APPENDICES
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This is to Certify that Mr./Ms./Dr./Professor
Prachi Tripathi has attended the
above Convention and presented a paper
entitled Chemical Examination and...... from
Stem of Bauhinia purpurea
(Paper no. ____).
ORG(0)-54

No TADA has been paid by the Society for
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Sincerely,

Professor P. L. Majumder
Honorary Secretary

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Organic Chemistry

Section
Phytochemical communication

A novel flavone glycoside from the stem of Bauhinia purpurea

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Abstract

A novel flavone glycoside, 5,6-dihydroxy-7-methoxyflavone 6-O-β-D-xylopyranoside (1) was isolated from the chloroform-soluble fraction of the ethanolic extract of Bauhinia purpurea stems. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Bauhinia purpurea; Flavonoids

Plant. Bauhinia purpurea L. (Caesalpiniaceae) stems were collected from the Sagar region (Dhamoni forest) in the month of August 1998 and identified by a taxonomist of the Department of Botany, Dr H. S. G. University, Sagar (M.P.) India. A voucher specimen (No. XIII) has been deposited in the Natural Products Laboratory, Department of Chemistry, Dr H. S. G. University, Sagar. The plant, commonly known as ‘Khairwal’ in Hindi, is found in sub-Himalayan tracts up to 1200 m above sea level.

Uses in traditional medicine. The bark is used as an astringent in diarrhoea [1,2].

Previously isolated classes of constituents. Chalcone glycosides [3], amino acids [4,5].

*Tel.: +91-7582-26465; fax: +91-7582-23236.
New-isolated constituents. 5,6-Dihydroxy-7-methoxyflavone 6-O-β-D-xylopyranoside (I), isolated from air-dried powdered stem in 0.065% yield following 95% EtOH extraction, partition with n-hexane, CHCl₃, EtOH and MeOH, and Si-gel CC of the CHCl₃ soluble fraction.

\[ \text{CH}_3\overset{O}{\text{O}} \]
\[ \text{RO} \overset{\text{O}}{\text{OH}} \]
\[ \text{K} \text{O} \]

\[ 1 \text{ R = xylosyl} \]

5,6-Dihydroxy-7-methoxyflavone 6-O-β-D-xylopyranoside (I). Light-yellow needles, m.p. 280–282°C (from Et₂O); UV max (MeOH): 250, 270, 317; (+ AlCl₃) 252, 271, 337; (+ AlCl₃·HCl) 252, 271, 338; (+ NaOMe) 248, 268, 367; (+ NaOAc) 270, 368; (+ NaOAc·H₂BO₂) 272, 319 nm; IR bands (KBr): 3600, 3350, 1640, 1625, 1510, 2785, 825 cm⁻¹; ¹H-NMR (300 MHz, DMSO-d₆): δ 7.40 (5H, brs, protons of ring B), 6.65 (1H, s, H-3), 4.01 (3H, s, OMe-7), 6.69 (1H, s, H-8), 4.83 (1H, d, J 8.1 Hz, H-1″), 3.83 (5H, brs, sugar protons); ¹³C-NMR (400 MHz, DMSO-d₆): 163.00 (C-2), 104.80 (C-3), 182.40 (C-4), 152.60 (C-5), 129.80 (C-6), 157.30 (C-7), 94.50 (C-8), 153.10 (C-9), 104.50 (C-10), 130.80 (C-1″), 125.40 (C-2″), 128.70 (C-3″), 132.00 (C-4″), 128.90 (C-5″), 125.50 (C-6″), 105.8 (C-1‴), 71.1 (C-2‴), 79.9 (C-3‴), 83.3(C-4‴), 65.2 (C-5‴), 60.10 (–OCH₃); EIMS m/z (% rel. int.): 416 [M]+* absent, 284 [M⁺–sugar moiety]** (50), 269 [aglycone*–Me]* (80), 266 [aglycone*–H₂O]** (50), 241 [aglycone*–CO–Me]* (60), 167 [A₁]** (5.5), 139 [A*–CO]** (12), 105 [B₁]+ (15), 102 [B₂]** (13) (see Mabry and Markham [6] for A₁, B₁ and B₂ nomenclature). Acid hydrolysis gave 7-methoxy-5,6-dihydroxy flavone [7] and xylose. Permethylation followed by acid hydrolysis yielded 2,3,4-tri-O-methyl-D-xylose [8].

