Journal of Chromatography, 261 (1983) 83–93
Elsevier Science Publishers B.V., Amsterdam — Printed in the Netherlands

CHROM. 15,669

REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF NUCLEOSIDES AND NUCLEOTIDES

DAVID L. RAMOS and ALLEN M. SCHOFFSTALL*
Department of Chemistry, University of Colorado at Colorado Springs, Colorado Springs, CO 80907 (U.S.A.)
(Received November 29th, 1982)

SUMMARY

The purpose of this work was to find a suitable reversed-phase high-performance liquid chromatographic method for analysing 2′-, 3′- and 5′-isomers of nucleotides and at the same time determining the free base, nucleoside, 2′,3′-cyclic nucleotide and the total diphosphates. The procedure developed was most effective for those nucleosides and their derivatives that displayed a range of retention times, allowing sufficient resolution for quantitative analyses. The isocratic analyses were particularly suitable for the determination of individual 2′-, 3′- and 5′-ribonucleotides and for 3′- and 5′-deoxyribonucleotides, which were very difficult to quantitate by cellulose thin-layer chromatography. The reversed-phase method enabled quantitative analysis of the nucleoside base, nucleoside, nucleotides and cyclic nucleotides derived from thymidine, deoxyadenosine, adenosine or guanosine. Overlapping elutions were observed for reversed-phase separations of derivatives of uracil and cytosine, but some quantitative results were obtained. The separation method was applied to the analysis of products of phosphorylation of nucleosides and deoxynucleosides in formamide solution.

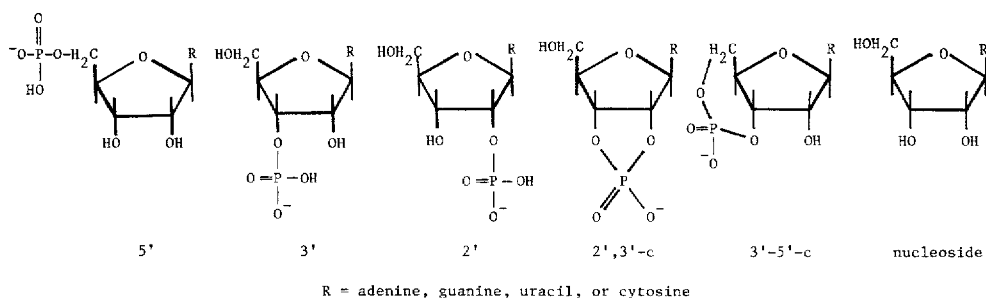
INTRODUCTION

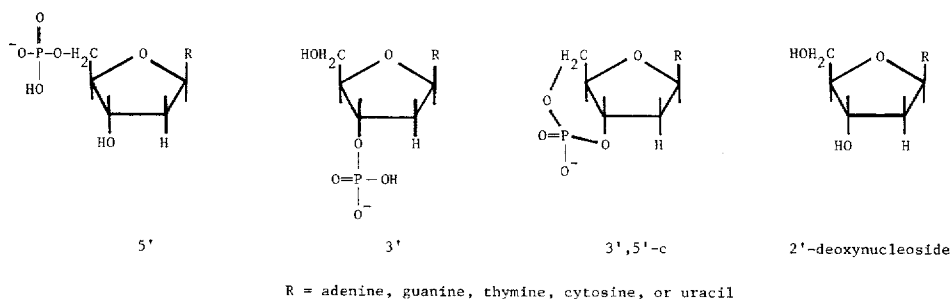
The separation of nucleotides by high-performance liquid chromatography (HPLC) has become common. The bulk of HPLC separations of nucleotides have involved the use of pelicular anion exchangers[1–10]. Cation exchangers have also found use[9,11,12]. Nucleosides and bases, including anomers and epimers[16], have been separated by reversed-phase HPLC[13–15]. A recent trend has been to separate nucleotides also by reversed-phase HPLC[8,17,18].

