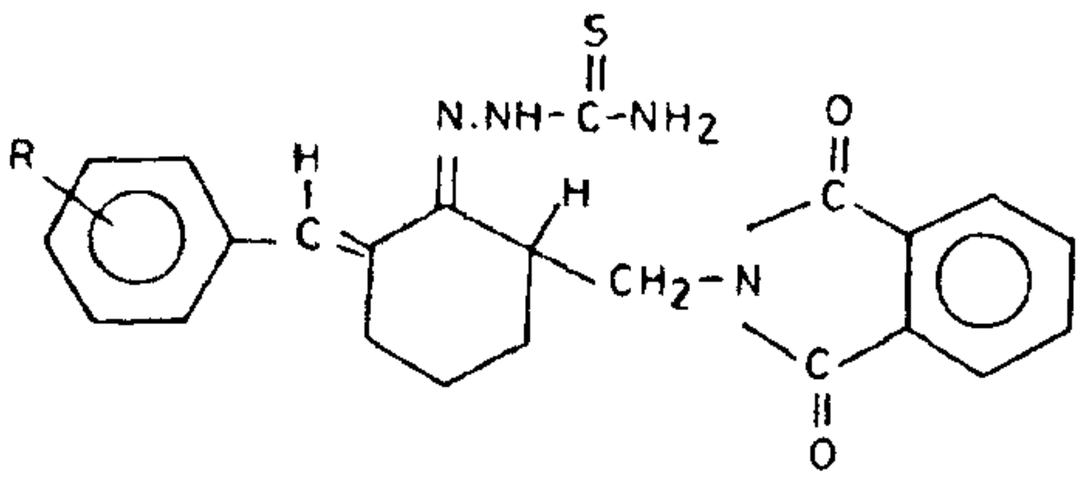
Table 1 *Antiviral activity of α-(methyl phthalimido)-α-(substituted styryl)-cyclohexanone-thiosemicarbazones against the Sunnhemp rosette virus*



Compound No	R	Percent inhibition of SRV In-vitro	In-vivo
1.	H	0	18[b]
2.	2-OH	59[a]	62[a]
3	4-OCH_3	38[b]	32[b]
4	2-OH,5-OCH_3	23[b]	40[b]
5	2-F	20[b]	43[b]
6	4-$(CH_3)_2N$	20[b]	47[b]
7.	4-Cl	52[a]	40[b]
8.	1-CH=CH-	64[a]	68[a]
9.	Styryl = CH_2	70[a]	42[b]

The concentration of compound used was 2 mg/ml.
Virus: Sunnhemp rosette virus
Test plant: *Cyamopsis tetragonoloba*
Data significance at 1 % level = ([a])
Data significance at 5 % level = ([b])

hibition respectively. But inhibition in the case of 4-Cl is decreased.

The *in vivo* activity of N-(methyl phthalimido)-cyclohexanone thiosemicarbazone is also decreased upto 42 percent. But in general percentage inhibition increases remarkably.

It is interesting to observe that the substituent (R, -CH=CH) profoundly increases the antiviral activity *in vivo* and *in vitro* both.

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A NEW ANTHRAQUINONE PIGMENT FROM THE STEM BARK OF *DIOSPYROS DISCOLOR*

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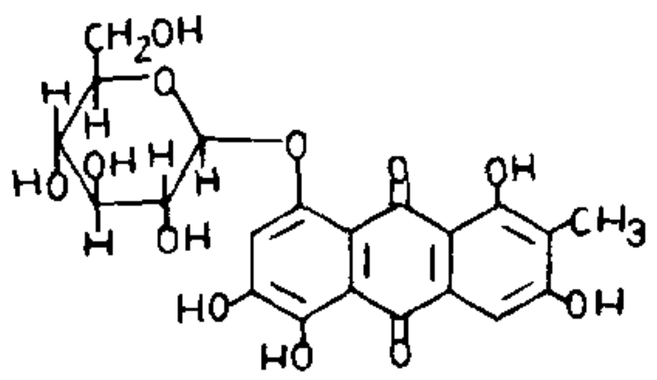
EVIDENCE is presented of the isolation and characterization of a new anthraquinone glycoside from the stem bark of *Diospyros discolor*. The structure was assigned as 1,3,5,6-tetra-hydroxy-2-methylanthraquinone-8-0-β-D-glucopyranoside by spectroscopic and chemical methods.

