Table 1: Antiviral activity of α-(methyl phthalimido)-α-(substituted styryl)-cyclohexane-thiosemicarbazones against the Sunn hemp rosette virus

<table>
<thead>
<tr>
<th>Compound</th>
<th>Percent inhibition of SRV</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>In-vitro</td>
</tr>
<tr>
<td>1. H</td>
<td>0</td>
</tr>
<tr>
<td>2. 2-OH</td>
<td>59a</td>
</tr>
<tr>
<td>3. 4-OCH₃</td>
<td>38b</td>
</tr>
<tr>
<td>4. 2-OH,5-OCH₃</td>
<td>23b</td>
</tr>
<tr>
<td>5. 2-F</td>
<td>20b</td>
</tr>
<tr>
<td>6. 4(CH₂)₂N</td>
<td>20b</td>
</tr>
<tr>
<td>7. 4-Cl</td>
<td>52a</td>
</tr>
<tr>
<td>8. 1-CH=CH-</td>
<td>64a</td>
</tr>
<tr>
<td>9. Styryl</td>
<td>CH₂</td>
</tr>
</tbody>
</table>

The concentration of compound used was 2 mg/ml.
Virus: Sunn hemp rosette virus
Test plant: Cyanopsis tetraragonoloba
Data significance at 1% level = (a)
Data significance at 5% level = (b)


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 FeCl₃, positive Borntrager reaction and a positive Molisch's test for an anthraquinone glycoside. The UV spectrum of the compound showed absorptions at 230, 280 and 430 nm and its IR spectrum (KBr) exhibited absorptions at 3350–3400 (br OH), 2950, 1635, 1610, 1580, 1440, 1290, 1120, 1090, 870, 820 and 750 cm⁻¹. Acid hydrolysis of the compound (7%, H₂SO₄) 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,
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), 2950, 1630 (chelated carbonyl groups), 1580, 1445, 1285, 1120, 1095, and 755 cm⁻¹. The PMR spectrum (d₄-DMSO, TMS, 60 MHz) of aglycone displayed signals at δ 2.50 (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 × CH₃)². It formed a penta methyl ether (Me₅Si-K₂CO₃), mp 182–84 and a penta acetate (Ac₂O-pyridine method), mp 130–32 (dec). The PMR spectrum of the acetate of the aglycone (d₄-DMSO, 60 MHz, TMS) displayed signals at δ 7.85 (s, 1H, H-4), 7.00 (s, 1H, H-7), 2.40 (s, 3H, 1 × CH₃), 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 hydroxy groups in the aglycone. The aglycone formed a red complex with ethanolic CuSO₄ showing the presence of 5-OH group.³ The aglycone gave an orange-red colour with 0.5 % methanolic magnesium acetate 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-8.⁴ The aglycone gave a positive colour reaction with conc. H₂SO₄ for 1.5 dihydroxy system.⁵ (IR 1630 cm⁻¹). 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 5,8 trihydroxy relationship as in the case of erythroglicin and trittiporin. The aglycone could be 1,3,5,6,8 pentahydroxy-2-methanthraquinone. 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.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 CH₃I (which methylates only β-OH group)⁸ followed by acid hydrolysis which yielded D-glucose (co-pc) and a partially methylated aglycone. The partially methylated aglycone gave positive test with conc. H₂SO₄ (1,5 dihydroxy groups)⁹ and zirconium nitrate (1,8 dihydroxy groups), 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-methanthraquinone-8-0-β-D-glucopyranoside. The aglycone and the glycoside are reported for the first time in nature.

