6. A. W. Sangster and K. L. Stuart, Chem. Rev., 65, 69 (1965).
7. I. A. Israilov, S. U. Karimova, M. S. Yunusov, and S. Yu. Yunusov, Khim. Prir. Soedin., 104 (1979).
8. O. Hoshino, H. Hara, M. Ogawa, and B. Umezawa, Chem. Pharm. Bull., 23, 2578 (1975).
9. H. Guinaudeau, M. Leboeuf, M. Debray, A. Cave, and R. R. Paris, Planta Med., 27, 304 (1975).

POTENTIOMETRIC ANALYSIS OF MIXTURES OF AMINO ACIDS WITH THEIR N-tert-BUTOXYCARBONYL DERIVATIVES IN MIXED SOLVENTS

T. Niyazkhonov, A. Ya. Veveris, and L. B. Kuznetsova

UDC 543.257:547.466

Potentiometric methods are proposed for the quantitative analysis of a mixture of a number of amino acids with their N-tert-butoxycarbonyl derivatives in mixed solvents. These methods can be used for the analysis of industrial samples of N-tert-butoxycarbonyl derivatives of amino acids.

N-tert-Butoxycarbonyl derivatives of amino acids, BOC-AAs, are a comparatively new class of derivatives of amino acids, AAs. They are obtained by replacing a hydrogen atom of the amino group of an AA by a N-tert-butoxycarbonyl group ($-\overset{\overset{O}{\|}}{C}-O-C(CH_3)_3$). BOC-AAS are used as protective groups in peptide synthesis, in increasing the molecular mass of biopolymers, in the production of physiologically active biochemical preparations, etc. [1, 2]. Reactions with the participation of BOC-AAs are performed in aqueous organic and organic solvents, and therefore the investigation of their acid–base properties in these media is of theoretical and practical interest. BOC-AAs are soluble in glacial acetic acid, dimethyl sulfoxide, dimethylformamide, and aliphatic nitriles and, with gentle heating, in water; on heating above 50°C, the action of strong acids and bases leads to the splitting out of the BOC group, which complicates the quantitative determination of the BOC-AAs.

There is information on the determination of individual amino acids and the analysis of their mixtures by acid–base titration of the NH_2 groups in protogenic solvents [3-5]. However, these solvents are unsuitable for the analysis of mixtures of BOC-AAs and AAs, since the majority of BOC-AAs do not contain NH_2 groups and, consequently, cannot be titrated as bases. The possibility of the differential titration of mixtures of BOC-AAs and AAs has scarcely been considered. There is likewise no information in the literature on the study of the acid–base properties of BOC-AAs and the values of their dissociation constants.

We have determined the dissociation constants, pK_a, of BOC-AAs in water and acetonitrile (AN) by Henderson's method using benzoic acid as standard:

	H_2O	AN
BOC-Glycine	3.74	20.44
BOC-Leucine	3.76	21.88
BOC-Valine	3.68	20.61
BOC-Serine	3.62	22.05
BOC-Glutamine	3.66	22.13
BOC-Proline	3.42	19.96
BOC-β-Phenyl-α-alanine	—	20.81
BOC-Tryptophan	3.62	21.96

As we see, in water, BOC-AAs exhibit approximately the same acidic properties and are stronger acids than benzoic. In AN, some differentiation of the acid properties of the BOC-AAs is observed: $\Delta pK_{(AN)}$ amounts to 1.5-2.0 units, in contrast to $\Delta pK_{(H_2O)}$, which is 0.25-0.3 units.

D. I. Mendeleev Moscow Institute of Chemical Technology. "Biokhimreaktiv" Scientific-Production Cooperative, Olaine. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 496-499, July-August, 1983. Original article submitted June 27, 1982.

0009-3130/83/1904-0467$07.50

The acid–base properties of the BOC-AAs in water differ substantially from the acid–base properties of the AAs corresponding to them. However, it was impossible to carry out a quantitative differential titration of mixture of BOC-AAs and AAs in individual solvents (H_2O, AN), since AAs are insoluble in the majority of organic solvents, including aliphatic nitriles, and in water they are weak acids and do not titrate.

On the basis of the results of the investigations performed, for the differential titration of mixtures of BOC-AAs and AAs we selected a mixed solvent H_2O–AN (1:10-15). The choice was due to the fact that the composition of the mixed solvent permitted the complete dissolution of the components of the mixture to be determined and, in addition to this, the solvent possessed a differentiating action in relation to the components titrated. Thus, on the curves of the potentiometric titration two potential jumps are observed, corresponding to the neutralization of the COOH groups of the BOC-AAs and of the AAs. The replacement of a hydrogen atom of the NH_2 group in the molecule of an AA by a BOC group strengthens its acid properties, and the BOC-AAs titrate first.

We also investigated the possibility of the quantitative analysis of a mixture of BOC-AAs and AAs in acetic acid and mixtures of it with AN. In acetic acid, BOC-AAs are fairly stable and do not interfere with the direct titration of the AAs by a 0.1 N solution of perchloric acid. The addition of AN increases the sharpness of the potential jump at the equivalence point.

EXPERIMENTAL

We used khr.ch. ["chromatographically pure"] BOC-AAs (Reanal), ch. ["pure"] AAs, and industrial samples of BOC-AAs from the "Biokhimreaktiv" Scientific Production Amalgamation. In the determination of dissociation constants we used khr.ch. BOC-AAs. Potentiometric titration was carried out on an apparatus consisting of a AVU-12 automatic burette, a pH 28 pH-meter with a system of a saturated calomel electrode and a glass electrode, a TTT-P automatic titrator, and a SRP-2c recorder. The amounts of each of the components of the model mixtures were varied within the range of 10-90 wt.%.

