Journal of Chromatography, 318 (1985) 373–377
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17,270

Note

Thin-layer chromatography of ecdysteroids

I. D. WILSON
Department of Drug Metabolism, Hoechst Pharmaceutical Research Laboratories, Walton Manor, Walton, Milton Keynes, Buckinghamshire MK7 7AJ (U.K.)

(Received September 24th, 1984)

In previous studies[1,2] the reversed-phase thin-layer chromatography (RP-TLC) of the ecdysteroids (insect moulting hormones) was investigated using C_2, C_8, C_{12} and C_{18} alkyl-bonded and paraffin-coated silica gel TLC plates. In this paper results from further studies on the TLC of the ecdysteroids undertaken using both normal- and reversed-phase TLC systems are described. Normal-phase (NP) TLC was performed on silica gel and aminopropyl bonded silica gel, whilst RP-TLC was investigated on C_8, C_{18}, aminopropyl bonded and paraffin-coated silica gel RP-TLC plates.

EXPERIMENTAL

Ecdysteroids were gifts from a number of sources. NP-TLC studies with silica gel as adsorbent were performed on 20 × 20 cm glass-backed TLC plates (Macherey Nagel and Co., Camlab, Cambridge, U.K.). Merck C_8, C_{18} and aminopropyl bonded TLC plates were a gift from BDH (Poole, U.K.) and were used as supplied. Paraffin-coated RP-TLC plates were prepared by coating the Macherey Nagel and Co. glass-backed silica gel TLC plates with a solution of heavy refined paraffin (Nujol 7.5%, v/v) in dichloromethane as described elsewhere[2]. All the TLC plates incorporated a fluorescence indicator (254 nm).

Chromatography was performed in glass chromatography tanks 25 × 25 × 5 cm. For NP-TLC the tanks were presaturated with the solvent before use.

Samples were applied as solutions in methanol (approximately 1 mg ml^{-1}) using 1-μl glass capillaries.

Following chromatography ecdysteroids were visualised by fluorescence quenching.

RESULTS AND DISCUSSION

In these investigations the chromatographic properties of NP- and RP-TLC systems were studied for ecdysone and 20-hydroxyecdysone for a range of solvent compositions. In addition the R_F values of 26 ecdysteroids were obtained in selected chromatographic systems.

0021-9673/85/$03.30

NP-TLC of ecdysteroids

NP-chromatography on silica gel is an important technique in ecdysteroid research and a large number of solvent systems have been described (reviewed by Morgan and Poole[3]). Many chloroform–ethanol based solvent systems have been used for the ecdysteroids and therefore the effect of ethanol concentration on the R_F of ecdysone and 20-hydroxyecdysone was investigated. For solvent systems containing between 20 to 40% ethanol the R_F values of ecdysone and 20-hydroxyecdysone varied between 0.21 and 0.15 to 0.63 and 0.56 respectively. However, the separation between the two compounds remained almost constant at 0.06 to 0.07 of an R_F unit. There is therefore, considerable scope for adjusting the R_F of these compounds if necessary whilst maintaining resolution. Above 40% ethanol in the solvent the separation rapidly decreased (Fig. 1).

One of the most commonly used solvent systems for ecdysteroid chromatography on silica gel is chloroform–ethanol (4:1, v/v). The R_F values for the 26 ecdysteroids in our collection were therefore obtained using this mobile phase and are given in Table I.

NP-TLC of ecdysone and 20-hydroxyecdysone was also investigated on aminopropyl bonded silica gel with chloroform–ethanol solvent systems. A comparison of the results obtained for the aminopropyl bonded TLC plates with ordinary silica gel showed that for the ecdysteroids there is little difference between them. These results are illustrated in Fig. 1.

RP-TLC of ecdysteroids

Variations in the R_F values of ecdysone and 20-hydroxyecdysone with methanol concentration in methanol–water based solvent systems were investigated for RP-TLC on C_8, C_{18}, aminopropyl and paraffin-coated silica gel TLC plates. The chromatographic properties of the C_8 and C_{18} bonded RP-TLC plates were found to be very similar, although the latter gave slightly better resolution. In previous

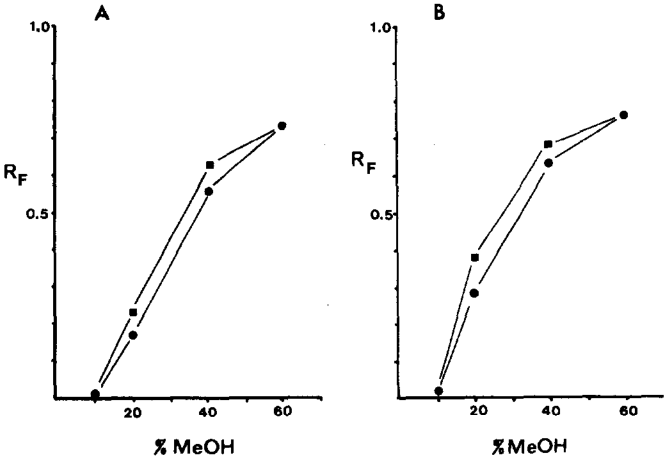

Fig. 1. Effect of methanol concentration on the R_F of ecdysone (■) and 20-hydroxyecdysone (●) in chloroform–methanol based solvent systems for NP-TLC on silica gel (A) and aminopropyl bonded silica gel (B) TLC plates.

