Steroids

XXIX. Separation and characterization of various classes of steroids by thin layer chromatography*

Thin-layer chromatography is becoming increasingly popular in the separation and characterization of steroids[2-4] but so far no published paper has reported the use of a single solvent system or two solvent systems which have been found to be satisfactory in the resolution of a large variety of steroids.

In this paper we report two solvent systems which have been constantly used in our laboratory for the detection and characterization of synthetic steroids. The solvent systems consisted of mixtures of benzene, methanol and ethyl acetate or chloroform, methanol and ammonia. The steroids reported range from simple cholestane and androstane series to complex heterocyclic steroids. Each of these compounds has been synthesized in our laboratory.

At least one of these solvent systems has always been found to be satisfactory for each steroid investigated. Neither of these solvent systems has been reported in the literature in connection with the separation of steroids. The second of these systems has been used by one of us[5] previously in the separation of pyrrolizidine alkaloids and is remarkably suitable for the polar aza-steroids.

Experimental

Reagents. All reagents used were of analytical grade. The chloroform used was "Baker Analyzed Reagent Spectrophotometric, grade" and was used as such. Other solvents were further purified by passing them through basic aluminum oxide, activity I (E. Merck).

Steroids. Those used in this investigation are listed with their chemical names in Tables I and II.

Method and results

(a) All chromatograms were run at room temperature on Silica Gel G (E. Merck). The plates were activated at 110° for one hour and stored in a desiccator over calcium chloride.

(b) The spreader used for coating the plates was manufactured by Desaga-Brinkman, U.S.A. The plates, 200 × 200 × 3.7 mm, were coated to 0.25 mm thickness.

(c) Solvent systems: (1) Benzene–methanol–ethyl acetate (85 : 10 : 5)
(2) Chloroform–methanol–ammonia (85 : 14 : 1)

Solutions of the steroid samples were made in chloroform or methanol and applied at the rate of 50–100 μg of steroid as a spot on a TLC plate. The spotted plate was developed in a Desaga (Heidelberg, Germany) jar in solvent systems No. 1 or No. 2. To ensure a saturated atmosphere the jar was lined with a filter paper (Whatman No. 1) at least 45 min before the development of the plates. The steroid samples were detected from the developed chromatogram by iodine vapors.

Each experiment was conducted by running a reference standard of 5-androsten-3β-ol-17-one along with other authentic samples. Tables I and II contain the R_F,

* For Part XXVIII, see ref. 1.

R_S, and R_M values of the steroid samples studied in solvent systems 1 and 2, respectively. These values are reported for reference and to illustrate the usefulness of these solvent systems.

