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Short Communication

Separation of the enantiomers of some potassium channel activators using an α_1-acid glycoprotein column

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ABSTRACT

The separations of the enantiomers of some 3,4-dihydro-2,2'-dimethyl-2H-1-benzopyrans and a related tetrahydronaphthalene on α_1-acid glycoprotein (Chiral-AGP) are presented, together with the results from an investigation of the effects of organic modifier and pH on the separations achieved The general utility of Chiral-AGP in separating the enantiomers of compounds from this class of antihypertensive agents is demonstrated in this paper

INTRODUCTION

3,4-Dihydro-2,2-dimethyl-2H-1-benzopyrans such as **1–5** and the related tetrahydronaphthalene **6** are potent antihypertensive agents which relax smooth muscle by activating potassium channels [1,2] These compounds contain two chiral centres but the relative stereochemistry of these is fixed in the *trans* configuration, so that each compound exists as only one pair of enantiomers The biological activity of cromakalim **1** is known to reside principally in one enantiomer [3], and it is therefore important to have analytical methods which can distinguish between the enantiomers of **1** and also between the enantiomers of its analogues High-performance liquid chromatography (HPLC) methods have been reported previously for the separation of the enantiomers of similar compounds on a hexahelicene phase [4] and using chiral ion pairs [5] but the former method required the presence of a nitro group in the aryl group of the benzopyran for enantioselectivity and the latter required the presence of a basic nitrogen group in the molecule Neither method was general, therefore The separation of the enantiomers of **1** has also been reported by gas chromatography [6] This study describes the separation of the enantiomers of **1–6** by HPLC on α_1-acid glycoprotein (Chiral-AGP), a column which appears to separate the enantiomers of a wide range of compounds from this class and thus provides a method more general than any previously described

EXPERIMENTAL

Chemicals

The synthesis of compounds **1–6** has been described [3,7,8] Methanol and propan-2-ol were from Romil Chemicals (Loughborough, UK), other chemicals were from Aldrich (Gillingham, UK) So-

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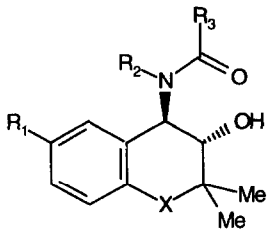

Compound	R_1	R_2	R_3	X
1	CN	-$(CH_2)_3$-		O
2	CN	-$(CH_2)_4$-		O
3	CN	H	Me	O
4	H	H	Me	O
5	CN	H	Ph	O
6	CN	H	Me	CH_2

dium phosphate buffers were prepared by dissolving the appropriate amount of sodium phosphate dibasic heptahydrate or sodium phosphate monobasic monohydrate in HPLC grade water to give 0 01 *M* solutions, adjusting to the required pH with concentrated orthophosphoric acid and filtering through a Millipore Durapore membrane (0 45 μm)

HPLC

All mobile phases were degassed with helium before use Chromatographic analyses were performed on a Waters 991 photodiode array system equipped with a Waters M600E pump and a Gilson 231/401 autosampler The primary detection wavelengths were 220 and 252 nm Analyses were carried out on a Chiral-AGP column (100 × 4 0 mm I D), supplied by Technicol (Stockport, UK) at a flow-rate of 0 9 ml/min Samples were prepared as 0 1 mg/ml solutions in eluent after prior dissolution in a small volume of methanol Injection volumes were 5 μl All analyses were performed at ambient temperature (approx 21°C) Capacity factors (k') for the peaks were calculated using the equation $k'_1 = (t_1 - t_0)/t_0$, where t_1 was the retention time of peak 1 and t_0 was the retention time of an unretained substance The latter was determined by injection of mobile phase with a slight difference in composition

