

Note

Chromatography of bis-quaternary amino steroids

II*. Separation on chemically bonded silica phases

M. GAZDAG*, K. VARSÁNYI-RIEDL and G. SZEPESI

Chemical Works of Gedeon Richter Ltd., 1475 Budapest (Hungary)

(Received July 8th, 1985)

In Part I¹, new thin-layer chromatographic (TLC) and high-performance liquid chromatographic (HPLC) methods were introduced for the separation of bis-quaternary amino steroids on silica stationary phases. It was found that the retention of the compounds investigated depends on the number of quaternary amino groups and also on the presence or absence of ester groups in the molecule. The HPLC system developed for the separation of pipecuronium bromide and its related steroids could be transferred to the TLC separation of the same components.

The HPLC separation of bis-quaternary amino steroids on chemically bonded silica phases has now been investigated. The main aim of this work was to study the retention behaviour of quaternary amines on chemically bonded phases in order to obtain more information about the possible interactions between the mobile phase composition (salt concentration, type of organic solvent, etc.) and sample molecules to elucidate the separation mechanism.

EXPERIMENTAL

A Liquochrom 2010 high-performance liquid chromatograph equipped with a variable-wavelength UV detector and a loop-type injector (Labor MIM, Esztergom-Budapest, Hungary) was used. The separations were performed on pre-packed Nucleosil CN, 10 μm ; Nucleosil $(\text{CH}_3)_2\text{N}$, 10 μm , and Nucleosil 10 C₁₈ (250 \times 4.6 mm I.D.) columns (Chrompack, Middelburg, The Netherlands). The compounds were detected at 218 nm.

The solvents used for the preparation of eluents were of HPLC grade and were obtained from E. Merck (Darmstadt, F.R.G.). All other reagents and solvents were of analytical-reagent grade and were obtained from Reanal (Budapest, Hungary).

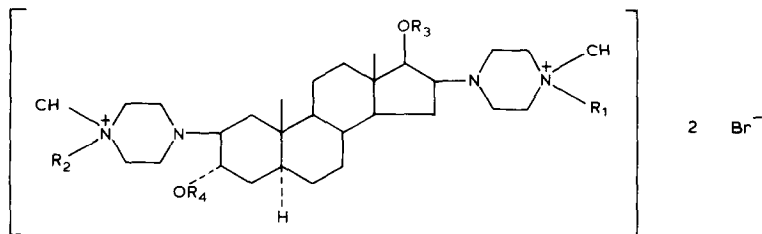
The compounds investigated were prepared at the Chemical Works of Gedeon Richter (Budapest, Hungary) and were of the highest possible quality.

* For Part I, see ref. 1.

RESULTS AND DISCUSSION

The compounds investigated were the same as used in Part I¹ and are shown in Table I. To investigate the separation of the model compounds, a medium polarity chemically bonded cyanopropylsilica stationary phase was used.

TABLE I
STRUCTURES OF THE COMPOUNDS INVESTIGATED



Compound	R ₁	R ₂	R ₃	R ₄
I	H	H	CH ₃ CO	CH ₃ CO
II	CH ₃	H	CH ₃ CO	CH ₃ CO
III	H	CH ₃	CH ₃ CO	CH ₃ CO
IV	CH ₃	CH ₃	H	CH ₃ CO
V	CH ₃	CH ₃	H	H
VI (pipercuronium bromide)	CH ₃	CH ₃	CH ₃ CO	CH ₃ CO

Effect of ammonium chloride and ammonium carbonate used in the eluent

Fig. 1 shows the change in the retention of the compounds investigated with variation in the ammonium chloride concentration of the eluent. In the absence of ammonium chloride, no separation was obtained. On increasing the salt concentration, the retention of the compounds showed a maximum (the maximum appeared at the lowest salt concentration applied), while the resolution calculated for VI and IV increased slightly. A satisfactory separation between the two mono-quaternary compounds II and III was achieved. The column efficiency is highly dependent on the salt concentration.

A similar dependence of retention on ammonium carbonate concentration in the eluent was observed, as illustrated in Fig. 2. When ammonium carbonate was used in the eluent, higher capacity ratios for bis-quaternary compounds and resolutions were obtained.

The best separation conditions were achieved when both ammonium chloride and ammonium carbonate were used in the eluent, in a 1:1 molar ratio. The influence of the total salt concentration on the chromatographic separation is demonstrated in Fig. 3. It can be seen that the eluent containing ammonium chloride and ammonium carbonate in 0.125–0.125 moles/dm³ concentrations seems to be the most suitable.

