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# Chiral high-performance liquid chromatographic separations of vinca alkaloid analogues on $\alpha_1$-acid glycoprotein and human serum albumin columns

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ABSTRACT

Separations of the stereoisomers of a series of tetracyclic and pentacyclic vinca alkaloid analogues having two or three chiral centres were performed on Chiral-AGP and Chiral-HSA high-performance liquid chromatographic columns. Phosphate buffers with pH 5–7 containing 5–35% acetonitrile or 2-propanol were used as mobile phases. The results were in accordance with previous binding data obtained with native AGP and on an HSA-Sepharose column. Whereas on Chiral-AGP the retention of the *trans* isomers having 1(*R*),12*b*(*S*)-indolo[2,3-*a*]quinolizidine or the corresponding 3(*S*),16(*R*)-eburnane absolute configurations was exceedingly high, on Chiral-HSA the *trans* isomers, independently of their absolute configurations, were more retained. Eburnane-type compounds could also be separated according to the configuration of the chiral centre at position 14. A comparison of the chromatographic properties of the vinca alkaloids on the Chiral-AGP and Chiral-HSA columns demonstrates that these compounds are bound with higher affinity to the AGP phase. The AGP column resolves a very broad range of vinca alkaloids compared with the HSA column. Higher stereoselectivity and a much better chromatographic performance were also obtained on the Chiral-AGP column.

## INTRODUCTION

It is well known that enantiomers of drugs have different biological activities [1,2]. Stereoselective binding to serum proteins, such as to $\alpha_1$-acid glycoprotein (AGP) and human serum albumin (HSA), can affect both the pharmacodynamics and the pharmacokinetics of the drug. The number of binding sites involved in the drug binding to AGP has been widely discussed. It is obvious that the experimental design influences the results to a large extent. From indirect drug displacement studies of basic drugs with isolated AGP, the results suggest that AGP contains one binding site [3–6]. However, studies performed with a direct method, such as Scatchard plots, indicate more than one and in most studies two classes of binding sites [7–10]. AGP immobilized on silica particles (the Chiral-AGP column) has also been used in binding studies of drugs of different character [11]. The results obtained using this technique demonstrate, in accordance with the results obtained with the native protein, using Scatchard plots, that basic, acidic and non-protolytic drugs are bound to one high-affinity site and there is at least one more site to which the drugs are bound with lower affinity. It has also been demonstrated in a large number of chromatographic studies, using immobilized AGP, that stereoselec-

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tive binding of drugs can be obtained for an extremely broad range of solutes [12–14].

HSA is assumed to have two main ligand binding sites, the endogenous bilirubin and the *L*-tryptophan sites [15,16], corresponding to the warfarin and benzodiazepine drug sites, respectively [17]. It has been demonstrated that enantiomers can be bound stereoselectively to native HSA [18]. When using a chromatographic column with immobilized proteins, even small differences in binding affinity (low α-values) of the enantiomers can give a resolution of the racemate, provided that a high enough separation efficiency is generated by the column. If the immobilized protein also maintains its original binding ability and the mobile phase components do not affect the chiral binding properties of the protein, chromatography of chiral compounds on serum protein bonded phases can conveniently provide relevant information on binding stereoselectivity. This has been demonstrated for HSA immobilized on Sepharose [19,20]. It was also demonstrated in one study for tryptophan and warfarin [21], and in another study for some benzodizepines, leucovorin and warfarin using HSA immobilized on silica [22].

The serum protein binding of a series of tetracyclic and pentacyclic vinca alkaloid analogues possessing two or three chiral centres was reported in a recent paper [23]. Their binding to AGP, measured by displacement of marker ligands, demonstrated very high stereoselectivities. The affinities of certain *trans* isomers were 20–100 times higher than those of the *cis* or the enantiomeric *trans* isomers. One of the purposes of this study was to investigate whether AGP bound to silica (Chiral-AGP) maintains this unique interaction.

The binding of the vinca alkaloids to HSA has also been characterized using an HSA-Sepharose column [23] and the binding affinities were found to be highly dependent on the chemical structure of the solutes. Separation factors up to 4 were reported with higher elution volumes for the *trans* isomers, independently of their absolute configurations. This paper also reports the separation of the stereoisomers of some of the vinca alkaloids on a Chiral-HSA high-performance liquid chromatographic (HPLC) column and a comparison with the previously reported *in vitro* results.