We selected reversed-phase HPLC as a method for the separation and quantitative analysis of adenine, adenosine, 2′-, 3′- and 5′-AMPs and 2′,3′-cAMP*. The

* Abbreviations: AMPs = adenosine monophosphates; ADPs = adenosine diphosphates; cAMP = adenosine cyclic phosphate. Similar abbreviations are used in this paper for guanosine (G), uridine (U), thymine (T), cytidine (C) and 2′-deoxyadenosine (dA) derivatives.

0021-9673/83/$03.00 © 1983 Elsevier Science Publishers B.V.





method was also useful for the separation of 2'-deoxyadenosine, thymidine and guanosine and their respective monophosphates. Uridine and cytidine derivatives were also separated, but gave considerable overlapping of peaks.

The method was used to discriminate between individual nucleotides produced by the phosphorylation of nucleosides which would not be otherwise resolved by cellulose thin-layer chromatography (TLC). For example, solutions of adenosine and KH_2PO_4 in formamide upon heating afforded a mixture of the phosphorylated products listed above. Reversed-phase HPLC was used to assess qualitatively the nature of the products formed and to quantitate the amounts of each component in the product mixture, including the individual 2'-, 3'- and 5'-nucleotides

EXPERIMENTAL

Reagents

Reagent-grade nucleotides, nucleosides and bases were purchased from Sigma (St. Louis, MO, U.S.A.) except for the following: 5'-ATP and 5'-ADP from Calbiochem (San Diego, CA, U.S.A.), 2'-deoxyadenosine, 3'-dAMP and 3',5'-cyclic dAMP from P & L Biochemicals (Milwaukee, WI, U.S.A.), 5'-AMP, 5'-TMP, and 5'-dAMP from Nutritional Biochemical Corporation (Cleveland, OH, U.S.A.) and adenine from Aldrich (Milwaukee, WI). The nucleotides, nucleosides, and bases were dissolved in reagent-grade formamide (Fisher Scientific, Fair Lawn, NJ, U.S.A.). The mobile phase used was 0.05 *M* $(NH_4)H_2PO_4$ in methanol–water (10:90) at pH 5.0. The methanol was HPLC reagent grade (J. T. Baker, Phillipsburg, NJ, U.S.A.) with a maximum UV cut-off of 205 nm.

Instrumentation

HPLC analyses were performed by use of two Altex (Berkeley, CA, U.S.A.) 110 solvent metering pumps equipped with Altex 110-19 pressure filters and controlled by an Altex 410 solvent programmer. Chromatography was carried out on a 250 × 4.6 mm I.D. Ultrasphere ODS column with a 5-μm particle diameter (Altex). Samples were introduced by an Altex 210 injection valve equipped with a 20-μl sample loop. Detection at 254 nm was done by an Altex–Hitachi 155 variable-wavelength spectrophotometer fitted with a 20-μl flow cell. The detector was coupled to a Soltec (Sun Valley, CA, U.S.A.) dual-channel recorder.

RESULTS

The method developed was similar to that of Anderson and Murphy[17] except that the mixtures of derivatives being studied were isocratically eluted with 0.05 *M* $(NH_4)H_2PO_4$ in methanol–water (10:90) at pH 5.0 using a flow-rate of 1.9 ml/min. Data given in Tables I–III are for standard solutions of components in formamide.

Fig. 1. shows the separations of a solution of adenosine and adenosine derivatives. The 5′-ATP and 5′-ADP were co-eluted as the first peak followed (in order) by 5′-AMP, 2′-AMP, 2′,3′-cAMP, 3′-AMP, 3′,5′-cAMP and adenosine. A separate analysis of adenine showed its retention to be between those of 2′-AMP and 2′,3′-cAMP (see Table I).

The separation of guanine and its derivatives is depicted in Fig. 2. Co-eluted as the first peak were 5′-GTP and 5′-GMP followed by 2′-GMP. Guanine and 2′,3′-cGMP constituted the third peak, and this was followed by 3′-GMP, 3′,5′-cGMP and guanosine. The elution order and the capacity factors (k') of these compounds are given in Table I. These compounds eluted in the same general pattern as their adenine-based counterparts, but approximately 1.7 times faster.

