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Original article

Inclusion complexes of 2-chloroethylnitrososulfamides (CENS) with β-cyclodextrin

Mekki Kadri [a], Nabila Dhaoui [a], Mohamed Abdaoui [a,*], Jean-Yves Winum [b], Jean-Louis Montero [b,*]

[a] *Laboratoire de chimie appliquée, Université de Guelma, BP 401, Guelma 24000, Algeria*
[b] *Laboratoire de chimie biomoléculaire, UMR 5032, Université Montpellier II, CNRS, laboratoires Mayoly Spindler, ENSCM, 8, rue de l'École-Normale, 34296 Montpellier cedex, France*

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Abstract

Host–guest association between four CENS (derivated from piperidine, dibenzylamine, dicyclohexylamine and methyl prolinate) and β-cyclodextrin was carried out in solution and solid state. Characterisation, stoichiometry, solubility, dissociation constants and stability of such complexes were studied, showing that the inclusion with β-CD appears as a promising mode of formulation of 2-chloroethylnitrososulfamides.

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1. Introduction

2-Chloroethylnitrosoureas (CENU) are an important family of alkylating agent with a broad spectrum of activity [1,2]. However, their contribution to cancer chemotherapy is limited by their toxic side effects which are related to the formation of carbamoylating species (isocyanate) during their decomposition [3,4].

2-Chloroethylnitrososulfamides (CENS) constitute a new family of oncostatic agents structurally related to 2-chloroethylnitrosoureas, but devoid of any carbamoylating activity. Promising agent prepared on this basis have demonstrated interesting cytotoxic activity and among them, some proved to be considerably more potent than the parent nitrosourea [5–12].

In previous papers [5–12], we reported the main approaches for the synthesis of the CENS and the general structural data of this new class of compounds (UV, IR, RX, ^{1}H, ^{13}C and ^{15}N NMR). The preliminary pharmacological studies showed that the hydro-liposolubility balance seemed to be in connection with the oncostatic potential. A comparative study between CENU and CENS families unambiguously demonstrated the subtle reactivity of the sulfamido class: indeed, the nature of the carrier linked to the nitrososulfamoyl group (primary vs secondary amine) clearly emerged among parameters concerning the stability limits of CENS.

Two approaches—not exclusive—should be envisioned concerning an improvement of their pharmacologic potential:

1. A systematic structural variation of the carrier group, followed by the determination of the stability and solubility factors of the resulting compounds.
2. A derivatization in a "prodrug" allowing an adequate formulation and a resistance against a precoce decomposition, especially in aqueous conditions.

In this paper, we report our results in this last option: our goal was the preparation of cyclodextrin-CENS inclusion complexes.

Beta-cyclodextrin (β-CD) is a cyclic oligosaccharide consisting of seven glucose units connected by α-1,4 glycoside bonds. This molecule is presented as an emblematic host molecule [13] able to form inclusion complex with various guest molecules. A considerable literature was devoted to β-CD and its applications, especially in the pharmacological area [14–22]. Several medicinal substances have been suc-

* Corresponding authors.
E-mail address: abdaoui_med@yahoo.fr (M. Abdaoui).

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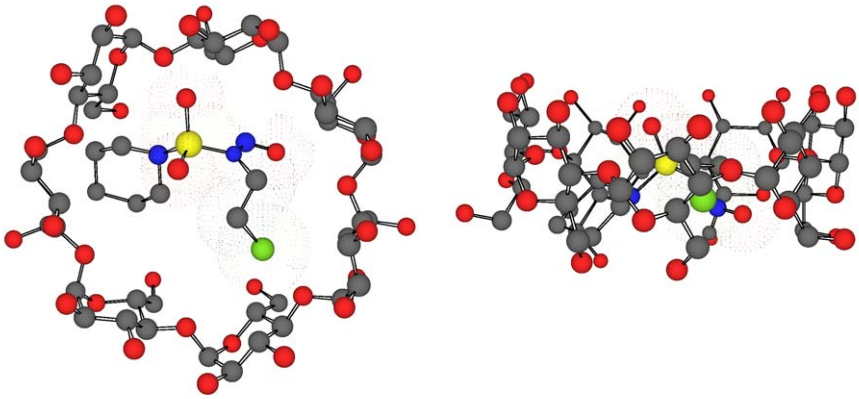

Fig. 1. Model of inclusion of **1** into β-CD.

