6. A. E. Zemlyakov, V. O. Kur'yanov, V. Ya. Chirva, and A. Ya. Khorlin, Bioorg. Khim., 12, No. 7, 929 (1986); A. E. Zemyakov, V. Ya. Chirva, and A.Ya. Khorlin, Bioorg. Khim., 12, No. 12, 1637 (1986).
7. R. U. Lemieux and H. Driguez, J. Am. Chem. Soc., 97, No. 14, 4069 (1975).
8. L. G. Rostovtseva, T. M. Andronova, V. P. Mal'kova, N. B. Sprokina, and V. T. Ivanov, Bioorg. Khim., 7, No. 12, 1843 (1981).

POTENTIOMETRIC DIFFERENTIATED TITRATION OF THE COMPONENTS OF NUCLEIC ACIDS AND THEIR DERIVATIVES.

VII. ACIDIMETRIC DETERMINATION OF SOME N-ACYL-2'-DEOXYRIBONUCLEOSIDES AND THEIR 5'-TRITYLATED DERIVATIVES

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The conditions have been investigated for the potentiometric titration of N-acyl-2'-deoxyribonucleotides and of trityl carbinol derivatives and their two-component mixtures with a nitromethane solution of perchloric acid. The influence of water, acetone, chloroform, and acetic acid on the conditions for the acidimetric analysis of tri-p-methyltrityl carbinol, p-monomethoxytrityl carbinol, and di-p-methoxy trityl carbinol in nitromethane have been shown. Procedures have been developed for the quantitative determination of N^6-benzoyl-5'-di-p-methoxytrityl-2'-deoxyriboadenosine, the 5'-tri-p-methyltrityl and 5'-p-monomethoxytrityl derivatives of N^4-benzoyl-2'-deoxyribocytidine, of N^6-benzoyl-2'-deoxyriboadenosine, and of N^2-isobutyryl-2'-deoxyriboguanosine by differentiated potentiometric titration. For the determination of the 5'-di-p-methoxy derivatives of N^4-benzoyl-2'-deoxyribocytidine and of N^6-benozyl-2'-deoxyriboadenosine a procedure is proposed which includes the use of two parallel titrations. Methods have been developed for the use of milligram amounts of substance.

In preceding papers [1-6], methods have been proposed for the potentiometric acidimetric titration of ribo- and 2'-deoxyribonucleotides, and of di-p-methoxytrityl carbinol and 5'-di-p-methoxytritylthymidine in nonaqueous solutions. The aim of the present work was to investigate the conditions for the potentiometric acidometric titration and the development of a procedure for the quantitative determination of N^6-benzoyl-2'-deoxyriboadenosine (dA^{Bz}), N^4-benzoyl-2'-deoxyribocytidine (dC^{Bz}), and N^2-isobutyryl-2'-deoxyriboguanosine (dG^{iBu}) and their 5'-di-p-methoxytrityl, 5'-monomethoxytrityl, and 5'-trimethyltrityl derivatives, preparations of which find wide use of oligonucleotide synthesis. Existing spectrophotometric and chromatographic methods for the quantitative determination of N-acyl-2'-deoxyribonucleosides and their 5'-tritylated derivatives possess inadequate accuracy and require for their performance the presence of standard samples of the substances to be analyzed, and therefore investigations directed to improving the analytical quality control are of practical interest.

Nitromethane (NM) was selected as the titration medium. Characteristic for this solvent is the wide extent of the absolute scale of acidity and a differentiation capacity with respect to weak bases [7, 8]. Furthermore, NM, like other nitroalkanes, is capable of ionizing trityl carbinol and its derivatives [9].

Figure 1 shows the most characteristic potentiometric titration curves. It can be seen that each of the curves for the titration of N-acyl-2'-deoxyribonucleosides has one potential jump which corresponds to the end of the quantitative protonation of the purine heterocycle (curves 1 and 3) or the pyrimidine heterocycle (curve 2). A comparison of these curves permits the conclusion that dA^{Bz} in NM posssesses a higher proton-accepting capacity than dC^{Bz}

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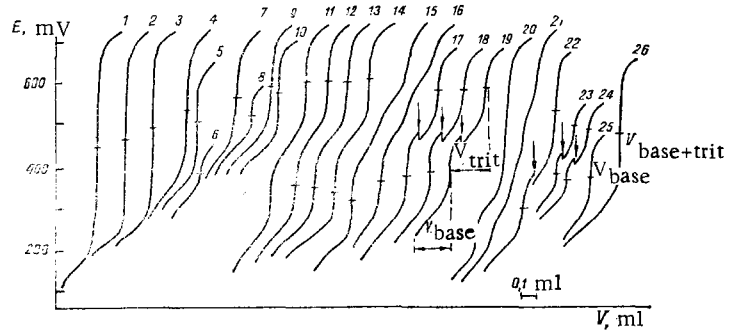

