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SEPARATION OF EPIMERS OF BUDESONIDE AND RELATED CORTICOSTEROIDS BY REVERSED BONDED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

Optimal conditions for the separation of the epimers of homologous 16α,17α-acetals of 6α,9α-difluoro-, 9α-fluoro- or non-fluorinated 16α-hydroxyprednisolone on μBondapak C_{18} have been evaluated. The separation factor increases strongly with increasing alkyl chain length at the asymmetrical carbon, C-22, the position where configurational differences of the molecules are located, and weakly with fluorine substitution at 6α and 9α positions. Acetate esterification of the C-21 hydroxy group does not influence the separation factor. Of the different types of organic modifier added to water in the eluents, ethanol gives the best results. Both the capacity and the separation factors increase with a decrease in the concentration of the organic modifier in the mobile phase, resulting in large improvements of separation of epimers. The retention mechanisms are discussed on the basis of molecular structures and current models of the chromatographic system.

INTRODUCTION

A great number of 16α,17α-acetals of 6α,9α-difluoro- (I), 9α-fluoro- (II) and non-fluorinated (III) 16α-hydroxyprednisolone have been synthesized and tested for anti-inflammatory and systemic activities by Brattsand *et al.*[1,2]. It has been reported[3] that the anti-inflammatory and corticoid activities of the 16α,17α-acetonide of triamcinolone are ten times greater than that of its precursor triamcinolone (II). The same enhancement was observed for the I, II and III 16α,17α-acetals[4].

The acetals were prepared by treating I, II or III with aldehydes, R–CHO, where R represents straight hydrocarbon chains with 1–9 carbon atoms. Accordingly, a new centre of chirality (C-22) was introduced into the molecule and two epimers were formed[2]. In 1969 Thalén[5] developed a method for separation and isolation of these epimers on a preparative scale by column chromatography on Sephadex LH-20, which made it possible to investigate the physiological characteristics of the separate epimers.

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At the same time, Thalén[5] found that the separation of the epimers increased in his chromatographic system with increasing chain length of the alkyl substituent at C-22. He also found that the separation factor for the epimers of 16α,17α-acetals with the same alkyl group at C-22 increased in the order I > II > III, *i.e.* with increasing fluoro substitution.

Structure elucidation of the epimers as well as of the epimeric mixtures has also been performed by Thalén and co-workers[1,2,5]. One of the acetals, budesonide, which is *ca.* 1:1 epimeric mixture of 16α,17α-(22*S*)- and 16α,17α-(22*R*)-propylmethylenedioxypregna-1,4-diene-11β,21-diol-3,20-dione, is now under clinical evaluation. The crystal and molecular structures of these epimers have been analysed by Albertsson *et al.*[6].

During the evaluation of budesonide there was need for an analysis system with high speed, linearity and sensitivity. A high separation efficiency is required as well, as in many cases the two epimers must be separated and individually determined. Preliminary investigations indicated that high-performance liquid chromatography (HPLC) system could fulfil most of the demands, whereas other chromatographic systems often used for corticosteroids, such as thin-layer chromatography (TLC), paper chromatography (PC) or gas–liquid chromatography (GLC) are inadequate with respect to speed, linearity and separation efficiency (TLC, PC) or are complicated by slow derivatization processes involving side-reactions and high-temperature operation (GLC).

As the use of GLC in the study of corticosteroids is limited, HPLC has become a very important tool for the separation and determination of these compounds, particularly in the pharmaceutical industry[7] and in clinical chemistry[8]. Adsorption chromatography on silica[9,10] or alumina[11], straight- and, especially, reversed bonded-phase chromatography[12–22] and liquid–liquid partition chromatography[22–28] have been used. A prediction of the relative merits of these different modes of chromatography is difficult, as systematic comparisons are often lacking in the literature and there are uncertainties concerning the mechanisms in adsorption and bonded-phase chromatography. However, with respect to the corticosteroids studied in this work, the retention mechanism of the reversed bonded-phase system was shown to be completely different from those operative in adsorption chromatography on silica[29]. Although the straight-phase mode was shown to be more effective for the separation in some cases, the reversed-phase mode was more selective for most of the compounds. The present work is thus devoted to studies of the latter system.

Retention mechanisms in reversed-phase chromatography, using a chemically bonded stationary phase of octadecylsilane, have been considered by several authors[14,30–41]. Karch *et al.*[34] pointed out similarities in the retention behaviour between an LC system and a GC system, based on octadecylsilane and graphitized carbon black as stationary phases, respectively. From this, they suggested an interaction of the solute with the non-polar stationary phase by dispersion forces as the main mechanism of retention. Locke[36], on the other hand, suggested that interactions between the solute and the octadecylsilane phase are weak and non-selective, and therefore the solute properties in the mobile phase are important for selectivity. Horváth *et al.*[40] and Karger *et al.*[39] used a solvophobic concept for the solute interactions in the chromatographic process, and Horváth[40] developed the concept in a thermodynamic quantitative way. On the other hand, Westerlund and Theodorsen[41] con-

sidered the influence of partition effects, as they found that a thin layer of methanol is spontaneously adsorbed on to the support (0.1 ml/g) when methanol–aqueous phosphate buffer mixtures, containing quaternary ammonium compounds, were used as a mobile phases.

