Spectrochimica Acta, Vol. 48A, No. 8, pp. 1091–1099, 1992
Printed in Great Britain

0584-8539/92 $5.00+0.00

FT-IR study of the conformation and proton acceptor ability of *N*-tertiobutoxycarbonylsarcosine *N'*-methylamide and *N*-tertiobutoxycarbonylsarcosine *N',N'*-dimethylacetamide

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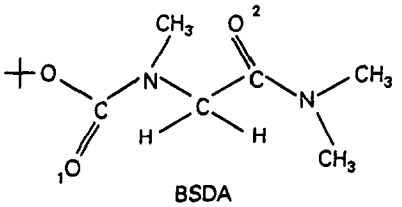
(*Received* 7 *January* 1992; *in final form* 24 *February* 1992; *accepted* 25 *February* 1992)

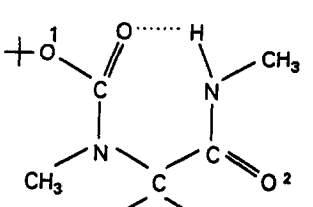
Abstract—Two model dipeptides, *N*-tertiobutoxycarbonylsarcosine *N'*-methylamide (BSMA) and *N*-tertiobutoxycarbonylsarcosine *N',N'*-dimethylamide (BSDA) are investigated by FT-IR spectrometry. The conformation of BSMA is very sensitive to the environment. In solvents of weak polarity (carbon tetrachloride, cyclohexane), BSMA accomodates the extended and seven-membered ring conformation, but in 1,2-dichloroethane, the C_7 conformers are greatly destabilized. Hydrogen bonding between BSMA or BSDA and phenols is studied in carbon tetrachloride. The thermodynamic data (equilibrium constants and enthalpies of complex formation) show that the BSMA complexes are stronger than the BSDA complexes. The spectroscopic data suggest that for BSMA, complex formation occurs at the O atom of the amide function while for BSDA about 50% of the complexes are formed on the O atom of the urethane group. The differences between the two sarcosine dipeptides are discussed in terms of cooperative and steric effects. It can be concluded that the global polarity of the medium exerts a greater influence on the conformation of the C_7 dipeptides than the specific interactions taking place on a given site of the molecule.

INTRODUCTION

IT IS A characteristic feature of cooperative transitions in polypeptides that the equilibrium between two possible conformations is highly sensitive to small changes of external parameters such as the polarity of the environment and steric conditions [1, 2]. Conformational changes involve processes in which the individual residues of the macromolecules are transformed from one conformation to another [3, 4]. The conformation of simple di- or tripeptides, very often considered as models for polypeptides, has been shown to be very sensitive to the polarity of the solvent [5–10]. Furthermore, the importance of specific interactions at the surface of membranes has been emphasized and it has been shown that the carbonyl groups are much more frequently involved in hydrogen bond formation than the NH bonds [11, 12]. It therefore seemed interesting to study the proton acceptor ability of model peptides. Few quantitative data on the interaction between proton donors and peptides are available in the literature. The interaction between phenol and *N*-acetylsarcosine *N',N'*-dimethylamide [13], phenol derivatives and tertiobutoxycarbonylglycine *N',N'*-dimethylacetamide [14] or tertiobutoxycarbonylproline *N'*-methylamide [15] and the bonding of some proton donors to polyproline [3, 4] or *N*-acetylpolysarcosine [13, 16] have been quantitatively investigated. In this work, two sarcosine derivatives, *N*-tertiobutoxycarbonylsarcosine *N'*-methylamide (BSMA) and *N*-tertiobutoxycarbonylsarcosine *N',N'*-dimethylacetamide (BSDA) are investigated by FT-IR spectrometry. BSMA can also accomodate the



BSDA



BSMA

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extended structure and its conformational state depends on the polarity of the environment. This will be discussed. The thermodynamic parameters (formation constants and enthalpies of complex formation) for the binding of phenols to BSMA and BSDA are determined in carbon tetrachloride. Furthermore, the study of the IR spectra allows us to determine the interaction sites, which are mainly the two carbonyl functions, and to detect eventual conformational changes brought about by hydrogen bond formation.

