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Cyclodextrin inclusion of four phenylurea herbicides: determination of complex stoichiometries and stability constants using solution ^{1}H NMR spectroscopy

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An inclusion complex formation between α- and β-cyclodextrin and four phenylurea analogues, namely metobromuron, monolinuron, monuron and fenuron, is reported. Complex formation was established using solution ^{1}H NMR spectroscopy. Complex stoichiometries were determined by the method of continuous variation using the chemically induced shifts of both the host and guest protons. An analysis of the spectroscopic data revealed the stoichiometry as 1:1 in all cases while a further analysis of the same data yielded values for the association constant *K* ranging from 208 to 2749 M^{-1}. From the observed chemical shifts it was deduced that in all cases, only the guest aromatic ring enters the host cavities, the substituted urea moiety protruding from the secondary rim in the case of α-cyclodextrin, but from the primary rim in the case of β-cyclodextrin.

Keywords: cyclodextrins; phenylurea herbicides; association constants; ^{1}H NMR; chemically induced shifts

Introduction

Discovered in the 1960s, phenylureas represent a large and an important class of herbicides used for the control of broadleaf weeds in cereals, vegetables and fruit trees through the action of inhibiting photosynthesis (*1*). These herbicides are not very toxic to animals but exposure to the UV light may significantly enhance their toxicity. A phototransformation may be induced by the absorption of light resulting in the formation of more toxic intermediates (*2*). In addition, it has been shown that phenylurea herbicides under suitable conditions, such as those present in humic soils, may undergo acid or base hydrolysis (*3*, *4*). However, owing to environmental conditions, these processes are slow and there is little accumulation of toxic by-products. Since these compounds are subject to slow transformation, with the mean persistence in the environment of 4–12 months, there are relevant risks (*5*). Phenylurea herbicides are also susceptible to thermal decomposition, which has significant implications for their storage, action and detection (*1*, *6–8*). The complexes formed between cyclodextrins (CDs) and pesticides are new entities and are endowed with properties that are in many instances superior to those of herbicides. The phenylureas investigated here for their affinity for CDs are metobromuron [3-(*p*-bromophenyl)-1-methoxy-1-methylurea], monolinuron [3-(*p*-chlorophenyl)-1-methoxy-1-methylurea], monuron [*N*′-(*p*-chlorophenyl)-*N*,*N*-dimethylurea] and fenuron (1,1-dimethyl-3-phenylurea) (Figure 1). This paper describes the complexation between the four phenylurea herbicides and the hosts α- and β-CD in solution, investigated by the ^{1}H NMR spectroscopy. The objective was to establish the solution-state stoichiometries and the association ('stability') constants and to infer possible geometries of guest inclusion in the host cavities.

Results and discussion

Solution ^{1}H NMR spectroscopy

The unique features of the hydrophobic cavity of CDs are the methine hydrogens (H_3 and H_5) situated inside the cavity and the methylene hydrogens ($H_{6a,b}$) located near the primary rim. These protons are extremely sensitive to inclusion complexation, undergoing measurable NMR shifts during this process. The guest aromatic protons may also undergo shifts upon inclusion. The measured chemically induced shifts (CISs henceforth) allow the extraction of association constants and stoichiometries of the formed complex. The stoichiometries are obtained from the observed changes in the CIS of a particular host or guest proton H_i using the method of constant variations (*9*). Changes in the chemical shifts of protons H_3 for α-CD and H_3, H_5, $H_{6a,b}$ for β-CD were monitored because they showed the largest CIS values, while for the guests, H_a and H_b were monitored as they showed the largest CIS values. Both of the guest protons H_a and H_b

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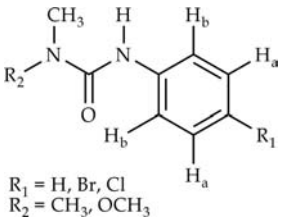

Figure 1. Chemical structure of the phenylurea herbicides. Subscripts a and b on the phenyl protons are used to identify the different chemical environments. Metobromuron: R_1 = Br, R_2 = OCH_3; monolinuron: R_1 = Cl, R_2 = OCH_3; monuron: R_1 = Cl, R_2 = CH_3; fenuron: R_1 = H, R_2 = CH_3.

