orthoformate, 172 ml. of absolute ethanol, and 8.6 ml. of 4*N* ethanolic hydrogen chloride were heated at reflux for 2 hr. The cooled solution was made slightly alkaline by the addition of a solution of sodium methoxide and then washed three times with 500 ml. of water. The dried benzene solution (potassium carbonate) was concentrated *in vacuo*. Residual benzene was removed by flushing *in vacuo* with 100 ml. of absolute ethanol. The crude residue was used without further purification for the next step.

The crude product may be purified by trituration with petroleum ether until crystalline, followed by recrystallization from methanol, m.p. 116–118° (solvated); 132–137° (desolvated). $[\alpha]_D^{23}$ −130° (ethanol).

Anal. Calcd. for $C_{21}H_{32}O_2$ (316.47); C, 79.69; H, 10.19. Found: C, 79.61; H, 9.99.

Semicarbazone of 17β-hydroxyandrostan-3-one (IVb). The crude dienol ether (II) was dissolved in 1875 ml. of absolute ethanol and hydrogenated with 8 g. of 5% palladium-charcoal catalyst under a hydrogen pressure of 40 p.s.i. The theoretical amount of hydrogen (0.347 mole) was absorbed in 10 hr. Hydrogenation was continued for a total of 19 hr., 104% of the theoretical amount of hydrogen being absorbed.

Two hundred milliliters of 2.5*N* hydrochloric acid[7] was added to the hydrogenation solution and the catalyst removed by filtration through a filter aid. The filtrate was refluxed for 20 min. A solution of 50 g. of sodium acetate in 100 ml. of water was added and the mixture stirred at 50–60° for 45 min., and to the warm (50–60°) solution of the steroid was added a warm solution of 37.5 g. (0.5 mole) of semicarbazide in 600 ml. of ethanol. The mixture was allowed to cool slowly to 20° and the filtered semicarbazone was washed with ethanol, then with ether, and dried *in vacuo*, yield 76.7 g. (64% from I), m.p. 249–251° dec., $\lambda_{max}^{CH_3OH}$ 228 mμ, ε 13,400. The melting point, mixed melting point and spectral data agreed with a sample of IVb prepared from authentic IVa.

5α-Androstan-17β-ol (V). Seventy four grams of semicarbazone, (IVb), 520 ml. of diethylene glycol, 52 ml. of 85% hydrazine hydrate, and 49 g. of powdered potassium hydroxide were heated in a round bottomed flask equipped with a thermometer, mechanical stirrer, and a vertical air condenser. The temperature was increased slowly to 200–210° and held at this temperature until the evolution of nitrogen had ceased (approximately 45 min.). The mixture was allowed to cool to 180–190° and poured into a well stirred mixture of 2 l. of water and 2 kg. of ice. The product was filtered and washed with water to neutrality. After drying at 70°, 54.8 g. (93%) of Va were obtained, m.p. 164–166.5. Recrystallization from ethanol and from heptane gave material, m.p. 165.5–166.5, $[\alpha]_D^{24}$ +12 ($CHCl_3$).

Anal. Calcd. for $C_{19}H_{32}O$ (276.45): C, 82.54; H, 11.66. Found: C, 82.48; H, 11.48.

The infrared spectrum in carbon disulfide corresponded favorably with the published spectrum.[8]

5α-Androstan-17β-ol acetate (Vb). Forty-one grams of Va and 185 ml. of acetic anhydride were heated and stirred on the steambath for 1 hr. The hot solution was diluted slowly with water until the excess acetic anhydride was decomposed. More water (total 750 ml.) was then added to precipitate the product. After filtration and drying, the crude acetate was dissolved in ether, treated with charcoal, and the ether removed *in vacuo*. The residue was recrystallized from 110 ml. of methanol to give 43 g. (91%) of Vb, m.p. 81–82.5, $[\alpha]_D^{24}$ +5 ($CHCl_3$).

Anal. Calcd. for $C_{21}H_{34}O_2$ (318.48): C, 79.19; H, 10.76. Found: C, 79.14; H, 10.84.

The physical properties of samples of Va and Vb made via the Clemmensen reduction of IVa[2a] were identical with those reported above.

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(7) The addition of acid before filtration coagulates the catalyst and prevents colloidal catalyst from passing into the filtrate.

(8) K. Dobriner, E. R. Katzenellenbogen, and R. N. Jones, *Infrared Absorption Spectra of Steroids*, Vol. I, Interscience Publishers, Inc., New York 1953, Spectrum No. 29.

Halochromism Studies on Prodigiosin

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Recently it was reported that prodigiosin perchlorate reacts with alcohols[1] causing spectral shifts from 536 to 542 mμ and that the solvent functioned as a Lewis base toward the pigment. During the course of experimentation in this laboratory it was observed that the pigment free base, which was yellow in nonpolar solvents or acetone, became red when shaken with water, acids, or alcohols. When red aqueous pigment solutions were extracted with water-insoluble solvents, the reaction seemed to reverse and the yellow base was extracted into the organic phase. Consequently, a determination of the acid dissociation constant was undertaken in an attempt to resolve this phenomenon. It was found that $K_a = 3.23 \times 10^{-8}$ ($pK_a = 7.51$) at 25°. This would indicate that protonated prodigiosin is about as weak an acid as hydrogen sulfide or hypochlorous acid, but still considerably stronger than water or alcohols whose pK_a values are 16 or greater. The halochromism phenomenon was finally traced to carbon dioxide in the water ($K_a = 3.5 \times 10^{-7}$ for carbonic acid) and to acid impurities in the solvents. Thus, if distilled water is boiled and cooled by passage of pure nitrogen or oxygen gas through it and an acetone solution of prodigiosin is added, there is no color change from yellow to red. If the solution is shaken or if the water is shaken or carbon dioxide passed through before addition of the pigment, the red color forms immediately because of reaction with protons derived from carbonic acid. Moreover, alcohols purified carefully in the usual fashion by drying with aluminum isopropoxide do not cause halochromism when used to dissolve the pigment.