The separation of uracil and its derivatives is shown in Fig. 3. Eluted first, 5′-UTP was followed closely by 5′-UMP and 2′-UMP. Co-eluted as the fourth peak 2′,3′-cUMP, uracil and 3′-UMP were followed by uridine and 3′,5′-cUMP. The elution order and retention times for these compounds are given in Table II.

TABLE I

CAPACITY FACTORS (k') FOR PURINE DERIVATIVES

Capacity factors determined using an isocratic elution with 0.05 *M* $(NH_4)H_2PO_4$ in methanol–water (10:90) (pH 5.0).

Compound	k'	*Compound*	k'
5′-ATP, 5′-ADP	0.1550*	5′-GTP, 5′-GMP	0.1692
5′-AMP	0.2329	2′-GMP	0.2414
2′-AMP	0.6370	Guanine, 2′,3′-cGMP	0.3793
Adenine	0.7465		
2′,3′-cAMP	0.9521	3′-GMP	0.7310
3′-AMP	1.3973	3′,5′-cGMP	0.7379
3′,5′-cAMP	1.7397	Guanosine	0.8759
Adenosine	2.0822		

* Estimated value.

TABLE II

CAPACITY FACTORS (k') FOR PYRIMIDINE DERIVATIVES

Capacity factors determined using an isocratic elution with 0.05 M $(NH_4)H_2PO_4$ in methanol–water (10:90) (pH 5.0).

Compound	k'	*Compound*	k'
5'-CTP }	0.0833	5'-UTP	0.0462
5'-CMP }		5'-UMP	0.1692
2'-CMP }		2'-UMP	0.2931
2',3'-cCMP }	0.1181	2',3'-cUMP }	
Cytosine }		Uracil }	0.4000
3'-CMP }		3'-UMP }	
Cytidine	0.3958	Uridine	0.7000
3',5'-cCMP	0.7500	3',5'-cUMP	1.0923

The separation of cytosine from its derivatives is represented in Fig. 4. Co-eluted as the first peak 5'-CTP and 5'-CMP were followed by the co-elution of 2'-CMP, 2',3'-cCMP, cytosine and 3'-CMP as the second peak. Cytidine and 3',5'-cCMP eluted last as the third and fourth peaks respectively. Table II shows the elution order and capacity factors for these compounds. Retention times were increased slightly when the percentage of methanol in the solvent was reduced to 5%. If methanol is omitted from the solvent, the growth of microorganisms occurs, clogging the column[17].

Attempts to improve the resolution in the separation of uracil derivatives and cytosine derivatives were met with difficulties. Reductions in the flow-rate (even minor ones) increased the amount of peak-broadening seen. Reducing the concentration of methanol in the mobile phase also did not improve the resolution. Both types of compound could probably be better separated using gradient elution or some other method. This process may also improve the resolution in the separation of guanine derivatives.

The separation of 2'-deoxyadenosine and its monophosphates is shown in Fig. 5. The 5'-dAMP eluted first followed by 3'-dAMP, adenine, 3',5'-cyclic dAMP and 2'-deoxyadenosine. Table III shows the retention data for this series of compounds.

Fig. 6 shows the separation of thymine, thymidine and thymidine monophos-

TABLE III

CAPACITY FACTORS (k') FOR SOME 2'-DEOXYRIBONUCLEOSIDES AND THEIR DERIVATIVES

Capacity factors determined using an isocratic elution with 0.05 M $(NH_4)H_2PO_4$ in methanol–water (10:90) (pH 5.0).

Compound	k'	*Compound*	k'
5'-dAMP	0.2759	5'-TMP	0.5571
3'-dAMP	0.3931	3'-TMP	0.8241
Adenine	0.7465	Thymine	1.3000
3',5'-c-dAMP	2.8897	3',5'-cTMP	1.8214
2'-dA	5.5103	Thymidine	2.8286