Owing to the favourable properties of the C_{18} system in separating corticosteroid derivatives[29], the present work deals with various ways to obtain optimal conditions within this system. In addition the retention behaviour is discussed on the basis of current theories and the molecular structures of the solutes.

EXPERIMENTAL

Liquid chromatography

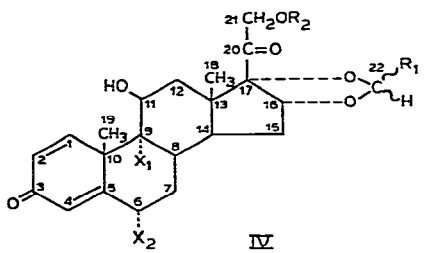
The delivery system consisted of a double-piston reciprocating constant flow pump Model M-6000 (Waters Assoc., Milford, Mass., U.S.A.). A flow-rate of 1.0 ml/min was used in all experiments, except when the HETP dependence on the flow-rate was studied. The column (30 cm × 4 mm I.D.) was packed with a microparticulate (10 ± 2 μm) octadecylsilane bonded phase (μBondapak C_{18}, Waters Assoc.). Further details concerning the packing material are given in ref. 29. A volume of 5 μl of solutions of the steroids, dissolved in ethanol or in the eluent at total concentrations of *ca.* 2 μg/μl, were injected into a U6K universal injector (Waters Assoc.). A UV absorption detector, Model 400 (Waters Assoc.), which monitors light absorption at 254 nm, was used for detection. The 12-μl flow cell has a conical shape that minimizes deflections in UV absorbance caused by changes in refractive index of the eluent.

Chemicals

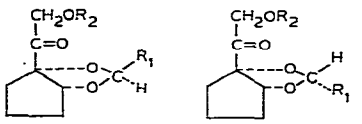
Eluents were prepared from glass-distilled water and various amounts of methanol, ethanol, 1-propanol, acetonitrile or tetrahydrofuran. The organic solvents were either of spectroscopic or pro analysi grade and they were used as received.

Corticosteroids investigated

The corticosteroids studied are described by the structural formula IV, in which $X_1 = X_2 = F$; $X_1 = F$, $X_2 = H$; and $X_1 = X_2 = H$ for the 6α,9α-difluoro, 9α-fluoro, and non-fluorinated derivatives respectively. For each of the acetals a pair of epimers, A and B, is defined by the configuration at C-22. By varying the alkyl chain length, R_1, at C-22 and by preparing esters, $R_{2,1}$ at the 21-hydroxyl, a great number of corticosteroids were synthezised and structural evidence was given by



IV



nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS)[1,2,5]. The derivatives studied here are summarized in Table I.

The UV absorptivities of the corticosteroids should be similar to those of the epimers of budesonide, which in ethanol were $1.5 \cdot 10^4$ and $1.2 \cdot 10^4$ at 242 ($\lambda_{max.}$) and 254 nm, respectively, as the structure of ring A is common to all substances. At 254 nm this corresponds to a detection limit of *ca.* 10 ng at reasonable values of the capacity factor.

TABLE I

FUNCTIONAL GROUPS OF THE 16α-HYDROXYPREDNISOLONE ACETAL DERIVATIVES

Structural formulae of the compounds are given in the text.

Compounds	R_1	R_2
Acetals of		
16α-hydroxyprednisolone	CH_3	H
	CH_2CH_3	H, CH_3CO
	$(CH_2)_2CH_3$	H, CH_3CO
	$(CH_2)_4CH_3$	H, CH_3CO
Acetals of 9α-fluoro-		
16α-hydroxyprednisolone	CH_3	H
	CH_2CH_3	H
	$(CH_2)_2CH_3$	H
	$(CH_2)_3CH_3$	H
Acetals of 6α,9α-difluoro-		
16α-hydroxyprednisolone	CH_3	H
	$(CH_2)_2CH_3$	H
	$(CH_2)_4CH_3$	H

Theory

The resolution, R_s, between two peaks 1 and 2 is a function of the number of theoretical plates, N, the separation factor, α, and the capacity factor, k', according to

$$R_s = \frac{1}{4}\frac{\alpha - 1}{\alpha}\sqrt{N}\frac{k_2'}{1 + k_2'\left(\frac{\alpha + 1}{2\alpha}\right)} \quad (1)$$

