THE SEPARATION OF 19-NOR-STEROIDS BY THIN-LAYER CHROMATOGRAPHY ON SILICA GEL*

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The application of thin-layer chromatography by several workers to the separation and identification of steroids and sterols has been reviewed by DEMOLE[1]. BARBIER *et al.*[2] have obtained good separations of less polar steroids on silica gel plates using different proportions of ethyl acetate in cyclohexane as developing solvent. We have studied the chromatographic properties of thirty-eight 19-nor-steroids by this technique during an investigation of the metabolites of 17α-ethynyl-19-nor-steroids in body fluids and tissues. Steroid spots on the chromatograms were made visible by spraying with antimony trichloride in chloroform[3]. This reagent gave specific colors with the various compounds in daylight and under ultraviolet light. These colors, together with the R_S values with reference to 17α-ethynyl-17β-hydroxy-5(10)-estren-3-one, can be used for preliminary identification of the individual steroids.

EXPERIMENTAL

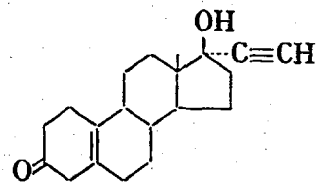
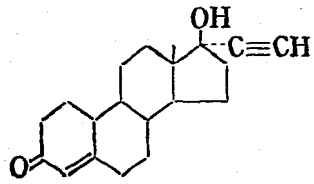
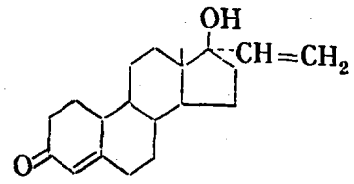
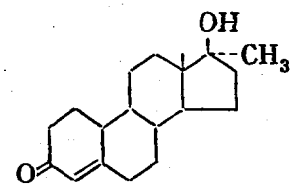
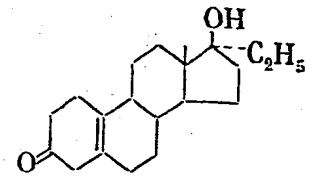
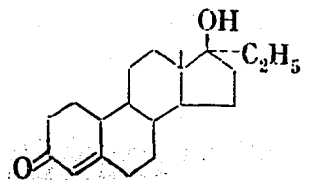
Glass plates 20 cm × 20 cm × 0.2 cm were used. Distilled water (70 ml) was added to a flask containing 30 g of silica gel G (Merck) and the flask was shaken vigorously for 30 sec. A layer 0.3 mm thick of the resulting suspension was applied to 5 glass plates using the Desaga applicator obtained from Brinkmann Instruments, Inc., Long Neck, N. Y. The plates were allowed to stand at room temperature for 30 min, and were then heated in an air oven at 110–120° for one hour. The plates were cooled in a desiccator until required for use.

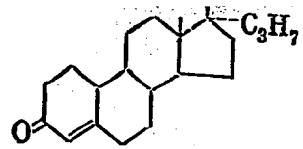
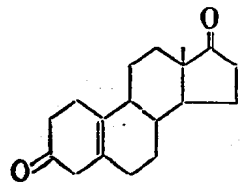
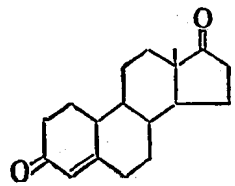
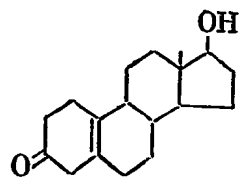
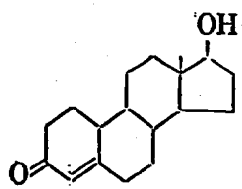
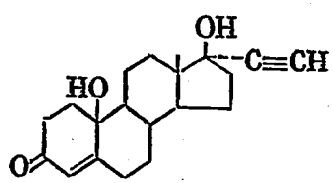
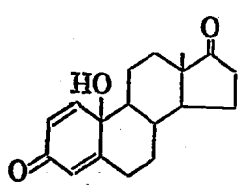
Steroids were applied in quantities of 50–100 γ at points 2 cm from the lower edge of the plates. Application was made in chloroform–methanol solution. Development was carried out in ethyl acetate–cyclohexane in the proportions of either (1:1) or (3:7). In either case the solvent was placed on the bottom of a rectangular tank (Brinkmann Instruments Inc.) to a height of 1 cm. The plates were placed in the tank and were removed when the solvent had ascended to a distance of 1 cm from the upper edge of the plate. The time of development was 90–105 min.