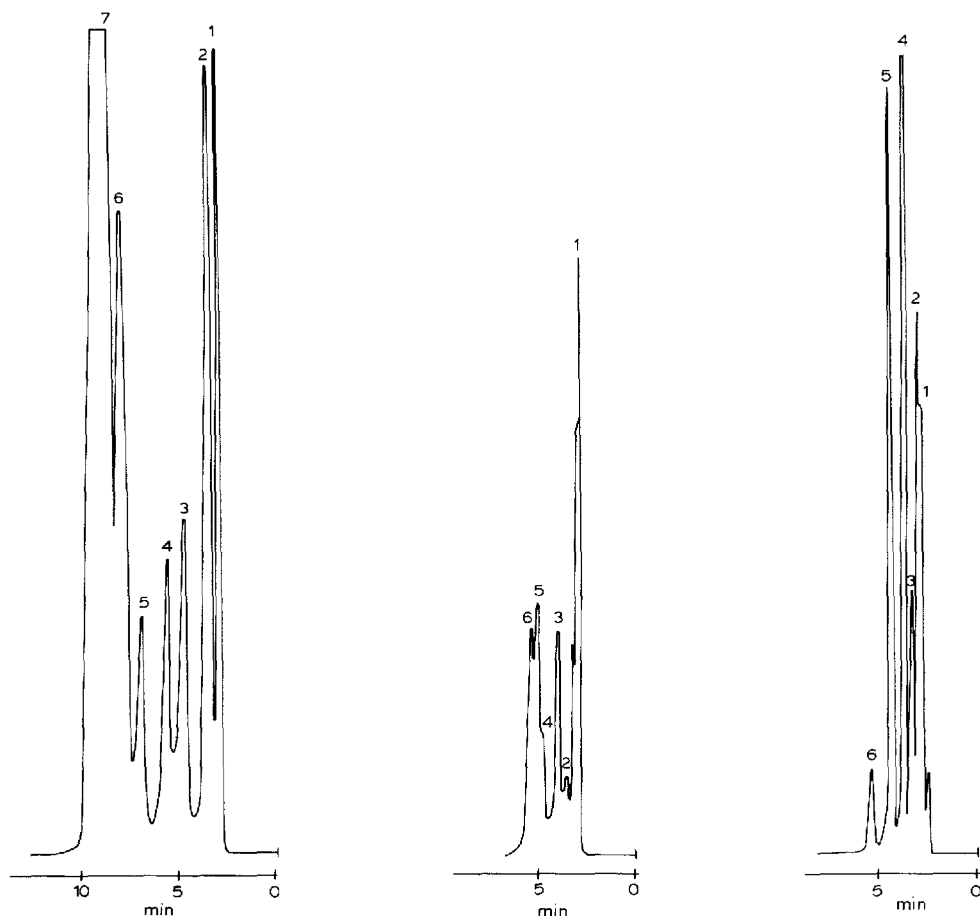


Fig. 1. Separation of a solution of adenosine and its derivatives by 0.05 *M* $(NH_4)H_2PO_4$ in methanol–water (10:90) (pH 5.0) at 1.9 ml/min. Peaks: 1 = 5′-ATP and 5′-ADP; 2 = 5′-AMP; 3 = 2′-AMP; 4 = 2′,3′-cAMP; 5 = 3′-AMP; 6 = 3′,5′-cAMP; 7 = adenosine.

Fig. 2. Separation of a solution of guanine and its derivatives by 0.05 *M* $(NH_4)H_2PO_4$ in methanol–water (10:90) (pH 5.0) at 1.9 ml/min. Peaks; 1 = 5′-GTP and 5′-GMP; 2 = 2′-GMP; 3 = guanine and 2′,3′-cGMP; 4 = 3′-GMP; 5 = 3′,5′-cGMP; 6 = guanosine.

Fig. 3. Separation of a solution of uracil and its derivatives by 0.05 *M* $(NH_4)H_2PO_4$ in methanol–water (10:90) (pH 5.0) at 1.9 ml/min. Peaks: 1 = 5′-UTP; 2 = 5′-UMP; 3 = 2′-UMP; 4 = 2′,3′-cUMP, uracil and 3′-UMP; 5 = uridine; 6 = 3′,5′-cUMP.

phates. The first peak was 5′-TMP and was followed by 3′-TMP, thymine, 3′,5′-cTMP and thymidine. The retention data for these compounds are given in Table III.

