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IDENTIFICATION OF 9α-FLUORINATED HOMOLOGUES OF HYDROCORTISONE AND PREDNISOLONE BY THIN-LAYER CHROMATOGRAPHY ON INSOLUBLE POLYVINYL-PYRROLIDONE

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SUMMARY

TLC on insoluble crosslinked polyvinyl-pyrrolidone and with dilute HCl solution as the developing solvent has been used successfully to separate the four related synthetic anti-inflammatory corticosteroids, hydrocortisone, prednisolone, 9α-fluorohydrocortisone and 9α-fluoroprednisolone, which differ in one fluorine atom and/or one double bond in position 1. The method is applicable to the identification of the fluorinated analogues in the presence of their respective unfluorinated alcohols merely by comparing their relative migration characteristics. The experimental ΔR_M values found for the 9αF atom are around +0.60.

INTRODUCTION

The characterisation of fluorine atoms in synthetic anti-inflammatory corticosteroids is a problem arising in the pharmaceutical practice for which chromatographic methods are being used. A good example is the identification of the 9α-fluorinated analogues of *hydrocortisone* (11β,17α,21-trihydroxypregn-4-ene-3,20-dione) and *prednisolone* (11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione) in the presence of the unfluorinated alcohols.

The halogen atom in such compounds does not appear to have a decided discriminative character when compared with other more polar functional groups (*i.e.* hydroxy or keto groups). The available literature showed that there was difficulty in obtaining ΔR_M (9αF) values greater than 0.25 by both partition and adsorption methods (see refs. 1–3 for PC methods; refs. 4–6 for TLC and ref. 7 for CC).

As it would be desirable to obtain higher and more specific values for the 9αF group which would be suitable for use as a rapid additional criterion for identifying the analogues mentioned above, we carried out some tests using various techniques, adsorbents and solvents common in TLC; in the systems tested, the mobilities of the analogues obtained under our experimental conditions were too close for such a purpose[8].

Eventually, after using a new chromatographic substrate and diluted aqueous

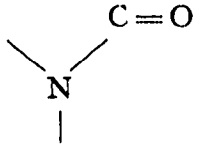
acidic developing systems, we succeeded in obtaining satisfactory results for the two pairs of the analogues, and we are endeavouring to extend this system to include others.

MATERIALS AND METHODS

Adsorbent

A commercially available product named *Polyclar AT**, which is a type of internally crosslinked polyvinyl-pyrrolidone, was used. It is insoluble in water, organic solvents, acids and alkalis, and can be heated to 150° in conc. H_2SO_4 without decomposition[9]. The polymer is supplied in 95% active form containing about 5% water.

This material is widely employed industrially for the adsorption of polyphenols in beer clarification processes; it has been used recently for the TLC of such polyphenols[10,11]. The active adsorbent site of the polymer is reported to be a strong proton acceptor amide group[12]:



The commercial product was ground in an "Alpine Kolloplex" mill and the resultant powder was screened to obtain a fraction of particles smaller than 40 μ. This fraction was used directly to prepare the TLC plates. For the uses indicated here, the material does not require any supplementary manipulation; mean particle sizes of insoluble PVP ranging between 30 and 60 μ have also been found to be suitable for the TLC of the steroids mentioned.

Preparation of the plates

Of the powder, 8 g were dispersed in 40 ml of isopropanol and the resultant suspension was distributed on 20 × 20 cm glass plates. A layer thickness of 250 μ was obtained by means of the "Stratomat Chemetron" automatic apparatus. The product shows a good adherence to glass and the stability of the layer is very good; it is considered intermediate between that of silica gel and cellulose. A descriptive report of these details is given in a previous paper[8].

Samples

Solutions (0.2%) of pure corticosteroids in methanol–chloroform (equal volumes) were used. The solutions were placed on the plates in a "Camag Template" with the help of a "Link" micropipette of 2 μl capacity (equivalent to 4 μg of steroid).

