restricted to less polar compounds, and the same limitation may also apply to the other TLC techniques for separating Δ^5–5α pairs. Reversed-phase TLC, by its nature, is only applicable to non-polar compounds, and a mixture of tomatidine and Δ^5-tomatidenol could not be resolved on silver nitrate-impregnated plates, even by continuous development[6].

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1 E. HEFTMANN, *Chromatographic Reviews*, Vol. 7, Elsevier, Amsterdam, 1965, p. 179.
2 R. TSCHESCHE, G. WULFF AND K. H. RICHERT, in A. T. JAMES AND L. J. MORRIS (Editors) *New Biochemical Separations*, Van Nostrand, London, 1964, p. 197.
3 M. MAUGRAS, C. ROBIN AND R. GAY, *Bull. Soc. Chim. Biol.*, 44 (1962) 887.
4 L. GÖLDEL, W. ZIMMERMAN AND D. LOMMER, *Z. Physiol. Chem.*, 333 (1963) 35.
5 R. D. BENNETT AND E. HEFTMANN, *J. Chromatog.*, 12 (1963) 245.
6 K. SCHREIBER, O. AURICH AND G. OSSKE, *J. Chromatog.*, 12 (1963) 63.
7 D. HEUSSER, *Planta Med.*, 12 (1964) 237.
8 T. M. LEES, M. J. LYNCH AND F. R. MOSHER, *J. Chromatog.*, 18 (1965) 595.
9 E. TRUTER, *J. Chromatog.*, 14 (1964) 57.
10 G. HESSE AND G. ROSCHER, *Z. Anal. Chem.*, 200 (1964) 3.
11 L. R. SNYDER, *J. Chromatog.*, 16 (1964) 55.
12 R. D. BENNETT AND E. HEFTMANN, *Phytochemistry*, 4 (1965) 577.
13 E. HEFTMANN, *Ann. Rev. Plant. Physiol.*, 14 (1963) 225.
14 R. D. BENNETT AND E. HEFTMANN, *J. Chromatog.*, 9 (1962) 353.
15 R. D. BENNETT AND E. HEFTMANN, *J. Chromatog.*, 9 (1962) 359.
16 D. I. CARGILL, *Analyst*, 87 (1962) 865.
17 D. L. AZARNOFF AND D. R. TUCKER, *Biochim. Biophys. Acta*, 70 (1963) 589.
18 R. IKAN, S. HAREL, J. KASHMAN AND E. D. BERGMANN, *J. Chromatog.*, 14 (1964) 504.
19 J. W. COPIUS-PEEREBOOM AND H. W. BEEKES, *J. Chromatog.*, 9 (1962) 316.
20 J. W. COPIUS-PEEREBOOM, *Z. Anal. Chem.*, 205 (1964) 325.
21 J. W. COPIUS-PEEREBOOM AND H. W. BEEKES, *J. Chromatog.*, 17 (1965) 99.
22 R. IKAN AND M. CUDZINOVSKI, *J. Chromatog.*, 18 (1965) 422.
23 L. J. MORRIS, in A. T. JAMES AND L. J. MORRIS (Editors), *New Biochemical Separations*, Van Nostrand, London, 1964, p. 295.
24 A. S. TRUSWELL AND W. D. MITCHELL, *J. Lipid Res.*, 6 (1965) 438.

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Response of steroids to sulfuric acid in thin-layer chromatography

Thin-layer chromatography is by now one of the most important analytical methods in the steroid field and a sizeable literature on this subject is now available[1]. Of the many detecting reagents in the literature sulfuric acid occupies a special place, because it reacts with all steroids to give colored and/or fluorescent zones and, after charring, permanent black-on-white chromatograms suitable for photographic or

TABLE I

SULFURIC ACID REACTION OF STEROIDS

Abbreviations: BE = blue; BK = black; BN = brown; BT = bright; DK = dark; DL = dull; ED = emerald; GN = green; GY = grey; LC = lilac; LT = light; OE = orange; PE = pale; PK = pink; PU = purple; RD = red; VY = very; YW = yellow.