For $\alpha \approx 1$, the equation is approximated by

$$R_s = \frac{1}{4}\frac{\alpha - 1}{\alpha}\sqrt{N}\frac{k_2'}{1 + k_2'} \quad (2)$$

which is the relationship generally given in the literature for the resolution of closely spaced peaks. By introducing the number of effective plates in the column, N_{eff}, the resolution eqn. 2 is reduced to:

$$R_s = \frac{1}{4}\frac{\alpha - 1}{\alpha} N_{eff}^{1/2} \quad (3)$$

as

$$N_{eff} = N\left(\frac{k_2'}{k_2' + 1}\right)^2 \quad (4)$$

RESULTS AND DISCUSSION

Optimization of N, k' and u

The dependence of the square root of the effective number of theoretical plates on k' for some of the 16α-hydroxyprednisolone derivatives is shown in Fig. 1. The continuous line was calculated from eqn. 4 for $N = 2100$. The close fit of experimental values to the curve indicates that N is independent of k'. As band broadening arising from slow mass transfer in the stationary phase, diffusion in a stagnant mobile phase and extra-column effects would be a function of k'[42–46], the result in Fig. 1 indicates that the band broadening originates mostly from combined mechanisms of flow and diffusion in the moving mobile phase[44,45]. These effects have been discussed in detail by Giddings[43] and by Knox and Saleem[46]. However, it should be pointed out that compared with the steroid derivatives, a larger plate number is obtained for smaller molecules, *e.g.* for benzene $N = 3400$ in the system used in Fig. 1. If band broadening originates from processes in the moving mobile phase, a reduced plate number with increasing molecule size is probably due to a slower diffusion for the larger molecules.

For the μBondapak C_{18} support the dependence of N on the linear flow-rate, u, is shown in Fig. 2. The shape of the curve is similar to that obtained by Karch

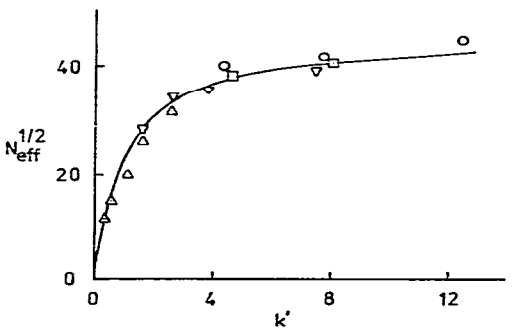


Fig. 1. Square root of N_{eff} plotted against k'. Support: μBondapak C_{18}. Mobile phases: ethanol–water, varied between 40:60 and 60:40 (v/v). Flow-rate: 1.0 ml/min. The alkyl chain at the chiral centre C-22 of the 16α-hydroprednisolone acetal derivatives was: △, CH_3 (epimer A and B); ⊡, $(CH_2)_2CH_3$ (epimer B); ▽, $(CH_2)_4CH_3$ (epimer B); ○, $(CH_2)_6CH_3$ (epimer A).

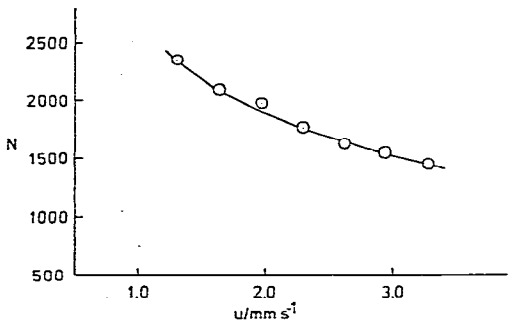


Fig. 2. Plate number plotted against linear flow-rate. Support: μBondapakC$_{18}$. Mobile phase: ethanol–water (42:58). Solute: epimer A of budesonide.

et al.[34] when *n*-pentanol, phenol and ethanol were chromatographed with water–methanol (1:1, v/v) as mobile phase on LiChrosorb SI-100, C_{18}, 10-μm particles. The column efficiency of the μBondapak C_{18} column for benzene ($H = 88\ \mu$m, $u =$ 1.7 mm/sec) is comparable with the LiChrosorb SI-100 column ($H = 86\ \mu$m, $u =$ 1.66 mm/sec) for *n*-pentanol[34]. As in our work they obtained a decrease in H with a smaller solute size, since a reduction of *ca.* 25% was obtained in going from *n*-pentanol to ethanol.

When the flow velocity is decreased, the plate number increases according to Fig. 2 and so does the effective plate number. A reduced flow-rate will lead to unrealistic long retention times, but this can be compensated for by increasing the strength of the eluent. However, if we select a definite retention time of the solute, k' decreases when flow decreases and, due to this, the effective plate number is reduced (see eqn. 4). Thus, for each retention time there is an optimal flow range with respect to the effective plate number, as shown in Fig. 3. The figure shows that the optimal range moves from high flow velocities to low when the retention time is increased. The efficiency increases with increasing analysis time.

