POTENTIOMETRIC DETERMINATION OF N-ACETYLGLUCOSAMINYL-N'-ACETYLMURAMYL-L-ALANYL-D-ISOGLUTAMINE AND THE STARTING MATERIALS AND INTERMEDIATES FOR ITS SYNTHESIS

B. A. Spintse, N. N. Podgornova, and A. Ya. Veveris

UDC 543.257.1:547.466

The conditions for the potentiometric acidimetric titration in nonaqueous solutions of N-acetylglucosaminyl-N'-acetylmuramyl-L-alanyl-D-isoglutamine (GMCP), its sodium salt, N-acetylglucosaminyl-N'-acetylmuramic acid, the γ-benzyl esters of the trifluoroacetate of D-isoglutamine, of N-tert-butoxycarbonyl-D-isoglutamine, and of N-tert-butoxycarbonyl-L-alanyl-D-isoglutamine, and also the o-nitrophenyl esters of N-tert-butoxycarbonyl-D-isoglutamine γ-benzyl ether and of N-tert-butoxycarbonyl-L-alanine have been investigated. Methods have been developed for the analysis of milligram amounts of GMDP and of the starting materials and intermediates for its synthesis by potentiometric titration with a ~0.025-0.030 N nitromethane solution of perchloric acid. The relative error of the determinations amounts to ±1.5% and the residual standard deviation to 1.3%.

A synthetic glycopeptide modeling the glycopeptides of bacterial cell walls – N-acetylglucosaminyl-N'-acetylmuramyl-L-alanyl-D-isoglutamine (GMDP) is a promising immunomodulator which possesses adjuvant properties [1]. The synthesis of GMDP includes a multistage process of obtaining the β-benzyl ester of L-alanyl-D-isoglutamine (DP) and its condensation with N-acetylglucosamimyl-N'-acetylmuramic acid (DS) in the presence of the Woodward reagent. In connection with the development of the technology of the industrial production of GMDP the necessity has arisen for creating methods for the analytical control of the individual stages of the process and the quality of the intermediates.

Our aim was to develop a procedure for determining the weight fraction of the main substance in preparations of GMDP, DS, and a number of intermediates in the synthesis of DP: the γ-benzyl esters of the trifluoroacetate of D-isoglutamine (I), of N-tert-butoxycarbonyl-D-isoglutamine (II) and of N-tert-butoxycarbonyl-L-alanyl-D-isoglutamine (III) and also the o-nitrophenyl esters of N-tert-butoxycarbonyl-D-isoglutamine γ-benzyl ester (IV) and of N-tert-butoxycarbonyl-L-alanine (V). GMDP and DS are in extremely short supply and expensive; their consumption in analysis must be in the smallest possible amounts, and therefore for the determination it was necessary to select methods permitting the analysis of milligram amounts. The alkalimetric method used previously for the analysis of N-tert-butoxycarbonyl derivatives of amino acids [2, 3] proved to be unsuitable for solving our problem (because of the instability of the titrant). We selected the method of potentiometric acidimetric titration in nonaqueous media. As the titrant we used a nitromethane solution of perchloric acid, and as the titration medium nitromethane (NM) or mixtures of it with acetic acid (AAc) and acetic anhydride (AAn).

As preliminary investigation showed, the different acid–base properties of the compounds being analyzed required a differentiated approach in the selection of the conditions for their analysis. Because of the inadequate solubility of (I) in NM, it was first dissolved in a small amount of AAc and titration was performed in the mixed solvent AAc–NM (1:30). As can be seen from Fig. 1, the neutralization of (I) is accompanied by a jump in potential by 80-100 mV (curve 1), which is sufficiently well defined for a determination of the equivalence point. However, mixtures of NM with AAc proved to be unsuitable for the determination of GMDP by the acidimetric method.