EXPERIMENTAL

Spectra and methods

The IR spectra were recorded on Perkin–Elmer 883 and Bruker FT-IR 88 spectrophotometers. Bruker Software (Pascal Program) was used for the deconvolution of the spectra registered at a resolution of 2 cm^{-1}. The equilibrium constants (K) were determined from the absorbance of the ν_{OH} stretching vibration of the phenols at concentrations of proton donor ranging from 0.005 to 0.008 mol l^{-1} and at concentrations of base between 0.001 and 0.03 mol l^{-1}. The error on K is 5%. The enthalpies of complex formation were determined from the K values at 298 and 323 K.

Products

BSMA was synthesized by the following procedure. To a solution of 19 mmol *t*-BOC-Sar (*t*-BOC = tertiobutyloxycarbonyl) in 60 ml dichloromethane, methylamine was slowly added. The solution was cooled to 0°C and 4.1 g of *N'*,*N'*-dicyclohexylcarbodiimide was added. The solution was stirred at room temperature overnight and the filtrate evaporated under low pressure. The residue was dissolved in chloroform and a great excess of *n*-hexane was added to allow BSMA to precipitate (m.p. = 92°C).

^{1}H NMR shifts (in $CHCl_3$ at 25°C): δ1.48 (*t*-Boc, 9H, singlet (s)), 2.84 ($NHCH_3$, 3H, doublet), 2.93 ($N-CH_3$, 3H, s), 3.84 (CH_2, 2H, s), 6.02 (NH, s).

BSDA was synthesized by the following method. To a solution of 3.9 g 1,1'-carbonyldiimidazole in 60 ml acetonitrile, 20 mmol *t*-Boc-sarcosine was added. When CO_2 was removed, dimethylamine was added to the reaction mixture and acetonitrile evaporated at low pressure. The reaction product was dissolved in chloroform and a great excess of *n*-hexane was added to yield BSDA, a pale syrup which could not be crystallized.

^{1}H NMR shifts (in $CDCl_3$ at 25°C): δ1.44 and 1.48 (*t*-Boc, 9H, 2s), 2.93, 2.95 and 2.98 ($N-CH_3$, 9H, 3s), 3.98 and 4.05 (CH_2, 2H, 2s). From the area of the two CH_2 signals, it can be deduced that 70% *trans* isomer is present in $CDCl_3$ solution.

N-t-Boc-sarcosine from Sigma, *N'*,*N'*-dicyclohexylcarbodiimide from Janssen Chimica and 1,1'-carbonyldiimidazole from Janssen Chimica were used without further purification. The solvents and phenols were carefully dried and purified by standard methods.

RESULTS AND DISCUSSION

Conformation and proton acceptor ability of BSMA

The wavenumbers ν_{NH} and $\nu_{C=O}$ stretching vibrations of BSMA dissolved in four organic solvents having dielectric constants between 2.02 and 10.7 are indicated in Table 1. Some spectra are reproduced in Figs 1 and 2. The two absorptions observed in carbon tetrachloride at 3452 and 3376 cm^{-1} are assigned, by comparison with literature data on sarcosine dipeptides [17–19], to the ν_{NH} vibration of the extended (ν_{NH}^{f}) and of the C_7 (ν_{NH}^{C7}) conformers. The extinction coefficient of the ν_{NH}^{f} band determined in very dilute carbon tetrachloride solution is 100 l mol^{-1} cm^{-1}. The extinction coefficient of the ν_{NH} vibration of an extended conformer is 160 l mol^{-1} cm^{-1} [20], near to the value of 170 determined with our FT-IR spectrometer for the ν_{NH} vibration of *N*-methylacetamide in a very dilute solution. From these data it can be deduced that in carbon tetrachloride, about 60% of the molecules are in an extended conformation. As judged by the relative intensity of the two NH vibrations, this percentage is higher in cyclohexane but is much lower in chloroform and 1,2-dichloroethane, where the ν_{NH}^{C7} band is hardly detectable.