Acknowledgements

The authors are thankful to the Director of CDRI, Lucknow for spectral analysis and to the Head, Dept. of Chemistry for providing laboratory facilities.

References

A NOVEL FLAVANONE GLYCOSIDE FROM
ALBIZZIA PROCERA BENTH

R. N. YADAVA and PRICHI TRIPATHI

Natural Products Laboratory, Dr. H. S. G. University,
Sagar-570 003 (M. P.)

(Received 8 March, 1999)

The plant Albizia procera Benth1,a (Family-Leguminosae) is commonly known as
Safed Siris in Hindi and is found throughout India. Its leaves are reported to be
useful as an insecticide and also used in treatment of ulcers. Earlier workersa,c have
characterised the occurrence of tritepenoid saponins and other compounds. The present
paper deals with isolation and characterization of a new flavanone glycoside, 5-hydroxy-
7,8-dimethoxyflavanone 5-O-α-L-rhamnopyranoside from the stem of Albizia procera
Benth.

EXPERIMENTAL AND DISCUSSION

The compound (I), C_{11}H_{13}O_{3}, mp 150, gave positive Shinoda4 and Molisch tests,
indicating (I) to be a flavonoid glycoside. In the UV spectrum in MeOH two maximum
absorbance was observed at λ 312 and 278 nm suggesting a flavanone skeleton6. Absence
of any characteristic shift with NaOAc indicated a blocked C-7 position by OMe group7.
It also responded negative Gibbs test, suggesting the location of another OMe at C-3 in
agreement with the observation of Sharif et al8.

The 1H-NMR spectrum of (I) was considered (ABX system) with resonance
signals at δ 5.25 as double doublet with J values of 5, 10 Hz due to H-2 proton and a

\[
\begin{align*}
\text{Compound (I)} & : R = \alpha-L-rhamnopyranoside \\
\text{Compound (II)} & : R = H
\end{align*}
\]

singlet of two proton intensity was observed at δ 3.0 assigned to C-3 protons which
confirmed the flavanone nucleus. Two sharp signals at δ 3.86 (3H) and δ 3.59 (3H)

\[\text{MnO} \quad \text{MeO} \]

\[\text{OH} \quad \text{OR} \]

\[\text{R} = \alpha-L-rhamnopyranoside \]

\[\text{R} = H \]

A NOVEL FLAVANONE GLYCOSIDE

indicated the presence of two methoxyl groups. The C-6 proton was observed at δ 6.13 and a broad singlet was appeared at δ 7.45 for the aromatic protons of ring B was unsubstituted. The anomic proton was appeared at δ 6.42 as doublet (J 2.0 Hz), sugar protons were appeared in the range of δ 5.65-5.3 as multiplet for four protons. The rhamnosyl methyl observed as doublet at δ 1.10 (J 6.0 Hz).

Acid hydrolysis of (I) yielded an aglycone (II), and rhamnose. The aglycone (II), mp 66-90°, M+ 300, analysed for C_{17}H_{18}O_{5}, (Found : C,68.02; H, 5.4 Clad. C,68.00; H, 5.33). The aglycone was identified as, 5-hydroxy-7,8-dimethoxylflavonone by comparison of its mp, UV, IR, 1H-NMR, MS with those reported in literaturea. The sugar was identified as rhamnose by Co-PC (Rf = 0.38).

Acetylation of compound (I) with Ac^+O/Pyridine afforded a triacetate derivative (Ia), mp 83-84°. M+ 572, C_{26}H_{28}O_{12}, (Found : C, 68.3; H, 4.25; Calcd : C, 68.4; H, 4.5%). 1H-NMR spectrum of (Ia) showed the resonance signals of three aliphatic acetoxyls and the remaining signals were same as of the compound (I), (See experimental).

Permethylation of compound (I), followed by acid hydrolysis yielded the aglycone (II), and 2, 3, 4-tri-O-methyl-L-rhamnose as according to Petek*, suggesting that C-1 OH of the rhamnose was involved in the glycosidation. It also confirmed that there was only one hydroxyl group in the aglycone.