Solvent

A solution of 10 parts of HCl (d = 1.19) and 90 parts of water (v/v) was used. This solvent results in demixing and the β-front can be seen without any difficulty under UV light.

* Brand of insoluble polyvinyl-pyrrolidone ("polyvinyl-polypyrrolidone"), a product supplied by General Aniline & Film Corp., New York.

Development

Development was carried out in 21 × 21 × 9 cm "Desaga" chambers in the usual way, at a temperature of 20° (± 0.5°). The chamber was equilibrated with the solvent for 24 h by lining the side walls with filter papers soaked in solvent. The height of the solvent in the chamber was about 1.2 cm and the degree of inclination of the plates was about 110°. The total development time was 4 h 30 min, with a length of run of about 17.5 cm. The chromatograms were air-dried at room temperature.

If a place for reference notes was required, a straight line was traced with a hard pencil 3 cm from and parallel to the upper edge of the plate before allowing it to develop, the chromatogram being removed after 4 h 30 min; in these cases, the α-front was not visible and the β-front was situated somewhat lower than in a normal development.

Detection of the spots

A solution of 0.5% tetrazolium blue in 60% methanol (1 vol.) was mixed with a solution of 20% NaOH in 60% methanol (2 vol.). After spraying the plates with the above reagent, blue-violet spots appeared on an almost white background.

TABLE I

MEAN R_F VALUES AND OTHER CHROMATOGRAPHIC VALUES FOR 4 HYDROCORTISONE DERIVATIVES ON A PVP SUBSTRATE USING HCl (d = 1.19)–WATER (1:9) AS THE ELUENT

Values are referred to the second front of the solvent (β-front) for a development of 4 h 30 min.

No.	*Corticosteroid*	*Mean R_F*	*Standard deviation (σ)*	*Mean R_M*	*$\Delta R_{M(9\alpha F)}$ 1–2 3–4*	*$\Delta R_{M(\Delta^1)}$ 1– 2–*
(A) Development of plates with α-front. Each sample spotted alone at the points of application						
1	Hydrocortisone	0.759	0.01	−0.499		
					+0.632	+0.321
2	9α-Fluorohydrocortisone	0.424	0.01	+0.133		
3	Prednisolone	0.601	0.01	−0.178		
					+0.610	+0.299
4	9α-Fluoroprednisolone	0.270	0.009	+0.432		
(B) Development of plates with α-front. The 4 samples spotted as a mixture						
1	Hydrocortisone	0.759	0.01	−0.499		
					+0.626	+0.329
2	9α-Fluorohydrocortisone	0.427	0.007	+0.127		
3	Prednisolone	0.597	0.009	−0.170		
					+0.604	+0.307
4	9α-Fluoroprednisolone	0.269	0.008	+0.434		
(C) Development without α-front. The 4 samples spotted as a mixture						
1	Hydrocortisone	0.753	0.01	−0.484		
					+0.605	+0.318
2	9α-Fluorohydrocortisone	0.429	0.01	+0.121		
3	Prednisolone	0.592	0.01	−0.166		
					+0.596	+0.309
4	9α-Fluoroprednisolone	0.271	0.01	+0.430		

R_F calculations

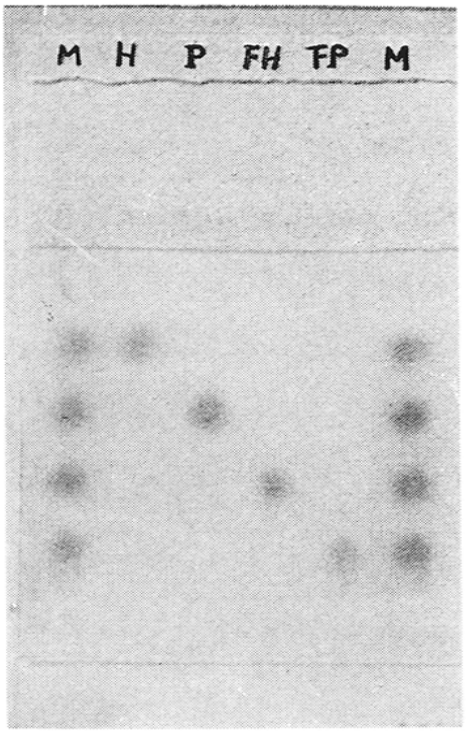
Since the value of k was about 0.7 in the chromatographic solvent system used[13], the mobility of the solutes was always referred to the β-front, which was situated about 12.5 cm from the starting line in an ordinary run and about 10 cm if the development was carried out without the α-front. The standard deviation of the mean values was calculated statistically.