Table 1. ν_{NH} and $\nu_{C=O}$ stretching vibrations of BSMA in different solvents (cm^{-1})

Solvent*	ν_{NH}^{f}†	ν_{NH}^{C7}	$\nu_{C1=O1}^{f}$	$\nu_{C2=O2}$	$\nu_{C1=O1}^{C7}$
C_6H_{12} (2.02)	3458 m	3381 m	1715 m	1701 m	1684 m
CCl_4 (2.23)	3452 m	3376 m	1706 sh	1691 s	1676 sh
$CDCl_3$ (4.82)	3448 s	3385 w	1700 sh	1679 s	—
$C_2H_4Cl_2$ (10.7)	3439	~3380 vw	1700 m	1684 s	—

* The dielectric constants are given in parentheses.
† vw = very weak, w = weak, m = medium, s = strong, sh = shoulder.

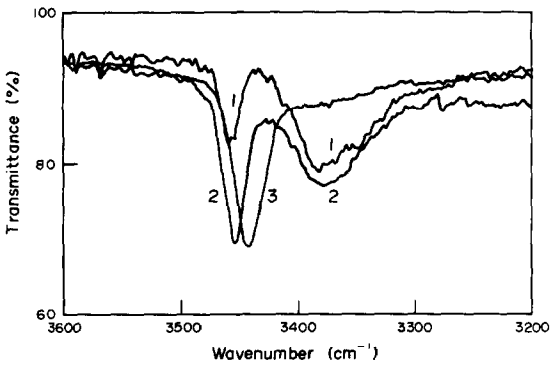

Fig. 1. FT-IR spectra (3600–3200 cm^{-1}) of BSMA. (1) In C_6H_{12} (saturated solution, $d = 0.05$ cm). (2) In CCl_4 ($c = 0.02$ mol l^{-1}, $d = 0.01$ cm). (3) In $C_2H_4Cl_2$ ($c = 0.01$ mol l^{-1}, $d = 0.1$ cm).

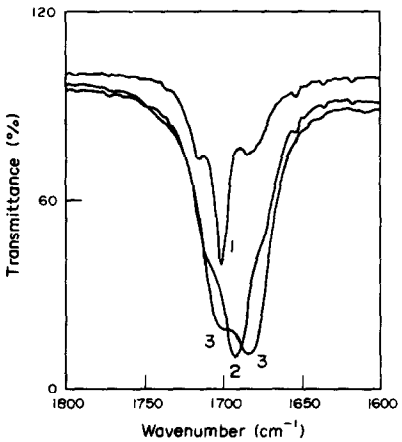

Fig. 2. FT-IR spectra (1800–1600 cm^{-1}) of BSMA. Same solvents and concentrations as in Fig. 1 ($S = C_6H_{12}$, $d = 0.01$ cm; $S = CCl_4$, $d = 0.05$ cm; $S = C_2H_4Cl_2$, $d = 0.1$ cm).

The band observed at 1691 cm^{-1} in carbon tetrachloride is assigned to the $\nu_{C2=O2}$ vibration [21] and the shoulders at 1706 and 1676 cm^{-1} to the $\nu_{C1=O1}$ vibrations of the extended and C_7 conformers. The low frequency shoulder disappears in chloroform and 1,2-dichloroethane, where the concentration of seven-membered species is very weak. In cyclohexane, where this concentration is higher and where the bands are less broad, the three bands are well resolved and the deconvolution of the spectra is shown in Fig. 3. The percentage of extended species, roughly estimated from the area of the 1795/1684 cm^{-1} absorption, is 45%. These results show that, as for other sarcosine, glycine or proline dipeptides [5–10, 22, 23], the C_7 conformation is appreciably depopulated in polar solvents. However, as judged by the relative intensities of the two ν_{NH} bands reproduced in Ref. [18], the proportion of C_7 conformers is higher in *N*-acetylsarcosine

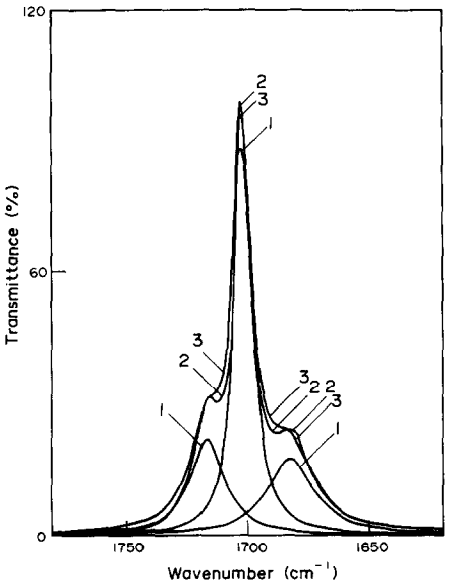

Fig. 3. Deconvolution of the FT-IR spectrum (1800–1600 cm^{-1}) of BSMA in C_6H_{12} ($c = 0.007$ mol^{-1}). (1) Separated components. (2) Sum of the three components. (3) Experimental spectrum.