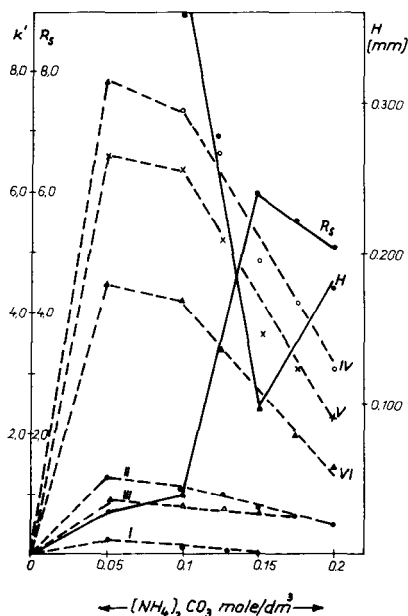
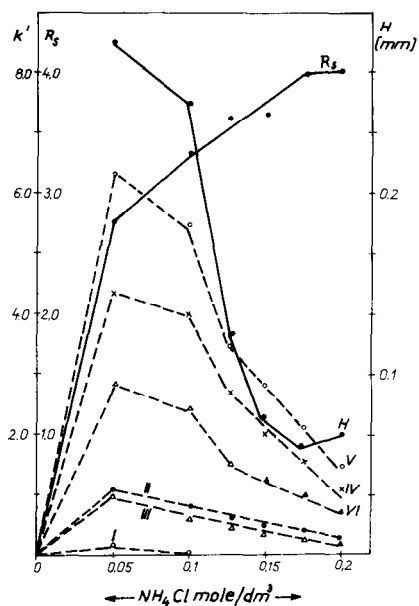


Fig. 1. Effect of ammonium chloride in the eluent on retention. Column, Nucleosil CN, 10 μ m (250 \times 4.6 mm I.D.); eluent, mixture of A and B (86:14), where A is methanol-acetonitrile (64:22) and B is ammonia solution (sp.gr. 0.880); flow-rate 1 cm³/min; detection, 218 nm. The R_s values were calculated for IV and VI and the H value for VI. Compound as in Table I.

Fig. 2. Effect of ammonium carbonate in the eluent on retention. Conditions as in Fig. 1.

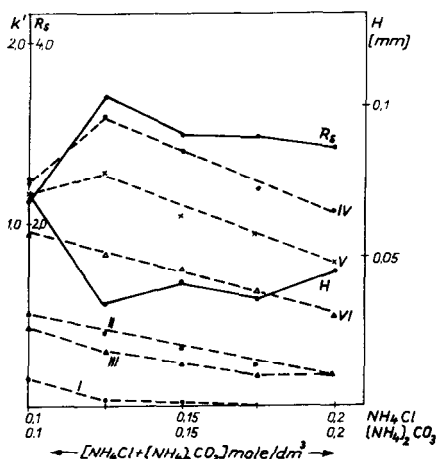


Fig. 3. Effect of ammonium chloride and ammonium carbonate in the eluent on retention. Conditions as in Fig. 1.

Effect of the nature and concentration of organic solvents on the selectivity and efficiency of the separation

Fig. 4 shows the dependence of capacity ratios, resolution and column efficiency on the ratio of methanol to acetonitrile in the eluent at a constant organic solvent concentration. The retention of the compounds shows a minimum (Fig. 5).

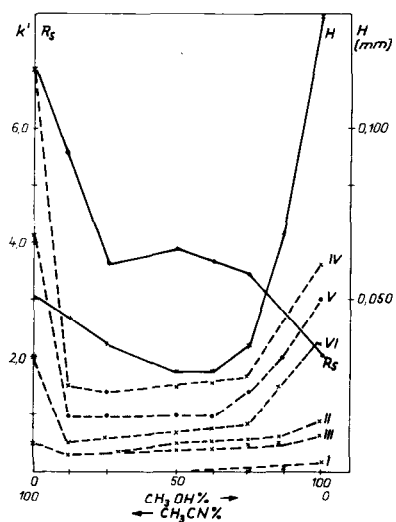


Fig. 4. Dependence of the retention on the ratio of methanol to acetonitrile. Eluent: mixture of A and B (86:14), where A is methanol-acetonitrile in different ratios and B is ammonia solution (sp.gr. 0.880) containing ammonium chloride and ammonium carbonate at concentrations of 0.125–0.125 moles/dm³. Other conditions and compounds as in Fig. 1.