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 l) 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, CH₂Cl₂, CHCl₃, EtOAc and Me₂CO. 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: Me₂CO (8:2) and crystallized as a brown coloured needles (Me₂CO: MeOH). [α]D = 50 (in MeOH), mp 224–25 (dec) [Found: C, 54.30; H, 4.29; C₂₇H₂_O₃ required: C, 54.31; H, 4.31 °].
Hydrolysis of the Glycoside: The glycoside (800 mg) was hydrolysed with 7% ethanolic \( \text{H}_2\text{SO}_4 \) (40 ml) for 4 hr under reflux as usual to yield the aglycone and the sugar, D-glucose \([R_f 0.18 \text{ in n-BuOH-HOAc- } \text{H}_2\text{O} ; 4:1:5 \text{ and co-pe}],[\alpha]^2 D_{20} + 45 \text{ (in MeOH)}\). [Found: C, 59.58; H, 3.30; C_{18}H_{16}O_{10} \text{ required; C, 59.60; H, 3.31} \text{ mol. wt.}] It formed a pentaacetate (100 mg of aglycone + 6 ml \text{Ac}_2\text{O} + 5 ml \text{pyridine}), mp 130–132°C (dec). [Found: C, 59.59; H, 3.90; OAc, 41.90; C_{22}H_{20}O_{12} \text{ required; C, 58.59; H, 3.90; 5 x OAc, 41.99 mol. wt.}] and penta methyl ether (80 mg of aglycone + 5 ml \text{Me}_2\text{SO}_4 + 2g \text{K}_2\text{CO}_3), mp 182–84°C [Found: C, 64.50; H, 5.33; OMe (Zeisel’s method), 41.62; C_{24}H_{24}O_{12} \text{ required; C, 64.51; H, 5.37; 5 x OMe, 41.66 mol. wt.}].

The authors are thankful to the Directors, Defence Laboratory, Gwalior and C.D.R.I., Lucknow for providing the spectral and micro-analytical data of the glycoside.

22 April 1985


**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 B1 and has been reported to be toxic to various species of experimental animals. Sterigmatocystin is considered to be the most prevalent mycotoxin contaminating foods. 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 Czapek–Dox agar slants by periodic subculturing. The liquid medium used for the isolation of sterigmatocystin was prepared as suggested by Rabie et al. Sterigmatocystin was extracted by the method of Vorster and Purchase 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 (Saccharomyces cerevisiae, 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 discs on nutrient slants 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. cerevisiae 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.
A New Anthraquinone Glycoside from the Stem Bark of Diospyros discolor

Santosh K. Srivastava and Sandhya Pitre

Received: May 15, 1985; Accepted: August 10, 1985

The alkaloids have been extracted and isolated according to a standard procedure (4–5). Their structures (8) have been determined on the basis of their physical constants (mp) and spectral data (UV, IR, MS, 1H-NMR) and confirmed by comparison with authentic samples (TLC, mmp) from the collection of our laboratory or provided by the C. S. I. R. O. (Department of Industrial Chemistry, University of Sydney, Australia).

The leaves yielded the furanoquinoline acryonine (0.02 % from the dried plant material) and benzamide (0.01 %) (9).

The stem bark yielded seven alkaloids. Three of them were the furanoquinoline derivatives acryonine (0.01 %), dictamine (0.005 %) and euvitine (0.005 %). The four remaining compounds were the acridones melicopine (0.02 %), melicopidine (0.02 %), 1,2,3-trimethoxy-10-methyl-acridan-9-one (0.01 %) and acronycone (0.08 %).

From a chemotaxonomic point of view, it is interesting to note that the major alkaloids isolated from the bark belong to the acridone series, as in the other species of this genus previously studied i.e., Sarcomecléa simplicifolia (Endl.) Hartley (2–4, 10) and Sarcomecléa leiocarpa (P. S. Green) Hartley (5). This fact is in full agreement with the botanical revision of this genus recently published by Hartley (1).

References

A New Anthraquinone Glycoside from the Stem Bark of Diospyros discolor

As brown coloured needles (MeCO: MeOH), C₆H₅O₆, m.p. 255–256° C (yield 1.4 g). (1) gave positive Bornträger reaction (3) and further Molisch's test for anthraquinone glycosides. (1) formed a heptaacetate (Ac₂O-C₂H₄N, m.p. 130–132° C (dec)). Its 1H-NMR spectrum (4) (DMSO-d₆, 60 MHz) displayed signals at δ 4.32 (d, J = 7.0 Hz, 1H, H–1, anomic proton): 2.08 (s, 6H, 2-OAc and 6-OAc), 2.15 (s, 3H, 3'-OAc), 1.94 (s, 3H, 4'-OAc), 3.5–3.80 (m, 6H, sugar protons), 7.84 (s, 1H, H–4), 7.00 (s, 1H, H–7), 2.40 (s, 3H, 1×CH₃), 3.90 (s, 3H, 1×OMe), 2.18 (s, 3H, 1×OAc), 2.10 (s, 3H, 1×OAc), 2.00 (s, 3H, 1×OAc). The UV-visible spectra (EtOH) of (1) showed the absorptions at 230, 280 and 430 nm, and its IR (KBr) spectrum exhibited principal absorptions at 3350–3340 (br, OH), 2980 and 1775 (methoxyl), 1635 (chelated >CO), 2950 (methyl) and 825 cm⁻¹ (glycoside), respectively. Acid hydrolysis of (1) (7% H₂SO₄) yielded r-glucose (C₆H₁₂O₆) and an aglycone (2), C₇H₄O₂, m.p. 150–153° C (dec).