Attachment of sugar to the aglycone was further confirmed by comparing UV spectra of the aglycone with that of the glycoside which showed a bathochromic shift of 15 nm in band I with AlCl3 (relative to MeOH) but no remarkable shift was observed in the case of glycoside suggesting that OH group at C-5 was free in the aglycone while substituted in the glycoside.

Enzymatic hydrolysis of compound (I) with ‘Takadiastase’ gave aglycone (II) and L-rhamnose suggesting <\-linkage between aglycone and sugar. The chemical shift and coupling constant values of anomic proton (δ 5.42, J 2.0Hz) also confirmed the <\-linkage between sugar and the aglycone. Quantitative estimation of sugar with Somogyi's method showed the presence of one mole of sugar per mole of aglycone.

Mass spectrum of acetylated glycoside (Ia) was in full agreement with the proposed structure. The molecular ion peak was absent. Base peak at m/z 300 (100%) was attributed to aglycone fragment resulting from the loss of acetylated sugar moiety from the acetylated glycoside. RDA fragments at m/z 197 and 104 was due to [A,]+ and [B,]+ fragments which further confirmed the presence of two methoxy and one hydroxy group on ring 'A' and unsubstituted B ring.

On the basis of above discussion structure of the compound (I) has been assigned as, 5-hydroxy-7, 8-dimethoxyflavanone-5-O-<\-L-rhamnopyranoside.

The mps were determined on thermoelectrical apparatus and are uncorrected. Electronic spectra were measured on a Perkin-Elmer Lambda 35 spectrophotometer, in
spectra on a Perkin-Elmer (FTIR) spectrometer, ¹H-NMR spectra on Bruker 300 MHz using solvent DMSO-d₆ TMS as an internal standard, and mass spectra on a JEOL-D-300 spectrometer.

Air dried and powdered stem part of the plant A. Procera was extracted with 95% ethanol four times. The combined concentrated ethanolic extract were extracted with benzene, ethylacetate, chloroform, acetone and methanol.

The ethyl acetate fraction of the ethanolic extract of the plant was subjected Si-gel CC using solvents ethyl acetate and methanol in gradient proportions. Fraction eluted with EtOAe-MeOH (7 : 3) afforded (I) which on crystallization with MeOH gave brownish yellow needles of (I), mp 125°, M⁺ 446 whence gave single spot on TLC in EtOAc : MeOH : CH₃COOH : H₂O (5 : 3 : 1 : 1).

**Compound I**: Crystallised from MeOH as brownish yellow crystals, mp 125°, M⁺ 446, 180 mg. (Found C, 61.68; H, 5.82; C₆H₅CH₃V₁₉, Caled, C, 61.70; H, 5.88%).

UV λₑₑₑ (MeOH) 278, 342; (+NaOMe) 276, 350; (+AlCl₃) 308, 358; (+NaOAc) 278, 340 nm, IR vₑₑₑ (KBr) 3430, 1625 (conjugated C=O), 1070-1025 (glycosidic C-O), 2785 (O-Me) cm⁻¹.

¹H-NMR of compound I: (300 MHz, DMSO-d₆, δ ppm) 5.25 (1H, dd, J 5, 10Hz, H-2), 3.0 (2H, s, H-3), 3.56 (3H, s, OMe-7), 3.89 (3H, s, OMe-8), 6.13 (1H, s, H-6), 7.45 (5H, br s, protons of ring B), 5.52 (1H, d, J 2.0 Hz, H-1'), 4.65-5.3 (3H, m, sugar protons), 1.10 (3H, d, J 6.0 Hz, rhamnosyl-CH₃).

¹³C-NMR (DMSO-d₆) 79.7 (C-2), 44.75 (C-3), 189-79 (C-4), 159.2 (C-5'), 97.2 (C-6), 164 (C-7), 112.87 (C-8), 160.2 (C-9), 105.9 (C-10), 138.87 (C-1'), 125.7 (C-2', 6'), 128.75 (C-3', 5'), 130.44 (C-4'), 100.1 (C-1''), 70.0 (C-2''), 70.4 (C-3''), 71.8 (C-4''), 68.3 (C-5''), 17.5 (C-6').

Acetylation of Compound I: Compound (I) was treated with Ac₂O/pyridine (1 : 2), 48 hrs. at 25° and usual workup afforded a triacetate derivative as brown amorphous powder, (Ia), mp 83-84°. (Found C, 63.3; H, 4.25; C₆H₅CH₃O₂₃, Caled C, 63.39, H, 4.30%).