The possibility was established of the successive protonation of the two secondary amino groups of GMDP during titration in the mixed solvent NM–AAn (2:1). The first of the

All-Union Scientific-Research Institute of Applied Biochemistry, and Biolar Scientific-Production Amalgamation, Olaine. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 568-571, July-August, 1987. Original article submitted January 13, 1987.

 0009-3130/87/2304-0476$12.50

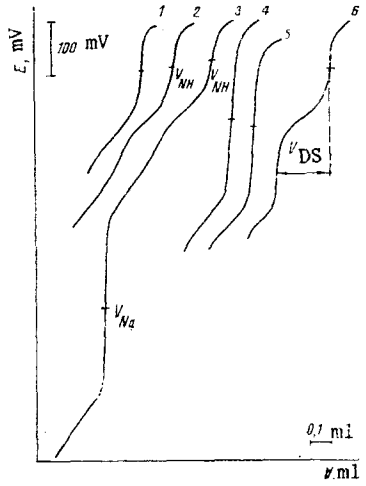

Fig. 1. Curves of the potentiometric titration with a 0.025 N nitromethane solution of perchloric acid in nitromethane (6) and mixtures of it with acetic acid (1), acetic anhydride (2, 3), and formic acid (4), and also in a mixture of formic acid and acetone (5): 1) (I); 2) GMDP: 3) sodium salt of GMDP; 4) (II); 5) (IV); 6) DS.

TABLE 1. Results of the Quantitative Determination of GMDP and of the Starting Materials and Their Intermediates for Its Synthesis (at n = 5, α = 0.95)

Substance analyzed	Taken, mg	Found, X, %	Relative error, %	Relative standard deviation, S_r,%
GMDP	4,18	100,4	+0,4	
	4,26	98,7	−1,3	1,2
	3,89	98,5	−1,5	
DS (trihydrate)	3,07	101,3	+1,3	
	2,63	99,0	−1,[illegible]	1,3
	2,75	98,8	−1,2	
I	5,62	99,3	−0,7	
	7,47	98,9	−1,1	0,9
II	4,78	98,5	−1,5	
	4,34	101,1	+1,1	0,6
III	4,98	99,4	−0,6	
	5,71	99,3	−0,7	0,5
IV	6,98	100,5	+0,5	
	5,64	100,3	+0,3	0,7
V	6,96	100,7	+0,7	
	8,45	99,2	−0,8	0,6

two potential jumps on curve 2 is ill-defined and is not recommended for determining the equivalence point, and therefore in the analyses we used the results of the total neutralization of the two protonatable groups (V_{NH}). When GMDP was titrated in the form of its sodium salt, three potential jumps appeared on the curve (curve 3), the first of them being most pronounced and corresponding to the titration of the sodium ions (V_{Na}). When the GMDP was converted completely into the salt form and the samples contained no by-products of the synthesis, the results of the titration were characterized by the following equivalence of the volumes of titrant used: $V_{Na} = 0.5\ V_{NH}$.

The method of direct acidimetric titration in mixtures of NM and AAn is inapplicable in the analysis of DS and of compounds (II-V) containing BOC protective groups. In the case of the titration of DS, there is no potential jump on the curve, and in the titration of the BOC derivatives the potential jumps are insufficiently pronounced (30-40 mV) to ensure the necessary accuracy of analysis. In these cases, the substances being analyzed were previously treated with acid. In the determination of the BOC-derivatives (II-V), weighed samples were dissolved in formic acid and the solutions were kept at 50-60°C for 30-40 min (until the N-protective group had been split out completely). The amino compounds formed possess pronounced basic properties in nonaqueous media and large jumps were observed on the potentiometric titration curves (curves 4 and 5) while in this case in place of NM it is possible to use acetone and acetonitrile as the titration media.