Retention behaviour and optimization of α

For a reversed-phase packing material consisting of octadecylsilane groups attached to a silica surface the retention behaviour can be controlled by varying the concentration of an organic modifier in water. As shown in Fig. 4, the retention of the 16α,17α-acetals of 16α-hydroxyprednisolone increases with a decrease in the concentration of ethanol in water. For individual epimers, log k' increases linearly with the concentration of water, and this relationship has been found earlier for other compounds[14,26,39,41]. Preliminary studies on a few of the steroids in the ethanol–water eluents have indicated a linear relationship between log solubility and the proportion of water, and also that the solubilities of the homologous steroids decrease with the alkyl chain length at C-22 at any given ethanol–water ratio. This is in agreement with the model of Locke[38], but more solubility data are required for a quantitative evaluation. Locke suggests that similar types of solutes are separated on the basis of their relative solubilities, as solute adsorption on to the stationary phase involves only weak dispersive forces of interaction[38]. The result also agrees with those of Horváth *et al.*[40], who state that the relative retention is governed by solvophobic

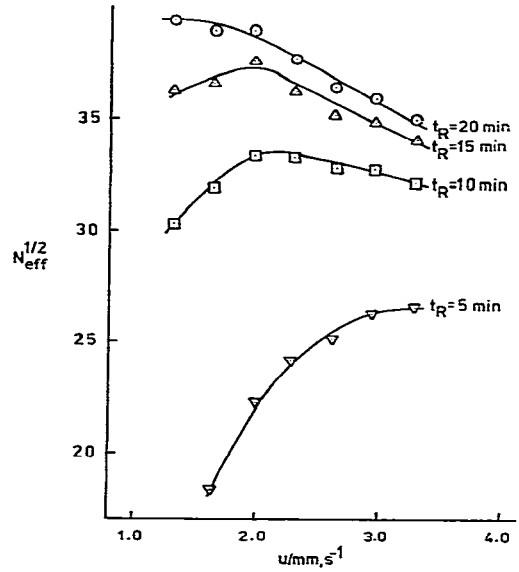


Fig. 3. Square root of N_{eff} plotted against flow-rate for various retention times of the solute. Support: μBondapak C_{18}. Solute: epimer A of budesonide. Mobile phases: ethanol–water, varied from 40:60 to 60:40 (v/v). N_{eff} values were calculated by use of Fig. 2 and eqn. 4.

forces acting on the solutes in the mobile phase. Actually, for given solutes, they predicted a linear dependence of log k' on the surface tension of the eluent. Within the mobile phase compositions reported in Fig. 4, the surface tension is a linear function of the ethanol concentration.

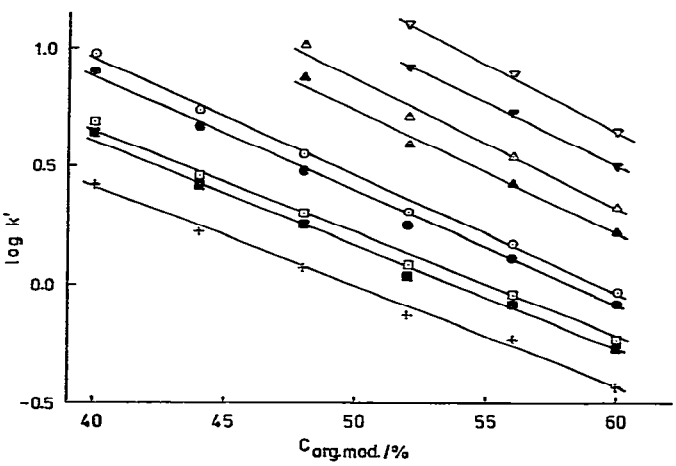


Fig. 4. Log k' plotted against concentration of ethanol in aqueous eluents (v/v). Support and flow-rate: see Fig. 1. Solutes: 16α-hydroxyprednisolone acetals with alkyl chains at the chiral centre C-22: +, CH_3 (epimer A and B); ■ □, CH_2CH_3 (epimer B and A, respectively); ● ○, $(CH_2)_2CH_3$ (epimer B and A, respectively); ▲ △, $(CH_2)_4CH_3$ (epimer B and A, respectively); ▼ ▽, $(CH_2)_6CH_3$ (epimer B and A, respectively).

