

CHROM. 3845

### Fluorescence and phosphorescence response of steroids to sulfuric acid in thin-layer chromatography at 77°K\*

The utility of low-temperature fluorimetry and phosphorimetry is discussed by MCGLYNN, NEELY AND NEELY<sup>1</sup>. Publications by CHOU AND LAWRENCE<sup>2</sup> and by HOOD AND WINEFORDNER<sup>3</sup> illustrate the applications of low-temperature luminescence in the thin-layer chromatography of organic compounds.

Although much work has been done concerning the response of steroids to sulfuric acid in thin-layer chromatography at room temperature, both under visible and ultraviolet light, little research has been carried out on the response at liquid-nitrogen temperature, 77°K. This note reports that the sulfuric acid derivatives of seven steroids on a Silica Gel G layer were observed to fluoresce and phosphoresce

TABLE I

STERIODS AND STEROID CONJUGATES INVESTIGATED

<i>Supplier's designation</i>	<i>Systematic name</i>
<i>Steroids<sup>a</sup></i>	
Androstane-3 $\beta$ -ol-17-one	3 $\beta$ -Hydroxy-androstan-17-one
$\Delta^4$ -Androstene-3,17-dione	Androst-4-ene-3,17-dione
$\Delta^4$ -Androstene-3,11,17-trione†	Androst-4-ene-3,11,17-trione
Androsterone†	3 $\alpha$ -Hydroxy-5 $\alpha$ -androstan-17-one
Corticosterone	11 $\beta$ ,21-Dihydroxypregn-4-ene-3,20-dione
Cortisone	17 $\alpha$ ,21-Dihydroxypregn-4-ene-3,11,20-trione
11-Dehydrocorticosterone†	21-Hydroxypregn-4-ene-3,11,20-trione
Dehydroisoandrosterone	3 $\beta$ -Hydroxyandrost-5-ene-17-one
Deoxycorticosterone	21-Hydroxypregn-4-ene-3,20-dione
(17 $\beta$ )-Estradiol†	3,17 $\beta$ -Dihydroxyoestra-1,3,5(10)-triene
Estriol†	3,16 $\alpha$ ,17 $\beta$ -Trihydroxyoestra-1,3,5(10)-triene
Estrone†	3-Hydroxyoestra-1,3,5(10)-triene-17-one
Etiocolanolone	3 $\alpha$ -Hydroxy-5 $\beta$ -androstan-17-one
Hydrocortisone	11 $\beta$ ,17 $\alpha$ ,21-Trihydroxypregn-4-ene-3,20-dione
5 $\alpha$ -Pregnane-3 $\beta$ ,20 $\beta$ -diol	3 $\beta$ ,20 $\beta$ -Dihydroxy-5 $\alpha$ -pregnane
5 $\beta$ -Pregnane-3 $\alpha$ ,20 $\alpha$ -diol	3 $\alpha$ ,20 $\alpha$ -Dihydroxy-5 $\beta$ -pregnane
5 $\beta$ -Pregnane-3 $\alpha$ ,20 $\alpha$ -diol diacetate†	3 $\alpha$ ,20 $\alpha$ -Diacetoxy-5 $\beta$ -pregnane
Progesterone	Pregn-4-ene-3,20-dione
Reichstein's substance S	17 $\alpha$ ,21-Dihydroxypregn-4-ene-3,20-dione
<i>Steroid conjugates<sup>b</sup></i>	
Androsterone glucuronide	
Androsterone sulfate, Na salt	
Oestriol-16 $\alpha$ -glucuronide, Na salt	
Oestriol-3 $\beta$ -glucuronide, Na salt	
Oestriol-17 $\beta$ -glucuronide, Na salt	
Pregnanediol glucuronide	
Pregnenolone sulfate, Na salt	
Testosterone glucuronide, Na salt	
Tetrahydrocortisone glucuronide, Na salt	

<sup>a</sup> A-grade chromatographic standards, purchased from Calbiochem, 3625 Medford St., Los Angeles, Calif. 90063.

<sup>b</sup> Obtained from the Steroid Reference Collection, Westfield College, London, England.

