During the conversion of anthranilic acid to indole, therefore, it seems probable that the pyrrole ring is also formed through the 1-position of the benzene ring.

References and Notes

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Paper Electrophoresis of Steroid Derivatives

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The preparation of pharmaceutical mixtures of estrogens, androgens, and progesterone in various concentrations and ratios has become a common practice. Owing to the lack of specificity of the available chemical and biological tests for the individual steroids in mixtures, there was a need for a technique that could be used in the identification of these compounds. A number of excellent publications have appeared on the use of partition and adsorption chromatography for the separation of the steroids, which may be used in conjunction with the technique described here. These papers have been referred to by Lieberman (*1*) and summarized by Block (*2*).

The steroids are insoluble in aqueous buffers and do not possess any appreciable charge; thus they do not lend themselves readily to a paper electrophoretic separation. Paul and Durrum (*3*) attempted to overcome the solubility problem by using nonaqueous solvents instead of an aqueous buffer. In spite of the low conductivity of such a system, they did find that the steroids moved toward the anode but failed to separate under the conditions employed. Voigt and Beckmann (*4*) esterified the steroids with succinic anhydride and were able to move dehydroandrosterone acetate, desoxycorticosterone, and dehydroisoandrosterone and to separate the last two by paper electrophoresis. Only desoxycorticosterone moved as one band, indicating that more than one product had formed during esterification. These authors also used their technique in a study of the neutral ketonic fraction in urine (*5*). They formed the hydrazones of the ketosteroids with Girard's reagent T (trimethylacethydrazide ammonium chloride) and then esterified this fraction. They do not mention any attempt to separate the hydrazones that would eliminate the esterification process.

In the present study, the hydrazones were prepared by refluxing 2.5 mg of the steroid with 7.0 mg of Girard's reagent in 2 ml of 10-percent acetic acid in methanol for 2 hr in a manner similar to that described by Zaffaroni (*6*). The paper electrophoresis apparatus and the technique of applying the samples to the paper were the same as those that I used in a study of serum lipoproteins (*7*). Several solvent systems were investigated, and the most useful one consisted of a 0.05*M* solution of sodium borate. The hydrazones were detected by viewing the completely dried electropherogram under a quartz-mercury lamp followed by dipping the papers into a solution of the Kraut-Dragondorff reagent (*2*).

The degree of separation obtained for a mixture of progesterone, testosterone, and estrone is shown Fig. 1. The excess Girard reagent has moved off the paper into the cathodic compartment ahead of the larger hydrazone molecules. The dihydrazone of progesterone that possesses two positively charged quaternary groups moves ahead of the monohydrazones that possess only one quaternary group. The monohydrazones of testosterone and estrone move at the same rate in either an acetate buffer at a *p*H of 4.5 or a diethylbarbiturate buffer at a *p*H of 8.6, but the hydrazone of estrone is less mobile than the hydrazone of testo-

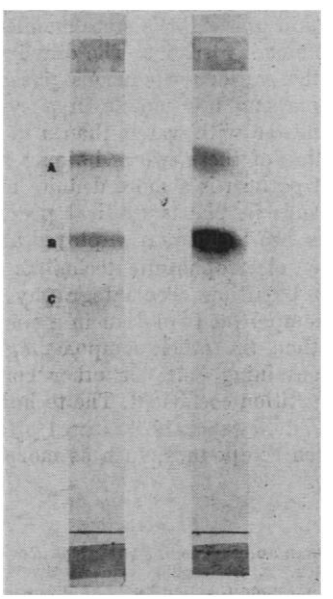

Fig. 1. The electropherogram on the left shows the hydrazones of progesterone (*A*), testosterone (*B*), and estrone (*C*). The electropherogram on the right shows the hydrazones of progesterone and testosterone. The progesterone being the most mobile has moved the greatest distance from the line of application (pencil line on the electropherogram). These electropherograms were run in 0.05*M* sodium borate for 18 hr at a potential of 200 v and a current of 1.5 ma/in. width of paper.

Table 1. Relative mobilities of steroid hydrazones expressed as a fraction of the distance moved by the progesterone derivative using a conducting medium of 0.05*M* sodium borate.

