The N-terminal serine appears to be a part of that peptide chain which in mammals ends in tyrosine. In light of the extreme variability of the fibrinopeptide N-terminals (Table I), the conserved mammalian tyrosine end group—common to both fibrinogen and fibrin—may have a specific function which results in its being selected for during evolution. It will be interesting to find where among the vertebrates the N-terminal tyrosine makes its first appearance.

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Steroids. CCXXIII*.
Color reagent for steroids in thin-layer chromatography**

Colorimetric reactions have proved valuable for quantitative and qualitative analysis. In the case of steroids which do not exhibit ultraviolet absorption, colorimetric reactions are indispensable for their location in chromatograms. Such reactions are also used as supporting evidence for structural groupings in steroids, although in general colorimetric reactions are not specific enough to be used as primary evidence.

The general, non-specific reagent described here uses vanillin in H_2SO_4–ethanol. Vanillin was used by FREREJAQUÉ[2] to detect digitalis glucosides on paper with formation of blue colors. MCALEER AND KOZLOWSKI[3,4] used vanillin in H_3PO_4 to detect sapogenins and 17-hydroxy-20-ketopregnanes on paper. The former produced various colors while the latter gave orange spots on a background of yellow. The latest reported use of vanillin for the detection of steroids employed this reagent in $HClO_4$ to detect pregnanetriols in paper chromatography[5].

The advent of thin-layer chromatography[6] has presented a medium in which

* See ref. 1 for steroids CCXXII.
** Contribution No. 283.

extremely corrosive reagents may be used without the disadvantages of paper. Thus, many of the above reagents probably avoid the use of H_2SO_4 because the detection was done on paper. A reinvestigation of this reagent revealed that in H_2SO_4 it had greater sensitivity and a greater variety of steroids reacted. The reactivity and sensitivity were enhanced by the addition of ethanol possibly due to increased solubilization, an effect similar to that reported by ZAFFARONI[7]. Reagents containing varying concentrations of vanillin and varying proportions of H_2SO_4–ethanol were tested. The best combination as spray reagent was 0.5% vanillin in H_2SO_4 – ethanol (4:1). The reagent when freshly prepared is yellow, but will turn greenish on being kept overnight. It was prepared fresh each day.

TABLE I

COLOR REACTION OF SOME STEROIDS WITH 0.5% VANILLIN IN H_2SO_4–ETHANOL (4:1)

No.	Steroid	Color	
		Ambient	100° (5 min)
1	4-Chloro-testosterone	lilac	grey
2	Cholestane	NC*	grey
3	Allopregnane-3,21-diol-11,20-dione	NC	brown
4	Allopregnane-3,17,21-triol-11,20-dione	NC	brown
5	Prednisolone	NC	brown
6	Prednisone	light rose	brown
7	Pregnenolone	light brown	brown
8	6β-Bromo-Δ^4-androstene-3,17-dione	lilac	brown
9	6β-Nitro-Δ^4-androstene-3,17-dione	yellow	brown
10	Allopregnane-3,17,21-triol-20-one	NC	red
11	Allopregnane-3,11,17,21-tetrol-20-one	light brown	red
12	Testosterone acetate	NC	red
13	Estrone	orange	orange
14	Progesterone	NC	orange
15	6β-Chloro-progesterone	orange	orange
16	6β-Fluoro-progesterone	brown	orange
17	11-Dihydro-corticosterone	yellow	orange
18	Cortisone	NC	orange
19	Androstane-3,17-dione	yellow	yellow
20	Androstane-3-one	yellow	yellow
21	Estrone 3-methyl ether	orange	yellow
22	Estradiol	orange	greenish yellow
23	Corticosterone	NC	greenish yellow
24	6β-Hydroxy-progesterone	yellow	dark green
25	11-Dihydrocortisone	yellow	dark green
26	11-Dihydrocortisone-21-acetate	NC	dark green
27	Allopregnane-3,11,21-triol-20-one	violet	violet to purple
28	Deoxycorticosterone	NC	violet to purple
29	11-Deoxy-17-hydroxycorticosterone	rose	violet to purple
30	17α-Ethynyl estradiol 3-methyl ether	purple	violet to purple
31	Pregnanediol	grey	violet to purple
32	Allopregnanolone	lilac	violet to purple
33	Testosterone	lilac	violet to purple
34	Androsterone	lilac	violet to purple
35	Androstane-17-one	violet	violet to purple
36	Δ^2-Androstene	violet	violet to purple

* NC = no color.

In solution, most steroids containing hydroxyl groups or double bonds change the color of this reagent to red at room temperature. The visual sensitivity is about 20–50 μg/3 ml of reagent. When this reagent was used as a spray, steroids on paper produced orange spots against a yellow background, but due to the poor contrast the sensitivity was low.

Entirely different results were obtained when this reagent was used to detect steroids in thin-layer chromatography. All steroids from hydrocarbons to highly substituted steroids were detected with this reagent. The colors produced ranged from grey or brown through all shades of the spectrum. Most produced color without heating. Maximum color was produced by heating in an oven at 100° for 5 min. In general, heating at 100° for 10–20 min changes most of the colors to brown.

There appears to be no difference between detection on silica gel G or neutral alumina thin layers with binder*. The steroids were applied to the plates as 100 μg/cm^2 spots in chloroform solution. Table I lists the results obtained with various steroids on silica gel G when sprayed with this reagent. In all cases tested, the sensitivity was at least 5 μg/cm^2. At concentrations of 5 μg/cm^2 the color did not change (except in intensity) from what it was at 100 μg/cm^2.

The wide range of colors produced, and with all types of steroids, makes it difficult to assign the formation of a particular color to a definite grouping. Slight changes in structure appear to result in distinctly different colors. For example, testosterone acetate (12) gives a red color upon heating even though one would expect the ester to be hydrolysed to give the same color as testosterone (33). Androstane-3-one (20) gives a yellow color, androstane-17-one (35) gives reddish purple, but androstane-3,17-dione (19) only gives a yellow color, so the effect is not additive. Since even small changes between steroids generally result in distinct colors or shades, this reagent should help to reveal differences which are not detectable with more specific reagents. For example, the nine corticoids listed in Table I (5, 6, 17, 18, 23, 25, 26, 28 and 29) are distinguishable from each other by considering the colors produced before and after heating. Thus corticoids 5, 18, 23, 26 and 28 produce no color at ambient temperature but upon heating for 5 min at 100°, each gives rise to a different color. The 3 pregnanetriols (4, 10 and 27) can also be distinguished from each other. This reagent used in conjunction with other more specific color tests should help in making tentative identifications possible.

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