^{1}H-NMR studies of acid-base properties and conformation of *N*-alkyl derivatives of aspartic acid

D. CVIKOVÁ, P. BUTVIN, P. BALGAVÝ, and J. MAJER

Department of Analytical Chemistry, Faculty of Pharmacy, Komenský University, 832 32 Bratislava

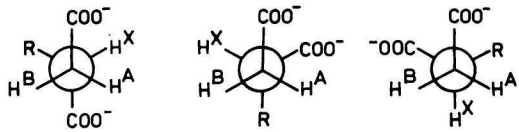
Received 21 August 1981

Dedicated to Professor Dr. Ing. J. Tomko, DrSc., in honour of his 60th birthday

Preparation and properties of *N*-alkyl derivatives of aspartic acid are described and the ^{1}H-n.m.r. spectra of these compounds are discussed. The protonization constant of the amine nitrogen and the conformation of the αCH—βCH$_2$ fragment in neutral and alkaline solution in D_2O were determined from the ^{1}H-n.m.r. spectral data. These results were considered when studying the influence of formation of supermolecular associates upon conformation of the fragment under investigation.

Описывается приготовление и изучение *N*-алкилпроизводных аспарагиновой кислоты. На основании спектров ^{1}H-ЯМР была определена константа протонирования аминного азота и конформация фрагмента αCH—βCH$_2$ изученных соединений в нейтральном и щелочном растворах в D_2O. Результаты обсуждаются с точки зрения влияния образования сверхмолекулярных ассоциатов на конформацию указанного фрагмента.

The ^{1}H-n.m.r. spectroscopy is a widely used tool for obtaining information on conformation of peptides and proteins [1]; it is of advantage to employ this method for conformational analysis of αCH—βCH$_2$ fragments of amino acid residues. The method is based on determination of population of three rotamers (*I*—*III*) of the αCH—βCH$_2$ fragment (Scheme 1) from experimental data of vicinal coupling constants [2]. Population of the individual rotamers p_1, p_2, and p_3 was estimated



I *II* *III*

Scheme 1

from the measured coupling constants J_{AX} and J_{BX} employing the following equations

$$J_{AX} = p_1 J_g + p_2 J_t + p_3 J_g \quad (1)$$

$$J_{BX} = p_1 J_t + p_2 J_g + p_3 J_g \quad (2)$$

$$p_1 + p_2 + p_3 = 1 \quad (3)$$

where J_g and J_t are the coupling constants of protons in position *gauche* and *trans*, respectively [3]. Changes in the chemical shift values of protons in the αCH—βCH$_2$ grouping make it possible to determine the location of proton dissociation at pH changes in the solution [4, 5]. These procedures were widely applied for studying the conformation of aspartic acid or its part in peptides [6—9]. The *N*-alkyl derivatives of aspartic acid were prepared in our program concerning the investigation of properties of the chelating tensides prepared from natural amino acids and complexones. Such compounds can form in aqueous solutions supermolecular aggregates — micelles, or tubular and lamellar species having the supermolecular structure as observed in liquid—crystal systems of a smectic type [10]. Here, the conformation of the αCH—βCH$_2$ fragment can be influenced, as it has been found in the case of *O*-alkyl-D,L-tyrosines [11], as well as the acid-base properties, being observed in the case of *N*-acyl derivatives of glycine, alanine, and glutamic acid [12]. This paper has been aimed to prepare aspartic acid derivatives and to examine the above-mentioned effects.

Experimental

Aspartic acid is a commercially available preparative (Lachema, Brno); its *N*-alkyl derivatives were obtained by reacting the monomethyl maleinate with primary amines in triethylamine [13].