Compound	*Color in daylight*			*Color in U.V.*
	Min.	*Initial color*	*Final color*	
C_{18} steroids				
$\Delta^{1,3,5(10)}$-Estratrien-3-ol	1.00	YW	DL-YW	BE
Δ^{4}-Estrene-3,17-dione	1.50	GN	GY-BN	LC
Δ^{4}-Estren-17β-ol-3-one	1.75	GY-GN	BN	BE
$\Delta^{5(10)}$-Estren-17β-ol-3-one	1.00	YW	OE	BT-BE
Estrone	1.25	OE	BT-OE	GN-BE
Equilin	0.50	YW	RD-OE	GN-BE
Equilenin	0.50	PE-YW	BN	DL-RD
Estradiol-17β	1.00	PE-OE	OE	GN-BE
Estradiol-17α	0.50	PK	BT-PK	BT-GN
$\Delta^{1,3,5(10)}$-Estratriene-3,16α-diol	1.00	PE-PK	OE-PK	BE
$\Delta^{1,3,5(10),6}$-Estratetraene-3,17β-diol	0.25	BT-GN-YW	OE	BT-GN
$\Delta^{1,3,5(10),6,8}$-Estrapentaene-3,17α-diol	1.50	PE-OE	RD	DL-RD
$\Delta^{1,3,5(10)}$-Estratriene-3,17β-diol-16-one	1.50	OE	OE	GN
Estriol	1.75	PE-PK	PK	DL-RD
16-Epiestriol	1.50	PE-OE	OE	DL-LC
C_{19} steroids				
5α-Androstan-17-one	3.00	PE-GY	GY	VY-PE-BE
5α-Androstan-17β-ol	1.00	LT-BN	DL-GY	PE-BE
5α-Androstan-3α-ol	0.75	BE-GY	PE-GY	VY-PE-BE
5α-Androstan-3β-ol	1.50	GY-GN	BN	VY-PE-BE
5β-Androstan-17β-ol-3-one	1.75	BT-YW	DK-YW	PE-BE
Androsterone	2.50	PE-YW	GY	BE
5α-Androstan-3β-ol-16-one	1.50	GY-YW	GY	BE
Δ^{1}-5α-Androsten-17β-ol-3-one	1.00	GY-GN	GY-GN	BT-BE
$\Delta^{1,4}$-Androstadien-17β-ol-3-one	2.00	PE-OE	OE	DL-OE
Testosterone	1.00	BE-GN	ED-GN	BE
Dehydroepiandrosterone	0.50	BT-PK	PU	BT-BE
5α-Androstane-3,16-dione	3.00	PE-GY	GY	BT-BE
5α-Androstane-3,17-dione	2.25	PE-GN	GN	DL-BE
5β-Androstane-3,17-dione	1.50	GN	GN	DL-GN
Δ^{1}-Androstene-3,17-dione	1.00	GN	GY-GN	DL-GN
Δ^{4}-Androstene-3,17-dione	1.50	BE-GN	ED-GN	PE-BE
Δ^{4}-Androstene-3,16-dione	1.25	PE-GN	GY	BE
$\Delta^{1,4}$-Androstadiene-3,17-dione	1.75	PK	OE	YW
5α-Androstane-3β,16β-diol	1.50	GY	OE	PE-BE
Δ^{5}-Androstene-3β,16β-diol	0.50	LT-PK	BE-PU	BE
5α-Androstane-3α,17β-diol	1.50	GY-GN	PU	DL-RD
5α-Androstane-3β,17β-diol	2.00	GY	GY-RD	DL-GY
Δ^{5}-Androstene-3β,17β-diol	0.50	GY-PK	PU	DL-BE
Δ^{4}-Androstene-3β,17β-diol	0.50	PK	GY-PU	BT-BE
Δ^{5}-Androstene-3β,17α-diol	0.50	PK	BE-PU	BE
Δ^{4}-Androsten-11β-ol-3,17-dione	0.75	BE-GN	BE	BE-GN
Δ^{4}-Androstene-11α,17α-diol-3-one	1.50	BE-GN	GN	GN
Δ^{5}-Androstene-3β,17β-diol-16-one	0.50	PK	DK-PK	GN-BE
5α-Androstane-3,11,17-trione	2.75	GY	GY	VY-PE-BE
Δ^{4}-Androstene-3,11,17-trione	1.00	PE-GY	PE-GY	VY-PE-BE

(*continued on p. 492*)

TABLE I (*continued*)