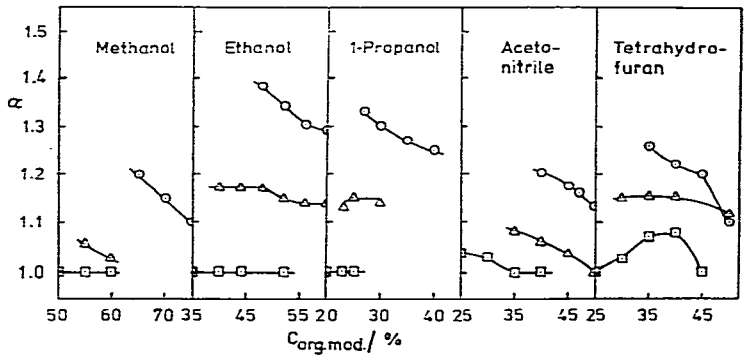


Fig. 5. Epimer separation factors plotted against the concentration of organic modifiers in aqueous mobile phases (v/v). Support and flow-rate: see Fig. 1. Solutes: as in Fig. 4 with alkyl chains at C-22: ⊡, CH_3; △, $(CH_2)_2CH_3$; ○, $(CH_2)_4CH_3$.

Separation factors obtained for the epimers of three different homologous steroids are plotted against the concentration of organic modifier in water in Fig. 5. It is seen that the levels in the values of the separation factors are different when various organic modifiers are used. This is in contradiction to the findings of Karger *et al.*[39], who found that the selectivity is roughly independent of the type of organic modifier when they separated *n*-alcohols on a chemically bonded octadecylsilane stationary phase. However, in accordance with our observations, they found that α increases with a decrease in the concentration of the organic modifier. In Fig. 5 this

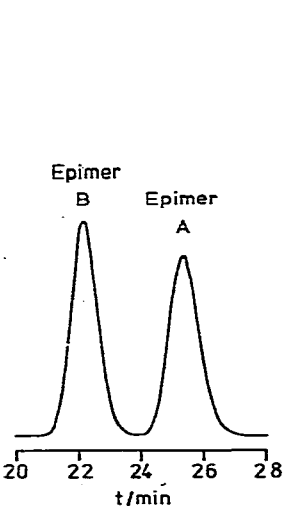


Fig. 6. Optimal separation of the epimers of budesonide. Support and flow-rate: see Fig. 1. Mobile phase: ethanol–water (40:60).

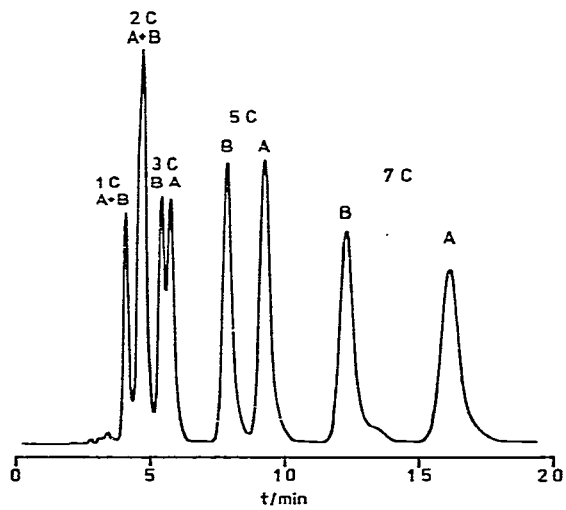


Fig. 7. Separation of 16α-hydroxyprednisolone acetal derivatives. Support and flow-rate: See Fig. 1. Mobile phase: ethanol–water (60:40). Solutes: the epimers and the number of carbons atoms in the alkyl chains at C-22 in the acetals are given in the figure.

effect is more pronounced the longer the hydrocarbon chain bonded to the asymmetric carbon at position C-22.

In the present work the selection of type and concentration of modifier is critical, as under optimal conditions a proper separation of the epimers of budesonide according to eqn. 3 ($R_s \geqslant 1.5$) is obtained when $\alpha \geqslant 1.16$. This α value is exceeded only in the ethanol–water system when the proportion of water is larger than 52% (see Fig. 5). An optimal separation of the epimers of budesonide is shown in Fig. 6.

The effect of increasing the proportion of water in the eluent is thus to improve the separation of epimers owing to the effect on k' (see eqn. 2), as well as to increases in the separation factor (see Fig. 5). This is demonstrated in Figs. 7 and 8, *e.g.* the resolution of the epimers of the hexylidenedioxy homologue, $R_1 = (CH_2)_4CH_3$, is increased from 1.8 to 3.3 when the ethanol concentration is decreased from 60 to 48% (v/v). The figures also show the drastic increase in separation of epimers with alkyl chain length and that epimer B is eluted before epimer A.