According to this method and starting from maleic anhydride (0.1 mol) and alkylamine (0.1 mol) following *N*-alkylaspartic acid β-methyl esters were prepared: *N*-butyl derivative in a 83% yield, *N*-octyl and *N*-decyl derivatives in 86 and 88% yields, respectively. These esters were saponified in two different ways.

a) *N*-Butylaspartic acid β-methyl ester (48 mmol) was dissolved in solution of $Ba(OH)_2 \cdot 8H_2O$ (60 mmol) in water (250 ml) and hydrolyzed at 80°C for 2 h. The hot reaction mixture was treated with the corresponding amount of 0.1 M-H_2SO_4, the precipitated $BaSO_4$ filtered off and the concentrated filtrate left to crystallize. The crystals obtained were recrystallized from water—ethanol—acetone 1 : 1 : 1, and dried with recovery 75%, m.p. 162°C (163°C, Ref. [14]).

b) *N*-octyl, or *N*-decylaspartic acid β-methyl ester (70 mmol) was dissolved in water (135 ml) containing 5 M-NaOH (34 ml) and saponified on a steam bath for 2 h. The mixture was acidified with 5 M-HCl after cooling. The precipitate was reprecipitated in an analogous way. Recovery and melting points of the respective esters were 88 and 94%, and 154—155 and 152°C. The identity and purity of the prepared compounds were verified by elemental

analysis, i.r. and ^{1}H-n.m.r. spectroscopy and potentiometric titration. Based upon the obtained results the purity of the triple-recrystallized *N*-butyl derivative, of the three times precipitated *N*-octyl derivative, and that of the *N*-decyl derivative was 97, 97, and 99%, respectively, which is sufficient for this kind of study. These compounds possess the chiral centre. They were obtained in ± form due to principle of their synthesis.

For ^{1}H-n.m.r. spectroscopy, these substances were dissolved in D_2O in a 0.2 mol dm^{-3} concentration (unless otherwise stated). The pD of solutions was adjusted with KOD and DCl in D_2O. The pD of the solution was measured with a pH-meter Radelkis (Hungary), model OP-205 and a combined electrode EA 125 (Metrohm, Switzerland) at 25°C. The pH readings were corrected into pD according to [15]. The ^{1}H-n.m.r. spectra were taken with a Tesla BS 487 A spectrometer operating at 80 MHz; internal reference was the deuterated *tert*-butyl alcohol $(CH_3)_3COD$ (TBA), or trimethylsilylpropionic acid (TSP). The chemical shift values are in p.p.m. (δ scale) relative to TSP ($\delta_{TBA} = 1.233$ p.p.m.).

Results and discussion

The ^{1}H-n.m.r. spectrum of aspartic acid (ASP) has already been reported [7]. Due to the nonequivalence of the βCH_2 protons (neighbouring to the chiral centre), the spectrum shows in alkaline and neutral pD range an ABX pattern. Spectrum of *N*-butylaspartic acid (BUASP) has the ABX system of αCH—βCH_2 protons overlapped in alkaline and neutral pD regions by protons of the *N*-butyl substitution ($\delta(CH_3) = 0.90$ p.p.m., $\delta(N—CH_2) = 2.80$ p.p.m., and $\delta(CH_2)_2 =$ 1.10—1.75 p.p.m. at pD 11.1). *N*-Decylaspartic acid (DASP) has signals of the αCH—βCH_2 grouping resembling a simplified ABC system: that of αCH is a triplet and that of βCH_2 a doublet. This spectrum is, similarly as with BUASP superposed by the signal of substituents ($\delta(CH_3) = 0.86$ p.p.m., $\delta(N—CH_2) =$ 2.90 p.p.m., $\delta(CH_2)_8 = 1.00$—1.80 p.p.m. at pD 11.1). The *N*-octylaspartic acid (OASP) reveals an analogous spectrum.

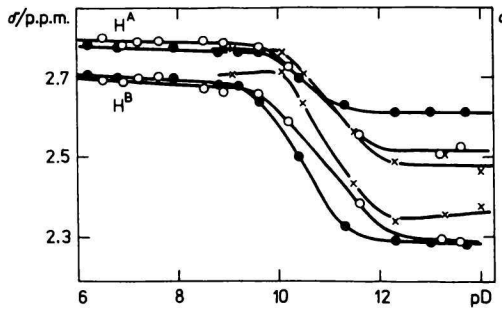


Fig. 1. Dependence of H^A and H^B chemical shift values upon pD.
● ASP; ○ BUASP; × OASP.