Compound	*Color in daylight*			*Color in U.V.*
	Min.	*Initial color*	*Final color*	
C_{21} steroids				
5α-Pregnan-3β-ol	1.00	GY-BN	BN	YW-BE
5β-Pregnan-3α-ol	1.50	GY-PK	PK-GY	VY-PE-BE
5α-Pregnan-3β-ol-20-one	1.25	GY-PK	GY-PK	BE
5α-Pregnan-20β-ol-3-one	2.00	PE-YW	GY	LT-BE
5β-Pregnan-3β-ol-20-one	2.50	PE-PK	GY-PK	PE-BE
Δ^5-Pregnen-3β-ol-20-one	0.75	PK	PK-PU	BE
Δ^{16}-5β-Pregnen-3β-ol-20-one	1.50	PE-PK	PK	YW
$\Delta^{5,16}$-Pregnadien-3β-ol-20-one	0.75	OE	BN	YW-GN
Δ^4-Pregnen-20α-ol-3-one	1.25	YW-PK	OE	BT-BE-GN
Δ^4-Pregnen-20β-ol-3-one	1.00	PE-YW	YW	BT-BE
5α-Pregnane-3,20-dione	1.75	GY-YW	YW-GY	BT-BE
5β-Pregnane-3,20-dione	2.50	PE-YW-GY	GY	PE-BE
Progesterone	1.50	GY-PK	GY	BE
$\Delta^{4,16}$-Pregnadiene-3,20-dione	1.75	YW	LT-BN	LC
5β-Pregnane-3α,20α-diol	1.25	GY-PK	GY-PK	VY-PE-BE
5β-Pregnane-3β,20α-diol	1.50	PK	GY-PK	VY-PE-BE
5α-Pregnane-3β,20β-diol	1.00	PK	RD-BN	DL-BE
5β-Pregnane-3β,20β-diol	1.75	PK	RD	PE-BE
Δ^4-Pregnene-3β,20β-diol	0.50	PE-PK	GY	PE-BE
Δ^5-Pregnene-3β,20β-diol	0.75	GY-PK	BE-PU	BT-BE
5β-Pregnan-12α-ol-3,20-dione	0.75	GN	GY	OE
Δ^4-Pregnen-6β-ol-3,20-dione	2.50	PE-YE	YW	BT-BE
Δ^4-Pregnen-11α-ol-3,20-dione	1.50	YW	BN-RD	BE
Δ^4-Pregnen-15α-ol-3,20-dione	1.50	YW	OE	BT-BE
Δ^4-Pregnen-17α-ol-3,20-dione	1.50	GY	BN	BE
Desoxycorticosterone	0.75	GN	DK-BE	DL-BE
5α-Pregnane-3β,16α-diol-20-one	1.50	PK	OE	DL-OE
5β-Pregnane-3β,16α-diol-20-one	1.50	YW	BN	YW-GN
5β-Pregnane-3β,17α-diol-20-one	2.25	LT-PK	OE	BT-LC
5β-Pregnane-3,11,20-trione	1.25	PE-YW	YW	BT-BE
Δ^4-Pregnene-3,11,20-trione	2.00	GY	GY	BE
Corticosterone	1.00	GN	DK-GN	BT-GN
11-Dehydrocorticosterone	2.00	PE-YW	OE	BE
11-Desoxycortisol	1.00	PK	PU	RD
Cortisone	2.50	YW	OE	BT-BE
Cortisol	0.75	OE-PK	BN	BT-GN
5β-Pregnane-17α,21-diol-3,20-dione	1.00	YW	BN	BE
5β-Pregnane-3α,17,20α,21-tetrol-11-one	1.50	GY-OE	OE	BT-BE
5β-Pregnane-3α,17,20β,21-tetrol-11-one	2.00	PE-GN-YW	GY	BT-BE
Δ^4-Pregnene-11β,17α,21-triol-3,20-dione	0.50	YW	BN	BT-GN-BE
$\Delta^{1,4}$-Pregnadiene-17α,21-diol-3,11,20-trione	1.50	YW	BN	DL-BE
5β-Pregnane-3α,11β,17,20α,21-pentol	1.00	GY	GY-GN	PE-BE
Cardenolides				
Uzarigenin	1.50	YW	GN	GN
Strophanthidin	0.50	YW	OE	GN
Digoxigenin	1.00	YW	OE	BT-BE
Digitoxigenin	1.00	GN	GN	BE
Gitoxigenin	1.00	PE-YW	RD-BN	BT-BE
Bile acids				
Cholanic acid	2.00	LT-GY	GY	DL-GN
Lithocholic acid	2.00	PE-PK	GY-PK	BE
Desoxycholic acid	1.50	GN-YW	YW	GN-BE

(*continued on p. 493*)

TABLE I (*continued*)