TABLE I

THIN-LAYER CHROMATOGRAPHY IN BENZENE–METHANOL–ETHYL ACETATE

Systematic name	R_F *value*	R_S *value*	R_M *value*
17α-Methyl-3,5-seco-4-nor-androstan-17β-ol-5-on-3-oic acid	0.48	1.09	0.033
N-Benzyl-3,5-seco-4-norcholestan-5β-ol-3-amide	0.50	1.25	0.000
4-Oxa-5α-cholestan-3-one	0.69	1.57	—0.347
4-Benzyl-4-aza-5-cholesten-3-one	0.68	1.55	—0.328
4,17α-Dimethyl-4-aza-5-androsten-17β-ol-3-one	0.27	0.61	—0.431
4-(β-Hydroxyethyl)-4-aza-5-cholesten-3-one	0.44	1.00	0.104
4-Phenyl-4-aza-5-cholesten-3-one	0.66	1.50	—0.276
4-Methyl-4-aza-5-cholesten-3-one	0.71	1.61	—0.388
4-Oxa-5α-pregnane-3,20-dione	0.57	1.29	—0.125
17α-Methyl-4-aza-5-androsten-17β-ol-3-one	0.16	0.36	0.280
4-Aza-5-cholesten-3-one	0.52	1.18	—0.022
4-Methyl-4-aza-5-pregnene-3,20-dione	0.45	1.02	0.086
4-Methyl-4-aza-5-cholestene	0.81	1.84	—0.569
4β,5β-Epoxypregnane-3,20-dione	0.68	1.49	—0.328
4α,5α-Epoxypregnane-3,20-dione	0.71	1.61	—0.388
4-Aza-5-pregnene-3,20-dione	0.42	0.95	0.170
3,5-Seco-4-norpregnan-20β-ol-5-on-3-oic acid	0.47	1.07	0.053
17α-Methyl-4-oxa-5-androsten-17β-ol-3-one acetate	0.64	1.45	—0.252
17α-Methyl-4-aza-5α-androstan-17β-ol-3-one	0.11	0.25	0.911
3-Aza-A-homo-5α-cholestan-4-one	0.29	0.66	0.389
4-Methyl-4-aza-5-pregnen-20β-ol-3-one	0.29	0.66	0.389
3,5-Seco-4-norcholestane-3,5β-diol	0.26	0.59	0.453
3-Aza-A-homo-5β-cholestan-4-one	0.37	0.84	0.230
2′-Aminothiazolo[*d*-3,2]-5α-cholest-2-ene	0.43	0.98	0.120
4-(β-Hydroxyethyl)-4-aza-5-pregnen-20β-ol-3-one	0.14	0.32	0.789
3β-Acetoxy-6-aza-B-homo-5α-cholestan-7-one	0.50	1.13	0.000
N-(β-Hydroxyethyl)-3,5-seco-4-norcholestan-5β-ol-3-amide	0.59	0.66	0.387
4-(Dimethylaminoethyl)-17α-methyl-4-aza-5-androsten-17β-ol-3-one	0.16	0.36	0.720
Thiazolo[*d*-3,2]-5α-cholest-2-ene	0.61	1.39	—0.194
4-Oxa-5α-cholestane	0.73	1.66	—0.432
6-Acetyl-6-aza-B-homo-5α-cholestan-3β-ol acetate	0.60	1.36	—0.180
Indolo[*b*-3,4]-5β-cholest-3-ene	0.70	1.59	—0.366
3,5-Cholestadien-7-one	0.33	0.75	0.307
Quinoxalino[*b*-2,3]-5α-cholestane	0.67	1.52	—0.310
2′-Aminothiazolo[*d*-3,2]-17α-methyl-5α-androst-2-en-17β-ol	0.28	0.64	0.389
1′-Nitrosoindolo[*b*-3,2]-5α-cholest-2-ene	0.67	1.52	0.310
g-Benzoquinoxalino[*b*-2,3]-5α-cholestane	0.71	1.61	—0.387
1′-Nitrosoindolo[*b*-3,4]-5β-cholest-3-ene	0.63	1.43	—0.236
1′-Aminoindolo[*b*-3,2]-5α-cholest-2-ene	0.64	1.45	—0.251
6-Methyl-6-aza-B-homo-5α-cholestan-3β-ol	0.41	0.93	0.158
6-Methyl-6-aza-B-homo-5α-cholestan-3β-ol methiodide	0.46	1.14	0.072
3,5-Seco-4-norandrostan-17β-ol-5-on-3-oic acid	0.23	0.52	0.550
1′-Methylindolo[*b*-3,2]-5α-cholest-2-ene	0.51	1.16	—0.018
1′-Methylindolo[*b*-3,4]-5β-cholest-3-ene	0.62	1.41	—0.215
7a-Aza-B-homo-5α-cholestan-3α-ol-7-one acetate	0.54	1.23	—0.076
4-Methyl-4-aza-5α-cholestan-3-one	0.57	1.30	—0.125
4-Methyl-3-phenyl-4-aza-2,5-cholestadiene	0.62	1.41	—0.208
3-Ethyl-4-methyl-4-aza-2,5-cholestadiene	0.65	1.48	—0.268
2,3-Seco-5α-cholestane-2,3-diol	0.33	0.75	0.332
B-Nor-3,5-cholestadiene	0.66	1.50	—0.276