Determined graphically, a plot of peak area *vs.* concentration for 2′,3′-cUMP gave a straight line from 0.2 to 2.2 mg/ml. Similar plots were made for other standards.

Analysis of reaction mixtures

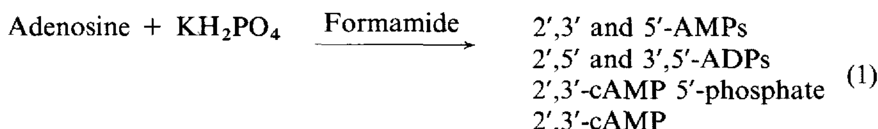
We have investigated the phosphorylation of nucleosides and deoxynucleosides

Fig. 4. Separation of a solution of cytosine and its derivatives by 0.05 *M* $(NH_4)H_2PO_4$ in methanol–water (10:90) (pH 5.0) at 1.9 ml/min. Peaks: 1 = 5′-CTP and 5′-CMP; 2 = 2′-CMP, 2′,3′-cCMP, cytosine, and 3′-CMP; 3 = cytidine; 4 = 3′,5′-cCMP.

Fig. 5. Separation of a solution of 2′-deoxyadenosine and its derivatives by 0.05 *M* $(NH_4)H_2PO_4$ in methanol–water (10:90) (pH 5.0) at 1.9 ml/min. Peaks: 1 = 5′-dAMP; 2 = 3′-dAMP; 3 = adenine; 4 = 3′,5′-cyclic dAMP; 5 = 2′-deoxyadenosine; S = solvent.

Fig. 6. Separation of a solution of thymidine and its derivatives by 0.05 *M* $(NH_4)H_2PO_4$ in methanol–water (10:90) (pH 5.0) at 1.9 ml/min. Peaks: 1 = 5′-TMP; 2 = 3′-TMP; 3 = thymine; 4 = 3′,5′-cTMP; 5 = thymidine; S = solvent.

using dihydrogen phosphates in formamide solution at various tempertures[19,20]. Several phosphorylated products were obtained as indicated from cellulose TLC separations. The reactants and products for the phosphorylation of adenosine are shown in eqn. 1:



$$\text{Adenosine} + KH_2PO_4 \xrightarrow{\text{Formamide}} \begin{array}{l} 2',3' \text{ and } 5'\text{-AMPs} \\ 2',5' \text{ and } 3',5'\text{-ADPs} \\ 2',3'\text{-cAMP } 5'\text{-phosphate} \\ 2',3'\text{-cAMP} \end{array} \quad (1)$$

The array of products obtained required a discriminating analytical method. In addition to the products shown, there was always some unreacted adenosine and occasionally some adenine present from deribosylation.

Quantitative TLC analyses of the reaction mixtures performed previously[19] were successful in providing the total percentage of nucleoside monophosphates, cyclic phosphate and nucleoside diphosphates. One major problem encountered was that isomeric nucleotide products, in particular the monophosphates, were not resolved. The reversed-phase HPLC method reported here gave separation of a particular base, its ribo- or deoxyribonucleoside and monophosphates and cyclic phosphates.

A number of reaction mixtures were analysed. In each case, a nucleoside or deoxynucleoside and KH_2PO_4 were dissolved in formamide and the solutions were heated for specified periods and analyzed using reversed-phase HPLC. Quantitative TLC analyses of the reaction mixtures performed previously were successful in pro-

TABLE IV

COMPARISON OF ANALYTICAL METHODS

(a) Adenosine + $KH_2PO_4 \xrightarrow[\text{Formamide}]{37°C,\ 6.5\ months}$ Products

Reaction A: 0.05 *M* 0.05 *M*
Reaction B: 0.05 *M* 0.50 *M*

Compound	*Products of reaction A (%)*		*Products of reaction B (%)*	
	*TLC**	*Reversed-phase HPLC***	*TLC**	*Reversed-phase HPLC***
Adenosine	85	87.5	83	84.9
5′-AMP	10	7.6	12	7.9
2′-AMP	5***	3.3	5***	4.4
3′-AMP	–	1.4	–	2.5
2′,3′-cAMP	0	Trace	0	0.31