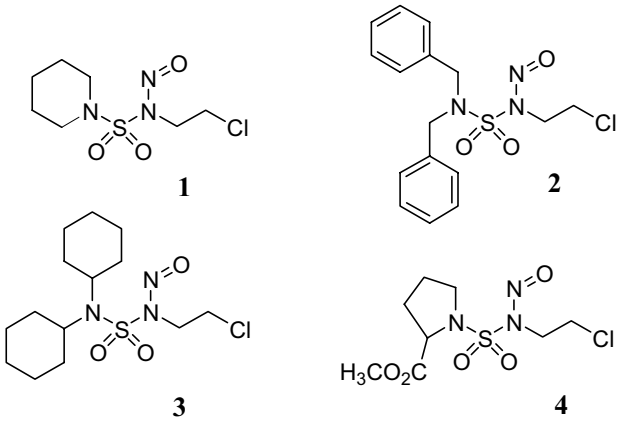

Fig. 2. CENS used in this study.

cessfully complexed by cyclodextrin. These complexes can increase the stability and improve the solubility and bioavailability of the substance and modify the pharmacokinetics of the resulting drugs with a subsequent reduction in adverse effects [14–22]. No mention, however, was reported on attempts of complexation with CENUs.

The most important structural feature of β-CD is its torus like shape with a hydrophobic interior cylindrical cavity and hydrophilic faces. In terms of relative size of β-CD-host and CENS-guest, the choice can be justified by the structural parameters independently established by X-ray diffraction studies. In the conical cylinder of cyclodextrin, the cavity diameter is estimated as 6.8 Å and the height of torus 8 Å. In terms of hydrophobic interactions, a good affinity could be expected between the osidic cavity and the lipophilic nitrososulfamide moiety. Fig. 1 shows the fit between the piperidine-CENS **2** and the cyclic polyholoside at the suitable scale.

The complexation between CENS **1–4** (Fig. 2) and β-CD was carried out following two different procedures, in liquid phase and at the solid state. Different spectroscopic and spectrometric techniques were used to characterize inclusion complexes (UV–Vis and ^{1}H NMR).

2. Results and discussion

2.1. Formation of solid state inclusion complexes

Solid state complexes between CENS and β-CD have been obtained, giving, respectively, the complexes **C-1** to **C-4**. The complexes exhibit the following physicochemical and spectral characteristics (Table 1).

The values of these characteristics are different from those of both CENS and the free β-CD. The formation of inclusion complexes was confirmed by examination of ^{1}H NMR spectra. The shifts (0.33 ppm) of H-3 and H-5, respectively, in the β-CD moiety of an inclusion complex from the original chemical shifts of β-CD itself indicated that the guest compound existed near H-3 and H-5 in the hydrophobic cavity. On the other hand, a displacement towards the low fields was observed in C**H**$_2$NNO and C**H**$_2$Cl of CENS associated with the β-CD.

2.2. Detection of host–guest complexes formation in solution

Upon complexation, several physicochemical properties of guests are modified. The high electron density prevailing inside the cyclodextrin cavity mobilises the electrons of the incorporated molecules [23], resulting in characteristic changes in various spectral properties of both guest and host.

In solution, the complexation was demonstrated by spectrophotometry at UV–Vis at 20 ± 0.1 °C. The solution concentrations used were 10^{-5} M in methanol. Our methodology consisted to compare the absorption spectra of β-cyclodextrin and CENS with the absorption spectra of their respective complexes under the same conditions. Initial attempts to use acetonitrile as solvent failed because of the limited solubility of our compounds (**C-1** and **C-2**), thus, the complexation was carried out in methanol medium. Fig. 3 represents the absorption spectra of the solutions of CENS, β-CD and of their complexes.

Nitrososulfamide moiety is a chromophoric well suitable for spectrophometric measurements in UV–Vis in the range of 225–245 nm (ε about 5000). The CENS spectra show

Table 1
Characterisation of the solid state inclusion complexes

Compounds	Mp (°C)	IR (KBr, ν cm^{-1})				λ (nm) [a]	ε (l/mol cm)
		N=O	SO_2	C=O	OH		
1	52	1575	1348 1150	–	–	244	40 000
2	44	1580	1360 1154	–	–	230	38 000
3	65	1550	1375 1175	–	–	227	35 000
4	98	1515	1365 1150	1710	–	243	42 000
C-1	200	1610	1330 1144	–	3500 Broad	252	52 660
C-2	110	1575	1358 1134	–	3500 Broad	247	41 390
C-3	150	1620	1380 1160	–	3500 Broad	237	35 320
C-4	178	1600	1373 1173	1730	3500 Broad	253	19 490

[a] Methanol as solvent.

maxima of absorption at 244, 230, 227 and 243 nm. The β-cyclodextrin spectrum presents two broad bands in the UV region, with maxima at 223 and 284 nm; on the other hand, their respective complexes have absorption spectra quite different. A comparison of absorption spectra of CENS (**1–4**) with their respective complexes (**C-1–C-4**) clearly showed, in each case, a bathochromic shift (from 5 to 10 nm) accompanied by isobestic points at 270 nm for **C-1**, and 272 nm for **C-4**. These results indicate the formation of new species probably inclusion complexes [24–26].