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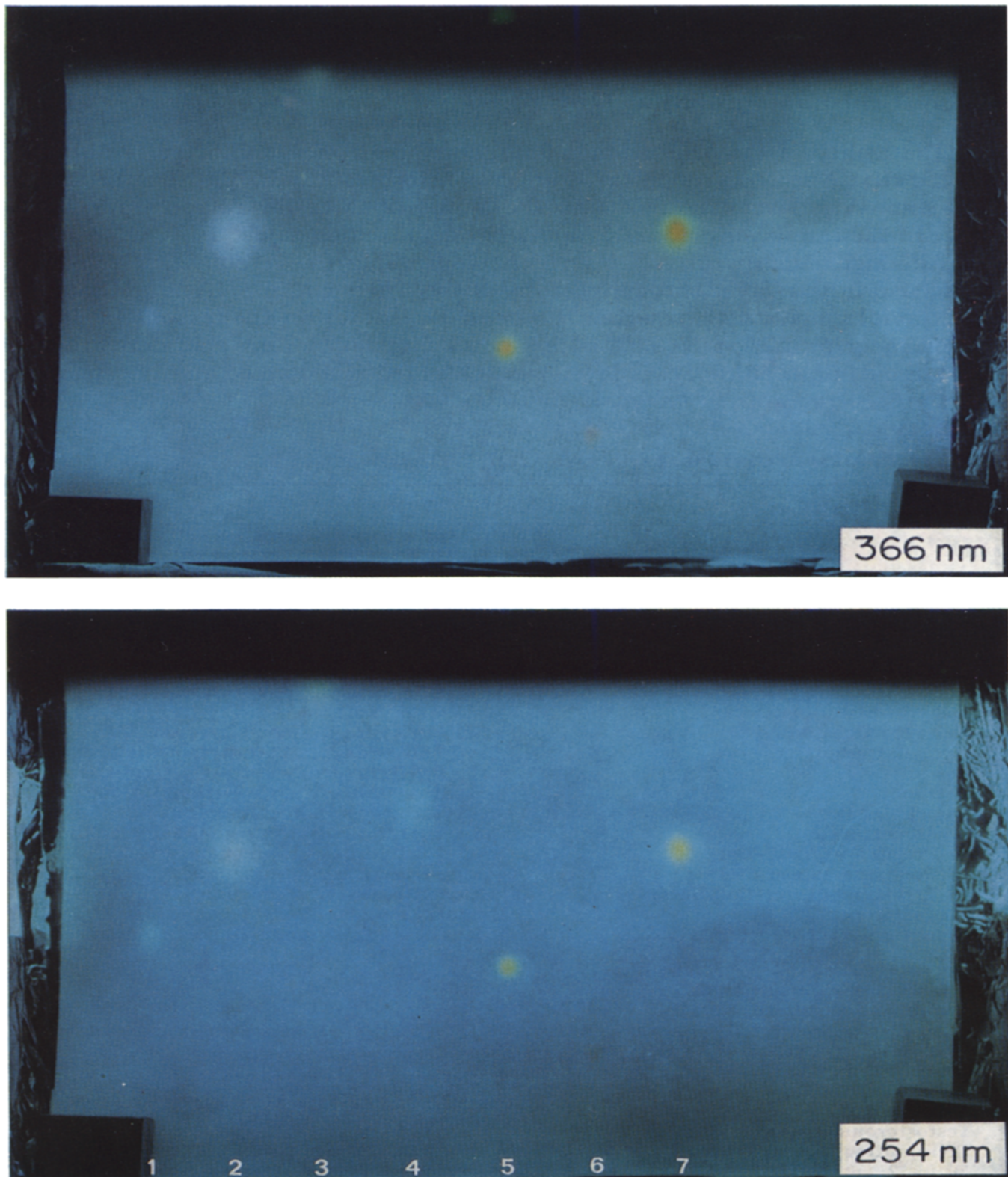


Fig. 1. Fluorescence of seven sulfuric acid-treated steroids at 77°K. 1 = 11-dehydrocorticosterone; 2 = androsterone; 3 = 5 $\beta$ -pregnane-3 $\alpha$ ,20 $\alpha$ -diol diacetate; 4 =  $\Delta^4$ -androstene-3,11,17-trione; 5 = (17 $\beta$ )-estradiol; 6 = estriol and 7 = estrone.