TABLE I

R_F AND $R_{ecdysone}$ VALUES OBTAINED FOR ECDYSTEROIDS* BY NP-TLC ON SILICA GEL TLC PLATES, USING CHLOROFORM–ETHANOL (4:1)

Compound	R_F	$R_{ecdysone}$	*Compound*	R_F	$R_{ecdysone}$
Poststerone	0.32	152	22-Isoecdysone	0.13	62
2-Deoxyecdysone	0.38	180	Calonysterone	0.42	200
Ecdysone	0.21	100	Pterosterone	0.32	152
20-Hydroxyecdysone	0.15	71	Kaladasterone	0.49	233
Muristerone A	0.27	129	Pinnasterol	0.56	267
Dacrysterone	0.27	129	20-Hydroxyecdysone 2-cinnamate	0.53	252
2-Deoxy-20-hydroxyecdysone	0.31	141	Polypodine B 2-cinnamate	0.56	267
Makisterone A	0.20	95	Acetylpinnasterol	0.68	324
Polypodine B	0.22	104	Ponasterone C	0.38	181
Inokosterone	0.17	77	Ponasterone C 2-Cinnamate	0.65	310
2-Deoxy-3-epiecdysone	0.44	209	Ponasterone A	0.42	200
Ajugasterone C	0.22	104	Carpesterol	0.86	410
Cyasterone	0.33	157			

* Structures as in ref. 4.

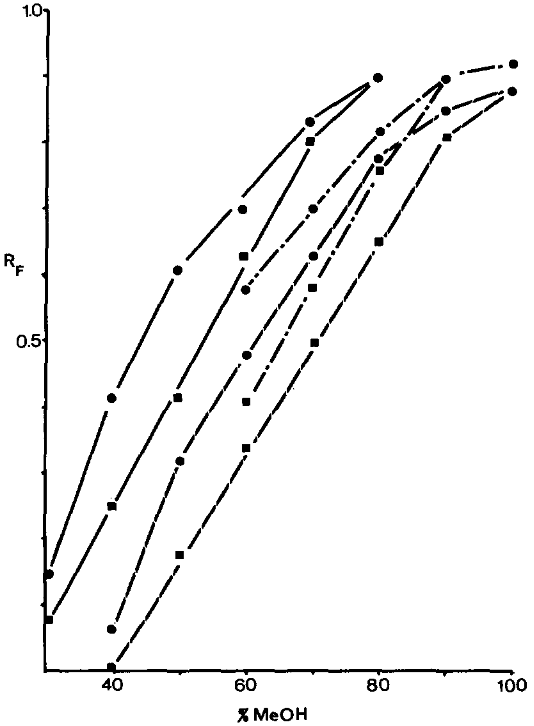

Fig. 2. Effect of methanol concentration on the R_F of ecdysone (■) and 20-hydroxyecdysone (●) in methanol–water based solvent systems for RP-TLC on paraffin-coated (——), C_{18} bonded HP-TLC (—·—) and C_{18} bonded TLC (— —) plates.

studies[1] on C_{18} bonded RP-HPTLC plates from the same manufacturer the range of solvent systems which could be used was limited by the hydrophobic properties of the adsorbent to those containing less than 40% water. The C_{18} bonded TLC plates examined in this study were not subject to this limitation and could be used with mobile phases containing from 0 to 100% of water. Further, whilst the use of solvent systems containing 40% water with the hydrophobic C_{18} bonded plates required long development times (1–2 h) the non-hydrophobic C_{18} RP-TLC plates developed rapidly with the solvent migrating at an average speed of 0.3 cm min^{-1} irrespective of mobile phase composition. The relationship between R_F and solvent composition for both types of C_{18} bonded plate is illustrated in Fig. 2.

