tion of the fungus the radial growth is measured. The temperature is kept between 24–28° during the whole period. The radial growth of the fungi at various concentrations of the thiocyanates and in the absence of the compound are given in Table I.

It is interesting to note that a methyl group at meta position and nitro group at ortho and para position to the thiocyanato group increases fungicidal activity of the thiocyanate. The 3-methyl 2 : 4 : 6 trinitro-phenyl thiocyanate is found to be the most active towards both the test fungi.

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TANNINS OF *CAESALPINIA PULCHERRIMA* BARK

The seeds[1,2] of *Caesalpinia pulcherrima* have been reported to contain a galactomannan. The stem bark of this plant is highly astringent and is widely used as an abortifacient and as an emmenagogue. It has now been investigated and found to contain gallic acid, ellagic acid, leucodelphinidin and a new tannin, which has been studied in detail.

Extraction

Following a general procedure for the extraction of polyphenols the bark was extracted with ethanol. The ether soluble fraction from the concentrated ethanolic extract was found to contain gallic acid, ethyl gallate and traces of ellagic acid. Ethyl acetate extracted a leucoanthocyanidin along with a tannin (A). The mother liquor was further concentrated to a viscous residue and macerated with acetone, which extracted some more of the tannin (A). From the residue free ellagic acid could be extracted out with ethanol containing traces of pyridine. The leucoanthocyanidin could be characterised as leucodelphinidin by its characteristic colour reactions and spectral studies. On acid treatment, it could be converted to its corresponding anthocyanidin delphinidin, which was found to be identical with an authentic sample isolated from *Solanum melongena* fruits, in its colour reactions, paper chromatography and λ_{max} (560 mμ, ethanolic HCl).

The isolation of ethyl gallate, which is usually isolated as an artefact formed as a result of alcoholysis of depside links present in tannins, during the extraction with ethanol, led us to modify the method of extraction. The bark was extracted with water at room temperature. The combined extract was demineralised over a mixed bed of cation and anion exchange resins to constant conductance and then concentrated under diminished pressure to a syrupy mass. Maceration with ether of the viscous residue gave some gallic acid and further extraction with ethyl acetate gave a mixture of gallic acid, leucodelphinidin and another tannin (B). Maceration with acetone of the remaining sticky residue gave some more amount of tannin (B). The acetone concentrate was charged over a silica gel (deactivated) column and eluted with benzene-acetone mixture. Final crystallisation of the last fractions from acetone-ether mixture gave a colourless semi-crystalline compound, which was found to be a homogeneous entity by paper chromatography and TLC.

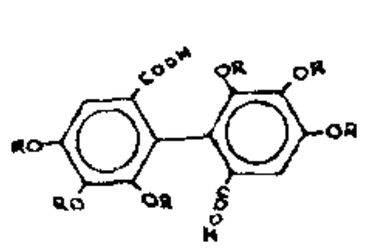
Tannins (A) and (B)

Both these tannins gave positive Molisch test and blue-black precipitate with ferric chloride, suggesting these to be polyphenolic glycoside, but positive colour reaction with aniline hydrogen phthalate reagent[3], confirmed their non-glycosidic nature. Tannin (A) on alkali hydrolysis gave D (+)-glucose, gallic acid and ellagic acid, suggesting thereby that these acids are possibly esterified with the glucose moiety.

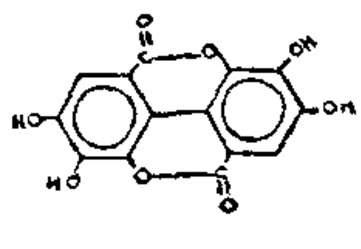
Tannin (B) on acid as well as alkali hydrolysis gave glucose, gallic acid and ellagic acid. The quantitative estimation of glucose shows the presence of 19% glucose. Ellagic acid precipitated out almost quantitatively during hydrolysis and could be directly weighed and found to be 30%. Gallic acid was found to be 55% by potentiometric titration. This calculates to 3 moles of gallic acid and one mole of ellagic acid per mole of glucose. The acetate of this tannin analysed for 15 acetyl groups ($-COCH_3$, 40·5%) per mole of acetate. Methylation was done with diazomethane. IR spectrum of the methyl ether confirmed the absence of any free hydroxyl group. The methyl ether on acid as well as alkaline hydrolysis gave three acids, which were identified as trimethyl gallic acid, 3 ; 4 dimethyl gallic acid and

hexamethoxydiphenic acid (I *b*). These three acids were found to be present in the ratio 2 : 1 : 1 respectively, as indicated by comparative paper chromatography carried out with an artificial mixture of the authentic acid.