Compound	*Color in daylight*			*Color in U.V.*
	Min.	*Initial color*	*Final color*	
Bile acids (continued)				
Chenodesoxycholic acid	1.25	PK	PE-PK	BE
Desoxycholic acid	1.00	GY-PK	GY	BE
Cholic acid	1.50	PE-GN-YW	YW	BE
Sterols				
$\Delta^{3,5}$-Cholestadiene	0.25	PK	DK-PK	GN
5α-Cholestan-3-one	3.00	PE-YW	LT-BN	BE
5β-Cholestan-3-one	2.25	LT-BN	LT-BN	PE-BE
Δ^{4}-Cholesten-3-one	2.25	PE-YW	GY	BE
Δ^{5}-Cholesten-3-one	0.75	PE-YW	PK	BE
$\Delta^{1,4}$-Cholestadien-3-one	2.00	GY-PK	BN	DL-RD
$\Delta^{4,6}$-Cholestadien-3-one	2.00	PK	PK-OE	BE
3,5-Cyclocholestan-6-one	1.00	PE-YW	YW	PE-BE
3,5-Cyclocholestan-6β-ol	1.25	PK	BN-RD	OE
5α-Cholestan-3β-ol	2.00	GY	BN	BK
5β-Cholestan-3α-ol	1.75	PE-GY-PK	PK-GY	VY-PE-BE
5β-Cholestan-3β-ol	1.75	GY-PK	LT-BN	BK
5α,6α-Epoxycholestan-3β-ol	1.00	DL-YW	RD	GN-BE
Cholesterol	1.00	GY-PK	PU	DL-BE
Δ^{7}-5α-Cholesten-3β-ol	1.00	YW	GY	YW-BE
7-Dehydrocholesterol	0.00	GY-GN	GY	BE
Desmosterol	0.50	PK	GY	DK-BN
5α-Cholestan-3β-ol-6-one	2.25	PE-YW-GN	YW-GN	BE
Δ^{5}-Cholesten-3β-ol-7-one	1.00	GY	GY	DL-GN
Δ^{4}-Cholestene-3,6-dione	0.50	PE-OE	OE-RD	BE
Δ^{5}-Cholestene-3β,4β-diol	1.25	GN	BN	DL-GN
Ergosterol	0.00	YW	BK	GN
Δ^{22}-Stigmasten-3β-ol	0.75	GY	BN	DL-BE
β-Sitosterol	1.25	GY-PK	PU	DL-RD
Stigmasterol	0.50	PK	GY	DK-BN
Lanosterol	0.50	YW	BN	GN-BE
Dihydrolanosterol	1.75	GY-PK	PK-BN	PE-DL-RD
Sapogenins				
Smilagenin	1.25	YW	OE	GN-BE
Sarsasapogenin	0.75	YW	OE	GN-BE
Tigogenin	1.00	PE-PK	OE	BE
Neotigogenin	1.25	PK	OE	PE-BE
Yamogenin	0.75	PK	GY	BE
Diosgenin	1.50	OE	GY	BT-BE
Hecogenin	1.00	PE-YW	YW	BT-BE
Gentrogenin	0.75	PK	RD	BE
Kryptogenin	0.50	DK-PK	DK-BN	BT-BE
Alkaloids				
Holaphyllamine hydrochloride	2.00	PE-YW	YW-GY	VY-PE-BE
Holamine	1.75	PE-PK	LT-BR	DL-RD
Holaphylline	1.50	PE-OE	LT-BN	BE
Solasodine	0.75	PK	PU	BE
Tomatidine	0.50	PK	GN	BT-BE
Δ^{5}-Tomatiden-3β-ol	0.50	PK	PU	BE
Solanidine	0.50	DK-PK	RD	BT-BE

other forms of documentation and quantitation. Although all steroids respond to this reagent, it is nevertheless highly selective and even minor structural alterations in steroids may produce distinct changes in the color or fluorescence response. A further advantage of the sulfuric acid reaction is that it is very sensitive, and the detection of a spot containing as little as 0.01 μg of many steroids in thin-layer chromatograms usually offers no difficulty.

The sulfuric acid test has been known and used in steroid analysis long before the advent of thin-layer chromatography, but the high sensitivity resulting from the concentration of steroids on the surface of fine granules of an inert, white adsorbent such as silica gel has added considerably to the value of the test. In the preparation of the reagent various authors have recommended various concentrations of sulfuric acid in water or alcohol and a variety of additives. Since the active ingredient in most cases is probably concentrated sulfuric acid, we prefer to spray the thin-layer plates with sulfuric acid–water (1:1, w/w) and then heat them on a hot plate in a dark hood.

The purpose of the present communication is to summarize our observations on the response of 141 representatives of the various classes of steroids to the sulfuric acid reagent. The results are listed in Table I. The spot tests were carried out as follows.

The steroids were dissolved in dichloromethane and 4 μg of each compound was spotted on a Silica Gel G layer, 250 μ thick. The spot area was approximately 20 sq. mm in each case. The plates were sprayed with 50% sulfuric acid and heated on a hot plate at a surface temperature of 78°. The time required for the initial appearance of a color reaction, the initial color in daylight, the color after heating for 10 min, and the color in long-wave (366 mμ) U.V. light were recorded.

The sensitivity of the sulfuric acid reaction was determined for 34 representative samples. Various amounts of each were spotted on Silica Gel G plates, 250 μ thick, and the plates were developed with dichloromethane or mixtures of dichloromethane and acetone so that the compounds travelled a distance of approximately 5 cm. The plates were then air-dried, sprayed with 50% sulfuric acid, heated on the hot plate at a surface temperature of 225°, and viewed in long-wave U.V. light. In all cases the lower limit of detection was between 0.01 and 0.05 μg.

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