RESULTS AND DISCUSSION

The results are given in Table I. The R_F values are the means of 24 chromatograms on different plates (one of the 25 was not considered) for the A and B series, and of 15 chromatograms for the C series. As can be seen from the values reported, the reproducibility in our experimental conditions is quite good.

The spots are circular, very well defined, without tailing and are about 5–8 mm in diameter.

The ΔR_M values obtained for the 9αF group are of the same order for both pairs of analogues and are quite large (around +0.60). The ΔR_M values obtained for the



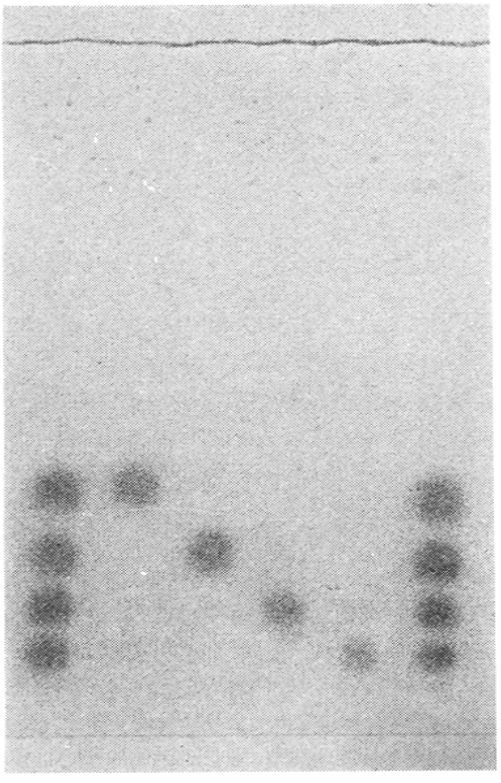


Fig. 1. A representative chromatogram showing the behaviour of the four synthetic corticosteroids hydrocortisone (H), prednisolone (P), 9α-fluorohydrocortisone (FH), and 9α-fluoroprednisolone (FP) alone and as a mixture (M), on insoluble PVP layers using the solvent system HCl (d = 1.19)–water (10:90) at 20 °. The length of the development was 16.0 cm with a β-front at about 11.4 cm.

Fig. 2. A chromatogram developed with distilled water as solvent on an insoluble PVP layer, showing a rather good separation of the four corticosteroids tested, placed on the plate in the same order as in Fig. 1. The length of the development was about 16.0 cm and the temperature 20 °.

1-double bond, although less, are also appreciable (around +0.30) considering the results reported in the literature, and are also of the same order for the two pairs of analogues. The ΔR_M values are sufficiently great to allow identification of any of the substances in the presence of others. However, the substitution by the 9αF atom is particularly discriminative.

Fig. 1 shows a typical chromatogram. In other aqueous systems—both neutral, acidic or alkaline—the corticosteroids tested behaved similarly, although the R_F values were generally lower (Fig. 2). In the usual "adsorption" and "partition" solvent systems containing proton-donor mobile phases, good spots can be obtained and fair to good separations can also be achieved for the 9αF derivatives with respect to the unfluorinated alcohols. The mechanisms involved in the migration of 11β-hydroxycorticosteroids on insoluble polyvinyl-pyrrolidone plates seem to be rather particular ones, worthy of further investigation; a preliminary discussion on this subject has been published[8].

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