The developed plates were heated to 100–110° and immediately placed in a fume hood and sprayed with a saturated solution of antimony trichloride in chloroform. The color of the spots was observed immediately after spraying, and after a period of 24 h at room temperature. The plates were examined under a long wave

* Supported by Grant No. 1 from the American Cancer Society (Massachusetts Division) Inc., and by a grant from Mrs. STANLEY MCCORMICK.

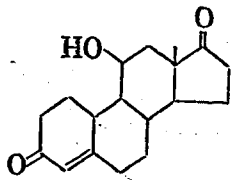
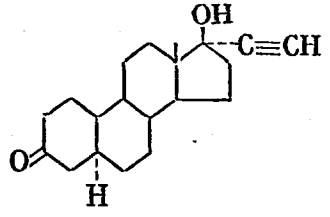
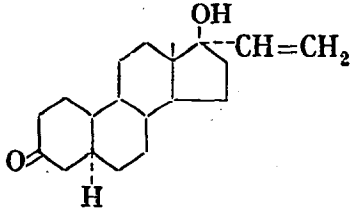
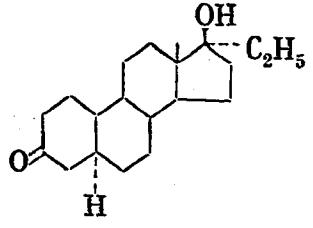
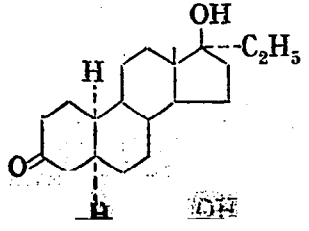
TABLE I

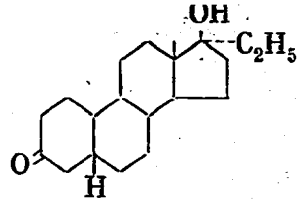
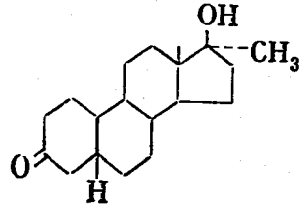
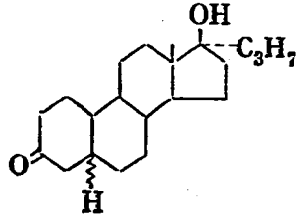
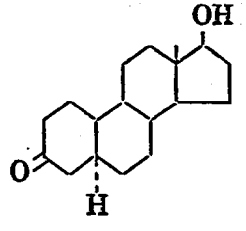
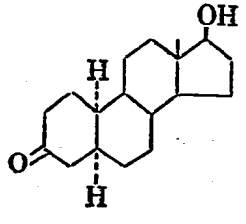
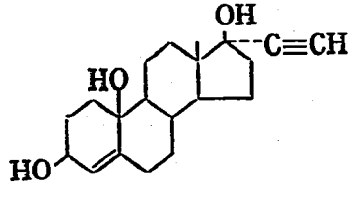
No.	Compound	Ethyl acetate/cyclohexane in mobile phase	R_F	R_S*	Standard error of R_F	Daylight color: After 10 min	Daylight color: After 24 h	Color under U.V. light after 10 min
1		1:1	0.74	1	±0.02	purple	gray-violet	purple-violet absorption
		3:7	0.51	1	±0.02			
2		1:1	0.55	0.74	±0.01	gray-violet	gray-violet	red absorption
		3:7	0.30	0.59	±0.01			
3		1:1	0.56	0.76	±0.03	red-purple-brown	red-brown	strong pink-red absorption
		3:7	0.31	0.60	±0.03			
4		1:1	0.40	0.54	±0.05	orange-red	red-violet	pink absorption
		3:7	0.21	0.41	±0.03			
5		1:1	0.68	0.92	±0.01	orange	pink-orange	bright yellow absorption with blue fluorescent border
		3:7	0.46	0.88	±0.01			
6		1:1	0.47	0.63	±0.03	purple-red	pink with violet border	pink to purple-red absorption
		3:7	0.27	0.52	±0.01			