(Figure 1) were monitored in the case of complexation with α-CD, while only H_b was monitored when the complexation was with β-CD. The Job plots for complexation with α-CD broadly maximise at $r = 0.5$ while those for the β-CD complexes are generally symmetrical, the maxima occurring at $r = 0.5$ (Figures 2 and 3, respectively) (*9*). This is indicative of the 1:1 stoichiometry for each complex within the range of concentrations investigated. The Job plot for the guest protons (H_a and H_b) of fenuron interacting with α-CD could not be determined as the spectra were not clearly resolved. The order of the CISs determined from the Job plots in Figure 2 for each guest with α-CD is $H_a > H_b > H_3$ for metobromuron and $H_3 > H_a > H_b$ for monolinuron and monuron. The fact that only proton H_3 of the host shows a significant CIS (Table 1) indicates that the guest in each case must be lodged near the

Table 1. Maximum observed CIS values for the complexation of the four guests with α-CD.

Guest	$\Delta\delta_{obs}^{max}H_3$ (ppm)	Guest $\Delta\delta_{obs}^{max}H_a$ (ppm)	Guest $\Delta\delta_{obs}^{max}H_b$ (ppm)
Metobromuron	0.079	−0.123	−0.097
Monolinuron	0.058	−0.026	−0.021
Monuron	0.065	−0.055	−0.042
Fenuron	0.005	−0.002	−0.005

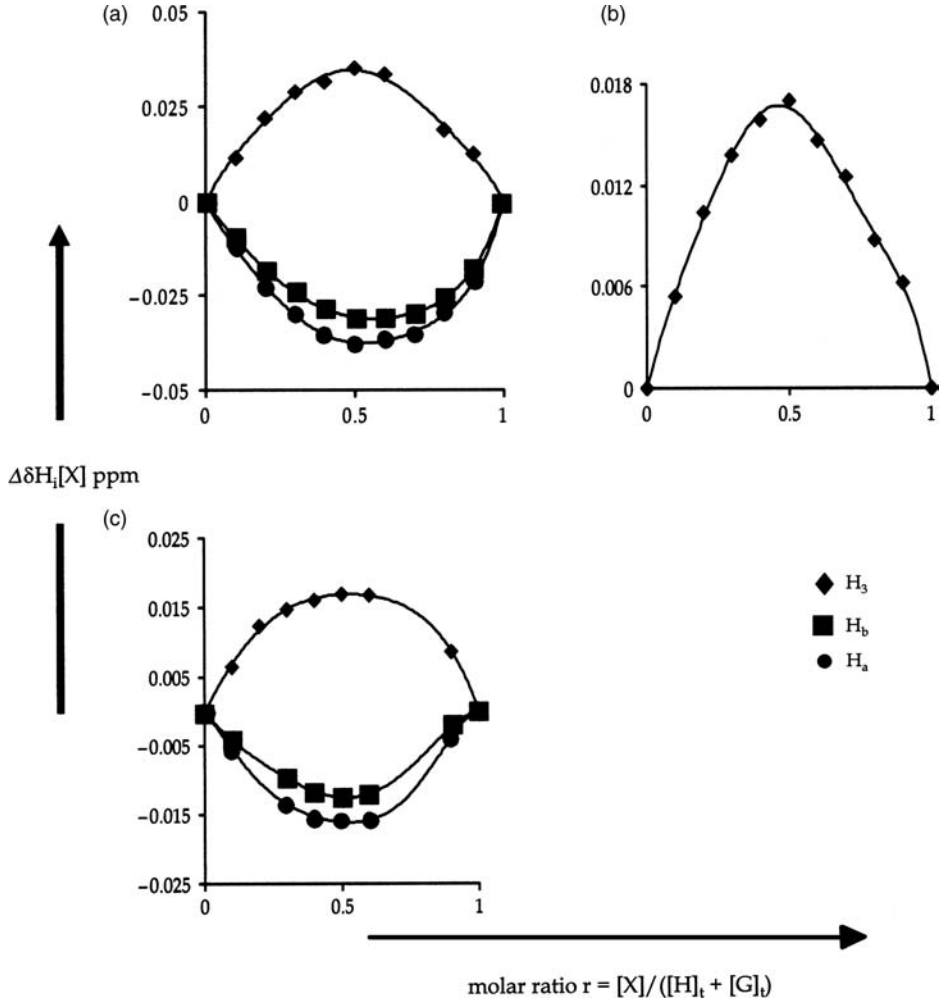

Figure 2. Job plots for α-CD complexation with (a) metobromuron, (b) monolinuron and (c) monuron.