Solute structures and retention

The molecular structures of the epimers A and B of budesonide in the solid crystalline state are shown in Fig. 9. A more detailed description of the structures is given in ref. 6. In both epimers the propyl chain at C-22 is disordered and in solution the chain probably rotates freely. In both epimers the chain extends α-axially with respect to the steroid nucleus. In epimer B the E-ring is in the half-chair conformation (conformation I) whereas in epimer A C-22 has been displaced towards the steroid nucleus (conformation II). The molecular structures of the two epimers are so close that they crystallize together in the same solid solution[6], and if the structures in Fig. 9 were unique even in the chromatographic system, a separation would not be likely. Let us, therefore, consider the possibility of formation of both con-

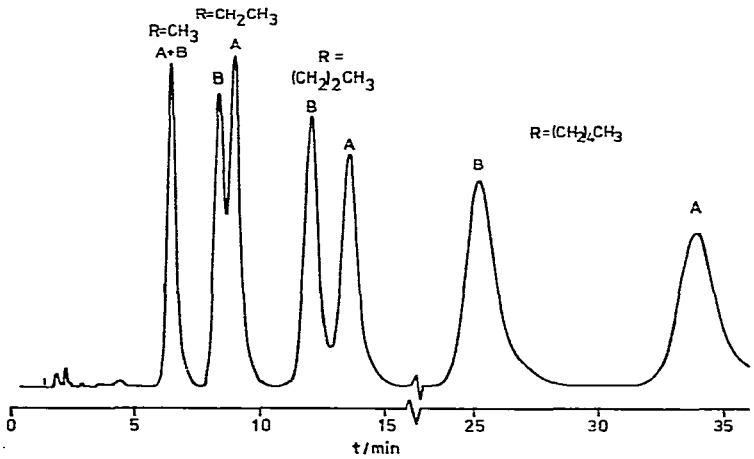


Fig. 8. Separation of 16α-hydroxyprednisolone acetal derivatives. Mobile phase: ethanol–water (48:52). For other conditions see Fig. 7.

formations of ring E for both epimers, *i.e.*:

$$\text{Epimer } A_I \rightleftarrows \text{Epimer } A_{II} \quad (5)$$

$$\text{Epimer } B_I \rightleftarrows \text{Epimer } B_{II} \quad (6)$$

where I and II denote the two conformations of ring E and where epimers A_{II} and B_I are those found in the solid state[6] and shown in Fig. 9.