Fig. 1. Curves of the potentiometric titration with an 0.025 N nitromethane solution of perchloric acid (1-22, 25, 26) and an 0.025 N acetonitrile solution of hydrogen chloride (23, 24) in NM with the following water contents: < 0.012% (4, 7, 9, 15, 17, 21, 23, 24) and 0.03% (8) and in mixtures of NM and acetic acid (15:1) (1-3, 5, 11-14, 20), of NM and acetone (20:1) (20), and of NM and chloroform (10:1) (10, 16, 18, 19); 1) dA^{Bz}; 2) dC^{Bz}; 3) dG^{iBu}; 4-6) DMTC; 7, 8) MMTC; 9, 10) TMTC; 11) dA^{Bz} + MMTC; 12) dC^{Bz} + MMTC; 13) dC^{Bz} + TMTC; 14) dG^{iBu} + TMTC; 15) 5'-MMTdA^{Bz}; 16) 5'-TMTdC^{Bz}; 17) 5'-MMTdA^{Bz}; 18) 5'-TMTdC^{Bz}; 19) 5'-TMTdG^{iBu}; 20) dA^{Bz} + DMTC; 21, 22) 5'-DMTdA^{Bz}; 23) 5'-DMTdC^{Bz}; 24) 5'-DMTdG^{iBu}; 25, 26), 5'-DMTdC^{Bz} or 5'-DMTdG^{iBu} [25) medium acetic acid—acetone (1:2); 26) acetic acid-NM (1:2)].

and dG^{iBu}. It is true, as follows from literature information [2-4, 10], that in water and in organic solvents cytidine and 2'-deoxyribocytidine (dC) possesses greater basicities than adenosine and 2'-deoxyriboadenosine (dA). Such changes in the difference in strength in the case of the N-benzoyl derivatives of dC and dA are explained by the electron-accepting action of the N-protective group which, possibly, leads to a change in the position of protonation in the dC^{Bz} heterocycle.

The simultaneous presence in the molecules of the corresponding 5'-tritylated 2'-deoxyribonucleosides of two groupings (heterocyclic base and trityl group) capable of entering into interaction with a strong acid predetermined the possibility of investigating the conditions for their differentiated titration. Artificial two-component mixtures of N-acyl-2'-deoxyribonucleosides with various derivatives of trityl carbinol — di-p-methoxytrityl carbinol (DMTC), mono-p-methoxytrityl carbonol (MMTC), or tri-p-methyltrityl carbinol (TMTC). As can be seen from Fig. 1, the latter also titrate with one potential jump on the titration curve (curves 4, 7, 9). It is characteristic that the potentiometric titration curves for MMTC and TMTC are extremely similar to one another (under identical conditions: the titrant was a 0.025 N solution of perchloric acid in NM, the moisture content of the NM being less than 0.012%), but with respect to the size of the potential jump they are inferior to the titration curves of DMTC. An increase in the amount of water in the NM led to a sharp deterioration in the conditions for the potentiometric titration of MMTC (curve 8) and of the other trityl carbinol derivatives. The addition to the NM of a number of cosolvents (for example, methylcellosolve, acetone) likewise had an adverse effect on the sizes of the potential jumps. For example, when 15% of acetone was present in the NM there were no potential jumps in the titration curves of MMTC and TMTC, while in the case of DMTC the potential jump on the curve was ill-defined (curve 6). It was also established that the addition to the NM of the same amounts of acetic acid and chloroform had a comparatively small effect on the sharpness of the potential jumps on the potentiometric titration curves of trityl carbinol derivatives (curves 5 and 10). Consequently, these solvents can be used in analysis if it is required to increase the solubility of the materials under investigation or to improve the conditions of differentiated titration.

Artificial mixtures containing MMTC and TMTC titrate with potential jumps on the curves. Figure 1 gives, as examples, the titration curves of the mixtures dA^{Bz} + MMTC, dC^{Bz} + MMTC, dC^{Bz} + TMTC, and dG^{iBu} + TMTC (curves 11-14). The first jumps on these curves are located in the ∿200-400 mV region and correspond to the titration of the heterocyclic base of the nucleoside. Two potential jumps are also observed on the curves for the potentiometric titration of N^6-benzoyl-5'-mono-p-methoxytrityl-2'-deoxyriboadenosine (5'-MMTdA^{Bz}), N^4-benzoyl-5'-tri-p-methyltrityl-2'-deoxyribocytidine (5'-TMTdC^{Bz}) (curves 15 and 16), and a number of other

5'-tritylated N-acyl-2'-deoxyribonucleosides. However, here the potential jumps on the curves corresponding to the titration of TMT and MMT groups were less pronounced than on the titration of artificial mixtures containing MMTC and TMTC. Such a deterioration of the titration conditions in the case of the 5'-tritylated N-acyl-2'-deoxyribonucleosides is explained by the influence of an intermediate process connected with the cleavage of the bond between the oxygen and the trityl group in position 5' under the action of a strong acid.