Steroid	Relative mobility
Progesterone	1.00
Androsterone	0.85
Desoxycorticosterone	.83
Methyltestosterone	.77
Ethisterone	.76
Testosterone	.75
Estrone	.53

sterone in the borate system. Dissociation of the hydroxyl group at C_3 of estrone in the borate system probably results in the net charge becoming less positive than that of the testosterone hydrazone, resulting in a slower movement toward the cathode.

The relative mobilities of several steroid hydrazones are recorded in Table 1. There does not appear to be any simple correlation of relative mobilities and charge per unit of weight.

Cortisone acetate was not run in the borate system, but it moves just behind testosterone in a diethylbarbiturate buffer made up in 20-percent methanol (*7*). Hydrocortisone moved as two spots in the borate system with mobilities similar to progesterone and testosterone, suggesting that both di- and monohydrazones were present.

A dihydrazone possessing a greater ratio of net charge per unit of weight is more mobile in an electric field than a monohydrazone. The dihydrazone is more soluble in the stationary aqueous phase on a paper chromatogram and less mobile in a system, such as butanol saturated with water, than a monohydrazone. A combination of these two techniques to give a two-dimensional pattern is a more definite means of identifying steroids in pharmaceutical preparations than either of the two techniques employed alone.

The paper electrophoretic technique is considered superior to partition chromatography for mixtures where one component is present in a much higher concentration than the other components, and also for solutions containing salts or other compounds that effect the partition coefficient. The technique described has been used to separate mixtures of other quaternary nitrogen compounds, such as morphine, codeine, and choline.

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Flowering Hormone in Relation to Blooming in Sweetpotatoes

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The Jersey varieties of sweetpotato bloom sparsely if at all under ordinary conditions in the continental United States. This handicaps the sweetpotato breeder. Many investigators (*1*) have tried to induce these varieties of sweetpotatoes to bloom but none of them have been successful. However, Cordner and Sorensen (*2*) reported inducing sweetpotatoes to bloom by growing them in gravel culture and Culbertson (*3*) induced blooming by grafting. Recently Kehr, Ting, and Miller (*4*) and Zobel and Hanna (*5*) reported success in inducing flowering of Jersey varieties by grafting them on *Ipomea carnea* and *I. purpurea*, respectively. This result has been attributed to the accumulation of carbohydrates in the sweetpotato scion when it is supported by a root system incapable of forming storage roots.

The purposes of our study were (i) to develop a technique for inducing flowering in Jersey varieties and (ii) to determine the factors that induce flowering in the grafted scions of sweetpotatoes. This study was initiated in September 1953. The cleft graft has been used and the success of the grafts has varied with the different species used as grafted stocks and scions. Briefly our technique has consisted of the following steps:

1) A strong terminal of a sweetpotato shoot about ½ to 1 in. long was cut slantingly on both sides as grafted scion.

2) The stem of the stock was split longitudinally downward about ¼ in. for insertion of the scion.

3) The graft was tightly tied with a sisal fiber until the union was established. The grafted plants were kept in a humid atmosphere in a propagating box for 4 to 10 days, depending on seasonal conditions. The plants were then removed and transplanted to 6-in. pots and kept under favorable growing conditions.

A number of tests have been conducted and four of these are reported in this paper.

Effect of root-stock species. Fourteen related species forming nonstorage roots were used as stocks. A breeding line sweetpotato (P-47) was also used. Orlis (a sport of Yellow Jersey) was used as grafted scion and 5 to 10 plants of each species were grafted. The results of this test (Table 1) suggest that the absence of storage roots in the understock per se will not assure blooming in the sweetpotato scion. All but one of the 15 species used in these grafting tests are of the nonstorage root type. Only two species, *I. tricolor* and *I. hederacea*, were found to be effective in inducing flowering. It seems that various stock species have different abilities to induce blooming.

Effect of defoliation of morning glory stock. Orlis scions were grafted on the morning glory stocks (*I. tricolor*) with varying number of leaves, that is, none, 3, and 7 or more. Five to ten grafts were made in each