¹H-NMR (300 MHz, DMSO-d₆) 2.05 (3H, s, OAc-2'), 2.10 (3H, s, OAc-3'), 2.15 (3H, s, OAc-4'), 3.87 (3H, s, OMe-7), 3.50 (3H, s, OMe-8), 5.42 (IH, d, J 2.0 Hz, H-1'), 4.65-5.3 (4H, m, sugar protons), 1.10 (3H, d, J 6.0 Hz, sugar methyl), 7.80 (2H, d, J 8.0 Hz, H-2', 6'), 7.61 (3H, Br t, H-3', 4' and 5'), 5.50 (1H, dd, J 5, 10Hz, H-2), 6.30 (1H, s, H-6), 2.97 (1H, s, H-3), 2.65 (1H, s, H-3). EIMS m/z (rel. int) [M⁺]+ absent, 300 [M⁺-acetylated sugar moiety] [aglycone]+ (100%), 299 [M⁺-H]+ (16.57), 257 (6.8), 197 [A⁺, H⁺] (25), 196 [A⁺]+ (75.5), 181 (95), 153 (93), 104 (25), 103 (18).

Acid Hydrolysis of (I): Compound (I) (2mg) was refluxed with solution of 3% H₂SO₄ (10 ml) for 2 hrs. at 100°. After cooling the reaction mixture, the resulting aglycone was crystallized from ether as brown amorphous powder (II) identified as 5-hydroxy-7,8-dimethoxy-flavanone by comparison of its mp and spectral data with reported literature.
A NOVEL FLAVANONE GLYCOSIDE

The aqueous hydrolysate was neutralized with BaCO₃ and subjected to PC using solvents: n-Butanol: Acetic acid: water (4:1:5) and was detected as rhamnose by spraying with aniline hydrogen phthalate. (Co-PC, Rf value: 0.38).

**Enzymatic Hydrolysis** of the Compound (I): Compound (I) with Takadiastase (10ml) were kept together in a round bottomed flask (100 ml) at 25º for 30 hrs. After addition of water it was extracted with n-butanol and subjected to CC over Si-gel to afford an aglycone (II) and rhamnose (by Co-PC, Rf: 0.38).

**Permethylaion of I following by acid hydrolysis:** Permethylaion of I by Kuhn’s procedure followed by acid hydrolysis gave the aglycone II, and 2,3,5,4-tetra-O-methyl-L-rhamnose (Co-PC and Co-PC and Co-TLC) indicated that C-1 OH group of the rhamnose is involved in the glycosidation.

The authors are thankful to Prof. S. P. Banerjee, Head, Department of Chemistry for laboratory facilities and to The Director, CDRI, Lucknow for spectral analysis data.

**REFERENCES**

A novel flavone glycoside from the stem of *Desmodium gangeticum*

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Natural Products Laboratory, Department of Chemistry, Dr H.S. Gour University, Sagar 470003, India.

Received April 6, 1998 - Accepted (revised) June 16, 1998.

SUMMARY. A novel flavone glycoside, 4',5,7-trihydroxy-8-prenylflavone 4'-O-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside (1), was isolated from the stem of *D. gangeticum*. Its structure was determined by chemical and spectral data.

Key words: *Desmodium gangeticum*; 4',5,7-trihydroxy-8-prenylflavone 4'-O-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside; flavonoids.

*Desmodium gangeticum* DC. (Fabaceae) commonly known as ‘Sarivan’ in Hindi and distributed in outer Himalayas up to 5000 ft and throughout India, is reported to be useful in diarrhoea, chronic fever, biliousness, cough, vomiting, dysentery, piles, asthma, snake bite and scorpion sting.1-3 Earlier reports on its chemical constituents include alkaloids, pterocarpanoids (gangetin and desmodin) and phospholipids.4,5 In the present paper, we report the isolation of a new flavone glycoside, 4',5,7-trihydroxy-8-prenylflavone 4'-O-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside (1).

EXPERIMENTAL

Plant material. *D. gangeticum*, stems collected from Sagar region in September, 1997 and identified by Taxonomist of Botany Department, Dr H.S. Gour University, Sagar (M.P.), India.