The investigations showed that the influence of this intermediate reaction can be eliminated by adding acetic acid to the solution being analyzed (curves 17-19). The best results were achieved if the addition took place after the neutralization of the heterocyclic base (in Fig. 1, the addition of acetic acid is marked by an arrow). For this purpose, after the first potential jump on the curve, titration was temporarily suspended, and after the introduction of the necessary amount of acetic acid, the solution undergoing analysis was stirred for 1-2 min.

By the use of quantitative determination of chromatographically homogeneous samples, it was established that the consumptions of titrant up to the first potential jump (V_{base}) (and from the first to the second potential jumps (V_{trit}) were equal to one another, and this equality can serve as one of the criteria for estimating the purity of preparations. Table 1 gives the results of determination in which the quantitative amount of 5'-tritylated N-acyl-2'-deoxyribonucleosides was calculated from two parameters (V_{base} and V_{trit}). It is characteristic that the results of determinations for V_{base} and V_{trit} agree well with one another and show the reliability of procedures based on the use of differentiated titration. The fact that the results of analysis of a number of samples were between 91 and 97% is explained by the presence of organic solvents in the samples being analyzed, because of the tendency of 5'-tritylated nucleosides to form solvents. When samples of 5'-trityl-N-acyl-2'-deoxyribonucleosides contained impurities participating in the titration process, the consumptions of titrant V_{base} and V_{trit} differed from one another: when dA^{Bz}, dC^{Bz}, or dG^{iBu} were present as impurities, $V_{base} > V_{trit}$, while when trityl carbinol derivatives were present, $V_{trit} > V_{base}$. The difference between V_{base} and V_{trit} can be used for the quantitative determination of impurities.

The possibilities of differentiated titration proved to be extremely limited in the determination of protected 2'-deoxyribonucleosides containing the 5'-DMT group. Two potential jumps appeared on the curves in the titration of mixtures of dA^{Bz} + DMTC and 5'-DMTdA^{Bz} (curves 21 and 22). On the titration of 5'-DMTdC^{Bz} and 5'-DMTdG^{iBu} with a nitromethane solution of perchloric acid, fairly clear transition between the first and second potential jumps on the curves was achieved. A more distinct transition was observed when an acetonitrile solution of hydrochloric acid was used as titrant (curves 23 and 24). However, when the jumps were ill-defined, the results of the determinations possessed a lower accuracy (the relative standard deviation reached 2-4%). More reliable results of the analysis of 5'-DMTdC^{Bz} and 5'-DMTdG^{iBu} were obtained by the use of a procedure including the combination of two parallel titrations. One of them was performed in a mixture of NM and acetic acid (13:2), when the consumption of titrant in the combined titration of the base of the nucleoside and the DMT group was determined (curve 26, $V_{base+trit}$) Since when more than 15% of acetone is present in NM the DMT group is not determined, the consumption of titrant V_{base} was found by a parallel titration in acetone—NM (1:5) (curve 25). The consumption of titrant V_{trit} in this case was calculated from the difference between the results of two titrations.

Thus, the possibilities of determining 5'-tritylated N-acyl-2'-deoxyribonucleosides by differentiated potentiometric titration have been investigated for the first time. The results of the titration permit the quality of the compounds being analyzed to be estimated by comparing two indices.

EXPERIMENTAL

The substances to be analyzed were synthesized under laboratory conditions. Before use their chromatographic homogeneity was checked and so was their quantitative composition by a known spectrophotometric method [11]. The procedures for purifying the NM and preparing the titrants were described in a preceding paper [6]. A RTS-882 titrator (Denmark) was used for performing the potentiometric titrations with a burette ensuring a dosing accuracy of about ±0.002 ml, and a glass-calomel electrode pair. The comparison electrode was previously filled with an aqueous solution of lithium perchlorate.

Analytical Procedures. In the determination of N^6-benzoyl-2'-deoxyriboadenoside (dA^{Bz}), N^4-benzyl-2'-deoxyribocytidine (dC^{Bz}), and N^2-isobutyryl-2'-deoxyriboguanosine (dG^{iBu}), weighed samples (between 3 and 5 mg) were each dissolved in 1 ml of acetic acid, and 15 ml

TABLE 1. Results of the Quantitative Determination of N-Acyl-2'-deoxyribonucleosides and Their 5'-Tritylated Derivatives