## EXPERIMENTAL

### *Chromatography*

The HPLC experiments were carried out using a system composed of a Jasco Model 880-PU pump, a Rheodyne Model 7125 injector (20-μl loop), a Jasco Model 875 UV–VIS detector set at 263 nm and a Hewlett-Packard integrator or JCL-6000 chromatographic software. The sample concentration was 0.02–0.04 mg/ml.

*Chiral-AGP stationary phase.* This was obtained from ChromTech (Norsborg, Sweden). Preliminary tests were performed on a guard column (10 × 3 mm I.D.) and the final chromatograms were recorded on an analytical column (50 × 4 mm I.D.). The mobile phase was 0.01 *M* phosphate buffer (pH 6.0, 6.5 and 7.0) containing 20–35% (v/v) of 2-propanol (IP) or acetonitrile (AN).

*Chiral-HSA stationary phase.* This was obtained from ChromTech. The chromatograms were recorded on a 100 × 4 mm I.D. column. The mobile phase was 0.1 *M* phosphate buffer of pH 7.0 containing 10% (v/v) of IP or AN, pH 6.15 containing 10% of IP and pH 5.00 containing 5% and 10% of IP.

### *Chemicals*

Compounds **1–9** were obtained from the Chemical Works of Gedeon Richter (Budapest, Hungary): **1a** = *cis*-(−)-alcohol; **1b** = *trans*-(−)-alcohol; **2a** = *cis*-(−)-ethyl (Et) ester; **2b** = *trans*-(−)-Et-ester; **3a** = *cis*-(−)-methyl (Me) ester; **3b** = *trans*-(−)-Me ester; **3c** = *trans*-(+)-Me ester (**3b** and **3c** are enantiomers); **4a** = *cis*-(+)-Et apovincaminate (Cavinton); **4b** = *trans*-(+)-Et apovincaminate; **5a** = *trans*-(−)-Me apovincaminate; **5b** = *trans*-(+)-Me apovincaminate (**5a** and **5b** are enantiomers); **6a** = *cis*-(−)-lactam; **6b** = *trans*-(−)-lactam; **7a** = *cis*-(−)-eburnamonine; **7b** = *trans*-(−)-eburnamonine; **8a** = 3(*R*), 16(*S*), 14(*S*)-E (E = 14,15-dihydro-14-hydroxymethyl-eburnamonine); **8b** = 3(*S*), 16(*R*), 14(*R*)-*E* (**8a** and **8b** are enantiomers); **8c** = 3(*S*), 16(*R*), 14(*S*)-E; **9a** = laevorotatory mixture of *trans*-vincamine and *trans*-epivincamine; **9b** = dextrorotatory mixture of *trans*-vincamine and *trans*-epivincamine (**9a** and **9b** are enantiomeric mixtures of epimers).

Chromatograms were recorded with the separate

stereoisomers and also with their mixtures. Only **9a** and **9b** were epimeric at position 14.

## RESULTS AND DISCUSSION

### *Separation on Chiral-AGP: effects of pH and organic modifier*

The chromatographic properties of the hydrophobic vinca alkaloids were investigated using mobile phases containing a phosphate buffer with pH between 6.0 and 7.0 and acetonitrile or 2-propanol at concentrations of 20–35% (v/v) as uncharged organic modifiers. It has been observed previously that the retention and enantioselectivity of acidic, basic and non-protolytic compounds can be affected to a large extent by both the pH of the mobile phase and the nature and the concentration of an uncharged modifier [24]. Previous binding studies with native AGP suggested high affinities for some of the vinca alkaloids [23], and therefore preliminary chromatographic screening was performed on an immobilized AGP guard column, which did reveal high retentions in accordance with the previous findings. Without using mobile phase modifiers (2-propanol or acetonitrile), certain stereoisomers could not be eluted within a reasonable time. Detailed chromatographic studies were performed on a short (50 × 4.0 mm I.D.) AGP column. The chromatographic data, *i.e.* capacity factors ($k'_1$ and $k'_2$) and separation factors ($\alpha$), for the vinca alkaloids are summarized in Table I; Figs. 1–3 show representative chromatograms.