Extraction and isolation. Dried and powdered stems (3.0 kg) were extracted with 90% EtOH at reflux temp. The extract was concentrated under reduced pressure to give a brown viscous mass which was successively partitioned with petrol, benzene, EtOAc, CHCl₃, acetone and MeOH. The acetone soluble fraction on Silic CC eluting with CHCl₃-MeOH 5:2 gave compound 1 (175 mg).

4',5,7-Trihydroxy-8-prenylflavone 4'-O-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside  (1). Pale yellow needles, mp 264-265°C (MeOH); M⁺ at m/z 646; IR bands (KBr): 3600, 3450-3200, 1640, 1625, 1510, 1420, 1360 cm⁻¹; UV max (MeOH): 275 (λg 4.26), 305 (4.13), 326 (4.18); (+NaOAc) 276, 317, 328; (+AlCl₃) 280, 307, 343 nm; ¹³C-NMR (100 MHz, DMSO-d₆): 164 (C-2), 103.8 (C-3), 182.4 (C-4), 157.9 (C-5), 99.2 (C-6), 162.6(C-7), 105.3 (C-8).

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161.9 (C9), 107.6 (C10), 122.9 (C1'), 128.8 (C2') 116.9 (C3'), 174.2 (C4'), 116.9 (C5'), 128.8 (C6'), 22.6 (C1'), 123.3 (C2'), 132.4 (C3'), 25.9 (C4'), 18.9 (C5') (prenyl group), 100.4 (C9'), 76.1 (C3'), 68.8 (C4'), 75.0 (C5'), 65.8 (C6'), (glucose), 99.9 (C1'''), 69.8 (C2''), 68.2 (C3''), 70.1 (C4''), 66.2 (C5''), 17-4 (C6''), (rhamnose); FABMS: m/z [M]+ absent, 338 (100%) [M]+ - sugar moieties, 323 ([M]+ - Me) (99%), 283 [M]+ - 55 (32), 205 [A]+ - Me (10) 165 (15), 118 [B]+. Aetate, mp 175-7°C (aq EtOAc); 1H-NMR (300 MHz, CDCl3): δ 1.89, 2.10 (18H, OAc x 6), 1.90-2.47 (6H, OAc x 2), 4.36 (1H, d, J=4.5Hz, H-1'''), 5.38 (1H, d, J=7.4Hz, H-1'''), 4.49-5.58 (12H, m, sugar protons), 1.12 (3H, d, J=6.0Hz, rhamnosyl methyl), 7.00 (2H, d, J=9.0Hz, H-3' and H-5'), 7.85 (2H, d, J=9.0Hz, H-2' and H-6'), 1.68 (3H, s, Me-5''), 1.83 (3H, s, Me-4''), 3.56 (2H, d, J=6.8Hz, CH2-1'''), 5.52 (1H, br t, J=6.8Hz, H-2'''), 6.62 (1H, s, H-3), 6.34 (1H, s, H-6); FABMS m/z: [M]+ present, 422 [M+-acetylated sugar moiety], 338 [422-2 x Ac] (Aglucose); 323 [M+-Me], 283 [M+-55], 205 [A]+ - Me, 118 [B]+.

**Acid hydrolysis of 1.** Compound 1 (2 mg) was refluxed with 10%HCl (5 ml) for 4 h. The mixture was then extracted with EtOAc to afford 4',5,7-trihydroxy-8-prenylflavone as needles, mp 240-243°C (from CHCl3-MeOH). The aq layer was shown by PC (n-BuOH-AcOEt-water 4:1:5) to contain glucose and rhamnose.

**Enzymatic hydrolysis.** A mixture of compound 1 (4 mg) and takadiastase (10 ml) was kept at 25°C for 24 h. After extraction with n-BuOH and Silica gel CC, a partial glycoside (2) was obtained, mp 246-247°C, C20H24O6: 1H-NMR (300 MHz, CDCl3): δ 6.60 (1H, s, H-3), 6.30 (1H, s, H-6), 1.70 (3H, s, Me-5''), 1.85 (3H, s, Me-4''), 3.52 (2H, d, J=6.8Hz, CH2-1'''), 5.31 (1H, br t, J=6.8Hz, H-2'''), 7.09 (2H, d, J=9.0Hz, H-3' and H-5'), 7.87 (2H, d, J=9.0Hz, H-2' and H-6'), 5.55 (1H, d, J=7.4Hz, H-1''', anomic proton); FABMS m/z 500 [M]+, 338 [500 - sugar moiety], 323 [M'-Me], 283 [M'-55], 205 [A]+ - Me, 118 [B]+.