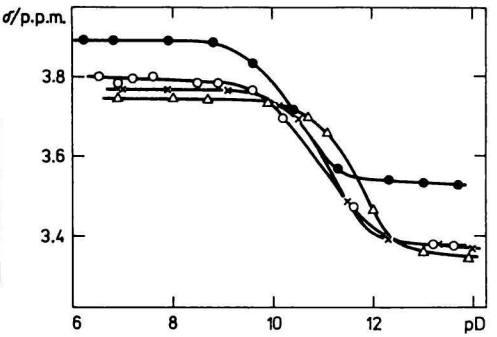


Fig. 2. Dependence of H^X chemical shift values upon pD.
● ASP; ○ BUASP; × OASP.

Relationships between the chemical shifts of βCH_2, αCH, and $N—CH_2$ protons and pD are plotted in Figs. 1—3. Considerable changes in all ASP derivatives are observed in the 9—13 pD range due to a protonization of nitrogen. Changes in chemical shift values due to this protonization and also the pK values taken from relationships shown in Figs. 1 and 2 are listed in Table 1. Were these chemical shift

Table 1

Difference of chemical shift values and pK values

Acid	$\Delta\delta$/p.p.m.				pK
	αCH	βCH^A	βCH^B	$N—CH_2$	
ASP	0.36	0.16	0.43	—	10.5
BUASP	0.43	0.28	0.38	0.64	10.8
OASP	0.41	0.31	0.35	0.59	11.1
DASP	0.41	—	—	0.58	11.9

values associated with changes in electron density during protonization of nitrogen only, then, due to the induction effect, they should be mostly pronounced with αCH^X and $N—CH_2$ protons, whilst with protons βCH^B and βCH^A they should be approximately equal. Nevertheless, the great changes $\Delta\delta(\beta CH^B)$ indicate that noticeable changes in orientation of this fragment occurred during protonization of nitrogen in relation to magnetically anisotropic bonds. A similar effect was observed with γ-aminobutyric acid, where the change in chemical shift of γCH_2 protons was smaller after protonization of nitrogen than with protons βCH_2 [5].

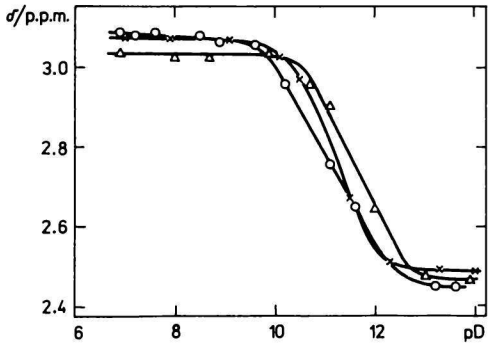


Fig. 3. Dependence of $N—CH_2$ group chemical shift values upon pD.
○ BUASP; × OASP; △ DASP.

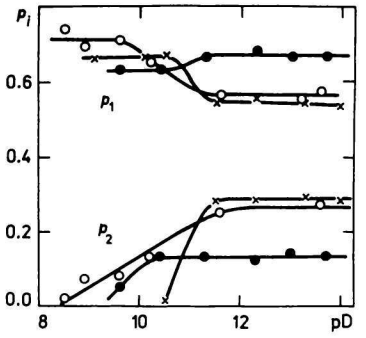


Fig. 4. Population dependence of rotamers *I* and *II* upon pD.
● ASP; ○ BUASP; × OASP.

The chemical shift value of the αCH^X proton decreases in the series ASP—BUASP—OASP—DASP both in neutral and alkaline medium evidently due to an increased electron density at the αCH^X proton; this behaviour is associated with substitution of aspartic acid with an aliphatic chain. The pK value increases in the same way. The obtained results do not allow at the time being to decide, whether this change is exclusively due to the change of acid-base properties leading to supermolecular aggregates.

Values of coupling constants calculated from the spectra in both alkaline and neutral medium are given in Table 2. For OASP in a neutral solution and for

Table 2

Dependence of coupling constants on the pD of solution

Acid	pD	J_{AB}/Hz	J_{AX}/Hz		J_{BX}/Hz
ASP	13.7	−15.28	3.77		9.58
	7.3	−17.65	1.79		10.41
BUASP	13.6	−15.33	5.31		8.64
	7.2	−17.70	1.65		11.05
OASP	13.7	−14.89	5.41		8.22
	7.0	—		11.98*	
DASP	13.9	—		13.80*	
	6.9	—		11.53*	

* Values $J_{AX} + J_{BX}$.

