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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(mulled in Nujol) showed the following major bands: no bands in the OH or NH region; 6.35 (S), 6.27 (S), 6.14 (S).

Aspidofiline picrate. The picrate was prepared by treating an ethereal solution of aspidofiline with picric acid in ether; the crystalline picrate was separated and recrystallized several times from acetone, m.p. 146° (capillary, non-corrected).

Anal. Calcd. for $C_{26}H_{25}N_5O_9$: C, 56.62; H, 4.57; N, 12.70. Found: C, 56.83; H, 4.7; N, 12.49.

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Extractives from the Dipterocarpaceae

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The Dipterocarpaceae are an important family of trees which grow in southeast Asia and are characterized by an abundant secretion of resins such as dammar and gurjun which possess economic importance. References to early researches on dammar are given by Glimmann[1] and recently a comprehensive investigation of the constituents of dammar has been carried out by Mills[2,3] who determined the constitution of the neutral triterpenes present. Among those triterpenes was hydroxydammarenone-II first isolated by van Itallie[4] from the balsams of *D. hasseltii* and *D. trinervis*. King[5] *et al.* isolated this compound from three Dipterocarpus woods, "gurjun," "yang," and "keruing" and established the identity of hydroxydammarenone-II with dipterocarpol isolated by Ourisson[6,7] from the balsams of several Dipterocarpus species—*D. Dyeri, D. alatus, D. intricatus* and *D. atrocarpifolius*.

From the acidic fraction of gum dammar we have isolated asiatic acid and will report our findings in a future communication. From two woods of the *Dipterocarpus* species, *D. verrucosis* and *D. grandiflorus* we have isolated dipterocarpol in yields of 0.12% and 0.16% respectively.

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EXPERIMENTAL

D. Verrucosis. The wood (4 lb.) in the form of shavings was extracted continuously with light petroleum for 24 hr. The extract was concentrated to give a resin (32.7 g.) which was hydrolyzed for 6 hr. with 10% methanolic potassium hydroxide (300 ml.). The hydrolysis liquor was filtered to remove a small amount of insoluble matter, diluted with much water, and extracted with ether to give a viscous oil (17.1 g.). Chromatographic analysis of the oil on alumina (500 g.) in light petroleum solution followed by elution with petrol (b.p. 60–80°) benzene mixtures, then by benzene gave eluates (2.5 g.) which did not contain triterpenoid material. Elution with benzene-ether, ether, and finally with ether containing methanol gave gums (12.5 g.) which when dissolved in methanol slowly deposited crystalline material, m.p. 118–123°. Repeated recrystallization from light petroleum (b.p. 60–80°) gave dipterocarpol, m.p. 132–134°, $[\alpha]_D^{20}$ +67° ($CHCl_3$; *c*, 1.09); infrared bands at 3500, 1695, 1440, 1370 and 815 cm.$^{-1}$ A mixed melting point with an authentic specimen of dipterocarpol kindly supplied by Dr. T. J. King of Nottingham University showed no depression and the infrared spectra of both specimens were identical.

D. grandiflorus. Wood shavings (4 lb.) of *D. grandiflorus* were extracted as above with light petroleum (b.p. 60–80°) and the extract (33.2 g.) when hydrolyzed with methanolic potassium hydroxide gave a non saponifiable fraction (20.2 g.) which was chromatographed as above. The eluates resulting from elution with benzene-ether and ether yielded gummy material which deposited dipterocarpol from methanol solution. Repeated recrystallization from light petroleum (b.p. 60–80°) gave dipterocarpol (2.9 g.), m.p. 132–134° identical with the material obtained above from *D. verrucosis; oxime*, m.p. 176–178° (Mills[8] gives m.p. 178–179°); *semicarbazone*, m.p. 203–205°. (Ourisson[7] gives m.p. 206–207°).

Dammarendiol-II. Reduction of dipterocarpol, isolated from *D. grandiflorus*, with lithium aluminium hydride followed by chromatography of the product on alumina gave dammarendiol-II, m.p. 130–133°, $[\alpha]_D^{20}$ +33° (*c*, 1.01). Mills[3] gives m.p. 131–133°, $[\alpha]_D^{20}$ +34°.

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9,11-Dihalosteroids

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Exploratory experiments directed to the development of a program for the systematic investigation of dihalosteroids, in particular those with fluorine at C-11, were undertaken in these laboratories in 1957. The 11β-fluoro-9α-halosteroids were made either by the use of an *N*-haloamide in anhydrous hydrogen fluoride containing about 30% pyridine or by the reaction of an 11β-hydroxy-9α-bromosteroid with this same solvent pair.