N'-methylamide than in the present case. The lower tendency of BSMA to form seven-membered rings can be explained by steric effects of the tertiobutoxy group and by the electron attracting effect of the oxygen atom. The $\Delta\nu_{NH}$ shift (88 cm^{-1}) is also greater in the *N*-acetyl than in the tertiobutoxy (78 cm^{-1}) homologue.

Table 2 lists the values of the equilibrium constants and the enthalpies of complex formation ($-\Delta H$) for the interaction between BSMA and phenols. It must be pointed out here that equilibrium constants determined from the intensity of phenolic OH stretch vibrations represent overall equilibrium constants. When two kinds of complexes are formed, they do not necessarily have the same *K* values. The same remark also holds for the enthalpies. Owing to the volatility of carbon tetrachloride, the $-\Delta H$ values have been determined at only two temperatures, 298 and 323 K.

Table 2. Thermodynamic data for the interaction between BSMA and phenols in CCl_4

Phenols*	$K^{298\,K}$ (1 mol^{-1})	$K^{323\,K}$ (1 mol^{-1})	$-\Delta H$† (kJ mol^{-1})
4-CH_3O-phenol (10.21)	100	51	23
Phenol (9.95)	108	52	24
4-Br-phenol (9.34)	260	110	26
3-Br-phenol (9.03)	328	128	27
3,4-DiCl-phenol (8.58)	572	237	29
3,5-DiCl-phenol (8.18)	815	303	31
3,4,5-TriCl-phenol (7.75)	1615	612	33

* The pK_a values of the phenols in water are given in parentheses [24].

† Calculated from the *K* values deduced from the log *K* vs pK_a correlations.

The logarithms of the equilibrium constants are linearly related to the pK_a of the proton donors:

$$\log K^{298\,K} = 6.99 - 0.49\ pK_a \quad (r = 0.996)$$

$$\log K^{323\,K} = 6.13 - 0.44\ pK_a \quad (r = 0.992).$$

The mean $-\Delta S°$ value is 44 J mol^{-1} K^{-1}. These results show that BSMA has a good acceptor ability. For phenols having pK_a between 10.30 and 7.75, the complex band

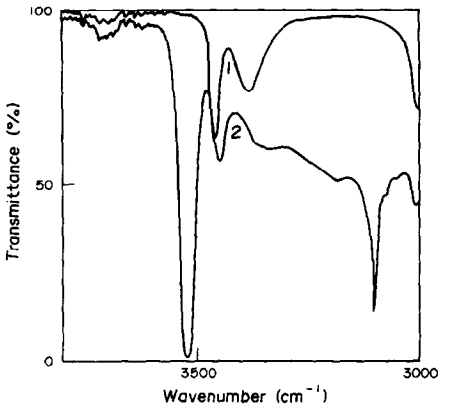

Fig. 4. FT-IR spectrum (3700–3000 cm^{-1}) of: (1) BSMA (c = 0.01 mol l^{-1}); (2) BSMA (c = 0.01 mol l^{-1}) and 2,6-diCl$_2$-4-NO$_2$-phenol (c = 0.02 mol l^{-1}). Solvent = CCl_4.

overlaps with the ν_{NH}^{C7} absorption. For the 2,6-diCl-4-NO_2 complex, the $\nu_{OH\ldots O}$ band is observed at lower wavenumber (about 3150 cm^{-1}) and the ν_{NH}^{C7} vibration is shifted downfield, by about 35 cm^{-1} (Fig. 4). There is also a small decrease in the intensity of the ν_{NH}^{f} absorption. These results must be taken with caution owing to the broadness of the $\nu_{OH\ldots O}$ absorption. The deconvolution of the spectrum in the $\nu_{C=O}$ region of the