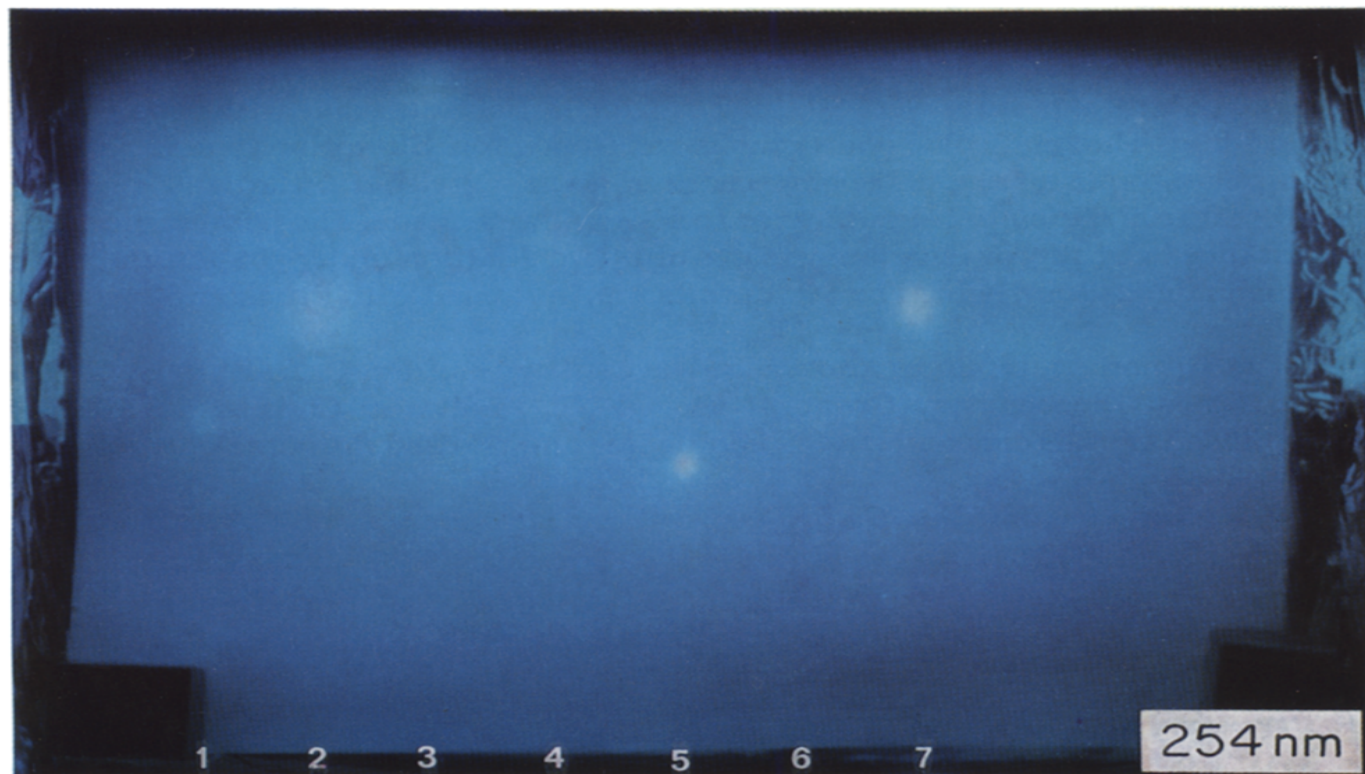
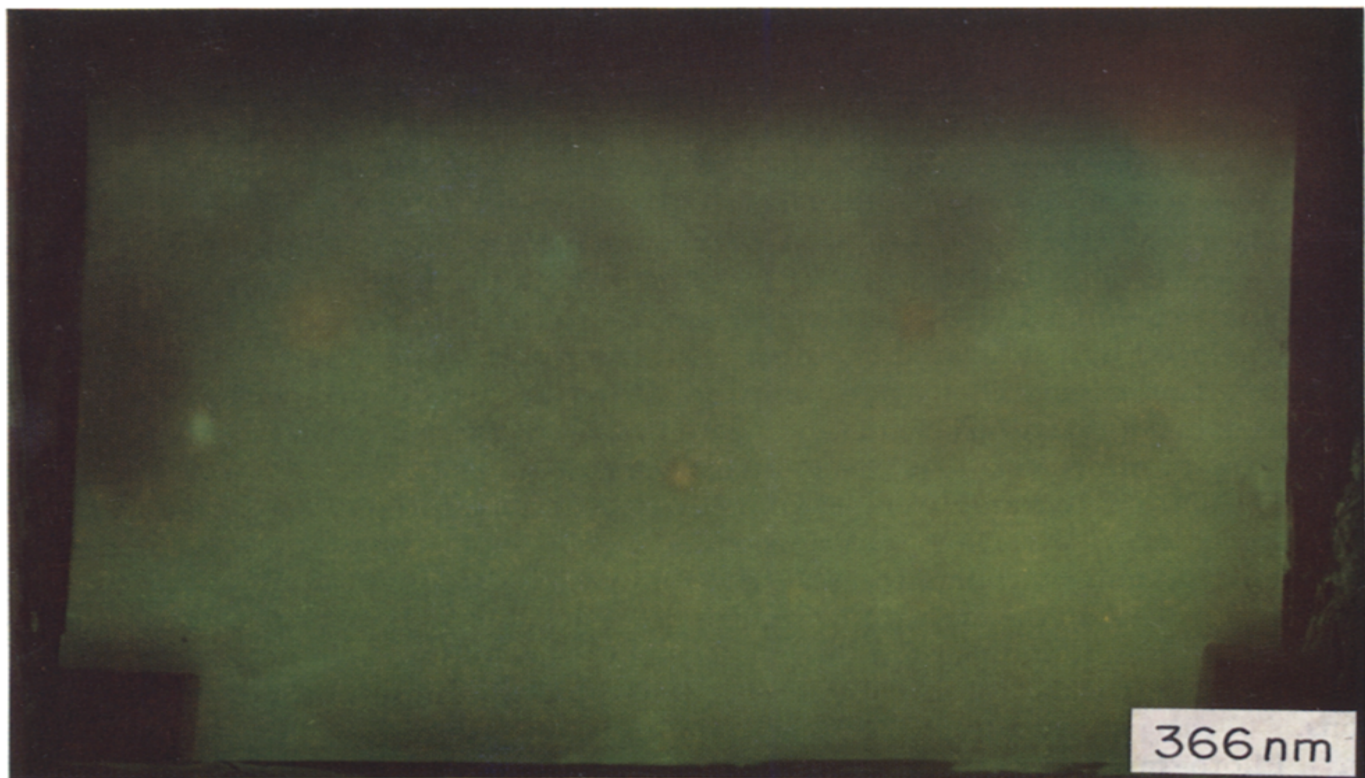