A decrease in the pH of the mobile phase from 7.0 to 6.0 results in a decrease in $k'$ values, which is in accordance with previous findings for basic compounds [24]. This effect was much more pronounced for the most retained stereoisomers. However, it is interesting to note the chromatographic behaviour of the lactam compound (**6**). A decrease in the the pH from 7.0 to 6.0 results in an increase in retention for the first-eluted enantiomer and a decrease or no change in retention for the last-eluted enantiomer. The first-eluted enantiomer behaves with respect to retention as an acidic compound normally does on the AGP column by decreasing the pH. The most retained enantiomer behaves as can be expected for a basic compound on decreasing the pH if AN is used as the modifier. If IP is used, however, the retention is almost unaffected by a decrease in pH, a behaviour which is normally observed for neutral compounds [24]. Obviously, the chiral binding properties of the protein are affected differently by IP and AN. Such observations have been made previously [25].

The stereoselectivity increases with increase in pH and decrease in modifier concentration for all the compounds studied. At the same modifier concentration a higher stereoselectivity is obtained with AN than IP.

It has been observed that both 1-propanol and AN are adsorbed to a high extent on immobilized AGP, forming a multilayer on the protein surface, and 1-propanol is bound with the highest affinity [25]. Increasing the concentration of these modifiers in chromatographic experiments results in a decreased retention of the solutes owing to competition between the modifier and the solutes for binding to the immobilized protein molecules. As can be seen from Table I, the decrease in the retention of the most retained enantiomers is more pronounced. The more hydrophobic IP gives lower retention compared with the same concentration of AN.

Considering the very high affinity for these types of compounds to AGP, optimum separations could be achieved at pH 6.0, with high concentrations [25–35% (v/v)] of either modifier. However, Herényi and Görög [26] utilized another approach for the resolution of some of the compounds that were investigated in this study, *i.e.*, the four stereoisomers of the hydrophobic ethyl apovincaminate (**4**), on a 100 mm Chiral-AGP column. They applied gradient elution with increasing IP concentration in the mobile phase, from 18 to 35% (v/v), resulting in the elution order *cis*-(+), *trans*-(−), *cis*-(−), *trans*-(+).

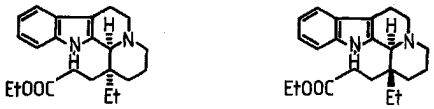
### *Chemical structure and stereoselectivity*

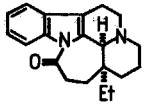
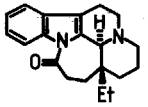
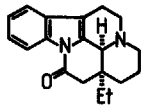
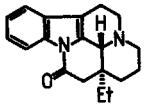
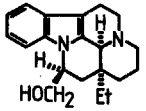
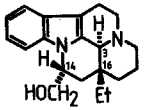
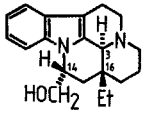
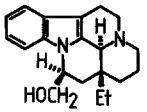
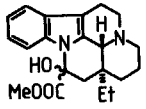
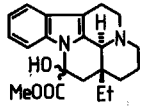
Similarly to the native protein, AGP immobilized on silica preferentially binds the compounds having 1(*R*), 12b(*S*)-indolo[2,3-*a*]quinolizidine (**1b**, **2b**, **3b**) or the analogous 3(*S*), 16(*R*)-eburnane (**4b**, **5b**, **6b**, **7b**, **8b**, **8c**, **9b**) absolute configurations. With compounds having the third chiral centre at position 14 (**8** and **9**), Chiral-AGP could also discriminate according to the absolute configuration of this centre (*cf.*, Figs. 2 and 3). Comparison between **8b** and **8c** suggests that the 14(*S*) absolute configuration is bound with the highest affinity, in agreement with data obtained when native AGP was used [23].

TABLE I

SEPARATION OF MIXTURES OF VINCA ALKALOID STEREOISOMERS ON CHIRAL-AGP COLUMN (50 × 4 mm I.D.)