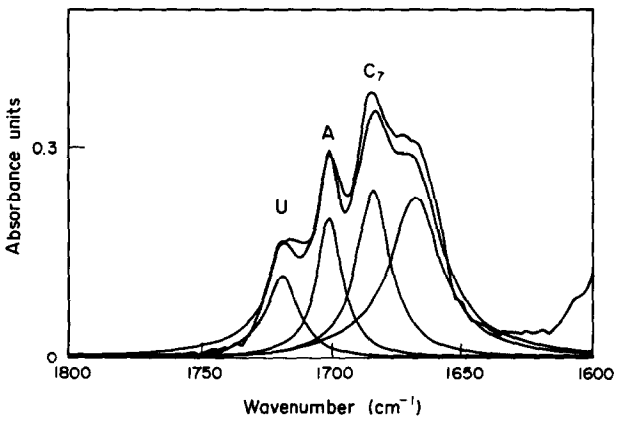

Fig. 5. Deconvolution of the FT-IR spectrum (1800–1600 cm^{-1}) of the complex between BSMA (c = 0.007 mol l^{-1}) and 3,5-dichlorophenol (c = 0.006 mol l^{-1}). Solvent = C_6H_{12}, d = 0.005 cm.

3,5-dichlorophenol complex in cyclohexane is shown in Fig. 5. The comparison with the spectrum of the binary mixture shows that a new complex band is observed at 1670 cm^{-1}. This absorption is assigned to the shifted $\nu_{C2=O2}$ band ($\Delta\nu$ = 31 cm^{-1}). Complexes between *N*-methylacetamide and phenols show similar shifts [25]. Furthermore, one can observe an intensity decrease of the $\nu_{C1=O1}^{f}$ vibration and an intensity increase of the $\nu_{C1=O1}^{C7}$ vibration. The results can also be treated more quantitatively. From the equilibrium constant determined in cyclohexane for the 3,5-dichlorophenol–BSMA complex (3120 l mol^{-1}), it can be computed that for the formal concentrations indicated below (Fig. 5), the concentration of complex is 5.1×10^{-3} mol l^{-1}. This concentration corresponds exactly to the concentration computed from the decrease in the intensity of the

$\nu_{C2=O2}$ band. Furthermore, the decrease in the concentration of the extended conformers (1.4×10^{-3} mol l^{-1}) does not markedly differ from the increase in the concentration of C_7 conformers (1.1×10^{-3} mol l^{-1}). Also, the band at 1670 cm^{-1} cannot be assigned to the shifted $\nu^{f}_{C1=O1}$ vibration. A shift of 45 cm^{-1} seems too high, at least for proton donors such as phenols. These results suggest that complex formation occurs almost exclusively at the amide carbonyl function and that complex formation brings about a small increase of the concentration of seven-membered species. The concentration of complexes formed on the C1=O1^{f} group must be very weak or zero. These deductions have been made by considering the concentrations of complexes and the IR spectra at 298 K. They should be about the same at the temperature of 323 K. It has indeed been shown by NEEL [17] that the conformation of dipeptides is almost insensitive to temperature and that conformational changes only appear when the temperature exceeds 343 K.

Interestingly, the Raman spectra of *N*-acetylglycine *N'*-methylamide dissolved in water show that C2=O2 . . . HO hydrogen bond formation brings about a strengthening of the intramolecular hydrogen bond. The downward shift of the ν^{C7}_{NH} vibration is 47 cm^{-1} [26]. This can be ascribed to cooperative effects and will be discussed in more detail in the last section.

Proton acceptor ability of BSDA

The thermodynamic data are listed in Table 3, which also indicates some values of the frequency shift of the ν_{OH} vibration. The correlation log K vs pK_a can be written:

$$\log K^{298\,K} = 7.12 - 0.52\,pK_a \quad (r = 0.996)$$
$$\log K^{323\,K} = 6.63 - 0.50\,pK_a \quad (r = 0.996).$$

The mean $-\Delta S°$ value is 45 J mol^{-1} K^{-1}. The results show that the proton acceptor ability of BSDA is weaker than that of BSMA. The $-\Delta H$ values for phenol and 4-CH_3O-phenol show a reverse trend, but this could be due to experimental errors on $-\Delta H$, which are ± 1.5 kJ mol^{-1}.