RESULTS AND DISCUSSION

Compounds **1–6** were chromatographed using eluents of three different pH values and with varying percentages of either methanol or propan-2-ol as organic modifiers to determine the best separation conditions for each racemate Example chromatograms for **2** and **3** are shown in Fig 1 and the results for all the compounds are summarized in Tables I, II and III The data in Table I show that the k' values, and usually also the enantioselectivities (α), increase with a decrease in the percentage propan-2-ol Compound **5** was so well retained at 5% propan-2-ol that experiments below this modifier concentration were not performed It can be seen from the data in Table I that all the racemates in the series can be separated using an eluent consisting of propan-2-ol and phosphate buffer at pH 7, although the enantiomers of the two cyclic amides (**1** and **2**) were less well separated than those of the other compounds The increase in retention for the series **3**, **1**, **2**, **5** appeared to correlate with the expected order of hydrophobicities of these compounds, the aromatic ring in **5** produced a particularly marked increase in retention However, the enantioselectivities observed for these compounds did not mirror the trend in retention —it appears that processes giving rise to retention for these compounds do not necessarily also give rise to chiral discrimination This can also be seen from a comparison of the behaviours of **3**, **4** and **6** For this range of similar compounds, **3** was the least retained in all conditions tried, but it consistently gave the best enantioselectivity Such observations further illustrate the documented dependence of the stereoselectivity of the Chiral-AGP column on small structural changes in the solute molecules [9,10]

Variation of mobile phase pH (using 1% propan-2-ol as organic modifier) gave the results shown in Table II For all compounds, reducing the pH lead to decreased retention and for most of the compounds it also lead to decreased enantioselectivity However, for **2**, decreasing the pH from 4 7 to 3 *increased* the enantioselectivity observed, in marked contrast to the result for the very similar compound **1** The effect of pH was also investigated for **5**, using 5% propan-2-ol as the organic modifier At pH 3 there was a decrease in retention compared to that