7		1:1 3:7	0.41 0.19	0.55 0.37	±0.02 ±0.01	pink-orange	pink-orange	orange yellow absorption
8		1:1 3:7	0.71 0.50	0.96 0.96	±0.03 ±0.01		bright yellow	strong sky-blue absorption
9		1:1 3:7	0.25 0.13	0.33 0.26	±0.03 ±0.03		bright yellow to blue	strong sky-blue absorption
10		1:1 3:7	0.51 0.29	0.70 0.57	±0.03 ±0.01	yellow	gray-brown	green-blue fluorescence
11		1:1 3:7	0.36 0.14	0.49 0.27	±0.04 ±0.02		bright blue, or by heating bright sky blue	bright blue absorption
12		1:1 3:7	0.28 0.10	0.37 0.13	±0.01 ±0.01	pink to brown-violet	pink-brown	pink-brown absorption with strong blue border
13		1:1 3:7	0.36 0.21	0.48 0.41	±0.05 ±0.03	brown-yellow	orange-brown	dark purple absorption

(continued on p. 324)

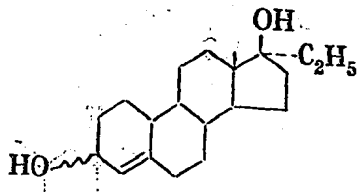
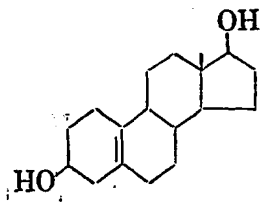
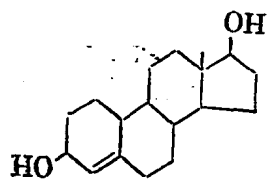
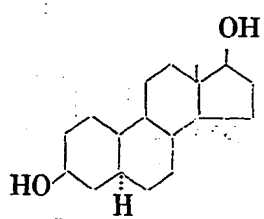
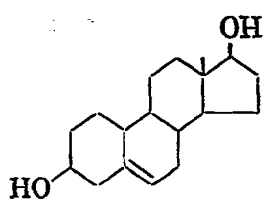
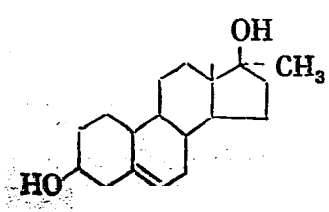
TABLE I (*continued*)