(b) Thymidine + $KH_2PO_4 \xrightarrow[\text{Formamide}]{100°C,\ 12\ h}$ Products

0.05 *M* 0.10 *M*

Compound	*Products TLC (%)**	*Products (Reversed-phase HPLC) (%)***
Thymidine	40§	34.7
3′,5′-TDP	18	16.1
5′-TMP	43§§	28.6
3′-TMP	–	17.9
Thymine	–	2.7

* Cellulose TLC analyses were performed directly following completion of the reaction. The eluting solvent was *n*-butanol–acetone–acetic acid–ammonia (conc.)–water (350:250:150:26:224).

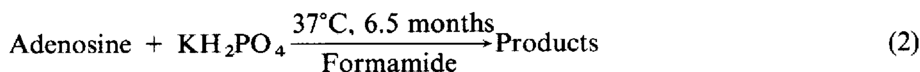
** Reversed-phase HPLC analyses were performed approximately 1 year after the TLC analyses. During this time, reaction mixtures were kept at room temperature.

*** This percentage is the total for 2′-AMP and 3′-AMP, which ran together on the TLC plate.

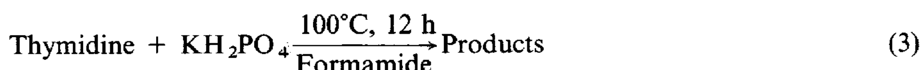
§ This percentage includes thymine.

§§ This percentage is the total for 3′-TMP and 5′-TMP, which ran together on the TLC plate.

viding total percentages of nucleoside monophosphates, cyclic phosphates and nucleoside diphosphates. A problem encountered with this mode of analysis was that some isomeric nucleotide products were not fully resolved, in particular the monophosphate products. We were primarily interested in the relative amounts of 2'-, 3'- and 5'-monophosphates[20]. Reversed-phase HPLC was selected as an analytical method that could overcome the separation difficulties encountered in determining individual nucleotides. Two of the reactions studied are shown in eqns. 2 and 3:



$$\text{Adenosine} + KH_2PO_4 \xrightarrow[\text{Formamide}]{37^\circ\text{C, 6.5 months}} \text{Products} \quad (2)$$



$$\text{Thymidine} + KH_2PO_4 \xrightarrow[\text{Formamide}]{100^\circ\text{C, 12 h}} \text{Products} \quad (3)$$

Reversed-phase HPLC results, along with, for comparison, data obtained earlier using quantitative TLC, are listed in Table IV. Individual nucleotides were not resolved well using the TLC method.

Our objective was to quantitate the amount of each nucleoside monophosphate produced in the respective reactions. The best separations of monophosphates were observed for the reaction products of adenosine, deoxyadenosine and thymidine. The method clearly delineated the amount of monophosphorylated products formed. However, diphosphates generally eluted just prior to the 5'-phosphates; triphosphates were not formed in these reactions. Our best cellulose TLC separations were not sufficient to resolve individual nucleotides. The reversed-phase HPLC method was superior in this respect as well as being a more rapid method.

TABLE V

SEPARATION OF ADENOSINE DERIVATIVES BY CELLULOSE TLC AND BY REVERSED-PHASE HPLC

Compound	*R_F values for adenosine on cellulose TLC**		*Reversed-phase HPLC data*	
	*Solvent A***	*Solvent B****	*Retention time (min)*§	*Retention time (min)*
2'-AMP	1.36	1.48	–	4.78
3'-AMP	1.21	1.05	–	7.00
5'-AMP	1.32	1.81	6.24	3.60
5'-ADP	1.57	2.29	3.69	2.92
5'-ATP	1.66	2.52	3.08	2.92
2',3'-cAMP	1.11	0.71	–	5.70
3',5'-cAMP	1.07	0.71	38.8	8.00
Adenine	0.21	0.62	14.2	4.96
Adenosine	1.00	1.00	36.4	9.00

* Ref. 19.
** Solvent A: isoamyl alcohol–5% aqueous sodium citrate (bottom layer) (1:1).
*** Solvent B: Saturated ammonium sulphate–1 *M* ammonium acetate–2-propanol (80:18:2).
§ Ref. 17.