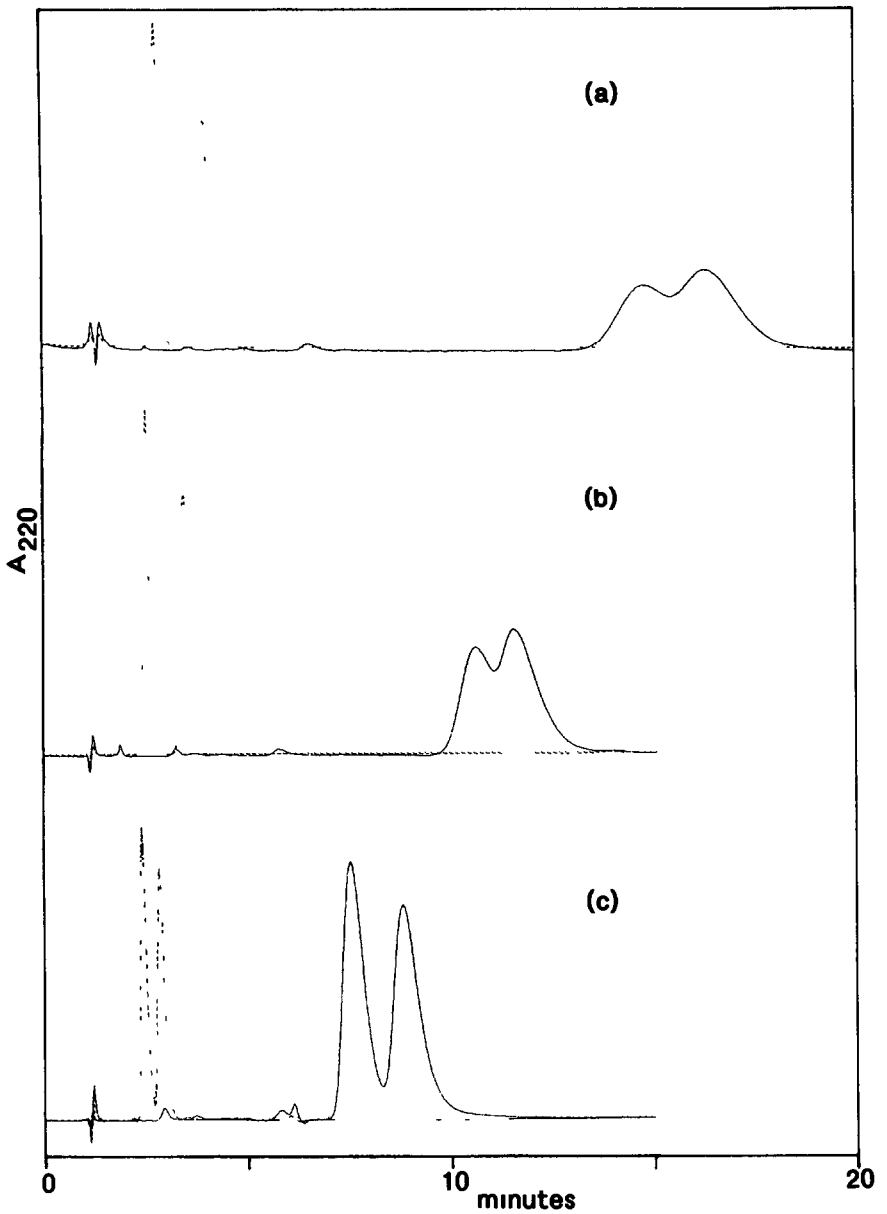

Fig 1 Example chromatograms for **2** (solid line) and **3** (broken line) with eluent conditions as follows (a) 1% propan-2-ol in aqueous phosphate buffer pH 7 0, (b) 1% propan-2-ol in aqueous phosphate buffer pH 4 7, (c) 1% propan-2-ol in aqueous phosphate buffer pH 3 0

observed at pH 7 (k'_2 = 6 35 at pH 3), but the decline in enantioselectivity was less marked (α = 1 29 at pH 3)

Changing the organic modifier to 10% methanol (Table III) produced, in most cases, approximately the same enantioselectivities as observed using 1% propan-2-ol, but the retention times were reduced, thus giving shorter analysis times Compound **2** was

TABLE I

INFLUENCE OF CONCENTRATION OF PROPAN-2-OL ON THE RETENTION AND ENANTIOSELECTIVITY FOR **1–6**

Column, Chiral-AGP (100 × 4 mm I D), mobile phase, 0 01 *M* phosphate buffer (pH 7 0) containing different concentrations of propan-2-ol, flow-rate 0 9 ml/min

Compound	5% Propan-2-ol		2 5% Propan-2-ol		1% Propan-2-ol	
	k'_2	α	k'_2	α	k'_2	α
1	0 82	1 00	1 86	1 10	4 08	1 20
2	1 59	1 00	4 42	1 14	10 66	1 11
3	0 45	1 19	0 95	1 51	1 86	1 84
4	0 75	1 19	1 96	1 33	4 69	1 49
5	10 32	1 34	NP[a]		NP[a]	
6	0 78	1 00	1 60	1 11	3 16	1 29

[a] NP = Experiment not performed

again an exception, the enantioselectivity observed for this compound was considerably reduced with methanol, except at pH 3 Again this contrasted with the behaviour of **1**, for which 10% methanol gave *improved* enantioselectivities, except at pH 3

From the results, it can be concluded that Chiral-AGP appears to be very useful column for the separation of the enantiomers of these antihypertensive agents and that their separations can be usefully manipulated by changing the eluent pH or the organic modifier A mobile phase pH of around 7 gives the best chance of obtaining a separation and either propan-2-ol or methanol can be used as the organic modifier, with methanol usually being preferred for the shorter analysis times it can provide (for the same selectivity) The series of compounds described in this study has shown some interesting contrasts in chromatographic behaviour and this aspect will be further investigated in the near future

TABLE II

INFLUENCE OF BUFFER pH ON THE RETENTION AND ENANTIOSELECTIVITIES FOR **1–6** WITH PROPAN-2-OL AS ORGANIC MODIFIER

Conditions as in Table I, with 1% propan-2-ol used as the organic modifier and variation of buffer pH

Compound	pH 7		pH 4 7		pH 3	
	k'_2	α	k'_2	α	k'_2	α
1	4 08	1 20	3 89	1 12	3 21	1 00
2	10 66	1 11	9 00	1 10	6 93	1 20
3	1 86	1 84	1 98	1 70	1 66	1 33
4	4 69	1 49	4 05	1 23	3 22	1 00
5	Experiment not performed					
6	3 16	1 29	3 10	1 17	3 00	1 06

TABLE III

INFLUENCE OF BUFFER pH ON THE RETENTION AND ENANTIOSELECTIVITIES FOR **1–6** WITH METHANOL AS ORGANIC MODIFIER

Conditions as in Table II, with methanol in place of propan-2-ol

Compound	pH 7		pH 4 7		pH 3	
	k'_2	α	k'_2	α	k'_2	α
1	3 03	1 25	2 37	1 20	2 09	1 00
2	7 1	1 00	4 93	1 00	4 47	1 15
3	1 56	1 79	1 33	1 80	1 24	1 37
4	3 88	1 41	2 97	1 30	2 55	1 10
5	Experiment not performed					
6	2 46	1 34	2 06	1 31	2 02	1 14

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