]^2} \quad (1)$$

$$\delta_{obs} = 5.2 + \{(a + b[\text{acid}]_{total}) - 5.2\}\,\frac{2[\text{dimer}]}{[\text{acid}]_{total}} \quad (2)$$

dimeric association,[12] where: a = limiting dimer chemical shift of carboxylic acid proton in 100% diluent ($\delta_{dim}^{solvent}$/ppm); b = the rate of change of δ_{dim} with concentration of acid (Δ/ppm dm^3 mol^{-1}); [dimer] = concentration of carboxylic acid dimer (mol dm^{-3}); [monomer] = concentration of carboxylic acid monomer (mol dm^{-3}); [acid]$_{total}$ = total concentration of carboxylic acid present (mol dm^{-3}); δ_{obs} = observed chemical shift of the carboxylic acid proton (RCO$_2$*H*; ppm). The [dimer] was determined iteratively using the proprietary general curve-fitting routine of *Kaleidagraph 3.0.5* (Abelbeck Software). This gave the dimerisation constant (K_{dim}^{obs}) and the parameters a ($\delta_{dim}^{solvent}$) and b (Δ).

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