The spectra in the $\nu_{C=O}$ region are reproduced in Fig. 6. The bands observed in carbon tetrachloride at 1696 and 1674 cm^{-1} are assigned to the $\nu_{C1=O1}$ and $\nu_{C2=O2}$ vibrations, by comparison with the spectra of carbamates [27] and amides [27]. As shown by the study of the IR spectra in the 900–700 cm^{-1} region, the shoulders observed at 1710 and 1660 cm^{-1} cannot originate from a Fermi resonance but are best explained by the presence of *cis* isomers in the solution. In chloroform, the percentage of *cis* isomers is 30% but this percentage must be lower in less polar solvents.

Complex formation brings about an intensity decrease of the two carbonyl absorptions. The new band at 1655 cm^{-1} is assigned to complexes formed on the oxygen atom of the C2=O2 function. The shift of 21 cm^{-1} is consistent with that observed in *N*,*N*-dimethylacetamide complexes [28]. As in the *N*-acetylsarcosine

Table 3. Thermodynamic data for the interaction between BSDA and phenols in CCl_4*—frequency shifts of the ν_{OH} stretching vibration

Phenols	$K^{298\,K}$ (l mol^{-1})	$K^{323\,K}$ (l mol^{-1})	$-\Delta H$ (kJ mol^{-1})	$\Delta\nu_{OH}$ (cm^{-1})
3,4-DiCH_3-phenol	62	28	25	260
4-CH_3O-phenol	73	32	25	
Phenol	92	42	26	
4-Br-phenol	236	100	27	300
3-Br-phenol	281	123	27	310
3,4-DiCl-phenol	532	233	27	330
3,5-DiCl-phenol	724	304	28	345

* Same remarks as below Table 2 apply.

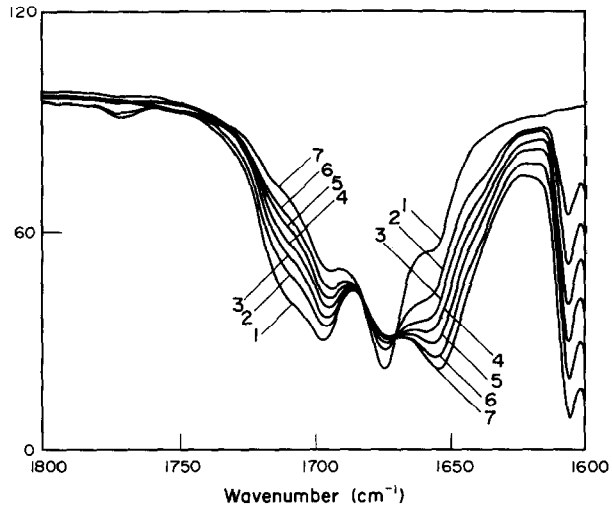

Fig. 6. FT-IR spectra (1800–1600 cm^{-1}) of BSDA ($c = 0.01$ mol l^{-1}). Concentrations of phenol (mol l^{-1}): (1) 0; (2) 0.012; (3) 0.015; (4) 0.021; (5) 0.029; (6) 0.036; (7) 0.054. $d = 0.05$ cm.

N',N'-dimethylacetamide–trifluoroethanol complex [13], there is an overlap between the shifted $\nu_{C1=O1}$ and the free $\nu_{C2=O2}$ absorptions. From the intensity decrease of the $\nu_{C1=O1}$ vibration and the total concentration of complexes computed from the formation constant, it can be computed that about 40% of the complexes are formed on the O_1 atom.

These deductions are in very good agreement with the observations in the ν_{OH} region shown in Fig. 7 for the BSDA–3,5-dichlorophenol complex (spectrum 4). The unusual broadness of the ν_{OH} band culminating at 3260 cm^{-1} can be attributed to the superposition of two absorptions. It can indeed be considered that BSDA results from the juxtaposition of two model molecules, *N,N*-dimethylmethylcarbamate CH_3–O–C–N–$(CH_3)_2$ (DMC) and *N,N*-dimethylacetamide (DMA). The ν_{OH} absorptions of the complexes involving these two proton acceptors and the same phenol are shown in Fig. 7 (spectra 1 and 2). The formal concentrations have been chosen in order to have the same concentrations of carbamate and amide complexes, their sum being equal to the concentration of BSDA complexes. Spectrum 3, which results from the addition of spectra 1 and 2, reproduces the experimental spectrum with good accuracy.