Substance analyzed	Taken, mg	Found, %				
		from V_{base}		from V_{trit}		by the comparison method [11]
		$\bar{X}$	Sr (n=5)	$\bar{X}$	Sr (n=5)	$\bar{X}$
dA^{Bz}	3,45	99,4	0,8	—	—	—
dC^{Bz}	3,90	98,6	1,0	—	—	—
dG^{iBu}	4,61	99,5	0,9	—	—	—
MMTC	4,63	—	—	98,5	1,1	98
TMTC	5,13	—	—	99,2	1,0	99
DMTC	4,06	—	—	97,3	0,8	97
5'- $MMTdA^{Bz}$	7,58	98,6	1,2	99,6	1,3	98
5'- $MMTdC^{Bz}$	7,74	93,7	1,1	94,3	1,3	94
5'- $MMTdG^{iBu}$	7,96	96,1	1,2	95,4	1,2	96
5'- $TMTdA^{Bz}$	8,27	97,7	1,4	97,1	1,2	96
5'- $TMTdC^{Bz}$	8,89	98,3	1,2	98,8	1,4	98
5'- $TMTdG^{iBu}$	8,04	94,5	1,1	95,6	1,2	94
5'- $DMTdA^{Bz}$	8,37	99,2	1,3	98,1	1,3	99
5' $DMTdC^{Bz}$	8,68	91,7	1,4	92,5	1,2	92
5' $DMTdG^{iBu}$	8,93	98,0	1,5	97,6	1,4	98

of NM was added. Potentiometric titration was performed with a 0.025 N nitromethane solution of perchloric acid until the appearance of a potential jump on the curve.

Weighed amounts of 5'-tri-p-methyltrityl-N^6-benzoyl-2'-deoxyriboadenosine (5'-$TMTdA^{Bz}$), 5'-tri-p-methyltrityl-N^2-isobutyryl-2'-deoxyguanosine (5'-$TMTdG^{iBu}$), and 5'-tri-p-methyltrityl-N^4-benzoyl-2'-deoxyribocytidine (5'-$TMTdC^{Bz}$) (7-9 mg) were each dissolved in 1 ml of chloroform that had previously been freed from ethanol, and then 10 ml of NM was added and potentiometric titration was performed. After the first potential jump, titration was suspended, 10 ml of a 10% solution of acetic acid in NM was added, and titration was continued until the appearance of the second potential jump on the titration curve.

Weighed amounts of 5'-mono-p-methoxytrityl-N^4-benzoyl-2'-deoxyribocytidine (5'-$MMTdC^{Bz}$), 5'-mono-p-methoxytrityl-N^2-isobutyryl-2'-deoxyriboguanosine (5'-$MMTdG^{iBu}$), 5'-mono-p-methoxytrityl-N^6-benzoyl-2'-deoxyadenosine (5'-$MMTdA^{Bz}$), and 5'-di-p-methoxytrityl-N^6-benzoyl-2'-deoxyriboadenosine (5'-$DMTdA^{Bz}$) (7-9 mg) were each dissolved in 10 ml of NM. The subsequent procedure was the same as in the analysis of the compounds containing the trimethyltrityl group.

In the analysis of 5'-di-p-dimethyltrityl-N^2-isobutyryl-2'-deoxyriboguanosine (5'-$DMTdG^{iBu}$) and 5'-di-p-methoxytrityl-N^4-benzoyl-2'-deoxyribocytidine (5'-$DMTdC^{Bz}$), weighed amounts (40-45 mg) were each dissolved in 25 ml of acetic acid in a measuring flask, and then the volume of the solution in the flask was made up to the mark with acetic acid. For titration, 5.00-ml portions of the solution to be analyzed were taken in two beakers. To one beaker was added 10 ml of acetone and titration was carried out with 0.025 N solution of perchloric acid until the appearance of a potential jump, and V_{base} was determined. To the other beaker was added 10 ml of NM and, after titration, $V_{base+trit}$ was determined.

CONCLUSIONS

The conditions for the potential titration of N-acyl-2'-deoxyribonucleosides and of tritylcarbinol derivatives and their two-component mixtures by a nitromethane solution of perchloric acid have been investigated. The influence of water, acetone, chloroform, and acetic acid on the conditions for the acidimetric analysis of tri-p-methyltrityl carbinol, mono-p-methoxytrityl carbinol, and di-p-methoxytrityl carbinol in nitromethane have been shown. The possibility has been demonstrated of using differentiated potentiometric titration in the analysis of 5'-tri-p-methyltrityl, 5'-mono-p-methoxytrityl, and 5'-di-p-methoxytrityl derivatives of N-acyl-2'-deoxyribonucleosides. Procedures have been developed which are distinguished by simplicity of performance and by fairly high accuracy and permit quantitative determinations to be carried out with the same reliability with respect to the neutralization both of the heterocyclic base and of the trityl group.

LITERATURE CITED

1. A. Ya. Veveris, U. Ya. Mikstais, and I. Ya. Sternberga, Khim. Prir. Soedin., 132 (1977).