Mobile phase, 0.01 *M* phosphate buffer with 2-propanol (IP) or acetonitrile (AN) modifier.

| Samples | pH | Modifier | | Elution order | $k'_1$ | $k'_2$ | $\alpha$ |
|---|---|---|---|---|---|---|---|
| | | Compound | Concentration (%) | | | | |
|  **1a** and **1b** | 7.0 | IP | 25 | a,b | 2.81 | 10.12 | 3.61 |
| | | AN | 25 | a,b | 2.73 | 23.08 | 8.46 |
| | 6.5 | IP | 25 | a,b | 2.65 | 6.03 | 2.28 |
| | | AN | 25 | a,b | 2.87 | 13.65 | 4.76 |
| | 6.0 | IP | 25 | a,b | 2.46 | 4.08 | 1.66 |
| | | AN | 25 | a.b | 2.29 | 5.59 | 2.45 |
|  **2a** and **2b** | 7.0 | IP | 25 | a,b | 2.84 | 36.38 | 12.81 |
| | 6.5 | IP | 25 | a,b | 2.77 | 21.07 | 7.61 |
| | 6.0 | IP | 25 | a,b | 2.56 | 11.60 | 4.54 |
| | | IP | 30 | a,b | 1.86 | 6.20 | 3.34 |
| | | AN | 35 | a.b | 0.87 | 4.14 | 3.61 |
|  **3a** and **3b** | 6.0 | IP | 25 | a,b | 2.42 | 11.57 | 4.77 |
| | | IP | 30 | a,b | 1.75 | 6.09 | 3.49 |
| | | AN | 35 | a.b | 0.76 | 3.44 | 4.55 |
|  **3c** and **3b** | 6.0 | IP | 25 | c,b | 2.81 | 11.57 | 4.11[a] |
| | | IP | 30 | c,b | 1.88 | 6.06 | 3.23[a] |
| | | AN | 35 | c,b | 0.78 | 3.56 | 4.56[a] |
|  **4a** and **4b** | 6.0 | IP | 30 | a,b | 1.33 | 7.72 | 5.80 |
| | | AN | 30 | a,b | 1.81 | 23.07 | 12.75 |
| | | AN | 35 | a,b | 0.77 | 8.54 | 11.04 |
|  **5a** and **5b** | 6.0 | IP | 30 | a,b | 1.97 | 7.30 | 3.70[a] |
| | 6.0 | AN | 35 | a,b | 0.78 | 8.91 | 11.42[a] |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
|  |  | 7.0 | IP | 25 | a,b | 1.39 | 3.24 | 2.33 |
| | | | AN | 25 | a,b | 2.48 | 7.60 | 3.07 |
| | | 6.5 | IP | 25 | a,b | 1.73 | 3.41 | 1.97 |
| | | | AN | 25 | a,b | 4.00 | 7.36 | 1.84 |
| **6a** and **6b** | | 6.0 | IP | 25 | a,b | 2.14 | 3.25 | 1.51 |
| | | | AN | 25 | a,b | 4.83 | 5.49 | 1.14 |
|  |  | 7.0 | IP | 25 | a,b | 1.14 | 1.28 | 1.12 |
| | | | AN | 25 | a,b | 1.56 | 2.11 | 1.35 |
| **7a** and **7b** | | | | | | | | |
|  |  | 6.0 | IP | 25 | a,b | 2.08 | 5.50 | 2.64[a] |
| | | | AN | 25 | a,b | 2.19 | 7.86 | 3.59[a] |
| | | | IP | 30 | a,b | 1.34 | 2.88 | 2.15[a] |
| | | | AN | 30 | a,b | 1.48 | 4.32 | 2.92[a] |
| **8a** and **8b** | | | | | | | | |
|  |  | 6.0 | IP | 25 | b,c | 5.50 | 11.03 | 2.00 |
| | | | AN | 25 | b,c | 7.86 | 15.88 | 2.02 |
| | | | IP | 30 | b,c | 2.88 | 5.86 | 2.03 |
| | | | AN | 30 | b,c | 4.32 | 8.34 | 1.93 |
| **8b** and **8c** | | | | | | | | |
|  | | 6.0 | IP | 20 | nd[b] | 2.04 | 2.92 | 1.43 |
| | | | AN | 20 | nd | 2.75 | 4.05 | 1.47 |
| | | | IP | 25 | nd | 1.36 | 1.76 | 1.29 |
| | | | AN | 30 | nd | 1.13 | 1.49 | 1.32 |
| **9a** | | | | | | | | |
|  | | 6.0 | IP | 20 | nd | 5.22 | 14.19 | 2.72 |
| | | | AN | 20 | nd | 12.93 | 31.27 | 2.42 |
| | | | IP | 25 | nd | 2.66 | 5.82 | 2.19 |
| | | | AN | 30 | nd | 3.26 | 6.69 | 2.05 |
| **9b** | | | | | | | | |

[a] These values refer to enantioselectivity.
[b] Not determined.