The compound gave green colour with $FeCl_3$, positive Borntrager reaction[1] and positive Molisch's test for an anthraquinone glycoside. The UV spectrum (EtOH) of the compound showed absorptions at 230, 280 and 430 nm and its IR spectrum (KBr) exhibited absorptions at 3350–3400 (br, OH), 2910, 1635, 1610, 1580, 1440, 1290, 1120, 1090, 825, 820 and 750 cm^{-1}. Acid hydrolysis of the compound (7 % H_2SO_4) yielded an aglycone and D-glucose. The sugar was identified by direct comparison (co-pc) with an authentic sample and also by preparation of osazone derivative (mp,

204–5°; lit. mp 205°). The aglycone responded to colour reactions characteristic of hydroxyanthraquinones and furnished 2-methyl anthracene on zinc dust distillation.

The UV spectrum (EtOH) of the aglycone gave absorptions at 235, 282, 433 and 500 nm; and its IR (KBr) spectrum showed absorptions at 3350–3415 (br, OH), 2912, 1630 (chelated carbonyl groups), 1580, 1445, 1285, 1120, 1095, and 755 cm^{-1}. The PMR spectrum (d_6-DMSO, TMS, 60 MHz) of aglycone displayed signals at δ12.50 (s, OH, C-8), 12.45 (s, OH, C-6), 12.40 (s, OH, C-5), 12.05 (s, OH, C-1), 12.00 (s, OH, C-3), 7.85 (s, 1H, H-4), 7.00 (s, 1H, H-7) and 2.40 (s, 3H, 1 × CH_3)[2]. It formed a pentamethyl ether (Me_2SO_4-K_2CO_3), mp 182–84° and a penta acetate (Ac_2O-pyridine method), mp 130–32° (dec). The PMR spectrum of the acetate of the aglycone (d_6-DMSO, 60 MHz, TMS) displayed signals at δ7.85 (s, 1H, H-4), 7.00 (s, 1H, H-7), 2.40 (s, 3H, 1 × CH_3), 2.18 (s, 3H, 1 × OAc), 2.10 (s, 6H, 2 × OAc), 2.00 (s, 3H, 1 × OAc), 1.98 (s, 3H, 1 × OAc) indicating the presence of five hydroxyl groups in the aglycone. The aglycone formed a red complex with ethanolic $CuSO_4$ showing the presence of α-OH group[3]. The aglycone gave an orange-red colour with 0.5% methanolic magnesium acetate[4] showing the presence of β-OH at position-3. It also formed a red complex with zirconium nitrate solution soluble in HCl showing the presence of hydroxyl group at position C-8[5]. The aglycone gave a positive colour reaction with conc. H_2SO_4 for 1,5 dihydroxyl system[6], (IR 1630 cm^{-1})[7]. The UV spectrum of the aglycone exhibited a specific absorption in the region 480–520 nm (*i.e.* at 500 nm) which is characteristic for 5,6,8 trihydroxy relationship as in the case of erythroglucin and tritisporin[8]. Thus the aglycone could be 1,3,5,6,8 pentahydroxy-2-methylanthraquinone. Chromic acid oxidation of the aglycone methyl ether afforded 3,4,6-tri-methoxy phthalic acid, mp 215–16° (lit. mp 216–17° and co-tlc) as one of the oxidation product corresponding to positions 5,6,8 in aglycone methyl ether which further supported the above proposed structure of the aglycone.

The attachment of D-glucose in glycoside was shown to be at 8-hydroxy group by comparative study of the colour reactions of the glycoside and aglycone. The glycoside as well as the aglycone gave positive tests for 1,3 and 1,5 dihydroxy system respectively[4,6]. The aglycone gave a red complex with zirconium nitrate while the glycoside did not show the presence of D-glucose at position-8. The glycoside was methylated with CH_2N_2 (which methylates only β-OH group)[9] followed by acid hydrolysis which yielded D-glucose (co-pc) and a partially methylated aglycone. The partially methylated aglycone gave positive test with conc. H_2SO_4 (1,5 dihydroxyl groups)[6] and zirconium nitrate (1,8 dihydroxyl groups)[5], confirming the presence of free OH at C-8 position. The periodate oxidation and enzymatic (emulsin) hydrolysis of the glycoside indicated D-glucose to be in β-linked pyranose form. Thus the structure of new glycoside was established as 1,3,5,6-tetra-hydroxy-2-methylanthraquinone-8-0-β-D-glucopyranoside. The aglycone and the glycoside are reported for the first time in nature.



The above proposed structure of the glycoside was further supported by PMr spectrum (d_6-DMSO, TMS, 60 MHz) of the acetylated glycoside (Ac_2O-pyridine method), [δ: 4.32 (d, J = 7.0 Hz, 1H, H-1′, anomeric proton); 2.08 (s, 6H, 2′-OAc and 6′-OAc); 2.15 (s, 3H, 3′-OAc), 1.94 (s, 3H, 4′-OAc) and 3.5 – 3.80 (m, 6H, sugar protons), 7.84 (s, 1H, H-4), 7.00 (s, 1H, H-7) and 2.40 (s, 3H, 1 × CH_3), 2.18 (s, 3H, 1 × OAc), 2.10 (s, 3H, 1 × OAc), 2.00 (s, 3H, 1 × OAc), 1.98 (s, 3H, 1 × OAc)][2,9].