2.2.1. Determination of stoichiometry

Most current stoichiometric ratios between β-CD and guest molecules reported in the literature are 1:1, 1:2 and 2:1 [27,28]. The method of molar ratios [29] was used to establish the stoichiometry of complexes. Abrupt variations of the slopes in plot absorbance against molar ratios, confirmed the formation of an association between CENS and β-CD. The results reported in Fig. 4 indicated that molar ratio of CENS/β-CD is 1:1 independently of CENS nature. However, when **C-2** was used, we cannot exclude the concomitant formation of another species with 2:1 ratio in small proportions. This can be attributed to a possible insertion of another group such as aromatic moiety, which are complementary in size to the cyclodextrin cavity.

The stoichiometries were confirmed by the ^{1}H NMR spectra. By comparison with the integral of resonance of CENS and β-CD, one CENS unit was found to be bound to a single β-CD.

2.2.2. Measurement of octanol–water partition coefficients

Information on how these CENS and their inclusion complexes are distributed between water and a non-miscible solvent is of primary interest. In fact, this property, expressed by octanol–water partition coefficient *P*, has become a standard method to carry out quantitative structure–activity relation (QSAR) studies. It was also shown that the biological behaviour of drugs could be correlated with their partition coefficients [30–33]. Partition coefficients of CENS and their complexes were determined by the shake-flask method [34].

The octanol–water partition coefficient *P* is defined as the ratio of a chemical's concentration in the octanol phase to its

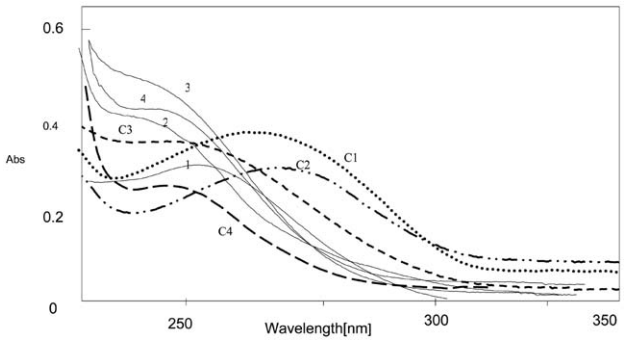

Fig. 3. UV–Vis absorption spectra of free CENS (10^{-5} M) and of their inclusion complexes in methanol

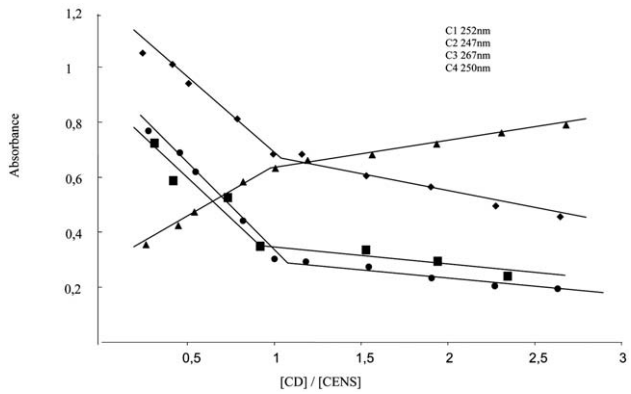

Fig. 4. Molar ratios method for determination of the stoichiometry.

Table 2
Values of partition coefficients of CENS and their complexes

CENS	Log*P*	CENS–β-CD	Log*P*
1	1.03	**C-1**	0.41
2	1.32	**C-2**	0.64
3	0.86	**C-3**	0.84
4	–0.19	**C-4**	–0.31

Molar ratio 1:1.

concentration in the aqueous phase of a two-phase octanol–water. The logarithm *P* is known as Hansch factor or sometimes lipophilicity.