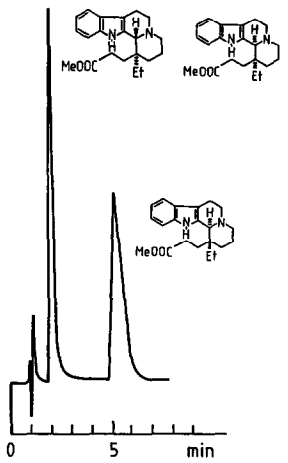



Fig. 1. Separation of the stereoisomers of **3** on a Chiral-AGP column (50 × 4 mm I.D.). Sample, mixture of **3a**, **3b** and **3c**. Mobile phase, 35% (v/v) AN in 0.01 *M* phosphate buffer (pH 6.0); flow-rate, 0.5 ml/min. Et = Ethyl; Me = methyl.

*Separations on Chiral-HSA*

Previous studies of vinca alkaloid analogues on an HSA-Sepharose column showed [23] that the binding affinities varied within a broad range ($K = 10^3$–$10^5$ l/mol) and the *trans* isomers, regardless of the configurations of the two chiral centres, had two to four times higher affinities than the corresponding *cis* isomers. In extreme cases, such as for the low-affinity alcohol (**1**) and the high affinity ethyl apovincaminate (**4**), the elution volumes did not show significant differences. For structure **8** the *R* configuration of centre 14 was bound with the highest affinity.

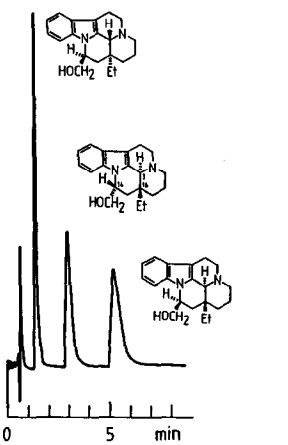



Fig. 2. Separation of the stereoisomers of **8** on a Chiral-AGP column (50 × 4 mm I.D.). Sample, mixture of **8a**, **8b** and **8c**. Mobile phase, 30% (v/v) AN in 0.01 *M* phosphate buffer (pH 6.0); flow-rate, 0.9 ml/min.

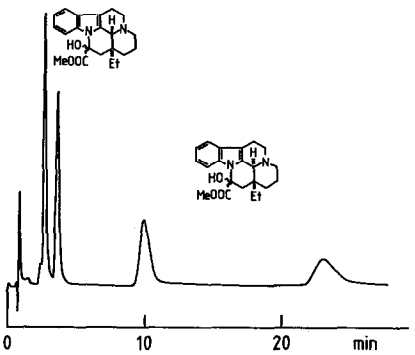



Fig. 3. Separation of the stereoisomers of **9** on a Chiral-AGP column (50 × 4 mm I.D.). Sample, mixture of **9a** and **9b**. Mobile phase, 20% (v/v) IP in 0.01 *M* phosphate buffer (pH 6.0); flow-rate, 0.5 ml/min.

Chromatographic data obtained on Chiral-HSA are summarized in Table II. The retention and stereoselectivity data obtained on the Chiral-HSA column were similar to those obtained on HSA-Sepharose gel. In general, the *trans* isomers are more retained than the *cis* species (see Fig. 4) and the configuration of the chiral centres in the *trans* isomer plays a minor role in determining the binding strength.

With the highly retained hydrophobic compounds **4** and **5** AN modifier produced the opposite elution order to IP. This is an interesting finding and this type of selectivity change has not been reported previously on HSA-bonded silica. As mentioned above, AN and 1-propanol are highly adsorbed on immobilized AGP [25], and it is also reasonable to assume adsorption of these modifiers on HSA. Thus, when adsorbed, the modifiers can compete with the enantiomers for binding to different hydrogen bonding groups in the binding sites. Accordingly, modifiers with different hydrogen bonding properties and different hydrophobicities affect the enantioselectivity in different ways. Another reasonable explanation for the modifier-induced changes in the enantioselectivity is that certain organic modifers, present at high enough concentra-

TABLE II

SEPARATION OF MIXTURES OF VINCA ALKALOID ANALOGUES ON A CHIRAL-HSA COLUMN (100 × 4 mm I.D.)