Aglycone (2) responded to the colour reaction characteristic of hydroxyanthraquinone and furnished 2-methylanthracene on zinc dust distilla-

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Inhibition of Tumour Growth in vitro by Bromelain, an Extract of the Pineapple Plant (Ananas comosus)

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Received: June 10, 1985; Accepted: July 12, 1985

Bromelain, an extract of the stem of the pineapple plant, (Ananas comosus, Merr.) with or without proteolytic and platelet aggregation inhibiting activity showed a dose dependent growth inhibition of 3 cell lines, Lewis lung carcinoma, YC-8 lymphoma and MCA-1 ascitic tumour cells.

Non steroidal anti-inflammatory drugs such as aspirin and indomethacin associated with inhibition of platelet aggregation (1, 2, 3) have been reported to retard tumour growth (4). Bromelain, a non toxic anti-inflammatory compound (5) also has platelet aggregation inhibiting properties (6, 7) and was therefore studied for its effect on tumour growth.

Fig. 1 Effect of heating Bromelain to 70°C and 100°C on the growth of Lewis lung carcinoma in cell culture. Bromelain concentration: 0.8 mg/ml. Incubation: 48 hrs at 37°C in 10% CO2 atmospheric.

Fig. 2 Retardation of tumour cell growth with increasing Bromelain concentrations. LLC = Lewis lung carcinoma; YC-8 = Lymphoma; MCA-1 = Ascitic tumor. The cells in all groups were incubated for 48 hrs at 37°C in 10% CO2 in air and were counted with Coulter counter (Model B). The graph represents the x of six individual experiments with 5 tumour cell cultures in each tumour line in each experiment.
which was recrystallised from petroleum ether—benzene, (60%), m.p. 182° (Found: C, 81.41; H, 5.40; N, 3.97. C₂₄H₁₉NO₉ requires: C, 81.58; H, 5.38; N, 3.96%).

3-Methyl-5-phenyl-3',4'-dihydro-spiro[2-isozaxoline-4,2'(1'H)-naphthalene-1-one] (3b): 2-Benzo-1-tetralone (0.01 mol), nitroethane (0.01 ml) and phenyl isocyanate (0.02 mol) were taken in dry benzene. The solution was cooled in an ice-water bath and stirred while few drops of triethylamine in benzene were added dropwise during 10 min. The reaction mixture was refluxed for 6 h while stirring. Separated diphenyl urea was removed by filtration. The filtrate was washed with water repeatedly and dried. The product was recrystallised from benzene, (40%), m.p. 196° (Found: C, 78.29; H, 5.81; N, 4.78. C₁₆H₁₇NO₄ requires: C, 78.35; H, 5.84; N, 4.81%).

**Antibacterial activity**: Antibacterial activity was assayed by employing Vincent and Vincent's agar-disc method. All the bacteria employed, both the gram-positive (Bacillus pURNILIS, Bacillus polymixa and Streptococcus albus) and gram-negative (Acetobacter acrogenes, Proteus vulgaris and Pseudomonas ovata) were found to be moderately sensitive to the compounds under investigation.

**Antifungal activity**: The fungicidal activity was studied by glass slide-humid chamber technique against four phytopathogen fungi (Fusarium oxysporum Schl., Curvularia lunata (Walker) Boed., Drechslera rostata and Alternaria alternata). The compounds exhibited strong fungicidal activity against all the four organisms and caused total inhibition at 200 µg ml⁻¹ concentration. The log ED₅₀ values for F. oxysporum, C. lunata, D. rostata and A. alternata were 2.1, 2.2, 2.06 and 2.08, respectively.

**Acknowledgement**

The authors are grateful to Prof. E. V. Sundaram, for facilities, and to Dr. K. Nagarajan, Director, Searle R & D Centre, Thane, for helpful discussion.

