THE DETECTION OF STEROIDS IN THIN-LAYER CHROMATOGRAPHY WITH TOLUENE-*p*-SULFONIC ACID

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ABSTRACT

A survey of the usefulness of 100% (w/v) aqueous toluene-*p*-sulfonic acid as a spray reagent for the detection of steroids on thin-layer chromatograms has been carried out. A broad spectrum of color reactions was observed over a wide structural range.

Solutions of strong acids have been widely used for the non-specific detection of steroids on thin-layer chromatograms[1,2]. However, one such acid, toluene-*p*-sulfonic acid, although in use in several laboratories, does not figure prominently among the spray reagents reported in the current steroid literature. Accordingly, a survey of the wide structural range of steroidal compounds detectable by toluene-*p*-sulfonic acid has been carried out in order to focus further attention on the advantages of this reagent and possibly promote its more widespread use.

A 20% solution of toluene-*p*-sulfonic acid in ethanol has been used as a spray reagent for the detection of steroids on paper[3] and thin-layer chromatograms[1], the spots

being observed in ultraviolet light. Application of this reagent to thin-layer chromatograms followed by heating at 100° for one hour or more resulted in the slow development of highly colored spots, thereby obviating ultraviolet examination of the chromatograms. With a more concentrated spray, 100% (w/v) aqueous toluene-p-sulfonic acid, similar colors became visible within 10 minutes at 100°.

The wide range of steroids detectable by this method, and the broad spectrum of colors observed, are illustrated in the Table. Even hydrocarbons can be detected but the appearance of color is often sluggish. For example, development of the cholestane (29) spot requires at least one hour at 100°. A correlation of color with structure is not possible although some steroids containing similar functional groups do give the same color reactions, as illustrated by compounds (6 - 11), all of which contain a 3-hydroxyl group and give a red color. However, small structural differences often give rise to different colors as exemplified by 11α-hydroxyprogesterone (21) and 12α-hydroxyprogesterone (35) which give yellow and purple spots respectively. This difference in color reaction between compounds of similar polarity, also shown by testosterone (32) and 19-nortestosterone (13), often enables such compounds to be distinguished even when the R_F values are virtually identical.

Silica-gel "G" plates were used throughout and the steroids were applied as 100 μg. samples in acetone or chloroform solution. The sensitivity of the method, with 100%

TABLE

Steroid Color Reactions with p-TsOH After 10 - 20 Min. at 100°

Steroid		Color
1.	17α,21-Dihydroxypregn-4-ene-3,11,20-trione	Brown
2.	11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	Brown
3.	3α,7α,12α-Trihydroxy-5β-cholanic acid	Yellow-Brown
4.	3α-Hydroxy-5β-cholanic acid	Purple-Brown
5.	3α,6α-Dihydroxy-5β-cholanic acid	Grey-Brown
6.	3β-Hydroxyandrost-5-en-17-one	Red
7.	3β-Hydroxypregn-5-en-20-one	Red
8.	Cholest-5-en-3β-ol	Red
9.	3β-Hydroxy-5α-androstan-17-one	Red
10.	3α-Hydroxy-5α-androstan-17-one	Red
11.	5α-Cholestan-3β-ol	Red
12.	Androsta-1,4-diene-3,17-dione	Orange-Red
13.	17β-Hydroxyestran-4-en-3-one	Orange
14.	17β-Hydroxy-A-homo-5α-androstan-4-one	Orange
15.	Estra-1,3,5(10)-triene-3,17β-diol	Orange-Yellow
16.	3-Hydroxyestra-1,3,5(10)-trien-17-one	Yellow
17.	17β-Hydroxy-5α-androstan-3-one	Yellow
18.	17β-Hydroxy-5β-androstan-3-one	Yellow
19.	5α-Androstan-3,17-dione	Pale Yellow
20.	Androsta-1,4-diene-3,11,17-trione	Yellow
21.	11α-Hydroxypregn-4-ene-3,20-dione	Yellow
22.	5α-Pregnane-11,20-dione	Pale Yellow
23.	5α-Cholestan-3-one	Yellow
24.	A-Homo-5α-cholestan-4-one	Yellow
25.	3,7,12-Trioxo-5β-cholanic acid	Pale Yellow
26.	3α,12α-Dihydroxy-5β-cholanic acid	Brown Yellow
27.	11β,21-Dihydroxypregn-4-ene-3,20-dione	Yellow-Green
28.	Androst-4-ene-3,17-dione	Green
29.	5α-Cholestane	Grey-Green*
30.	3α,6α,7α-Trihydroxy-5β-cholanic acid	Grey-Green
31.	17β-Hydroxyandrost-4-en-3-one oxime	Blue-Green
32.	17β-Hydroxyandrost-4-en-3-one	Green-Blue
33.	21-Hydroxypregn-4-ene-3,20-dione	Blue
34.	5α-Androstan-17β-ol	Violet
35.	12α-Hydroxypregn-4-ene-3,20-dione	Purple
36.	17α,21-Dihydroxypregn-4-ene-3,20-dione	Purple

*After 1 hour at 100°

aqueous toluene-p-sulfonic acid as the spray reagent followed by heating at 100° for 10 - 20 minutes, was at least 2 μg./cm.^{2} using testosterone as the standard. The intensity of the color observed was dependent both on the concentration of the steroid and the amount of spray applied.

In contrast to several of the spray reagents containing strong acids currently in use and which give color reactions with steroids[1,2,4], solutions of toluene-p-sulfonic acid are relatively non-corrosive, are not unpleasant to handle, and are stable[4] indefinitely.

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