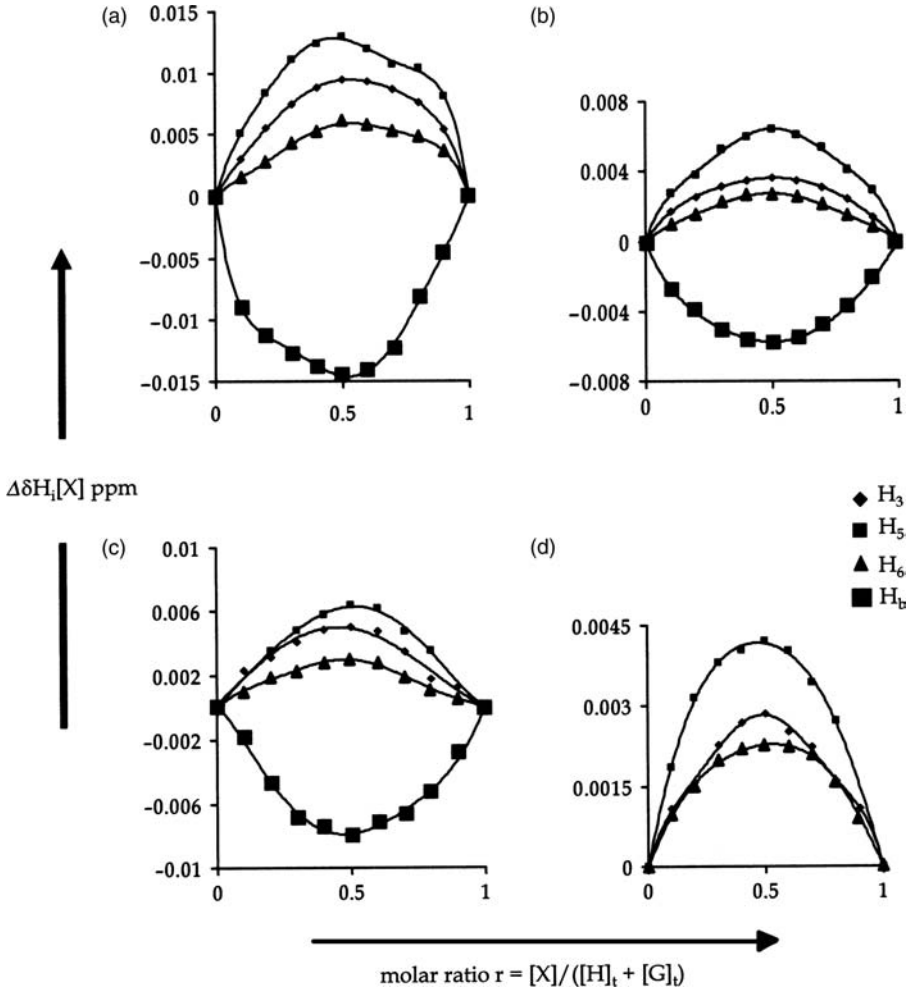

Figure 3. Job plots for β-CD complexation with (a) metobromuron, (b) monolinuron, (c) monuron and (d) fenuron.

secondary rim and not located deep within the cavity (Figure 4(a)). Also, the depth of penetration of each guest is slightly different. The variations in the magnitudes of the CISs indicate the extent of intrusion into the α-CD cavity. On the other hand, the magnitudes of the CIS values (Table 2) for the β-CD cavity protons H_3, H_5 and $H_{6a,b}$ (Figure 3) are in the order $H_5 > H_3 > H_{6a,b}$, suggesting that for each complex, the guest molecule is located near the primary rim (*10*). The data imply that the guest is located deep within the cavity (Figure 4(b)) and