**References**

**3,3'-Di-O-methyllellagic acid-4-O-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside from Stem Bark of Diospyros discolor Wild.**

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Masterpiece received 7 October 1985, revised 19 May 1986, accepted 13 November 1986

**The plant Diospyros discolor** (syn. D. mabola) is a medicinal plant, employed in the indigenous system of medicine. The previous study on the stem bark of D. discolor disclosed the presence of a new anthraquinone glycoside and a tannin. Our further examination on the stem bark of this plant has now revealed the presence of a new glycoside, 3,3'-di-O-methyllellagic acid-4-O-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside, characterised by spectral and chemical analyses.

The glycoside, m.p. 220-222° d, gave a yellow colour with alkali, a dark bluish green precipitate with FeCl₃, and positive Greissmeyer's reaction, suggesting that it to be an ellagic acid derivative. Its ir (KBr) spectral bands at 3 440-3 410 (br, OH), 1 730 and 1 720 (lactone), 2 870 and 1 715 (methoxy), 1 590, 1 560 and 1 470 (aromatic) and 820 cm⁻¹ (glicoside), and uv (EtOH) characteristic bands at 249, 355 (sh) and 370 nm substantiated this assumption.

Acid hydrolysis of the glycoside gave an aglycone and a mixture of sugars identified as D-galactose and D-glucose by direct comparison with authentic samples (co-pc); 2, m.p. 273-274°, C₁₂H₁₀O₄, λₘₚₐ₅ (EtOH) 248, 359 (sh) and 372 nm analysed for two methanol groups (Zeisel's). Its uv spectra exhibited a bathochromic shift of 33 nm in presence of sodium ethylate but no bathochromic shift was discernible with sodium acetate indicating that the aglycone is 3,3'-di-O-methyllellagic acid, which was confirmed by direct comparison (m.m.p. and co-tlc).

The glycoside on methylation with diazomethane and subsequent acid hydrolysis yielded 3,3',4',5'-tri-O-methyllellagic acid, m.p. 287-88° (m.m.p. and co-tlc) which gave a monosaccharide, m.p. 260-62° (m.m.p. and co-tlc). This observation confirmed that both the sugar units are linked at position-4 of the aglycone in the form of a disaccharide. The partial acid hydrolysis of the glycoside yields D-galactose (co-pc) and 3,3'-di-O-methyllellagic acid-4-O-glicoside, m.p. 213-15°. This is borne out by the fact that the D-Galactose was thus proved to be the terminal sugar in 1.

The structure was finally established by per-methylation (Hakomori's method) of the glycoside followed by acid hydrolysis which gave 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-glucose (R₅ values and co-pc) and 3,3',4',5'-tri-O-methyllellagic acid (m.m.p. and co-tlc). This confirmed the 1→4 inter-sugar linkage. The periodate
NOTES

oxidation of glycoside which liberated one mole of HCO₃⁻ with consumption of three moles of sodium metaperiodate supported the pyranose form of both the sugars. The almond enzymatic hydrolysis of the glycoside afforded 2 (m.m.p. and co-tic), d-galactose and d-glucose (co-pec), confirming the β-linkage in both the sugars and the aglycone. This led to the formulation of new glycoside as 3,3'-di-O-methyllelagic-acid-4-O-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside. The 60 MHz ¹H nmr spectra (CDCl₃) of the acetylated glycoside also supported the formulation (I) for glycoside, δ 4.90 (1H, d, J 7Hz, H-1”), 4.70 (1H, d, J 7Hz, H-1”), 4.00 (3H, s, 1×OMe), 3.90 (3H, s, 1×OMe), 7.20 (2H, s, H-5 and 5’), 2.0 (3H, s, 1×OAc), 2.06 (6H, s, 2×OAc and 3’-OAc), 2.08 (3H, s, 6’-OAc), 2.04 (6H, s, 2’-OAc and 3’-OAc), 2.02 (3H, s, 4’-OAc), 2.10 (3H, s, 6’-OAc) and 3.50–3.80 (12H, m, sugar-protons).

Acknowledgement

The authors are thankful to the Director, C.D.R.I., Lucknow, and Defence Laboratory, Gwalior, for the spectral and analytical data, and to Ms. A. Jain, Chemistry Department of this University for the authentic samples.