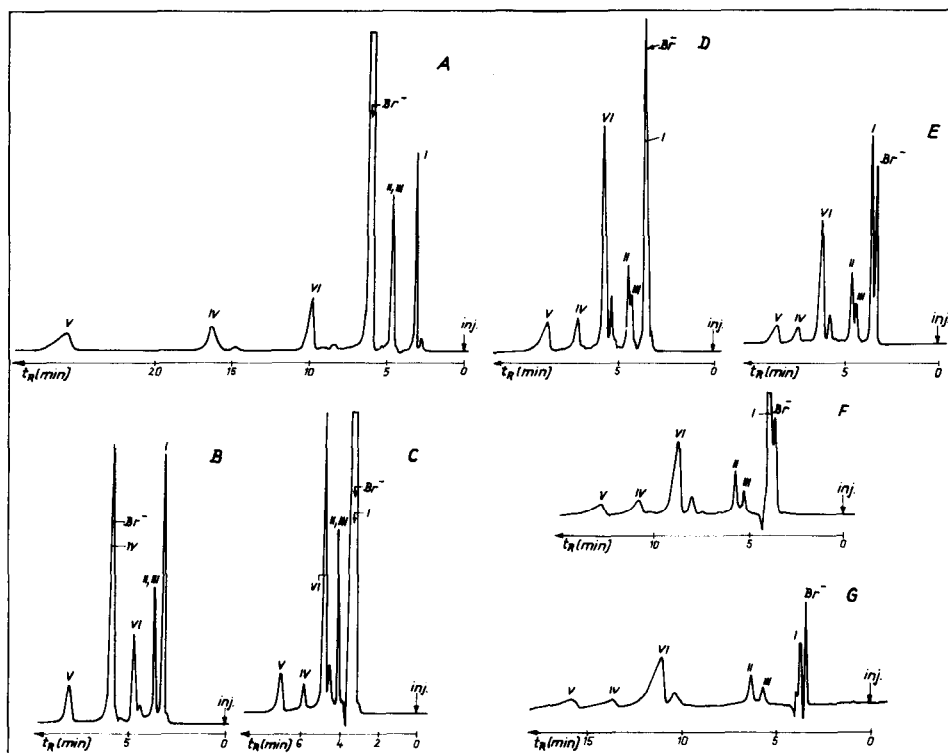


Fig. 5. Dependence of the separation on the ratio of methanol to acetonitrile. Conditions as in Fig. 4, the methanol concentrations relative to the total organic solvent concentration being (A) 0%, (B) 12.8%, (C) 25.6%, (D) 50%, (E) 74.4%, (F) 87.2% and (G) 100%.

When only acetonitrile is used in the eluent as an organic solvent, no separation of the mono-quaternary compounds II and III was achieved. Bromide ion also had a significant retention in this eluent system (Fig. 5A). On increasing the methanol concentration to 11% (Fig. 5B) the retentions of the bis-quaternary compounds (IV, V and VI) decreased dramatically and IV could not be separated from the bromide ion. The retention of bromide ion did not change significantly.

When the methanol concentration was 22% (Fig. 5C), the separation was more effective, except for II and III. With a 1:1 methanol to acetonitrile ratio (Fig. 5D) the mono-quaternary compounds could be distinguished from each other; with decreasing acetonitrile concentration the separation of mono-quaternary compounds was much better, but the peak shape of the bis-quaternary compounds deteriorated (Fig. 5E and F). When only methanol was used in the eluent the chromatogram was completely unsuitable for analytical applications (Fig. 5G).

From the chromatograms shown in Fig. 5, it can be concluded that the best separation can be achieved with an acetonitrile to methanol ratio in the range 1:1-1:2.

Fig. 6 illustrates the influence of the organic solvent concentration on the retention of the compounds (with a ratio of methanol to acetonitrile of 1:1). The best separation was achieved when 86% of organic solvent was used in the eluent.

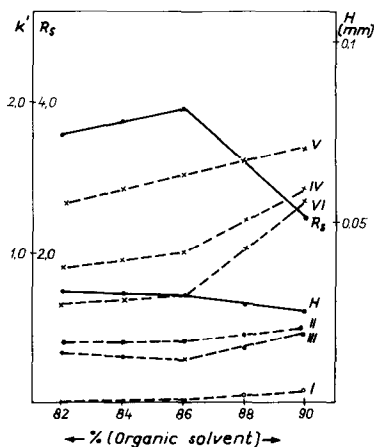


Fig. 6. Influence of organic solvent concentration on retention. Conditions as in Fig. 4; ratio of methanol to acetonitrile, 1:1.

Influence of stationary phases on the separation

Separations on octadecylsilica and dimethylaminosilica columns were also studied. On octadecylsilica, no successful separation was obtained when a reversed-phase eluent (less than 80% of organic solvent) and an aqueous buffered system (between pH 2 and 10) were used. Acceptable separations were obtained only when similar eluent systems were applied to those used on cyanopropylsilica, as shown in Fig. 7.