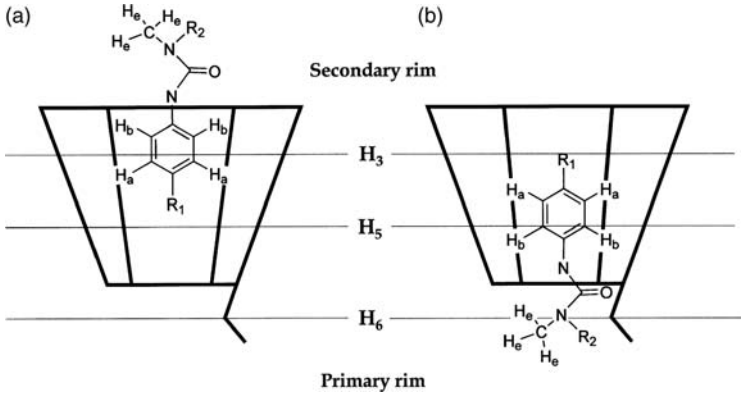

Figure 4. Schematic of the inferred general modes of inclusion of the guests in the cavity of (a) α-cyclodextrin and (b) β-cyclodextrin.

Table 2. Maximum observed CIS values for the complexation of the four guests with β-CD.

Guest	$\Delta\delta_{obs}^{max}H_3$ (ppm)	$\Delta\delta_{obs}^{max}H_5$ (ppm)	$\Delta\delta_{obs}^{max}H_{6a,b}$ (ppm)	Guest $\Delta\delta_{obs}^{max}H_b$ (ppm)
Metobromuron	0.030	0.051	0.015	−0.046
Monolinuron	0.017	0.027	0.010	−0.021
Monuron	0.023	0.036	0.010	−0.029
Fenuron	0.011	0.019	0.010	−0.006

an indication of the depth of insertion is provided by the fact that the signals for both H_3 and H_5 undergo a significant shift (*11*). For the guest, the aromatic protons show a significant downfield movement, and it is therefore concluded that the phenyl moiety interacts with the CD. Additionally, the protons belonging to the methyl and methoxyl groups (for each guest molecule) show only small shifts in the range of 0.001–0.009 ppm in absolute units. This indicates that the methyl and methoxyl moieties protrude from the torus (*12*), as indicated schematically in Figure 4. It is interesting to note that the experiments have shown analogous modes of inclusion for all the four guest compounds with α-CD. Similarly, the complexes with β-CD have more or less the same mode of inclusion, but different from that with α-CD.

The predominant stoichiometry for these complexes is 1:1. The association constants *K* are listed in Tables 3 and 4 along with the correlation coefficient (*R*) and the error loss function (*E*). It is generally accepted that the hydrophobicity of a guest favours its inclusion (*13*). It must, therefore, directly or indirectly influence the size of the binding constant. On this basis, it may be used as an indication of the strength of interaction between the host (H) and the guest (G). The aqueous solubilities of the guests are in the order fenuron > monolinuron > metobromuron > monuron. However, the association constants *K* for the α-CD complexes do not follow this order; they are, instead, in the order metobromuron > monolinuron > monuron, with the association constant for fenuron interaction with α-CD not determined. The order of the association constants with β-CD differs from those with α-CD and is metobromuron ≫ monuron ≈ fenuron > monolinuron. In fact, no trend is apparent from the data presented.

A recent study by Dupuy et al. (*14*) of the inclusion of the phenylureas isoproturon, fenuron, monuron and diuron in β-CD has been performed using the ^{1}H NMR spectroscopy. In order to compare our results for the β-CD complexation with the two common guests monuron and fenuron with those reported by Dupuy et al., it is necessary to clarify some of the theoretical aspects used in our interpretation of the data. Firstly, the chemical shift variation $\Delta\delta_{obs}$ is defined as $\Delta\delta_{obs} = \delta_{free} - \delta_{obs}$ in the present work, whereas Dupuy et al. reported the CIS as $\Delta\delta = \delta_{complex} - \delta_{free}$. The latter authors did not define the term $\delta_{complex}$ in their paper. We assume that it is equivalent to δ_{obs} for a particular H:G ratio which, however, is not stated. According to our definition, the quantity $\Delta\delta_{complex} = \delta_{free} - \delta_{complex}$ is obtained as a fitting parameter from which we calculate the theoretical $\delta_{complex}$. This value represents the chemical shift change only if the pure complex is present in solution. Secondly, it is important to know that if the NMR signal is shifted to lower δ values, then the shift is upfield; conversely, if the signal is shifted to higher δ values, then we have a downfield shift. Thus, in order for us to compare our results with those reported in Ref. (*14*), it is necessary to change the sign of the published $\Delta\delta_{obs}$ values. Thus, if $\Delta\delta_{obs} > 0$, then we have an upfield shift and if $\Delta\delta_{obs} < 0$, then the shift is downfield and this is valid only if we define $\Delta\delta_{obs}$ as $\Delta\delta_{obs} = \delta_{free} - \delta_{obs}$.