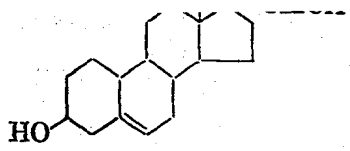
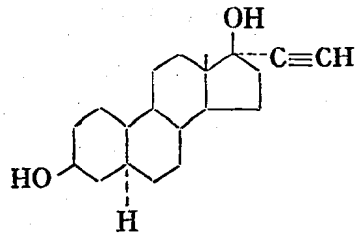
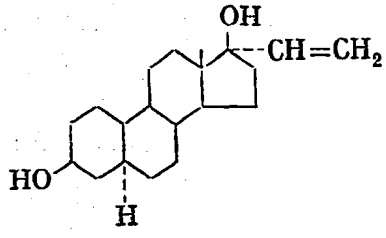
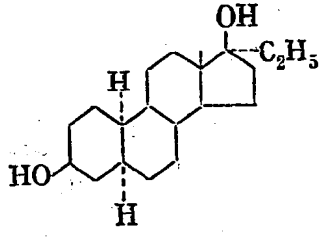
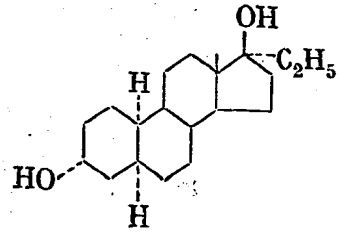
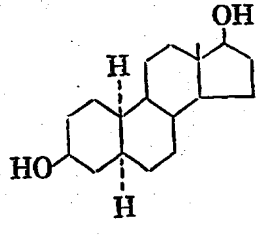
No.	Compound	Ethyl acetate/cyclohexane in mobile phase	R_F	R_S*	Standard error of R_F	Daylight color		Color under U.V. light after 10 min
						After 10 min	After 24 h	
14		1:1	0.25	0.33	±0.01		bright green-yellow	strong sky-blue fluorescence
		3:7	0.11	0.21	±0.01			
15		1:1	0.72	0.97	±0.05	brown-yellow	gray-violet	brown absorption
		3:7	0.42	0.82	±0.05			
16		1:1	0.70	0.94	±0.03	orange-yellow-brown	orange-brown	orange-brown absorption
		3:7	0.41	0.79	±0.02			
17		1:1	0.64	0.86	±0.03	orange-brown	orange-brown	bright brown absorption
		3:7	0.39	0.75	±0.03			
18		1:1	0.63	0.85	±0.03	brown-pink	red-brown	bright brown absorption
		3:7	0.38	0.74	±0.03			

19		1:1 3:7	0.65 0.39	0.87 0.76	±0.04 ±0.03	brown-orange	orange-brown	bright brown-red absorption
20		1:1 3:7	0.59 0.36	0.79 0.69	±0.02 ±0.01	pink-brown with violet border	orange-brown	blue-pink fluorescence
21		1:1 3:7	0.76 0.53	1.03 1.03	±0.05 ±0.02	pink-brown	red-brown	blue-pink fluorescence
22		1:1 3:7	0.59 0.36	0.79 0.70	±0.02 ±0.02		bright orange-yellow	weak blue absorption
23		1:1 3:7	0.63 0.40	0.84 0.77	±0.03 ±0.02		yellow	weak blue absorption
24		1:1 3:7	0.16 0.05	0.22 0.10	±0.01 ±0.01	0′ grass green 5′ sky blue 10′ ink blue	dark blue-violet	blue absorption

(continued on p. 326)

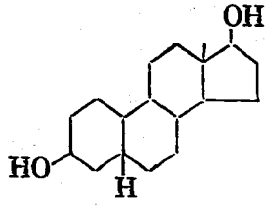
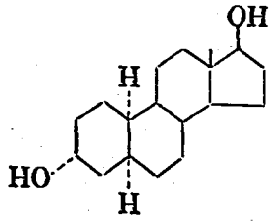
TABLE I (*continued*)

No.	Compound	Ethyl acetate/cyclohexane in mobile phase	R_F	R_S*	Standard error of R_F	Daylight color		Color under U.V. light after 10 min
						After 10 min	After 24 h	
25		1:1 3:7	0.53 0.27	0.71 0.52	±0.04 ±0.04	grey-green violet to purple	grey-green violet	grey-green-blue absorption
26		1:1 3:7	0.36 0.21	0.48 0.41	±0.04 ±0.02	pink-purple	grey-violet	dark brown red absorption
27		1:1 3:7	0.33 0.16	0.44 0.33	±0.04 ±0.01	pink-red to purple-red	red-purple	dark red absorption
28		1:1 3:7	0.39 0.25	0.52 0.45	±0.04 ±0.02	purple-red	red-purple	dark red absorption
29		1:1 3:7	0.38 0.23	0.51 0.45	±0.03 ±0.02	purple-violet	purple	purple absorption
30		1:1 3:7	0.43 0.26	0.59 0.51	±0.02 ±0.02	grey-green	grey-brown	grey-green absorption