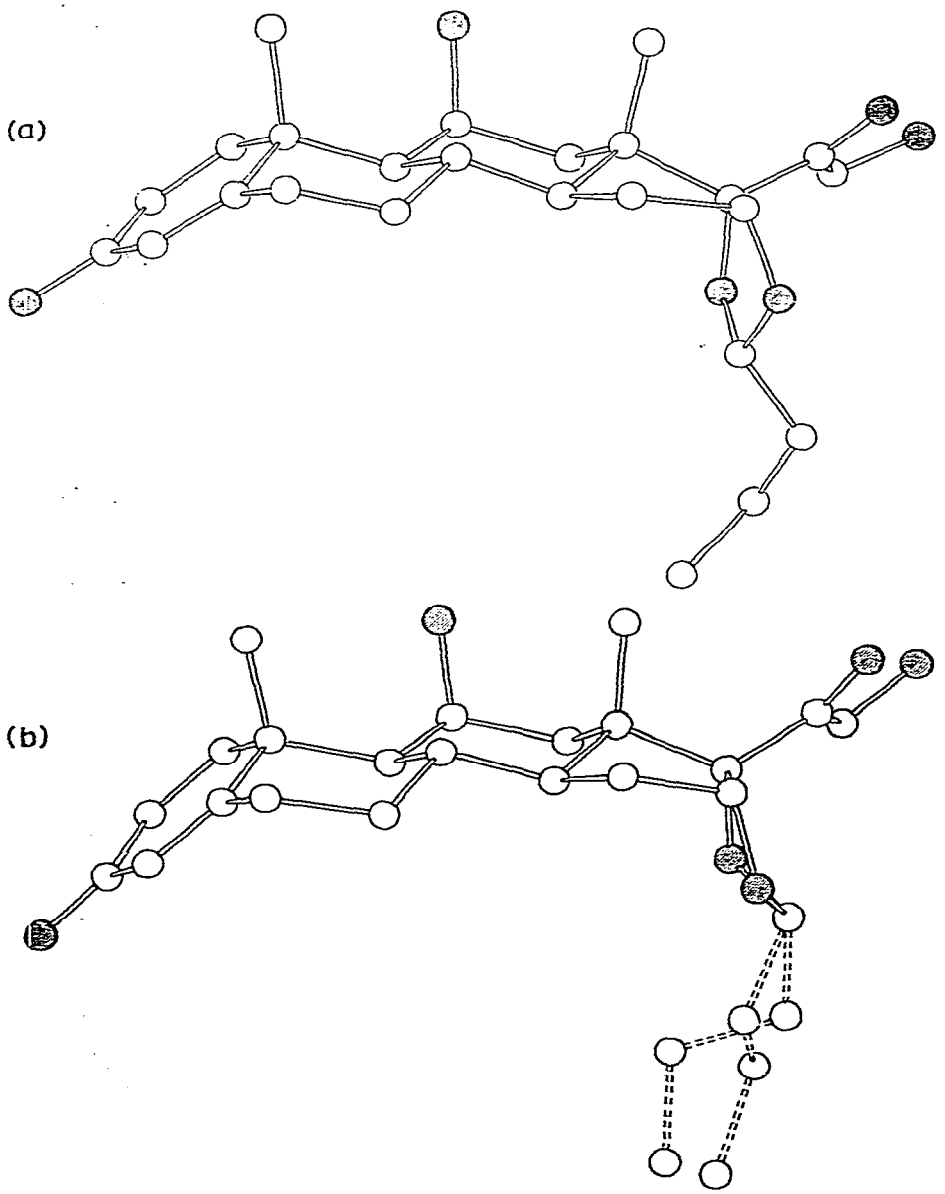


Fig. 9. Molecular structures of epimers A (a) and B (b) of budesonide. Hydrogen atoms are not shown. ○, Carbon atoms; ●, oxygen atoms.

In epimer B_{II} the propyl chain at the chiral centre C-22 would be directed towards the steroid nucleus and the distance between the two molecular parts would probably be too close. It seems therefore likely that a flipping over from B_I to B_{II} would require a high activation energy and probably equilibrium 6 is shifted to the left even in a polar solvent such as water. In epimer A, however, there is no steric hindrance for a changeover from A_{II} to A_I. In the latter the propyl chain will extend along the main molecular direction, which favours the lipophilic character of this molecule, compared to epimer A_{II} and epimer B_I, which are more bulky. Therefore, the polarity of the surrounding media might discriminate between the two conformations A_I and A_{II} and the equilibrium 5 might be shifted to the left when the lipophilic character is increased. The extension of the alkyl chain along the molecule in epimer A_I favours a close contact between the lipophilic α-face of the steroid and the C_{18}-chain. In epimer B_I the contact area in the complex should be smaller, owing to the curved form of the molecule, (see Fig. 9). This would explain the larger retention for epimer A compared with epimer B (see Figs. 7 and 8). There is some analogy between the structural effect discussed and the effect of alkyl chain branching on retention for isomeric alcohols in reversed-phase systems. Alcohol isomers were studied by Karch *et al.*[34] and by Callmer *et al.*[47]. For instance, on μBondapak C_{18} with methanol–water (5:95) as mobile phase, the elution order for the isomeric alcohols was: tertiary, secondary, normal[47].

Horváth *et al.*[40] stated that the chromatographic process for a chemically bonded reversed phase is governed by solvophobic interactions in which the repulsion between the polar solvent molecules and the non-polar parts of the solute is of importance. They derived an expression for the capacity factor, k', which for closely related substances could be simplified to:

$$\ln k' = A'' + \frac{N}{RT} \Delta A \gamma \tag{7}$$

where A'' expresses the sum of different electrostatic effects and Van der Waals interactions between the solute and the C_{18}-ligand, γ is the surface tension of the eluent and N, R and T have their usual meanings. The contact area between the C_{18}-chain and the solute is designated by ΔA, which is a fraction of the hydrocarbon surface area (HCSA). For a homologous series, we therefore expect a linear relationship between $\log k'$ and the number of carbons in the alkyl chain at C-22, and this is shown in Fig. 10 for the 16α-hydroxyprednisolone derivatives in various eluents. It is seen that the slopes of the lines are different for the two epimers and that the slopes increase with increasing proportion of water in the eluent. Derivatization of eqn. 7 with respect to ΔA and insertion of the numerical constants yields

$$\frac{\mathrm{d} \log k'}{\mathrm{d}\Delta A} = \frac{\gamma}{940.7} \tag{8}$$

Derivative values calculated from eqn. 8 as well as the slopes obtained from Fig. 10, using the HCSA of 18.1 Å^2 for one methylene group[48], are given in Table II for the two epimers at different compositions of the eluent. Comparisons of the values indicate that for epimer A the contact area per methylene group varies from *ca.* 34

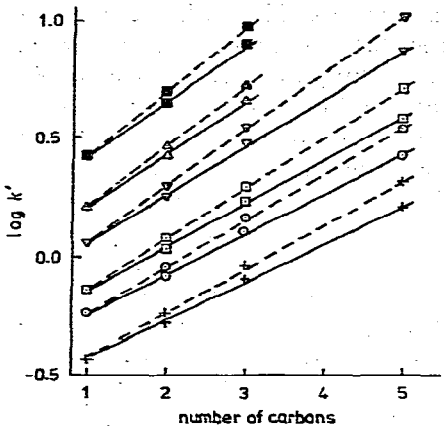


Fig. 10. Log k' plotted against the number of carbon atoms in the alkyl chain at chiral centre C-22 of epimers A (– – –) and B (———) of 16α-hydroxyprednisolone acetals. Support and flow-rate: See Fig. 1. Mobile phases: ethanol–water, (+) 60:40, (⊙) 56:44, (⊡) 52:48, (▽) 48:52, (△) 44:56, (■) 40:60).