DASP in alkaline and neutral solutions only the sum $J_{AX} + J_{BX}$ could be obtained from the spectra. Population of rotamers *I*—*III* (R = *N*-alkyl) plotted in Figs. 4 and 5 was obtained from equations (*1*—*3*) and values $J_g = 2.4$ Hz and $J_t = 13.3$ Hz published in [3]. Population of the rotamer *III* is the only one to be calculated for DASP. Population of the rotamer has varied mostly by 5%, which is in limits of an experimental error. Acids ASP, BUASP, OASP, and probably also DASP have the highest population of rotamer *I* in an alkaline medium. The population of rotamer *III* of all compounds under investigation increases in solution with protonization of nitrogen in the series BUASP—OASP—DASP.

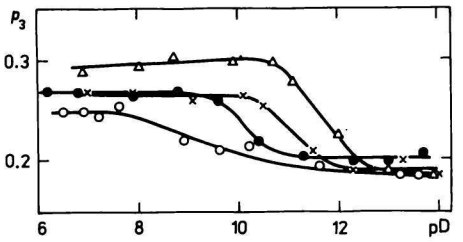


Fig. 5. Population dependence of rotamer *III* upon pD.
● ASP; ○ BUASP; × OASP; △ DASP.

Alkyl derivatives BUASP, OASP and probably also DASP reveal after protonization the increase of p_1 in contrast to ASP. The enhanced population of rotamers *I* and *III* after protonization is accompanied by a dramatic drop in population of the rotamer *II* down to the zero value.

Considering the bulkiness of the polar group and the relatively short aliphatic chains, it could be anticipated that *N*-alkyl derivatives of ASP are associated in the micelles at the given concentrations. The lone electron pair and the amine proton are likely oriented in the plane of Stern layer during association into micelles in an alkaline solution. This orientation results from the fact that the difference of pK values and particularly of chemical shifts of protons of the N—CH_2 group is not substantially influenced by the length of the aliphatic chain. The high stability of the rotamer *I* in this orientation is then caused by two factors: by the electrostatic repulsion of the negatively charged carboxyl groups (similarly as with ASP acid), and/or by a parallel orientation of carboxyl groups with the Stern layer, in which the total energy of the supermolecular aggregate is decreased by the solvation of these polar groups. The enhanced population of the rotamer *II* in an alkaline medium when compared with that of ASP acid also relates to solvation, since the carboxyl group in this conformation goes back from the Stern layer towards the aqueous phase. Such a stabilization effect due to solvation of carboxyl group has already been observed with *O*-hexyl-D,L-tyrosine and *O*-octyl-D,L-tyrosine [11]. The increase in population of rotamer *II* of BUASP and OASP derivatives was by 0.14 and 0.16, respectively, in respect to ASP acid. The same increase of *O*-octyl-D,L-tyrosine was found to be 0.17, when compared with that of L-tyrosine [11]. The population of rotamer *II* equaled zero, or it was close to zero in monoprotonized forms of all derivatives under investigation. The decrease of rotamer *II* population, accompanied with an increase of population of rotamers *I* and *III* evidences the importance of intramolecular interaction from the point of view of their stability. Both rotamers *I* and *III* are apt for an electrostatic interaction of the positively charged quaternary nitrogen with the negatively charged β-carboxyl group and possibly also for formation of an intramolecular hydrogen bonding. It is noteworthy that population of rotamer *I* increases during protonization of alkyl derivatives of ASP, whilst it slightly decreases with the ASP acid itself. One of the possible explanations is that the bulky β-carboxyl group in conformation *III* hinders sterically the association of compounds in the region of aliphatic chains. This effect is indicated by the relative turn of population of the rotamer *III* from BUASP through OASP to DASP. The increasing length of the aliphatic chain is associated with an increasing interaction energy among molecules so that the steric effect is less significant with the increasing aliphatic chain length.