31		1:1 3:7	0.54 0.29	0.73 0.57	±0.02 ±0.02	purple	grey-violet	dark purple absorption
32		1:1 3:7	0.53 0.31	0.71 0.60	±0.02 ±0.01	canary yellow	yellow-green-brown	grey-yellow brown absorption
33		1:1 3:7	0.53 0.30	0.71 0.59	±0.02 ±0.01	orange	orange-brown	orange absorption
34		1:1 3:7	0.65 0.41	0.88 0.79	±0.02 ±0.01	orange	orange-yellow	orange-yellow absorption
35		1:1 3:7	0.54 0.32	0.72 0.61	±0.02 ±0.01	orange	orange-brown	orange absorption
36		1:1 3:7	0.48 0.25	0.65 0.49	±0.01 ±0.01		bright brown	weak gray-blue absorption

(continued on p. 328)

TABLE I (*continued*)

No.	Compound	Ethyl acetate/cyclohexane in mobile phase	R_F	R_S*	Standard error of R_F	Daylight color		Color under U.V. light after 10 min
						After 10 min	After 24 h	
37		1:1 3:7	0.54 0.28	0.71 0.54	±0.03 ±0.02	bright brown		weak grey-blue absorption
38		1:1 3:7	0.42 0.24	0.57 0.47	±0.03 ±0.01	bright brown		weak grey-blue absorption

* R_S values determined with reference to 17α-ethynyl-17β-hydroxy-5(10)-estren-3-one (compound No. 1).

ultraviolet light 10–20 min after spraying. The lamp used was a "Blak-Ray", obtained from Ultra-Violet Products Inc., San Gabriel, Calif., and emitted mainly at about 3660 Å.

RESULTS

The steroids examined were 19-nor-ketones and alcohols many of which possessed 2-carbon side chains at position 17. The R_F and R_S values, together with the colors given with the antimony trichloride reagent are listed in Table I for the individual compounds. Typical chromatograms obtained with most of these compounds in the two solvent systems are shown in Figs. 1 and 2.

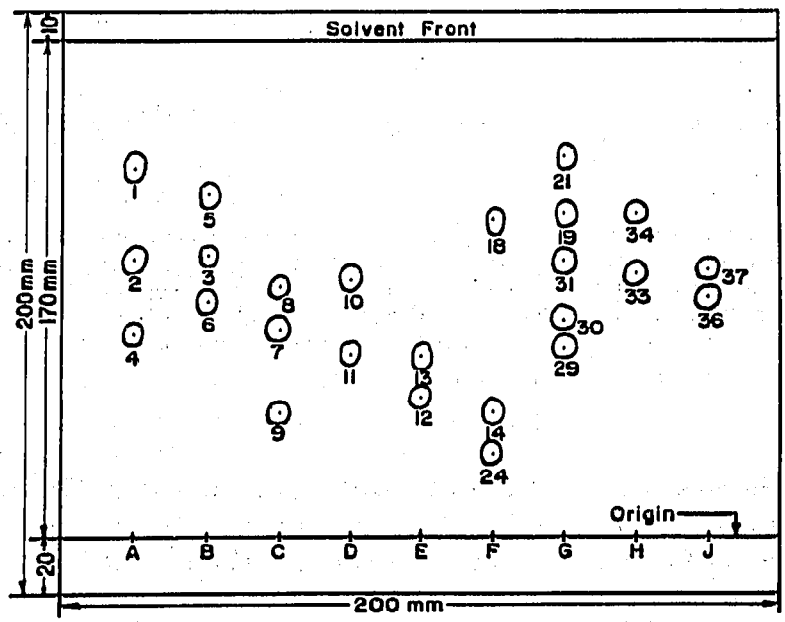


Fig. 1. Example of chromatogram obtained on 0.3 mm silica gel plate in the system ethyl acetate–cyclohexane (1:1). Numbers refer to steroids in Table I. (A) 0.15 mg of mixture of 1,2 and 4; (B) 0.15 mg of mixture of 3, 5 and 6; (C) 0.15 mg of mixture of 7, 8 and 9; (D) 0.10 mg of mixture of 10 and 11; (E) 0.10 mg of mixture of 12 and 13; (F) 0.15 mg of mixture of 14, 18 and 24; (G) 0.25 mg of mixture of 19, 21, 29, 30 and 31; (H) 0.10 mg of mixture of 33 and 34; (J) 0.10 mg of mixture of 36 and 37.