TABLE II

DERIVATIVE VALUES CALCULATED FROM EQN. 8 COMPARED WITH SLOPES OF THE LINES FOR EPIMERS A AND B ESTIMATED FROM FIG. 10

Mobile phase, ethanol–water (v/v)	*d log k'/dΔA (Å⁻²)*	*Slope*, epimer A (Å⁻²)*	*Slope*, epimer B (Å⁻²)*
60:40	0.0292	0.0100	0.0086
56:44	0.0302	0.0109	0.0093
52:48	0.0311	0.0117	0.0100
48:52	0.0321	0.0132	0.0113
44:56	0.0330	0.0144	0.0122
40:60	0.0339	0.0156	0.0130

* A HCSA value of 18.1 Å² for one methylene group[48] was used in the calculations.

to 45% of the HCSA when the ethanol concentration is changed from 60 to 40%. Corresponding variation of the contact area of epimer B is 29.5 to 38.5% of the HCSA value. The higher values for epimer A are plausible if, as discussed above, there is a flipping over from epimer A_{II} in the mobile phase to epimer A_I, representing a larger area, in the stationary phase. The increase in the percentage contact areas for both epimers with decrease in the ethanol concentration may be a consequence of a diminishing HCSA, *e.g.* a folding of the alkyl chain, when the hydrophobic forces increase.

In Fig. 11, log k' is plotted against the number of carbons in the alkyl chain at C-22 for the 16α-hydroxyprednisolone and its 9α-fluoro and 6α,9α-difluoro derivatives. The increasing differences in retention with increasing alkyl chain length, resulting in various slopes for the lines of the two epimers, is clearly seen for all

derivatives. However, the slope of the line for each of the epimers A and B is constant irrespective of the substitutions of hydrogen for fluorine at 6α and 9α positions and also of the acetate esterification of the 21-hydroxy group. Introduction of fluorine in 6α and 6α,9α-positions might reduce the total HCSA, which results in smaller values of log k'. The figure also shows that the substitutions influence the two epimers slightly differently so that the separation factors are increased by 0.02 and 0.04 log units for the separation of 9α and 6α,9α derivatives, respectively. As the alkyl chain in none of the conformations of epimers A or B will sterically interfere with the acetate ester group, esterification at the C-21 hydroxyl does not influence the separation factors of epimers. However, a constant contribution to the lipophilic character of all epimers results in larger log k' values for the esters (see Fig. 11). The figure also shows that acetonides that correspond to the non-fluorinated, 9α-fluorinated and the 6α,9α-difluorinated 16α-hydroxyprednisolone derivatives fit into the straight lines of epimer A if a constant carbon number of 1.4 is taken. This would indicate that the acetonides conformationally behave more like epimer A than epimer B in the chromatographic system.

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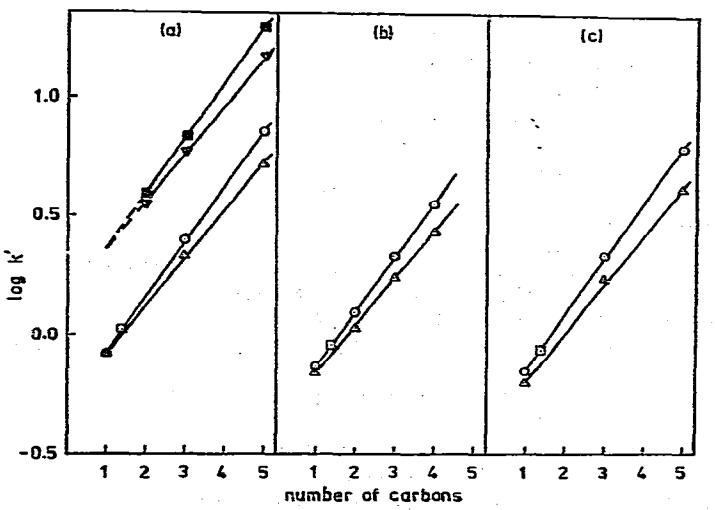

Fig. 11. Log k' plotted against the number of carbon atoms in the alkyl chain at C-22 of steroidal acetals and acetonides. Mobile phase: ethanol–water (50:50). Support and flow-rate: See Fig. 1. Solutes: (a) non-fluorinated, (b) 9α-fluoro- and (c) 6α,9α-difluoro-16α-hydroxyprednisolone acetals (△, ○, ▽, ■) or (□) acetonides. (△), (▽) and (○) (■) refer to epimers B and A, respectively, and (▽) (■) are 21-acetate esters.

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