References


Experimental

Extraction: The air-dried and powdered stem bark (3 kg) of D. discolor procured from the United Chemical and Allied Product, Calcutta, was exhaustively extracted with rectified spirit under reflux for 30 days. The total spirit extract (40 dm³) was concentrated (300 ml) and poured into distilled water (1 dm³). The water-soluble fraction was concentrated to a syrupy mass and successively extracted with petroleum ether, C₆H₆, CHCl₃, EtOAc, Me₆CO and MeOH. The MeOH extract was passed through a column of silica gel and eluted from Me₆CO–MeOH (1:1). The eluate was concentrated and the product on crystallisation from MeOH–Et₂O mixture afforded brown coloured crystals of 1 (0.5 g) (Found: C, 51.39; H, 4.60; C₆H₅O₂SO₃H calculated for: C, 51.39; H, 4.58%). Acetylation of 1 with Ac₂O/P roughly gave an acetate, m.p. 180–182°C (Found: C, 53.29; H, 4.60; C₆H₄O₃OAc calculated for: C, 53.33; H, 4.64%).

Acid hydrolysis of the glycoside: The glycoside (800 mg) was refluxed with aqueous H₂SO₄ (7%, 40 ml) for 3 h, cooled and extracted with ether. The ether extract was evaporated and the residue crystallised (MeOH–Et₂O) to yield 2 as pale yellow crystals (lit. m.p. 274°C) (Found: C, 58.12; H, 3.02; OMe, 18.75. C₆H₆O₃SO₃H calculated for: C, 58.18, H, 3.03; OMe, 18.78%). The aqueous layer after neutralisation (BaCO₃) was shown to be a mixture of d-galactose and d-glucose (co-pec).

Microdetermination of Phosphate with 2-Carboxy-2'-hydroxy-3',5'-dimethylazo-benzene-4-sulphonic Acid

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2-CARBOXY-2'-hydroxy-3',5'-dimethylazobenzene-4-sulphonic acid (CHDMAS, C₆H₇N₂O₄S) has been described as a reagent for the spectrophotometric studies of Pu³⁺, Cu³⁺, Ni²⁺, Fe³⁺ and Al³⁺. Bokare et al.* determined the stepwise stability constant of CHDMAS-Pu²⁺ complex using a potentiometric technique. The reagent has also been employed for the determination of fluoride in water*1. The decrease of absorbance of the Mg²⁺-CHDMAS complex on addition of phosphate at the λmax has been found to be quantitative, and a method has been evolved for the determination of phosphate in trace quantities.

Experimental

Mg²⁺ and phosphate solutions were prepared by dissolving magnesium sulphate (A.R.) and potassium dihydrogen orthophosphate (A.R.), respectively,

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Surprisingly, in the spectra of 4 and 5, the 14-H signal was a broadened singlet while in the case of 2 and 3 pairs of doublets were present.

The isolation of the lactone 1 may be an indication that the unusual lactones of *Mikania* species are all derived from germacr-1(10)Z,4E-dien-12,8a-olide, which could be transformed by oxidation to all the known lactones from this genus.

### Material and Methods

#### Methods and Instruments

The air dried aerial parts (800 g, collected near Tobati, Depto. Cordillera, Paraguay, voucher 678, deposited in the US National Herbarium) were extracted with EtO/petroleum ether/methanol, 1:1:1, and the extract was separated as reported previously (4). The defatted extract was first separated by CC (SiO2) into three fractions [1: EtO/petroleum ether (PE), 1:9:2; EtO/PE, 1:1:1; and 3: EtO]. PTLC of fraction 1 (SiO2, PE 254, EtO/PE, 1:9) gave 7 mg taxaraxesteryl acetate and 10 mg coumarin. HPLC (RP 8, CH3OH/H2O 7:3, ca. 100 bar) of fraction 2 gave 5 mg mikanolide, (R, 3.8 min), 3 mg mikanodenin (R, 4.8 min), and 3 mg 2 and 3 (R, 7.0 min). HPLC of fraction 3 (same conditions) gave 5 mg 4 (R, 4.7 min), 5 mg 5 (R, 4.4 min), and a mixture (R, 4.0 min) which gave by PTLC (PE/EtAc, 1:1) 15 mg 1 (R, 0.18) and 2 mg deoxyxymikanolide (R, 0.41). Known compounds were identified by comparing the 400 MHz 1H-NMR spectra with those of authentic material.