DISCUSSION

The chromatographic data and color reactions described above have proved useful in our laboratory in the detection and preliminary identification of 19-nor-steroids and their metabolites in body fluids and tissues following the administration of these compounds to humans and animals. The color given with antimony trichloride, while specific for each steroid, varied in shade and intensity with the concentration of the steroid and the time of heating the chromatogram before spraying. Care must be taken to compare the color of unknowns with standard spots of approximately the same intensity on the same chromatogram. The R_F values of the steroids varied somewhat on different chromatograms as shown in the standard errors in Table I. In all cases, the R_S values, based on the running speed relative to that of 17α-ethynyl-17β-hydroxy-5(10)-estren-3-one were much less variable than were the R_F values.

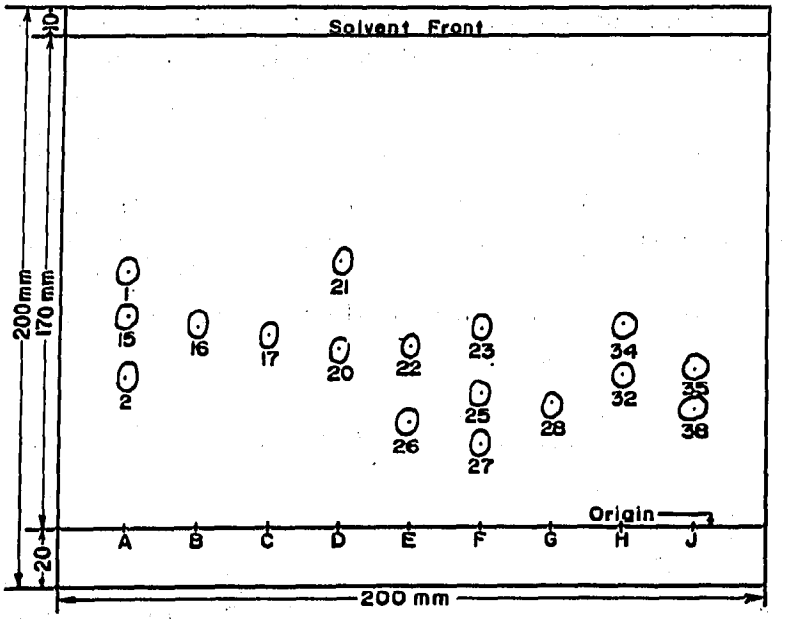


Fig. 2. Example of chromatogram obtained on 0.3 mm silica gel plate in the system ethyl acetate–cyclohexane (3:7). Numbers refer to steroids in Table I. (A) 0.15 mg of mixture of 1, 2 and 15; (B) 0.05 mg of 16; (C) 0.05 mg of 17; (D) 0.10 mg of mixture of 20 and 21; (E) 0.10 mg of mixture of 22 and 26; (F) 0.15 mg of mixture of 23, 25 and 27; (G) 0.05 mg of 28; (H) 0.10 mg of mixture of 32 and 34; (J) 0.10 mg of mixture of 35 and 38.

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SUMMARY

The separation of 19-nor-steroids by thin-layer chromatography on silica gel, and subsequent identification of the individual compounds by spraying the chromatograms with antimony trichloride in chloroform is described. Chromatographic mobilities and colors developed with the antimony trichloride reagent are listed for thirty-eight 19-nor-steroids.

REFERENCES

1 E. DEMOLE, *J. Chromatog.*, 6 (1961) 2.
2 M. BARBIER, H. JAGER, H. TOBIAS AND E. WYSS, *Helv. Chim. Acta*, 42 (1959) 2440.
3 R. NEHER AND A. WETTSTEIN, *Helv. Chim. Acta*, 34 (1951) 2778.