For this purpose, into a series of flasks, 2.5 ml of 10^{-4} M aqueous solutions of each compound (CENS or their complexes) were, respectively, mixed with the same volume of octanol at room temperature. The system was vigorously shaken under sonication until equilibrium. After centrifugation, the two phases were separated and the absorbances were measured at the appropriate wavelengths. The experimental results of partition coefficients measurements are shown in Table 2.

It can be shown that in the octanol–water solvent system, the hydrophobicity of CENS is relatively low and decrease in the following order: 4 > 3 > 1 > 2. As expected, this property was significantly modified with association of CENS–β-CD, especially with **C-1** and **C-2**, where the hydrophobicities were enhanced five times. On the basis of our results, it can be concluded that the hydrophobic properties and bioavailability of CENS could be improved by encapsulating them into β-CD.

2.2.3. Determination of stability constants

Because the solubility of CENS in water is generally poor, the determination of stability constants was carried out in a methanol/water mixture (10:90, v/v).

Solutions were prepared at a fixed concentration of CENS (10^{-5} M) and at a concentration of β-CD ranging from 2×10^{-5} to 2×10^{-4} M. The absorbances of the different solutions at suitable wavelength were processed by the method reported by Benesi–Hildebrand [35]. Plotting measured values of [CENS]/absorbance vs the reciprocal of the cyclodextrin concentrations (1/[β-CD]) and data gives the stability constant *K*. Experimental values of stability constants for the different complexes are reported in Table 3.

The stability of complexes increase in the order: **C-1 <C-3 <<C-4 <C-2**. The stability constants obtained depend on the nature of the carrier group for the CENS moiety. Although attention has been paid to the role of solvent in the interaction between the host and the guest, these stability constants values are higher then those for inclusion complexes with small and medium-sized organic molecules [14–18,36–38], however, they are in the same order of magnitude, compared to literature reports for similar guests [39].

3. ^{15}N NMR study

In previous works [10], we demonstrated the interest of using ^{15}N NMR in solution for the structural study of CENS. Thus, it seems that ^{15}N-MAS (magic angle spinning) NMR technique could be useful for analytical studies of inclusion complexes in solid state. In order to shorten experimental times, a selective radiolabelling of CENS was performed on nitroso group. The different labelled compounds were prepared in a four steps synthesis using $Na^{15}NO_2$ during the nitrosation step.

In methanol, the ^{15}N signal was observed at 175–185 ppm with nitromethane as internal standard for chemical shifts measurements (550 ppm when ammonia was used). The chemical shifts obtained from MAS-NMR were quite similar. During these experiments, an accumulation of eight scans (realized in 3 min) is sufficient to obtain an easily interpretable ratio: signal/noise, against 15 000 accumulations for 48 h with the natural abundance compounds.

The samples of inclusion complexes CENS–β-CD exhibited a particular stability since for 6 months the decomposition was not significant, whereas for the same period, the compounds **C-1/C-4** were denitrosed at 50%.

The mass spectroscopic studies fully agree with these findings. In previous work [10], it was noted that no signal corresponding to NO appears, in all cases, the molecular peak of CENS was represented by [M–NO]. However, according to our results, when inclusion complexes CENS–β-CD were used, in addition to the signal of β-CD, the molecular peak of CENS with its NO appears.

4. Conclusion

The inclusion complexes of β-cyclodextrin with four CENS have been investigated both in solid and in liquid states by UV–Vis, ^{1}H NMR and ^{15}N NMR techniques. The stoichiometry, binding constants and coefficient distribution

Table 3
Stability constants of 1:1 CENS/β-CD complexes

CENS–β-CD	**C-1**	**C-2**	**C-3**	**C-4**
K (l mol^{-1})	6038 ± 100	25 219 ± 150	9784 ± 100	24 337 ± 150
$\varepsilon \times 10^{-3}$ (l/mol cm)	60.48	43.46	17.38	31.27

were determined. The association one:one/host:guest complexes were formed. The resultant inclusion complexes exhibit relatively high binding constants and were more hydrophobic than the free CENS. Moreover, an increase in the stability of CENS was observed. Our results confirm the great potential of adduct with β-CD which appears as a promising mode of formulation of 2-chloroethylnitrososulfamides. Active investigation in this field is being carried out in our laboratory.