In an earlier study[2] we examined the use of a non-hydrophobic C_{12} bonded TLC plate for the RP-TLC of the ecdysteroids. A comparison of the results for the C_{12} and C_{18} bonded non-hydrophobic TLC plates shows that the latter gives better

TABLE II

R_F AND $R_{ecdysone}$ VALUES OBTAINED FOR ECDYSTEROIDS BY RP-TLC ON C_{18} BONDED AND PARAFFIN-COATED SILICA GEL TLC PLATES

Compound	*C_{18} bonded (methanol–water, 65:35)*		*Paraffin-coated (methanol–water, 50:50)*	
	R_F	$R_{ecdysone}$	R_F	$R_{ecdysone}$
Poststerone	0.42	120	0.45	128
Ecdysone	0.35	100	0.35	100
20-Hydroxyecdysone	0.47	134	0.49	140
Muristerone A	0.37	106	0.37	106
Dacrysterone	0.39	111	0.37	106
2-Deoxy-20-hydroxyecdysone	0.28	80	0.29	83
Makisterone A	0.40	114	0.38	109
Polypodine B	0.47	134	0.50	143
Inokosterone	0.47	134	0.49	140
2-Deoxy-3-epiecdysone	0.15	43	0.23	66
Ajugasterone C	0.36	103	0.50	143
Cyasterone	0.44	126	0.59	169
22-Isoecdysone	0.16	46	0.11	31
Calonysterone	0.28	80	0.32	91
Pterosterone	0.37	106	0.36	103
Kaladasterone	0.26	74	0.28	80
Pinnasterol	0.10	28	0.10	29
20-Hydroxyecdysone 2-cinnamate	0	0	0	0
Polypodine B 2-cinnamate	0	0	0	0
Acetyl pinnasterol	0	0	0	0
Ponasterone C	0.33	94	0.34	97
Ponasterone C 2-cinnamate	0	0	0	0
Ponasterone A	0.19	54	0.21	60
Carpesterol	0	0	0	0
2-Deoxyecdysone	0.19	54	0.12	60

resolution of ecdysone and 20-hydroxyecdysone and a greater spread of R_F values (from 0 to 0.88 as opposed to 0 to 0.55).

The effect of the methanol concentration of the solvent on the R_F values of ecdysone and 20-hydroxyecdysone on paraffin-coated plates is also illustrated in Fig. 2. Compared to the C_{18} bonded plates the paraffin-coated plates required rather less organic modifier in the mobile phase to achieve the same R_F. Like the non-hydrophobic C_{18} bonded plates the paraffin-coated plates could be used with solvent systems containing from 0 to 100% water.

The R_F values obtained for the 26 ecdysteroids on the non-hydrophobic C_{18} bonded and paraffin-coated RP-TLC plates are given in Table II. When the results were compared the selectivities of the two types of plate were remarkably similar. Therefore, with the appropriate adjustment of the mobile phase the two types of plate are equivalent.

The aminopropyl bonded TLC plates proved to be unsuitable for the RP-TLC of ecdysone and 20-hydroxyecdysone, both of which chromatographed with the solvent front under all the conditions tested.

CONCLUSIONS

The combination of NP- and RP-TLC systems provides considerable scope for the separation of this class of compounds. However, the resolution of some ecdysteroids by TLC remains difficult. For example ponasterone A and 2-deoxyecdysone have identical chromatographic properties in both normal- and reversed-phase systems. In such cases the techniques of high-performance liquid[5,6] or gas–liquid chromatography[7] may be more appropriate. Despite such limitations TLC provides a simple and convenient method for the separation of ecdysteroids.

Of the systems examined in this and other studies[1,2], NP-TLC on unmodified silica gel, and RP-TLC on either C_{18} bonded or paraffin-coated silica appear to be the most suitable for ecdysteroid TLC. Compared with the C_{18} bonded RP-HPTLC plates used in our earlier study[1] the C_{18} bonded TLC plates used here have a number of advantages including the extended range of solvent systems possible and the high rate of migration of the solvent for all mobile phase compositions. Similar advantages are provided by the paraffin-coated plates, and for most applications, the two types of RP-TLC plate should be interchangeable (with appropriate modifications to the mobile phase).

REFERENCES

1 I. D. Wilson, S. Scalia and E. D. Morgan, *J. Chromatogr.*, 212 (1981) 211.

2 I. D. Wilson, C. R. Bielby and E. D. Morgan, *J. Chromatogr.*, 242 (1982) 202.

3 E. D. Morgan and C. F. Poole, in J. E. Treherne, M. J. Berridge and V. B. Wigglesworth (Editors), *Advances in Insect Physiology 12*, Academic Press, London, New York, 1976, p. 17.

4 C. Hetru and D. H. S. Horn, in J. A. Hoffmann (Editor), *Progress in Ecdysone Research*, Elsevier/North Holland Biomedical Press, Amsterdam, 1980, p. 13.

5 R. Lafont, G. Somme-Martin, B. Mauchamp, B. F. Maume and J.-P. Delbecque, in J. A. Hoffmann (Editor), *Progress in Ecdysone Research*, Elsevier/North Holland Biomedical Press, Amsterdam, 1980, p. 45.

6 I. D. Wilson, C. R. Bielby and E. D. Morgan, *J. Chromatogr.*, 238 (1982) 97.

7 E. D. Morgan and I. D. Wilson, in J. A. Hoffmann (Editor),*Progress in Ecdysone Research*, Elsevier/North Holland Biomedical Press, Amsterdam, 1980, p. 29.