Mobile phase, 0.1 *M* phosphate buffer (pH 7.0) with 10% of 2-propanol (IP) or acetonitrile (AN) modifier.

| Sample | Modifier | Elution order | $k'_1$ | $k'_2$ | $\alpha$ |
|---|---|---|---|---|---|
| **1a, 1b** | IP | a,b | 1.41 | 2.01 | 1.42 |
| | AN | a,b | 1.52 | 1.98 | 1.30 |
| **2a, 2b** | IP | a,b | 2.42 | 2.90 | 1.20 |
| | AN | a,b | 2.23 | 2.63 | 1.18 |
| **3a, 3b** | IP | a,b | 2.34 | 2.89 | 1.23 |
| | AN | a,b | 2.18 | 2.56 | 1.17 |
| **3a, 3c** | IP | a,c | 2.34 | 3.29 | 1.41 |
| | AN | a,c | 2.18 | 2.65 | 1.21 |
| **4a, 4b** | IP | a,b | 26.50 | 34.80 | 1.31 |
| | AN | b,a | 23.17 | 29.00 | 1.25 |
| **5a, 5b** | IP | a,b | 22.33 | 35.67 | 1.60[a] |
| | AN | b,a | 26.50 | 30.67 | 1.16[a] |
| **6a, 6b** | IP | a,b | 13.55 | 19.42 | 1.43 |
| | AN | a,b | 9.37 | 13.56 | 1.45 |
| **7a, 7b** | IP | a,b | 9.71 | 20.53 | 2.11 |
| | AN | a,b | 6.52 | 11.25 | 1.73 |
| **8a, 8b** | IP | a,b | 1.84 | 3.32 | 1.80[a] |
| | AN | a,b | 1.72 | 3.39 | 1.97[a] |
| **8c, 8b** | IP | c,b | 1.70 | 3.32 | 1.95 |
| | AN | c,b | 1.65 | 3.39 | 2.05 |
| **9a, 9b** | IP | b,a | 1.68 | 1.88 | 1.12 |
| | AN | b,a | 2.05 | 2.14 | 1.04 |

[a] These values refer to enantioselectivity.

tion, induce small reversible changes of the secondary structure, transformation of parts of the peptide chain with $\beta$-comformation or an unordered structure into an $\alpha$-helical form. The secondary structure of AGP in solution, with and without IP, has been studied by circular dicroism (CD) in an attempt to correlate modifier-induced changes in the enantioselectivity with changes in the secondary structure of the protein [25]. These studies were performed using AGP solutions containing 40% IP, but no clear indications of the formation of an $\alpha$-helix was found. However, a reinvestigation of the old data together with new ones gave weak indications of the formation of an $\alpha$-helix [27]. If the changes are small they can be difficult to detect by CD. However, it is reasonable to assume that even small local changes in the secondary structure, hardly detectable by CD, can induce large changes in the enantioselectivity, in line with the findings on the HSA column reported above for **4** and **5**. So far, no CD studies of

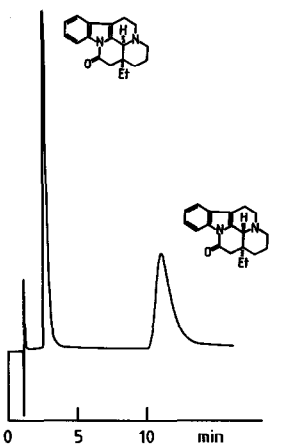



Fig. 4. Separation of a mixture of **7a** and **7b** on a Chiral-HSA column (100 × 4 mm I.D.). Mobile phase, 10% (v/v) IP in 0.1 *M* phosphate buffer (pH 5.0); flow-rate, 0.9 ml/min.

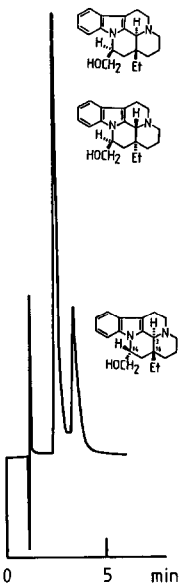



Fig. 5. Separation of the stereoisomers of **8** on a Chiral-HSA column (100 × 4 mm I.D.). Sample, mixture of **8a**, **8b** and **8c**. Mobile phase, 10% (v/v) IP in 0.1 *M* phosphate buffer (pH 6.15); flow-rate 0.9 ml/min.