The preliminary treatment of DS was performed with perchloric acid in NM at temperatures of 75-80°C. It was established that under these conditions there was a quantitative formation of product (possibly oxazoline derivatives) possessing more pronounced basic properties than DS. Potentiometric titration was carried out after the end of the treatment and

neutralization of the perchloric acid with an excess of tertiary alkylamine (triethylamine, tributylamine). Two potential jumps were observed on the titration curve (curve 6). The first of them corresponded to the neutralization of the excess of tertiary amine, and the consumption of titrant between the first and second jumps (V_{DS}) was used for the calculation of the amount of DS present.

Table 1 gives the results of the quantitative determination of the compounds mentioned, which indicate the adequate reliability of the proposed procedures.

EXPERIMENTAL

Samples of the compounds under investigation were synthesized under laboratory conditions and subjected to the methods of elementary analysis, high-performance liquid chromatography, and thin-layer chromatography.

Weighings were performed on a Mettler ME-22 microbalance (Switzerland). An RTS-822 titrator (Denmark) ensuring an accuracy of the metering of the titrant of ±0.002 ml, a 2.5-ml burette, and a glass-calomel electrode pair were used. The comparison electrode was filled with a saturated aqueous solution of lithium perchlorate. The titrant was a ~0.020-0.025 N solution of perchloric acid in NM. The NM was redistilled after being kept over calcined molecular sieves.

Analytical Procedures. Weighed samples of GMDP or its sodium salt (4-6 mg) were dissolved in 0.5 ml of glacial acetic acid, and 10 ml of nitromethane and 5 ml of acetic anhydride were added. Potentiometric titration was carried out with a 0.025-0.030 N solution of perchloric acid in nitromethane, and the consumptions of titrant V_{NH} and V_{Na} were determined from the titration curve. A control experiment was carried out in parallel.

To determine (I), a weighed sample (4-8 mg) was dissolved in 0.5 ml of acetic acid, and then 15 ml of NM was added and potentiometric titration was performed. A control experiment was carried out in parallel.

A weighed sample of DS was first treated with 2 ml of a 0.025-0.030 N solution of perchloric acid in NM and the mixture was kept at a temperature above 75-80°C for 2 h. Then 3 ml of a 0.025-0.030 N solution of tributyl- or triethylamine in NM and 10 ml of NM were added. Potentiometric titration was carried out until two potential jumps had appeared on the curve. A control experiment was carried out in parallel. The consumption of titrant V_{DS} was determined from the titration curve and the amount of DS present was calculated.

Weighed samples of compounds (II-V) (4-7 mg each) were first dissolved in 1 ml of formic acid and the solutions were kept at 50-60°C for 30-40 min. Then 15 ml of NM or acetone was added to the solution to be analyzed and potentiometric titration was performed. A control experiment was carried out in parallel.

CONCLUSION

The conditions for the potentiometric acidimetric titration of N-acetylglucosaminyl-N'-acetylmuramyl-L-alanyl-D-isoglutamine and the intermediates in its synthesis in organic solvents have been investigated.

A procedure has been developed for determining the N-tert-butoxycarbonyl derivatives of D-isoglutamine and L-alanine which includes the preliminary elimination of the N-protective groups by the action of formic acid.

Preliminary treatment with perchloric acid in nitromethane at 75-80°C is proposed for the acidimetric determination of N-acetylglucosaminyl-N'-acetylmuramic acid. The methods developed are distinguished by reliability in use and require only small amounts (2-7 mg) of samples from one determination. The time of analysis is from 15 min to 2.5 h.

LITERATURE CITED

1. L. I. Rostovtseva, T. M. Andronova, V. P. Mal'kova, et al., Bioorg. Khim., 7, 1843 (1981).
2. A. Z. Lusis, A. Ya. Veveris, and U. O. Kalei, in: Abstracts of Lectures at the 3rd All-Union Conference on Methods of Obtaining and Analyzing Biochemical Reagents, [in Russian], Olaine (1979), p. 33.
3. T. Niyazkhonov, A. Ya. Veveris, and L. B. Kuznetsova, Khim. Prir. Soedin., 496 (1983).