TABLE II

THIN-LAYER CHROMATOGRAPHY IN CHLOROFORM–METHANOL–AMMONIA

Systematic name	R_F *value*	R_S *value*	R_M *value*
3α-Amino-5α-cholestane hydrochloride	0.93	1.16	—1.154
N-Benzyl-3,5-seco-4-norcholestan-5β-ol-3-amide	0.84	1.05	—0.924
4-(β-Hydroxyethyl)-4-aza-5-cholesten-3-one	0.77	0.96	—0.523
17α-Methyl-4-aza-5-androsten-17β-ol-3-one	0.71	0.89	—0.387
4α,5α-Epoxypregnane-3,20-dione	0.90	1.12	—0.959
4-Benzyl-17α-methyl-4-aza-5-androsten-17β-ol	0.79	0.99	—0.568
17β-Hydroxy-4-(β-hydroxyethyl)-17α-methyl-4-aza-5-androstene	0.51	0.64	—0.018
17α-Methyl-4-aza-5α-androstan-17β-ol-3-one	0.67	0.84	—0.310
N,N-Bis-(β-chloroethyl)-3β-amino-5α-cholestane hydrochloride	0.32	0.40	0.326
N-(β-Hydroxyethyl)-3,5-seco-4-norcholestan-5β-ol-3-amide	0.62	0.77	—0.215
6-Methyl-6-aza-B-homo-5α-cholestan-3β-ol methiodide	0.20	0.25	0.602
4-(Dimethylaminoethyl)-4-aza-5-pregnene-3,20-dione methiodide	0.20	0.25	0.602

The R_M values have been calculated according to the definition given by BATE-SMITH and WESTALL[6].

$$R_M = \log\left(\frac{1}{R_F} - 1\right)$$

The R_S value of each steroid is calculated as:

$$\frac{\text{Distance from starting point to the center of the spot}}{\text{Distance from starting point to the reference substance}}$$

As can be seen from Tables I and II, this study has the following interesting features:

(1) All of the steroids studied have been efficiently chromatographed either by solvent No. 1 or solvent No. 2.

(2) Separation of 4β,5β-epoxypregnane-3,20-dione and 4α,5α-epoxypregnane-3,20-dione has been accomplished by solvent No. 1.

(3) Strongly polar aza-steroids such as 4-benzyl-17α-methyl-4-aza-5-androsten-17β-ol, 17β-hydroxy-4-(β-hydroxyethyl)-17α-methyl-4-aza-5-androstene, and 4-aza-5-cholestene, which barely moved with solvent system No. 1, gave satisfactory R_F values with solvent system No. 2.

Acknowledgements

We thank Mr. DAVID DILLON for his competent assistance and we wish to express appreciation for the support of this program received from the National Institutes of Health (Grant No. AI-06798).

Department of Pharmaceutical Chemistry, School of Pharmacy, University of Mississippi, University, Miss. 38677 (U.S.A.)

NORMAN J. DOORENBOS
RAMESHWAR K. SHARMA

1 N. J. DOORENBOS AND S. S. VAIDYA, *Chem. Ind. (London)*, (1967) 704.
2 B. P. LISBOA, *J. Chromatog.*, 13 (1964) 391.
3 B. P. LISBOA AND E. DICZFALUSY, *Acta Endocrinol.*, 40 (1962) 60.
4 T. GOLAB AND D. S. LAYNE, *J. Chromatog.*, 9 (1962) 321.
5 R. K. SHARMA, G. S. KHAJURIA AND C. K. ATAL, *J. Chromatog.*, 19 (1965) 433.
6 E. C. BATE-SMITH AND R. G. WESTALL, *Biochim. Biophys. Acta*, 4 (1950) 427.

Received January 18th, 1967