For the complexation between monuron and β-CD, the data presented here as well as those reported earlier (*14*) show upfield shifts for all the protons of β-CD. From our results, there is a clear distinction in magnitude between $\Delta\delta_{obs}$ for H_3, H_5, $H_{6a,b}$ and $\Delta\delta_{obs}$ for H_1, H_2, H_4. In fact, there is an order-of-magnitude difference between them, and it clearly indicates the occurrence of an interaction between the guest and the interior of the host in solution. On the other hand, the data in Ref. (*14*) are all of the same magnitude and therefore do not provide any clear indication of complexation. Similar trends are apparent for the signals obtained in our study and those in Ref. (*14*) for the guest protons, even though the magnitudes of the shifts are different. It is also worth noting that the $\Delta\delta_{complex}$ (H_b) value (0.073 ppm) obtained from the fitting procedure used in our work is close to the hypothetical value (0.1 ppm) reported earlier (*14*).

In the case of complexation between fenuron and β-CD, the authors of the previous study reported that all the proton signals of the host move upfield, except for the proton H_2 which does not move at all. In our study, all the proton signals are shifted, those for H_1 and H_4 moving downfield while those for H_2, H_3, H_5 and $H_{6a,b}$ move upfield. The shift variation reported in Ref. (*14*) for the

Table 3. *K*, *E* and *R* values for the complexation with α-CD at 298 K.

	Metobromuron	Monolinuron	Monuron	Fenuron
K (M^{-1})	1519	1085	208	–
R	0.9977	0.9991	0.9965	–
E	7.09×10^{-4}	4.3×10^{-5}	2.0×10^{-4}	–

Table 4. *K*, *E* and *R* values for the complexation with β-CD at 298 K.

	Metobromuron	Monolinuron	Monuron	Fenuron
K (M^{-1})	2749	368	726	715
R	0.9984	0.9997	0.9987	0.9990
E	4.8×10^{-5}	1.3×10^{-6}	9.8×10^{-6}	2.3×10^{-7}

H_4 proton of the host is larger than our reported shifts for H_3, H_5 and $H_{6a,b}$ protons. This is reflected in our results as the shift magnitudes for H_2 and H_4 are also larger than those for H_3, H_5 and $H_{6a,b}$. The only differences between the previously reported shifts for the guest protons and ours are the magnitudes of the shifts and the fact that no observable shift was previously reported for H_b (Table 5).

The two sets of data show very similar trends as far as the direction of the shift is concerned, the only exception being H_b. A possible reason for the reported differences may be related to the concentrations of the solutions, as most, if not all, other conditions are similar (see Materials and methods). However, the concentrations used in the previous work (*14*) were not reported and we are therefore unable to draw any further conclusions. From the reported 2D NMR experiments (*14*) for the interaction between β-CD and the guests monuron and fenuron, correlation cross-peaks were observed between H_3 and H_5 of the host and the *ortho*- and *meta*-protons of the guest. It was also observed that the correlation band for H_3 has a greater intensity than that of H_5. Dupuy et al. (*14*) concluded that the phenyl moiety is included in the torus while the methyl and methoxyl moieties protrude from the cavity. These results correlate very well with the conclusion that we arrived at based on the ^{1}H NMR data.

In conclusion, we believe that the present study has convincingly elucidated the nature of inclusion of the four phenylurea herbicides in α- and β-CD in solution under well-defined conditions. This information, together with the reliable stoichiometric data and especially the values of the association constants, will be of significant value in future considerations of the agrochemical application of the phenylureas in the form of their CD inclusion complexes.

Table 5. Comparison of shift variations for complexation between fenuron and β-CD with those reported previously (*14*).