**Micontholate (1)**

Colourless oil; IR νmax cm⁻¹: 3600 (OH), 1760 (γ-lactone); MS m/z [rel. int.]: 247 [M – OH]+ (5), 233 [M - CH₃OH]⁺ (6), 55 (100); ¹³C-NMR (CDCl₃, C-1-C-15): 125.2 d, 31.2 t, 29.7 t, 138.3 s, 127.1 d, 28.7 t, 46.9 d, 84.2 d, 35.7 t, 139.6 s, 140.0 s, 169.6 s, 120.3 t, 66.6 t, 61.8 t.

**Micontholate-15-O-isobutyrate and -methacrylate (2 and 3)**

Colourless oil; MS m/z (rel. int.): 246 [M - RCO₂H]⁺ (4), 228 [246 - H₂O]⁺ (6), 215 [246 - CH₃OH]⁺ (5), 71 [CH₃CO]⁻ (70), 69 [CH₂CO]⁻ (62), 57 (100).

**Micontholate-15-O-[2,3-dihydropyridosobutyrate (4)**

Colourless oil; IR νmax cm⁻¹: 3400 (OH), 1760 (γ-lactone), 1740 (CO₂R); MS m/z (rel. int.): 246.125 [M - RCO₂H]⁺ (12) (calc. for C₂₅H₂₂O₄: 246.126), 228 [246 - H₂O]⁺ (21), 215 [246 - CH₃OH]⁺ (17), 55 (100).

#### Acknowledgements

We thank Dr. R. M. King for identification of the plant material and the Deutsche Forschungsgemeinschaft for financial support.

#### References


### Two New Anthraquinones from the Seeds of *Peganum harmala*

Sandra Pitre1 and Santosh K. Srivastava2

Received: May 21, 1986

**Abstract:** Two new anthraquinones have been isolated from the seeds of *Peganum harmala* Linn and the structures established as 3,6-dihydroxy-8-methoxy-2-methylanthaquinone (peganone: 1) and 8-hydroxy-7-methoxy-2-methylantraquinone (peganone: 2) by spectral correlations and chemical evidence.

**Introduction**

*Peganum harmala*, L. (Rutaceae) is used in the Indian indigenous system of medicine (1). Previous investigations on the seeds of *P. harmala* has led to the isolation of sterols (2), and alkaloids (3). We reported herein two new anthraquinones named as peganone (1) and (2), respectively.

**Results and Discussion**

Peganone (1), C₁₉H₁₃O₄ (M⁺ 284), m.p. 190–192°C and peganone (2), C₁₉H₁₅O₄ (M⁺ 268), m.p. 269–270°C showed UV-visible maxima at 218, 225, 430 nm and 220, 230, 415 nm, respectively. Both gave a positive Borntrager reaction (4), characteristic for anthraquinones. The IR spectrum of 1 and 2 showed characteristic absorptions for hydroxyl, methoxyl, and methyl groups, respectively. The ¹H-NMR spectrum (6 ppm scale) of 1 displayed signals for two unchelated hydroxyls (12.00 and 12.05), one methoxyl (4.00), one methyl (2.40) [5] and two separate aromatic meta-coupled signals (8.00 and 7.00, each d, J = 2.5 Hz) were due to the presence of one proton each at C-5 and C-7, as in the case of emodin and its related derivatives (6). Further 1 showed a two separate singlets (7.30 and 7.90) corresponding to one proton each at the C-1 and C-4 positions, respectively. Compounds 1 and 2 exhibited no absorptions in the region 480–520 nm, indicating the absence of two α-hydroxyl groups in a 1,4- or 5,8-relationship (7). Compound 1 formed a trimethyl ether, m.p. 130–133°C and diacetate, m.p. 120–122°C, confirming the presence of two -OH and one -OMe groups in 1. Thus the structure of peganone 1 was assigned as 1.

The ¹H-NMR spectrum (6 ppm scale) of 2 exhibited signals for one chelated hydroxyl (15.00), especially at the C-8 position, which was also supported by its positive colour test with zirconium nitrate (8). In addition to this, the ¹H-NMR spectrum of 2 further showed signals for one methoxyl (3.83), one methyl (2.69)
Essential Oil from *Oriğanum dictamnus*

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Abstract: The essential oil of *Oriğanum dictamnus* L. was obtained by steam distillation with a yield of 0.8% of dry material. The oil was investigated after fractionation on a silica gel column and separation of phenols by combination of GLC-mass spectrometry. In the analysis, 30 components were found accounting for 98.28% of the oil, the predominating compounds being carvacrol, γ-terpinene, p-cymene, caraphyllene, borneol, terpin-1-en-4-ol, and carvacrol methyl ether.