It can be seen that a reversed elution order was obtained; first the bis-quaternary compounds (VI, V and IV), then the mono-quaternary compounds (III and

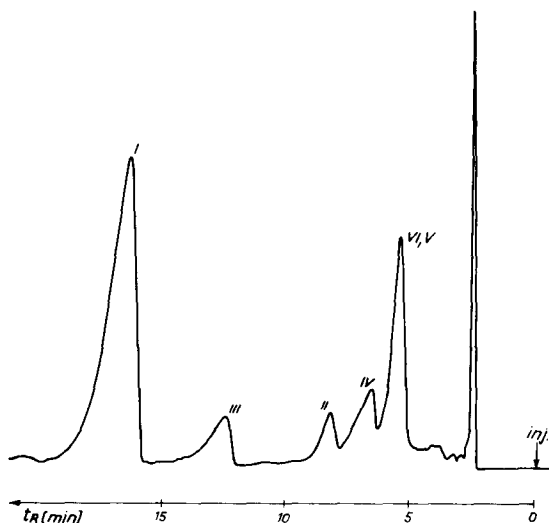


Fig. 7. Separation of bis-quaternary amino steroids on octadecylsilica stationary phase. Column, Nucleosil 10 C₁₈ (250 × 4.6 mm I.D.); eluent, mixture of A and B (86:14), where A is methanol-acetonitrile (64:22) and B is ammonia solution (sp.gr. 0.880) containing ammonium chloride and ammonium carbonate at concentrations of 0.125–0.125 moles/dm³. Other conditions and compounds as in Fig. 1.

II) and finally the tertiary amino derivative were eluted. However, the separation efficiency was significantly lower than on cyanopropylsilica or bare silica columns.

We found that on dimethylaminosilica stationary phase no successful separation can be obtained. However, if the column is decomposed by using an adverse solvent system, the remaining silanol groups can help the separation and similar results were achieved to those on a silica column.

Retention principles

Regarding the separation mechanism, from the experiments reported here and in Part I¹, the following conclusions can be drawn.

An eluent system containing a high concentration of organic solvents (acetonitrile and methanol) and ammonium salts dissolved in ammonia solution is suitable for the separation of bis-quaternary amino steroids on both silica and chemically bonded silica phases. First an ion-pair adsorption mechanism based on the migration of the uncharged ion pair formed by using chloride ion was assumed. This concept was similar to that described by de Zeeuw and co-workers^{2,3}. However, this premise was in contrast to some of our experiences, as the use of ammonium carbonate instead of ammonium chloride results in a similar separation, the separation can be performed not only on a silica column, etc.

Based on the results obtained, we consider that the retention principle may be similar to "salting-out" chromatography. The salts used in the eluent should have combined effects on the separation. On the one hand, the salts can decrease the polarity of a quaternary amino group, suppressing its dissociation. This assumption seems to be supported by the significant dependence of the retention on the nature and concentration of the organic solvents used in the eluent. On the other hand, the

presence of an inorganic salt in the eluent can decrease the silanophilic effect of the stationary phase supported by the influence of the salt concentration on the retention of the compounds. Both effects of the inorganic salts can increase the hydrophobic interaction of the compounds with the stationary phase.

Based on this concept, we assume that on bare silica or cyanopropylsilica stationary phases the retention mechanism is normal-phase hydrophobic interaction chromatography and on octadecylsilica it is reversed-phase hydrophobic interaction chromatography.

CONCLUSION

The best separations can be obtained on bare silica and cyanopropylsilica stationary phases. On cyanopropylsilica more efficient separations were obtained and on unbonded silica an increased selectivity could be achieved. A reversed elution order was obtained by using octadecylsilica but the selectivity and efficiency of the separation were significantly lower. Dimethylaminosilica itself cannot be used to separate bis-quaternary amino compounds unless it is partly hydrolysed. The separation characteristics obtained in the presence of a high concentration of silanol groups is similar to that obtained on unbonded silica. Based on the results obtained, hydrophobic interaction is suggested as the separation mechanism.

REFERENCES

- 1 M. Gazdag, G. Szepesi, K. Varsányi-Riedl, Z. Végh and Zs. Pap-Sziklay, *J. Chromatogr.*, 328 (1985) 279.
- 2 R. A. de Zeeuw, P. E. W. van der Laan, J. E. Greving and F. J. W. van Mansvelt, *Anal. Lett.*, 9 (1976) 831.
- 3 R. A. de Zeeuw, F. J. W. van Mansvelt and J. E. Greving, *J. Chromatogr.*, 147 (1978) 255.