DISCUSSION

Our experience using cellulose TLC, together with data from Anderson and Murphy[17], suggested to us that conditions similar to those used by these authors for the separation of isomeric nucleotides could prove effective. Cellulose TLC data and the reversed-phase HPLC data of Anderson and Murphy for adenosine derivatives are given in Table V together with our HPLC data. In as much as the solute selectivity using reversed-phase HPLC was high, we set out to use a similar set of conditions to separate the 2'-, 3'- and 5'-AMPs. We considered the use of an ion-exchange method, but the diversity of substances being analysed (bases, nucleosides, nucleotides and nucleoside diphosphates) led us to choose reversed-phase HPLC.

Solvent

Selection of the proper solvent system is very important in reversed-phase HPLC[21]. A mixture of .05 M $NH_4H_2PO_4$ and methanol–water (10:90) has been used in earlier studies[17,18] for gradient elution of ATP, ADP, 5'-AMP, adenine, adenosine and 3',5'-cAMP. This solvent mixture gave good selectivity in our isocratic analyses, particularly for derivatives of adenosine, deoxyadenosine and thymidine. An important factor influencing the retention behavior was the concentration of methanol in the mobile phase. As the concentration of methanol was increased, retention was decreased. The use of acetonitrile showed a similar effect to that of methanol, but to a greater extent. This influence of organic modifiers in the mobile phase was also noted by Anderson and Murphy[17]. Solvents such as acetonitrile and methanol compete for sites on the stationary phase with the nucleosides and nucleotides; this speeds their elution but not their order of separation.

Mechanism

Compounds were eluted generally in the same order as their polarities[22] and water solubilities[23]. The more highly retained substances are those which are more hydrophobic. Stacking of bases is also thought to be an important hydrophobic interaction, particularly in acidic media[24]. Other factors affecting selectivity among nucleoside derivatives are their acidities and basicities. The dispersion forces and hydrophobic interctions are more important for purines than for pyrimidines. Guanine has one more polar substituent than adenine: this contributes to shorter retention times for guanine derivatives. Deoxyadenosine derivatives gave the greatest retention times. Thymidine gave results similar to those for deoxyadenosine derivatives. For the purposes of distinguishing between 3'- and 5'-monophosphates in our work, thymidine and deoxyadenosine gave the clearest separations as no other substances had retention times falling between the 3'- and 5'-monophosphates. Adenosine derivatives afforded the greatest selectivity whereas guanosine derivatives showed less selectivity; however, these latter derivatives were readily quantified. Uridine derivatives were more difficult to analyse and cytidine derivatives could only be analysed in groups rather than individually. An ion-exchange method would be more desirable for cytidine derivatives. Differences in retention of the 2'-, 3'- and 5'-nucleotides are linked to differences in the polarities of these solutes, with polarity differences being the most important factor in determining the retention behavior of these ionized materials. Stacking is important for neutral substances having greater retention times

such as bases and nucleosides[24]. The polarity order is 5′ > 2′ > 3′ for each nucleotide series studied. For deoxynucleotides the polarity ordere is 5′ > 3′.

Conformational differences may play a large role in determining the differences in retention between 2′,3′-cyclic and 3′,5′-cyclic nucleotides. The 3′,5′-cyclic nucleotides have a six-membered ring fused to a five-membered ring. The 2′,3′-cyclic nucleotides have a strained five-membered phosphodiester ring fused to a five-membered sugar ring[25]. Both cyclic phosphates are less polar than the mononucleotides and both behave similarly on cellulose TLC indicating that they have similar polarities. In reversed-phase HPLC, the 3′,5′-cyclic nucleotides show greater solvophobicity than the 2′,3′-cyclic nucleotides.

CONCLUSIONS

(1) Isomeric monoribonucleotides and monodeoxyribonucleotides were separated using an isocratic reversed-phase HPLC technique. All but the cytidine derivatives were separated and analysed quantitatively. Cytidine derivatives gave low selectivity and lesser separation.