Introduction

The plant occurs wild and is also cultivated on the island of Crete, Greece and is widely used as a tea. It is known under many names from which “dictamos” and “erontas” are the most common. Its use has endured through the ages since it is mentioned even in mythology. Healing wounds (1) and gastric ulcer, inducing childbirth and abortion, stopping bleeding (2), were among the properties attributed to this plant. It stimulates the nervous system and shows a tonic action.

The essential oil is secreted in the glandular hairs of the leaves which develop from a single protodermal cell (3). Although the plant is of a very wide

Material and Methods

Melting points were taken in a melting point apparatus (Toshniwal, India) and were uncorrected. The UV-visible spectra in EtOH were obtained on a Perkin-Elmer model 202 recording spectrophotometer and the IR spectra were determined on a Perkin-Elmer model 157 spectrophotometer in KBr pellets. The 1H-NMR spectra were recorded in CDCl3 and DMSO-d6 at 60 MHz and 90 MHz, respectively, on a FT-NMR spectrometer with TMS as an internal standard and chemical shifts recorded in δ units. The mass spectra were taken with Hitachi RMU-6E mass spectrometer fitted with direct inlet system and operating at 70 eV.

Isolation of anthraquinones

The powdered seeds of *P. harmala*, procured from United Chemical and Allied Products Calcutta, India, were extracted with ethanol under reflux for 180 h on a water bath. The ethanol extract was segregated into water soluble and insoluble fractions. The benzene extract of the water-soluble fraction gave I, which was purified over neutral alumina column, eluted from CH3, CHCl3, C6H6 (8:2) and crystallized as red coloured needles (CHCl3, MeOH, 6:4). 1: yield 900 mg; IR (ν): 3100-3200 (br, OH), 2940 and 1450 (C = O), 2850 and 1170 (OMe), 1675 (CO), 1560, 1280, 1200, 1020 and 740; MS: m/e = 228 (100), 267 (90), 172 (85), 269 (80), 135 (80), 266 (65), 284 (50), 151 (40), 107 (25), 256 (25), 253 (22), 123 (18). Compound 1 formed a trimethyl ether (100 mg 1 + 5 ml Me3SO + 2 g K2CO3) and a diacetate (100 mg 1 + 5 ml Ac2O + 5 ml CH3OH); Acetate 1H-NMR: 8.00 (d, J = 2.5 Hz, 1H, H-5), 7.00 (d, J = 2.5 Hz, 1H, H-7), 7.00 (s, 1H, H-1), 7.30 (s, 1H, H-4), 4.00 (s, 3H, OMe), 2.45 (s, 3H, Me), 2.10 (s, 3H, OAc), 2.00 (s, 3H, 1× OAc).

The benzene extract of the water-insoluble fraction yielded 140 mg, which was purified over neutral alumina column, eluted from benzene and crystallized as red coloured needles (C6H6, MeOH 5: 5). 2: yield 950 mg; IR (ν): 3050-3150 (br, OH), 2950 and 1450 (C=O), 2880 and 1160 (OMe), 1670 and 1625 (C = O), 1565, 1200, 1020 and 740; MS: m/e = 212 (100), 251 (90), 156 (80), 119 (80), 253 (75), 266 (60), 250 (60), 151 (30), 240 (25), 239 (20), 123 (20), 91 (20). Compound 2 formed a dimethyl ether (100 mg 2 + 4 ml Me3SO + 2 g K2CO3) and monoacetate (100 mg 2 + 5 ml Ac2O + 5 ml C6H6); Acetate 1H-NMR: 8.00 (d, J = 8.0 Hz, 1H, H-4), 7.95 (d, J = 8.0 Hz, 1H, H-6), 6.75 (dd, J = 1.5 Hz and 8.0 Hz, 1H, H-3), 6.95 (d, J = 1.5 Hz, 1H, H-1), 3.85 (s, 3H, OMe), 2.70 (s, 3H, OAc).

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References


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