Proton	(Δδ) (*14*)	(Δδ) Present study
H_a	−0.01 downfield	−0.0017 downfield
H_b	Unaffected	0.0022 upfield
H_c	−0.02 downfield	−0.0060 downfield
H_e	0.01 upfield	0.0004 upfield

Materials and methods

NMR experiments

The D_2O (deuterium content 99.7%) was purchased from National R&D Institute for Cryogenics and Isotopic Technologies (INCD-ICSI) Rm. Vălcea, Romania. α- and β-CD were obtained from Cyclolab (Budapest, Hungary). Metobromuron, monuron and fenuron were obtained from ChemService (West Chester, PA, USA), while monolinuron was obtained from Riedel-deHaën, Sigma-Aldrich (Steinheim, Germany). All compounds were used as received. The NMR experiments were performed at 300 and 400 MHz with Varian-Gemini 300 and Bruker AMX 400 spectrometers, respectively. The ^{1}H NMR spectra were recorded in D_2O solution at 293 ± 0.5 K. The typical conditions were as follows: 16 K (32) data points; sweep width 4500 Hz, giving a digital resolution of 0.28 Hz/point. The 90° pulse width was 13 μs and the spectra were collected by co-addition of 32 or 64 scans. The pulse width was 5 μs (45°) and the spectra were collected by co-addition of 32 or 64 scans. In some cases, an appropriate Gaussian function was applied before Fourier transformation to enhance the spectral resolution. The stoichiometries of α- and β-CD complexes in solution were determined in the following way. Equimolar stock solutions of the host (H) and the guest (G) were prepared in D_2O. The stock solutions were mixed together ensuring a constant final volume. Changing the proportions of [H] and [G] ensured that the complete range ($0 < r < 1$) of ratios $r = [X]/([H]_t + [G]_t)$ was sampled. In the preceding definition, [X] is equivalent to the concentration of the host or guest for the sample, and $[H]_t$ and $[G]_t$ are the total concentrations of the host and guest, respectively. Thus, the total concentration of H and G was maintained constant ($[H]_t + [G]_t = M$, where M is the total concentration and $M = 1$ mM) for each sample. The association constants were calculated using the program CONSTEQ (*15*). From the NMR data, the quantity $\Delta\delta_{obs}\cdot[X]$ was obtained by subtracting the observed chemical shift value for a given sample from the shift of the free X. The $\Delta\delta_{obs}\cdot[X]$ was then plotted against r in a Job plot, the maximum of the curve corresponding to the complex stoichiometry (*11*). The association constants for the 1:1 complexes were evaluated by a nonlinear least-squares curve-fitting regression analysis of the observed chemical shift changes of the guest and β-CD NMR lines, as a function of concentration according to Equation (1).

The equation involves no approximations and correlates the total concentration of the host and guest molecules with the observed difference in the chemical shift, $\Delta\delta_{obs}$:

$$\Delta\delta_{obs}^{(X)} = \frac{\Delta\delta_c^{(X)}}{2[X]_t} \times \left\{ [M] + \frac{1}{K} \pm \left[\left([M] + \frac{1}{K} \right)^2 - 4[H]_t[G]_t \right]^{1/2} \right\}. \quad (1)$$

The program CONSTEQ (*15*) is based on an iterative procedure following specific algorithms in order to fit the experimental values of $\Delta\delta_{obs}$ to the appropriate equation. Each iteration step sets up a quadratic function to determine the direction of the search and calculates the loss error function E (Equation (2)):

$$E = \sum_{i,j} \left(\Delta\delta_{obs}^{(i,j)} - \Delta\delta_{calc}^{(i,j)} \right)^2. \quad (2)$$

E is defined as the sum of the squares of the deviations of the predicted values, until the search converges (where i counts the sample number and j the studied proton). The fitting procedure reaches convergence when the difference between two consecutive E values is less than 10^{-6} (Equation (2)). The treatment of the entire set of protons studied produces a single K value for the entire process and a set of calculated $\Delta\delta_{calc}$ values. $\Delta\delta_{calc}$ represents the chemical shift difference (for a given proton) between the free molecule and the pure complex (*16*). The program is quite flexible as both the host and the guest can be observed for spectroscopic perturbations, allowing up to 15 protons to be used in the fitting process.

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