HSA have been performed in the presence of AN and IP. On the HSA-Sepharose column both **4a** and **4b** and also **5a** and **5b** showed high affinities without a significant difference between the enantiomers. When the protein provided a high degree of stereoselectivity, the elution order was not influenced by mobile phase modifiers. In most instances the stereoselectivity was higher using IP as organic modifier. Higher capacity factors were also obtained for most compounds with IP. These findings are contrary to the findings on the AGP column. HSA, in contrast to AGP, prefers the *R* configuration at centre 14 in structure **8** (compare Figs. 5 and 2). Compounds **9a** and **9b** have low capacity factors and no separation could be detected according to centre 14.

The influence of the pH on the retention and the stereoselectivity was also studied for some of the compounds, **1a**, **1b**, **5a**, **5b**, **6a**, **6b**, **7a**, **7b**, **8a**, **8b** and **8c**, and the results are summarized in Table III. It can be noted that the separation factor for **7** was highly influenced by the pH in the range 5–7. The highest separation factor, 6.35, was obtained at pH

TABLE III

INFLUENCE OF pH ON THE CAPACITY FACTORS AND THE SEPARATION FACTORS OF VINCA ALKALOID STEREOISOMERS

Column, Chiral-HSA (100 × 4 mm I.D.); mobile phase, 0.1 *M* phosphate buffer with 2-propanol (IP) modifier.

| Sample | pH 5.00, 5% IP | | pH 5.00, 10% IP | | pH 6.15, 10% IP | | pH 7.00, 10% IP | |
|---|---|---|---|---|---|---|---|---|
| | $k'$ | $\alpha$ | $k'$ | $\alpha$ | $k'$ | $\alpha$ | $k'$ | $\alpha$ |
| **1a** | 0.81 | 1.00 | 0.48 | 1.10 | 1.06 | 1.22 | 1.40 | 1.39 |
| **1b** | 0.81 | | 0.53 | | 1.29 | | 1.94 | |
| **5a** | 3.55 | 1.93[a] | 2.06 | 1.72[a] | 12.55 | 1.91[a] | 21.18 | 1.71[a] |
| **5b** | 6.86 | | 3.55 | | 24.00 | | 36.31 | |
| **6a** | 2.61 | 1.00 | 1.50 | 1.03 | 6.69 | 1.48 | 13.24 | 1.60 |
| **6b** | 2.61 | | 1.55 | | 9.92 | | 21.18 | |
| **7a** | 2.01 | 7.37 | 1.32 | 6.35 | 5.35 | 3.32 | 8.71 | 2.30 |
| **7b** | 14.82 | | 8.38 | | 17.77 | | 20.05 | |
| **8a** | 0.64 | 1.64[a] | 0.43 | 1.65[a] | 1.08 | 1.74[a] | 1.69 | 1.79[a] |
| **8b** | 1.05 | | 0.71 | | 1.88 | | 3.03 | |
| **8c** | 0.65 | 1.62 | 0.43 | 1.58 | 1.08 | 1.73 | 1.59 | 1.91 |
| **8b** | 1.05 | | 0.68 | | 1.87 | | 3.04 | |

[a] These values refer to enantioselectivity.

5.0 where *trans*-**7** also was least retained. The stereoselectivity of **1** and **6** increased on increasing the pH from 5 to 7. The retention of all the compounds increased at higher pH, as expected.

## CONCLUSIONS

AGP and HSA immobilized on silica were used successfully for the separation of the stereoisomers of a series of vinca alkaloids. The native proteins, especially AGP, discriminate well between the stereoisomers of the vinca alkaloids. It has been demonstrated that the immobilized proteins follow the binding tendencies obtained by *in vitro* methods with the native proteins. Under certain chromatographic conditions the stereoselectivities, using immobilized proteins, can be significantly increased in comparison with the native proteins.

A comparison of the Chiral-AGP and Chiral-HSA columns concerning the retention of the vinca alkaloids demonstrated that these compounds are bound with higher affinity of the AGP phase. The Chiral-AGP column also gives higher stereoselectivity and better resolution owing to a much better chromatographic performance on the AGP phase.

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