As hexamethoxydiphenic acid (I *b*) is one of the products of hydrolysis of the methylated tannin it is assumed that hexahydroxy diphenic acid (I *a*) must be linked to the glucose core through ester linkages involving its two carboxyl groups. Moreover, hydrolysis of the tannin itself gave ellagic acid (II). It is well known that hexahydroxydiphenic acid easily cyclises to form its dilactone ellagic acid under the conditions of the hydrolysis.



(I) (a) R=H, (b) R=CH_3



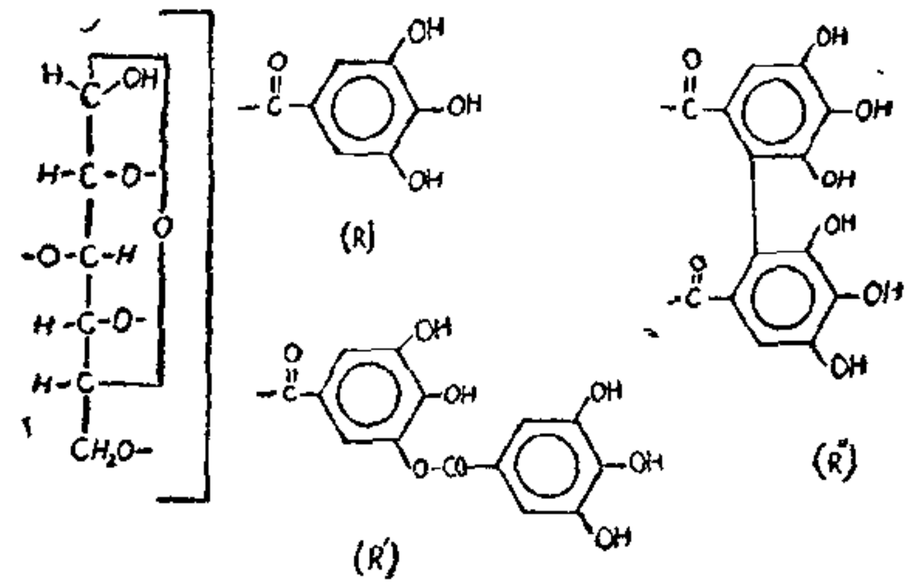
(II)

The proportion of trimethyl gallic acid, dimethyl gallic acid and hexamethoxyldiphenic acid, obtained by hydrolysis of the methylated tannin as 2 : 1 : 1 respectively, shows that one hydroxydiphenic acid, one gallic acid and one *m*-digallic acid are esterified with 2, 3, 4 and 6th carbons of glucose. Two of these hydroxyls may be involved in ester linkages with the two carboxyls of hexahydroxydiphenic acid. These hydroxyls may be 2 and 4, or 4 and 6 or 3 and 6. Out of the remaining two hydroxyls one is esterified with gallic acid and the other with a digalloyl chain.

On controlled methanolysis the tannin (B) gave traces of gallic acid, methyl gallate and another tannin, which compared on paper chromatography and TLC with the one obtained from the ethanolic extract of the bark (Tannin A). This tannin on hydrolysis gave gallic acid, ellagic acid along with glucose. Gallic acid estimation (43%) of this tannin suggested that there may be two gallic acid units per mole of glucose. This suggested that the remaining one gallic acid must be linked with a depside link. As no *m*-digallic acid could be detected during methanolysis, the possibility of a trigalloyl chain is completely excluded.

A number of alternative structures can be assigned to this tannin based on the above data, as it is difficult to assign definite positions to gallic acid, digalloyl and hexahydroxydiphenic acid units on the glucose moiety. Therefore, the distribution of R, R' and R" is of course random.

This tannin from *C. pulcherrima* stem bark is special in containing *m*-digalloyl unit in addition to hexahydroxydiphenic acid. A polygalloylated chain has not so far been reported to be present in any of the naturally occurring ellagitannins. Moreover, the presence of both leucodelphinidin and gallotannin in the same source has also some biogenetic significance, as probably this plant specialises in biosynthesising pyrogallol unit.



(R) (R') (R")

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A NEW SPRAY REAGENT FOR THE IDENTIFICATION AND DETERMINATION OF ORGANO-PHOSPHORUS INSECTICIDES BY THIN LAYER CHROMATOGRAPHY

Various reagents are described in literature for the detection of organo-phosphorus insecticides on thin layer chromatographic plates. Most common ones are palladium (II) chloride[1,5], bromine-fluorescein-silver nitrate[2,3], Congo red[4], mercuric nitrate-diphenylcarbazone[6], mercurous nitrate[7], reduction. diazotisation and coupling with N-1-naphthylethylene-diamine[8], ferric chloride-sulfosalicylic acid[9], etc. To a large extent they are not sensitive and are susceptible to impurities ; some are non-specific. Though some of these reagents[1,2,4,8,10], are sensitive with detection limit as low as 1 to 5 mcg.,