Comparison between BSMA and BSDA

As already outlined, the formation constants are higher for BSMA than for BSDA complexes. These results are somewhat unexpected because the substitution of the hydrogen atom of an N–H bond by a methyl group increases the proton acceptor ability of closely related bases. The experimental findings can be explained by the fact that in BSMA, the great majority of the complexes are formed on the amide carbonyl, while in BSDA about 40% of the complexes are formed on the urethane carbonyl. A comparison with model molecules shows that the K values of the BSDA complexes are intermediate between those of MMC and DMA. For phenol as reference acid, the K values are as follows:

$$K_{BSDA}^{298\,K} = 92\ l\ mol^{-1}; \qquad K_{MMC}^{298\,K} = 36\ l\ mol^{-1}\ [27]; \qquad K_{DMA}^{298\,K} = 130\ l\ mol^{-1}\ [28].$$

For the BSMA complex, the stability constants are somewhat higher than for the *N*-methylacetamide complex:

$$K_{BSMA}^{298\,K} = 108\ l\ mol^{-1}; \qquad K_{NMA}^{298\,K} = 95\ l\ mol^{-1}\ [25].$$

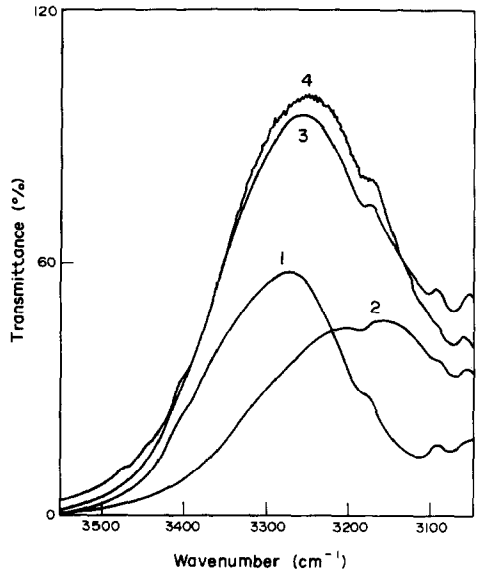

Fig. 7. FT-IR spectrum in the ν_{OH} region of complexes of 3,5-dichlorophenol and: (1) MMC ($c_{phenol} = 0.0078$ mol l^{-1}, $c_{MMC} = 0.0034$ mol l^{-1}); (2) DMA ($c_{phenol} = 0.0078$ mol l^{-1}, $c_{DMA} = 0.011$ mol l^{-1}). (3) Sum of spectra 1 and 2. (4) Experimental spectrum of the BSDA–3,5-dichlorophenol complex ($c_{phenol} = 0.016$ mol l^{-1}, $c_{BSDA} = 0.023$ mol l^{-1}). Solvent = CCl_4, $d = 0.4$ cm.

The difference between the two studied sarcosine dipeptides can be explained by the fact that in BSMA, the intramolecular hydrogen bond lowers the electronic density at the O_1 atom but increases the density at the O_2 atom. It must be pointed out, however, that part of the complex is formed at the O_2 atom of the extended species. The $\nu_{C2=O2}$ vibration seems, however, very insensitive to the conformational state of free BSMA and this also seems to be the case in the complexes. In BSDA, the availability of the electronic pairs of the O_2 atom is lowered owing to the vicinity of the CH_3 group bonded to the N_1 atom. Steric hindrance seems to be a leading factor in this case.

CONCLUSION

The results of this work show that the conformation of BSMA is very sensitive to the environment. The percentage of extended species varies from 45% to 100% on going from cyclohexane ($\varepsilon = 2.02$) to 1,2-dichloroethane ($\varepsilon = 10.7$). In contrast, the seven-membered ring is not broken by the formation of a hydrogen bond on the exocyclic oxygen atom. These results show that the global polarity of the solvent exerts a greater influence on the conformation of a C_7 dipeptide than the specific interactions taking place on a given site of the molecule. In five-membered ring dipeptides, complex formation brings about a partial breaking of the intramolecular hydrogen bond. The difference can be explained by the weakness of the intramolecular hydrogen bond in the C_5 conformers [14].

Acknowledgements—The authors thank the University of Leuven and the National Funds of Research of Belgium for financial support.

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