Plant material: Plant material of *D. discolor Willd* was procured from the United Chemicals and Allied Products, Calcutta, India.

Extraction and Isolation: The air-dried and powdered stem bark (3 kg) of *D. discolor* was extracted with ethanol under reflux for 30 days on a water bath. The ethanolic extract (40 lit) was filtered, the filtrate was concentrated under reduced pressure to 300 ml and segregated into water soluble and insoluble fractions. The water insoluble fraction was successively extracted with pet. ether, C_6H_6, $CHCl_3$, EtOAc and Me_2CO. The excess of solvent was removed from EtOAc extract under reduced pressure and the residue on TLC examination showed the presence of only one compound. It was purified over a column of silica-gel, eluted with EtOAc : Me_2CO (8 : 2) and crystallized as a brown coloured needles (Me_2CO.MeOH). $[\alpha]_D^{25}$ + 50 (in MeOH), mp 224–25 (dec) [Found, C, 54.30; H, 4.29; $C_{21}H_{20}O_{12}$ required; C, 54.31; H, 4.31%].

Hydrolysis of the Glycoside: The glycoside (800 mg) was hydrolysed with 7% ethanolic H_2SO_4 (40 ml) for 4 hr under reflux as usual to yield the aglycone and the sugar, D-glucose [R_f 0.18 in n-BuOH-HOAc- H_2O, 4 : 1 : 5 and co-pc].

Characterization of the aglycone: The aglycone on repeated crystallization from MeOH : Me_2CO mixture afforded brown-coloured needles, mp 308–10° (dec), $[\alpha]_D^{25}$ + 45° (in MeOH), [Found: C, 59.58; H, 3.30; $C_{15}H_{10}O_7$ required, C, 59.60; H, 3.31%]. It formed a pentaacetate (100 mg of aglycone + 6 ml Ac_2O + 5 ml pyridine), mp 130–132° (dec): (Found: C, 59.59; H, 3.90; OAc, 41.90; $C_{25}H_{20}O_{12}$ required; C, 58.59; H, 3.90; 5 × OAc, 41.99%) and penta methyl ether (80 mg of aglycone + 5 ml Me_2SO_4 + 2g K_2CO_3), mp 182–84°, [Found; C, 64.50; H, 5.33, OMe (Zeisel's method), 41.62; $C_{20}H_{20}O_7$ required, C, 64.51; H, 5.37; 5 × OMe, 41.66%].

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ANTIMICROBIAL ACTIVITY OF MYCOTOXIN STERIGMATOCYSTIN PRODUCED BY *ASPERGILLUS VERSICOLOR*

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STERIGMATOCYSTIN, a major secondary metabolite of *Aspergillus versicolor*, is a biogenetic precursor of aflatoxin B_1 and has been reported to be toxic to various species of experimental animals[1,2]. Sterigmatocystin is considered to be the most prevalent mycotoxin contaminating foods[3–5]. In the present investigation studies on the anti-bacterial action of sterigmatocystin have been carried out and a method evolved to assay sterigmatocystin by microbiological assay.

A. versicolor strain isolated in this laboratory from contaminated wheat maintained on Czapak-Dox agar slants by periodic subculturing. The liquid medium used for the isolation of sterigmatocystin was prepared as suggested by Rabie *et al*[6]. Sterigmatocystin was extracted by the method of Vorster and Purchase[7] and purified using preparative TLC. It was crystallized using acetone and the product was compared with the authentic sterigmatocystin supplied by Medical Research Council, South Africa.

The isolated sterigmatocystin and authentic sterigmatocystin were tested for their growth inhibiting effects using different micro-organisms like: yeast (*Sacharomyces carlsbergensis, S. cerevisiae*) bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and fungi (*Penicillium crustosum, P. cyclopium* and *P. patulum*). Microbiological assay was carried out by impregnating antibiotic assay discs with different concentrations of authentic and isolated sterigmatocystin followed by placement of the disc on nutrient agar plates inoculated with the test organisms. The plates were incubated for 12 hr at 30°C.

Table 1 represents the antibiotic action of the isolated and authentic sterigmatocystin. It can be seen from the table that sterigmatocystin acts as a mild antibiotic for both *S. carlsbergensis* and *S. cerevisiae* as well as for all the three strains of fungi used (*P. crustosum, P. patulum* and *P. cyclopium*). However the bacteria *S. aureus* and *B. subtilis* are more sensitive and are inhibited at a low concentration of 150 mcg of the toxin. A concentration of more than 200 mcg of sterigmatocystin per 20 ml medium inhibits growth