(2) In the presence of other 2′-deoxyadenosine derivatives 3′-dAMP and 5′-dAMP were separated quantitatively. The same held for thymidine derivatives.

(3) The determination of 2′-, 3′- and 5′-ribonucleotides was carried out in the presence of 2′,3′-cyclic nucleotides. The 2′,3′-cyclic nucleotides have retention times that are similar to those of the nucleotides. Greater retention times were observed for 3′,5′-cyclic nucleotides than for 2′,3′-cyclic nucleotides.

(4) Reversed-phase HPLC and cellulose TLC are two independent methods for quantitative analysis of nucleosides and nucleotides and their derivatives.

(5) The isocratic reversed-phase HPLC method was more effective than cellulose TLC analysis of the deoxynucleotides and nucleotides of individual bases. The reversed-phase HPLC method was faster and gave greater accuracy than the cellulose TLC method.

REFERENCES

1 P. R. Brown, *J. Chromatogr.*, 52 (1970) 257.
2 P. R. Brown, R. E. Parks, Jr. and J. Herod, *Clin. Chem.*, 19 (1973) 919.
3 P. R. Brown and R. E. Parks, Jr., *Advan. Exp. Med. Biol.*, 41B (1974) 799.
4 P. Virkola, *J. Chromatogr.*, 51 (1970) 195.
5 W. P. Kennedy and J. C. Lee, *J. Chromatogr.*, 51 (1970) 203.
6 C. Garrett and D. V. Santi, *Anal. Biochem.*, 99 (1979) 268.
7 J. X. Khym, J. W. Bynum and E. Volkin, *Anal. Biochem.*, 77 (1977) 446.
8 J. R. Miksic and P. R. Brown, *J. Chromatogr.*, 142 (1977) 641.
9 A. C. Burtis, M. N. Munk and F. R. MacDonald, *Clin. Chem.*, 16 (1970) 667.
10 Y. Iida, *Nippon Eiser-Guka Fasski (Jap. J. Hyg.)*, 33 (1978) 410.
11 F. R. Blattner and H. P. Erickson, *Anal. Biochem.*, 18 (1967) 220.
12 E. Junowicz and J. H. Spencer, *J. Chromatogr.*, 44 (1969) 342.
13 P. J. M. van Haastert, *J. Chromatogr.*, 210 (1981) 229.
14 R. A. Hartwick and P. R. Brown, *J. Chromatogr.*, 126 (1976) 679.
15 C. W. Gehrke, K. C. Kuo, G. E. Davis, R. D. Suits, T. P. Waalkes and E. Borek, *J. Chromatogr.*, 150 (1978) 455.
16 J. Montgomery, H. J. Thomas, R. D. Elliott and A. T. Shortnacy, in R. E. Harmon, R. K. Robins and L. B. Townsend (Editors), *Chemistry and Biology of Nucleosides and Nucleotides*, Academic Press, New York, 1978, pp. 37–53.

17 F. S. Anderson and R. C. Murphy, *J. Chromatogr.*, 121 (1976) 251.
18 J. L. Hodge and E. F. Rossomando, *Anal. Biochem.*, 102 (1980) 59.
19 A. M. Schoffstall, *Origins Life*, 7 (1976) 399.
20 A. M. Schoffstall, R. J. Barto and D. L. Ramos, *Origins Life*, 12 (1982) 143.
21 S. R. Bakalyar, R. McIllwrick and E. Roggendorf, *J. Chromatogr.*, 142 (1977) 353.
22 K. Karch, I. Sebestian, I. Halász and H. Engelhardt, *J. Chromatogr.*, 122 (1976) 171.
23 B. L. Karger, J. R. Gant, A. Hartkopf and P. Weiner, *J. Chromatogr. Sci.*, 128 (1976) 65.
24 P. R. Brown and E. Grushka, *Anal. Chem.*, 52 (1980) 1210.
25 C. Jardetsky, *J. Amer. Chem. Soc.*, 84 (1962) 62.