5. Experimental

5.1. Materials

β-CD was recrystallized twice from water; all CENS were synthesized as previously described [5]. Melting points were determined in open capillary tubes on a thermotechnical apparatus and are uncorrected. Solvents used in physicochemical measurements have been used without further purification. ^{1}H and ^{15}N NMR (400 MHz) spectra were recorded on a Bruker spectrometer AC400 using DMSO-d_6 as solvent. Fast atom bombardment mass spectra (FAB-MS) were recorded in positive ion mode on a JEOL DX 300 mass spectrometer and the matrix was GT (thioglycerol). All spectrophotometric measurements were performed on a Jasco (Tokyo, Japan) double beam UV–Vis spectrophotometer V530 connected to PC computer fitted with spectra analysis program.

5.2. Inclusion complexes preparation

To a stirred saturated solution of β-CD in water (2%) was added dropwise a solution of CENS (1 equiv.) in suitable solvent (dichloromethane or ether). The mixture was stirred vigorously for 24 h at room temperature. The solution became turbid and the resulting solid was separated and dried under vacuum.

5.3. Characterisation of complexes

5.3.1. ***C-1***

^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.75–5.65 (br s, 14H, OH_2 + OH_3 of β-CD), 4.85 (d, 7H, H_1 of β-CD), 4.35 (t, 7H, OH_6 of β-CD), 4.15 (t, 2H, CH_2NNO), 3.85 (t, 2H, CH_2Cl), 3.70–3.50 (m, 28H, ($H_{6(a,b)}$ + H_3 + H_5) of β-CD), 3.35–3.25 (m, 14H, (H_4 + H_2) of β-CD), 3.20 (t, 4H, CH_2-Npip), 1.70–1.40 (m, 6H, CH_2cycl); MS (FAB > 0, GT) *m/z*: 1135 [β-CD + H]$^+$ (10%), 256 [CENS + H]$^+$ (10%), 227 [(CENS-NO) + H]$^+$ (95%).

5.3.2. ***C-2***

^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.25 (m, 10H, Ar–H), 5.80 (d, 7H, OH_2 of β-CD), 5.70 (s, 7H, OH_3 of β-CD), 4.75 (d, 7H, H_1 of β-CD), 4.50 (t, 7H, OH_6 of β-CD), 4.40 (t, 2H, CH_2NNO), 4.35 (s, 4H, CH_2Bn), 3.75–3.66 (m, 28H, ($H_{6(a,b)}$ + H_3) of β-CD), 3.55 (t, 2H, CH_2Cl), 3.45–3.25 (m, 21H, (H_5 + H_4 + H_2) of β-CD); MS (FAB > 0, GT) *m/z*: 1135 [β-CD + H]$^+$ (15%), 368 [CENS + H]$^+$ (15%), 339 [(CENS-NO) + H]$^+$ (70%).

5.3.3. ***C-3***

^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.70–5.60 (br s, 14H, OH_2 + OH_3 of β-CD), 4.80 (d, 7H, H_1 of β-CD), 4.40 (t, 2H, CH_2N), 4.10 (t, 7H, OH_6 of β-CD), 3.90 (t, 2H, CH_2Cl), 3.75–3.25 (2m, 28H, ($H_{6(a,b)}$ + H_5 + H_4) of β-CD), 3.15 (m, 2H, N-CHcycl), 1.80–1.0 (m, 20H, CH_2cycl); MS (FAB > 0, GT) *m/z*: 1135 [β-CD + H]$^+$ (15%), 352 [CENS + H]$^+$ (10%), 323 [(CENS-NO) + H]$^+$ (75%).

5.3.4. ***C-4***

^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.8–5.6 (br s, 14H, OH_2 + OH_3 of β-CD), 4.85 (d, 7H, H_1 of β-CD); 4.50 and 4.40 (2m, 1H, C*H), 4.15 (t, 7H, OH_6 of β-CD), 3.95 (t, 2H, CH_2NNO), 3.75 (t, 2H, CH_2Cl), 3.70–3.50 (m, 31H, OCH_3, ($H_{6(a,b)}$ + H_3 + H_5) of β-CD), 3.45 (t, 2H, CH_2Npro), 3.40–3.25 (m, 14H, H_4 + H_2 of β-CD), 2.30–1.80 (2m, 1H + 3H CN_2Pro); MS (FAB > 0, GT) *m/z*: 1135 [β-CD + H]$^+$ (15%), 300 [CENS + H]$^+$ (10%), 270 [(CENS-NO) + H]$^+$ (80%).

5.4. Measurement of octanol–water partition coefficients

2.5 ml of 10^{-4} M aqueous solutions of each compound (CENS or its complexes) were, respectively, mixed with the same volume of octanol at room temperature. The system was shaken vigorously until equilibrium. After centrifugation, the two phases were separated and the absorbances were measured at the appropriate wavelength.

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