Analytical Procedure. Differential Titration. A weighed sample of an industrial BOC-AA (0.0500-0.0700 g) containing a residual amount of AA was dissolved in 2 ml of distilled water, and then 25-30 ml of AN was added and it was titrated potentiometrically with a 0.1 N solution of tetraethylammonium hydroxide (TEAH) in AN. The time of analysis was 10 min. The accuracy of the determination of the components of the mixture was ±0.5-1.5 wt. % when their ratio was from 1:9 to 9:1.

Combined Methods. A. A weighed sample of a mixture containing a BOC-AA and an AA was dissolved in the mixed solvent CH_3COOH–AN (4:1), and the AA was titrated with a 0.1 N solution of perchloric acid in glacial acetic acid. The amount of BOC-AA was found by difference from the weight of material taken and the amount of AA found.

B. A weighed sample of a mixture of BOC-AA and an AA was treated with AN and the resulting solution titrated with a 0.1 N solution of TEAH. Under these conditions the AA did not dissolve in the AN and did not interfere with the quantitative determination of the BOC-AA. The amount of AA was found from the difference between the weight of sample taken and the amount of BOC-AA found.

The methods permit reproducible results to be obtained when the amounts of AA or BOC in the mixture are low (0.5-1.0 wt. %).

The differential titration and combined methods give results in good agreement (see Table 1). The statistical treatment of the results of the potentiometric titration of mixtures of BOC-AAs and AAs was carried out in accordance with [6].

SUMMARY

1. Potentiometric methods for the quantitative analysis of mixtures of a number of amino acids with their N-tert-butoxycarbonyl derivatives in mixed solvents – H_2O–AN and CH_3COOH–AN – have been proposed.

TABLE 1. Results of the Potentiometric Titration of Mixtures of BOC-AAs and AAs (n = 6, t = 2.57, P = 0.95)

Composition of the mixture	Differential titration			Combined methods		
	$\bar{C}$, r	$S_r \cdot 10^2$	$\pm\sigma \cdot 10^2$	$\bar{C}$, r	$S_r \cdot 10^2$	$\pm\sigma \cdot 10^2$
β-Phenyl-α-alanine + BOC-β-phenyl-α-alanine	0,0404	5,53	14,22	0,0619	5,05	12,99
	0,1034	3,46	8,89	0,1004	3,56	9,15
Proline + BOC-proline	0,0401	4,98	12,82	0,0654	4,07	12,77
	0,1004	3,12	8,02	0,0968	4,00	10,28
Serine + BOC-serine	0,0501	4,55	11,60	0,0689	5,05	13,02
	0,1031	3,47	8,92	0,1054	4,16	10,68
Valine + BOC-valine	0,0502	4,79	12,33	0,0593	6,03	15,50
	0,1038	3,88	9,97	0,1027	4,46	11,46
Tryptophan + BOC-tryptophan	0,0680	3,83	9,85	0,0818	4,64	11,92
	0,1123	3,98	10,23	0,1418	2,78	7,16
Leucine + BOC-leucine	0,0512	5,59	14,37	0,0763	4,78	12,14
	0,1073	3,00	12,68	0,1370	3,16	8,13

2. The methods can be used for the analysis of industrial samples of BOC-AAs.

LITERATURE CITED

1. V. F. Pozdiev, N. N. Podgornova, N. K. Zentsova, et al., Khim. Prir. Soedin., 543 (1979).
2. J. M. Stewart and J. D. Young, Solid-Phase Peptide Synthesis, W. H. Freeman, San Francisco (1969).
3. A. P. Kreshkov, N. Sh. Aldarova, and G. V. Turovtseva, Dokl. Akad. Nauk SSSR, 169, No. 5, 1093 (1966).
4. N. Sh. Aldarova and G. V. Turovtseva, in: Problems of Analytical Chemistry [in Russian], Moscow, Vol. I (1970), p. 108.
5. N. Sh. Aldarova and M. V. Ermakov, in: Problems of Analytical Chemistry [in Russian], Moscow, Vol. 1 (1970), p. 229.
6. E. Ya. Neiman and B. Ya. Kaplan, Zh. Anal. Khim., 33, No. 3, 607 (1978).

SYNTHESIS OF DINITROPHENYLTETRAPEPTIDES AS CHROMOPHORIC SUBSTRATES OF ENDOPROTEINASES

S. V. Kulikov, N. Yu. Sokolova, S. V. Rodin, and M. A. Samartsev

UDC 577.156

The synthesis has been performed of ten tetrapeptides of the general formula Dnp-Gly-Gly-X-Arg-OH, where X = Val, Phe, Abu, Asp(OBu^t), Asp, Met, D-Phe, Ser, Thr, or Trp. The synthesis was carried out with Dnp-Gly-Gly-ONp, activated esters of protected amino acids, and arginine with an unprotected carboxy group. The coefficients of molar extinction of the tetrapeptides at 660 nm are given. It has been shown that the peptides can be used to determine the activities of neutral and alkaline proteinases from various sources, the peptides with X = Phe, Met, and Abu exhibiting the highest sensitivity to enzymatic hydrolysis.

The activities of proteolytic enzymes are determined both with the use of protein substrates and in relation to low-molecular-weight peptide substrates of definite structure. In the latter case, the possibility exists of differentiating proteinases with different specificities and also of achieving standardization of the conditions of the determination more easily. To increase the sensitivity of spectrometric analysis it is possible to introduce into the substrates a chromophoric grouping shifting the absorption spectrum of the peptide into the visible region; for example, an aromatic azo group [1] or a 2,4-dinitrophenyl resi-

All-Union Scientific-Research Institute of Ultrapure Biopreparations, Leningrad.

Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 499-504, July-August, 1983. Original article submitted June 15, 1982.