Fig. 2. Phosphorescence of seven sulfuric acid-treated steroids at 77°K. Arranged as in Fig. 1. (Number 3 also phosphoresces after excitation by 366 nm light but was not detected photographically because of its greater distance from the light source.)

when the layer was immersed in liquid nitrogen and excited with ultraviolet light, an observation that seems not to be recorded in the literature. At room temperature, these derivatives fluoresce but do not phosphoresce.

Table I lists the reference steroids and steroid conjugates investigated. The steroids were chromatographed as methylene chloride solutions at  $\sim 2\text{-}\mu\text{g}/\mu\text{l}$  concentration; the steroid conjugates were used as aqueous solutions.

The chromatograms were prepared by spotting  $2\text{-}\mu\text{l}$  portions of the solutions on  $250\text{-}\mu$ -thick Silica Gel G (Merck, "according to Stahl") layers freshly prepared on Mylar film. (Gelman ITLC-SA medium was also satisfactory.) The chromatograms were developed in a saturated chamber with chloroform-methanol (97:3, v/v) to 15 cm above the origin. They were removed, dried in air, sprayed with a 50-v/v% aqueous solution of sulfuric acid, and heated on a hot plate at a surface temperature of  $78^\circ\text{C}$  for 10 min, a technique used by other researchers<sup>4</sup>.

The chromatograms were examined immediately for fluorescence and phosphorescence at  $77^\circ\text{K}$ . They were placed in a Styrofoam tray that was lined with aluminum foil and precooled to  $77^\circ\text{K}$ , covered with  $\sim 1/2$  in. of liquid nitrogen, and viewed in a Chromato-vue cabinet (Ultraviolet Products, Inc., San Gabriel, Calif.) under both 366-nm and 254-nm ultraviolet light. The sulfuric acid derivatives of the seven steroids marked (†) in Table I were found to phosphoresce under these conditions.

Photographs were made of both the fluorescence (Fig. 1) and the phosphorescence (Fig. 2) of the seven steroids on exposure to 366-nm and 254-nm ultraviolet light. A 4 in. by 5 in. view camera provided with a lens of 135-mm focal length was used. The camera was mounted rigidly above the viewing port of the Chromato-vue cabinet. The best exposures were established from initial tests made with type 55 P/N Polaroid film. The final color photographs were taken on Ektacolor type L film. To obtain photographs of the phosphorescence at each wavelength, the lens aperture was f 4.7: fifteen sequential exposures of 10 sec each were made. The fluorescence was photographed at f 11 from single exposures of 15-sec duration under 366-nm and 6-sec duration under 254-nm light. The color prints were obtained with a master filter pack.

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