(1) A. J. Castro, A. H. Corwin, F. J. Waxham, and A. L. Beilby, *J. Org. Chem.*, **24**, 455 (1959).

Prodigiosin, however, has a great affinity for water and will partially remove water of crystallization from such materials as magnesium sulfate heptahydrate or copper sulfate pentahydrate. Thus an acetone solution of prodigiosin to which crystals of these materials have been added exhibits immediate reddening or color shift from yellow toward the red part of the spectrum. The effect is apparently related to the ability of the salt in question to ionize with formation of protons, as the color shift is strongest with salts like copper, ammonium, or zinc sulfate, intermediate with magnesium sulfate, and scarcely preceptible with sodium sulfate. On prolonged standing over a week with the exclusion of moisture, copper sulfate pentahydrate was dehydrated to the grey anhydrous salt.

These observations lead us to conclude that the halochromism is independent of the nature of the solvent and is caused by the oxonium ion which functions as a Lewis acid towards the pigment.

EXPERIMENTAL

Various methods for the extraction of the pigment were examined. Cells undergo lysation completely in formic or acetic acids, or pyridine within a matter of minutes. This lysate can be rapidly extracted with immiscible solvents to obtain the free prodigiosin. Thus colonial growth of *S. marcescens* on Difco Peptone agar slants was dissolved by addition of 90% formic acid. The lysate was poured into distilled water, extracted three times with petroleum ether, and the organic phase dried over anhydrous sodium sulfate. The aqueous phase contains a blue pigment, R_f 0.2, previously reported and chromatographed by Williams and Green[2] and is identical with their material. An accompanying purple pigment is an artifact caused by acid decomposition of prodigiosin. The organic phase was evaporated and the residue extracted with warm water giving a red aqueous phase and an orange residue which was not further characterized. The red aqueous phase was re-extracted with petroleum ether, evaporated, and taken up into acetone giving yellow prodigiosin, identified by paper chromatography[2] (R_f 0.7, ether-petroleum ether 1:2) and ultraviolet and visible spectra.[1]

Sufficient acetone prodigiosin solution was added to Sorenson buffers of gradient *p*H from 6 to 8 to given an optical density from 1 to 1.5. The visible region absorption curves as a function of *p*H were determined using a Cary Model 14 spectrophotometer. Two other absorbancies, at *p*H 2 and *p*H 10, were also determined over a concentration range to verify Beers Law, but the absorption in strong base was inconstant because of destruction of the pigment. Consequently a solution of free prodigiosin in acetone was used for the Beers Law verification in the basic region. These data were treated as described by Tobey[3] to obtain the pK_a and K_a.

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Aspidofiline, the Phenolic Alkaloid of Aspidosperma Pyrifolium Mart

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Aspidosperma pyrifolium Mart., a member of the family *Apocynaceae*, is a tree reaching a height of (5/m.)., with grey bark, and a fruit resembling that of the European pear tree. It is found in certain places in the drought region in the north-east of Brazil, generally the most arid part, and is commonly called "pereiro." The plant material was collected by J. Santa Rosa in the Municipio of Acari, Rio Grande do Norte, and botanically identified by the late J. G. Kuhlmann. From the leaves, a new base, Aspidofiline, was isolated, m.p. 186–187°, picrate, m.p. 146°.

Aspidofiline and its picrate analysed very well for a $C_{20}H_{22}N_2O_2$ compound. The new base is closely related to other *N*-acetyldihydroindoles, such as aspidospermine[2] and spegazzinine[3] isolated earlier from other Aspidosperma species. The ultraviolet absorption spectrum of aspidofiline is very similar to those of aspidospermine and spegazzinine, and characteristic of an *N*-acetyldihydroindole nucleus. The infrared spectrum shows an amide band at 6.14μ as in aspidospermine and spegazzinine. As in the case of other phenolic bases of the dihydroindole type, such as vomicine,[2] demethylaspidospermine,[2] and haplophytine,[4] aspidofiline shows no band in the OH or NH region, as a consequence of the strong hydrogen bonding of the phenolic hydroxyl with a carbonyl.

EXPERIMENTAL

The powdered leaves of A. pyrifolium (2/Kg.) were extracted with alcohol in a modified soxhlet. The extract was concentrated under reduced pressure and the viscous mass treated with 5% hydrochloric acid, the resinous part separated and the clear acid solution extracted with ether, basified with ammonia and again extracted with several portions of ether. The collected ether fractions were in turn extracted with dilute alkali.

The alkaline solution was acidified with hydrochloric acid and then treated with concentrated ammonia, and the precipitated base extracted with ether. In total, 2 g. of crystalline material was obtained.

After several crystallizations from ether, aspidofiline was obtained in well crystallized needles, m.p. 186°–187° (Kofler).

Anal. Calculated for $C_{20}H_{22}N_2O_2$: C, 74.51; H, 6.88; N, 8.69. Found: C, 74.47; H, 7.07; N, 8.62.

The ultraviolet absorption spectrum (in 95% ethanol) showed max at 258 mμ (log ϵ 3.85), a min at 242 mμ (log ϵ 3.67), inflection at 282 mμ. The infrared spectrum

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