The last factor to be mentioned is a significant broadening of lines of αCH, βCH_2, and N—CH_2 protons in the spectrum in a narrow concentration range and therefore, no splitting of signals could be observed (Fig. 6); with DASP it makes

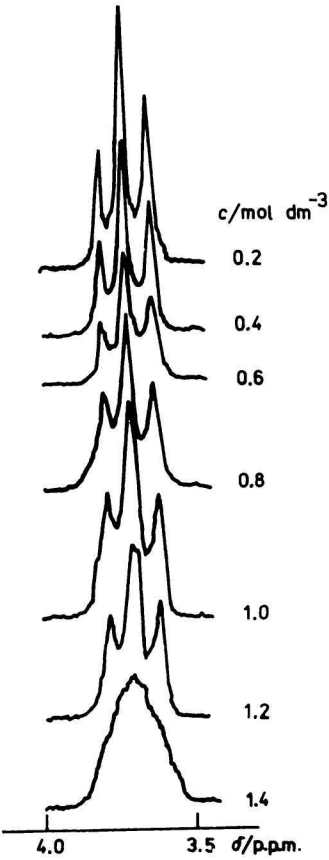


Fig. 6. Influence of concentration of *N*-decylaspartic acid on the shape of the H^x proton signal.

1.2—1.4 mol dm^{-3}. In the 0.2—1.2 mol dm^{-3} concentration range in an alkaline medium the shape of the spectrum of DASP acid is virtually concentration independent. Several ionogenic surface-active substances showed in an above-micellar concentration an alteration of the micelle shape from a spherical to a tubular one. The typical example of such an effect are the changes in light scattering and in the ^{1}H-n.m.r. spectra of aqueous solutions of alkylammonium salts [16—19]. It is probable that in the 1.2—1.4 mol dm^{-3} concentration range also the shape of supermolecular aggregates of *N*-decylaspartic acid shall undergo a similar alteration from a spherical to a tubular micelle. Due to such a change the velocity of the rotational motion of the supermolecular aggregate may lower, and, as a consequence, an increase of the increment of the anisotropic dipole—dipole interaction to the line broadening occurs.

The study of supermolecular aggregates of DASP acid is being in progress in our laboratory using both the e.p.r. spectroscopy and stable radicals as spin probes in these systems and the ^{13}C-n.m.r. spectroscopy.

References

1. Deslauriers, R. and Smith, I. C. P., *Biol. Magn. Resonance 2*, 243 (1980).
2. Feeney, J., *J. Magn. Resonance 21*, 473 (1976).
3. Martin, R. B., *J. Phys. Chem. 83*, 2404 (1979).
4. Jardetzky, O. and Jardetzky, C. D., *J. Biol. Chem. 233*, 383 (1958).
5. Taddei, F. and Pratt, L., *J. Chem. Soc. 1964*, 1553.
6. Pachler, K. G. R., *Z. Anal. Chem. 224*, 211 (1967).
7. Kainosho, M. and Ajisaka, K., *J. Amer. Chem. Soc. 97*, 5630 (1975).
8. Hansen, P. E., Feeney, J., and Roberts, G. C. K., *J. Magn. Resonance 17*, 249 (1975).
9. Rowe, J. J. M., Hinton, J., and Rowe, K. L., *Chem. Rev. 70*, 1 (1970).
10. Brown, G. H., Doane, J. W., and Neff, V. D., *A Review of the Structure and Physical Properties of Liquid Crystals*. Butterworths, London, 1971.
11. Menger, F. M. and Jerkunica, J. M., *Tetrahedron Lett. 52*, 4569 (1977).
12. Ptak, M., Egret-Charlier, M., Sanson, A., and Bouloussa, O., *Biochim. Biophys. Acta 600*, 387 (1980).
13. Laliberté, R. and Berlinquet, L., *Can. J. Chem. 40*, 163 (1962).
14. Zilkha, A. and Bachi, M. D., *J. Org. Chem. 24*, 1096 (1959).
15. Mikkelson, K. and Nielsen, S. O., *J. Phys. Chem. 64*, 632 (1960).
16. Henriksson, U., Ödberg, L., Ericksson, J. C., and Westman, L., *J. Phys. Chem. 81*, 76 (1977).
17. Ulmius, J. and Wennerström, H., *J. Magn. Resonance 28*, 309 (1977).
18. Ozeki, S., Tsunoda, M., and Ikeda, S., *J. Colloid Interface Sci. 64*, 28 (1978).
19. Maeda, H., Ozeki, S., Ikeda, S., Okabayashi, H., and Matsushita, K., *J. Colloid Interface Sci. 76*, 532 (1980).

Translated by Z. Votický