**Permethylation followed by acid hydrolysis.** Permethylation (DMF, CH3I, AgO) of compound 1 (20 mg) followed by acid (10%HCl) hydrolysis gave a partially methylated aglycone which underwent a butachloremic shift of 52 nm in band I with NaOEt. The methylated sugars were identified as 2,3,4-tri-O-methyl-β-D-glucose and 2,3,4-tri-O-methyl-a-L-rhamnose.

Acknowledgements. Sincere thanks are due to the Director, CDRI, Lucknow for spectral analysis and to Prof. V.K. Saxena, Department of Chemistry, for fruitful discussions.

REFERENCES

Ref. No. 9es/246/2000  Date 31.5.2000

From: Professor P. L. Majumder
Honorary Secretary

Ms. Prachi Tripathi
Natural Products Laboratory
Dr. H. S. Gour University
Sagar - 470 003

Dear Ms. Tripathi,

I am pleased to inform you that you have been duly selected the Dr. B. N. Mankad Awardee, 1999 in Organic Chemistry Section for your paper presented at the last Annual Convention of Chemists held at Calcutta in December, 1999.

Please note that the cash award of Rs.250/= (Rupees Two hundred and Fifty only) and a certificate will be presented to you at the Annual General Meeting of the Society to be held on 17th November 2000 at Gurukula Kangri University, Hardwar during the Annual Convention of Chemists, 15-28, November 2000.

You are, therefore, requested to be present at the said meeting to receive the above noted award. If, however, you are unable to attend, you may authorize some one to receive the award on your behalf. If that is also not possible the award will be sent to you by Registered Post after the Annual General Meeting. Kindly note that due to financial constraints the Society is not in a position to pay you TA/DA for attending this meeting.

I am enclosing an Information Circular of the forthcoming 37th Annual Convention of Chemists to be held in November 2000.

Thanking you,

Yours faithfully,

(P. L. Majumder)
To Whom It May Concern

This is to certify that Professor/Dr./Mr./Ms. Prachi Tripathi has attended the International Conference on Chemistry and Thirtysixth Annual Convention of chemists, 1999 and presented a paper entitled “Chemical Examination...” A. processeb (Paper no. 0RGCA-11).

No TA/DA has been paid by the Society for the purpose.

Signed

Professor P. L. Majumder
Honorary Secretary

Scientist-in-Charge

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Organic Chemistry : Pahup Singh

Inorganic Chemistry : Nityananda Saha
Physical Chemistry : Dulal C. Mukherjee
Industrial & Environmental Chemistry : D. C. Rupalwari
To,

S.L. Gargh
Research Journal of Chemistry and Environment
Sector - A/80, Sch No. 54, Vijay Nagar, A.B. Road
INDORE 452 010 (M.P.) INDIA

Respected Sir

Herewith I am sending two copies of research paper entitled "Chemical examination and anti-inflammatory action of the extract from the stem of Albizzia procera Benth." for publication in your esteemed journal.

Kindly do the needful.

Thanking you

Yours Sincerely

Dr. R.N. Yadava
CHEMICAL EXAMINATION AND ANTI-INFLAMMATORY ACTION OF THE EXTRACT FROM THE STEM OF ALBIZZIA PROCERA BENTH.

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ABSTRACT
A novel flavonol glycoside was isolated from the acetone soluble fraction of ethanolic extract of stem of A. procera Benth. which was identified as 5, 2', 4'-trihydroxy-3, 7, 5'-trimethoxyflavonol-2'-O-β-D-galactopyranosyl-(1→4)-O-β-D-glucopyranoside by various chemical degradations and spectral analysis. The acetone soluble fraction has found to show moderate anti-inflammatory action on albino rats by using non-immunological carrageenan induced hind paw odema method.

KEY WORD INDEX:
Albizia procera Benth.; Leguminosae; stem; flavonoid glycoside; 5, 2', 4'-trihydroxy-3, 7, 5'-trimethoxy flavonol-2'-O-β-D-galactopyranosyl (1→4)-O-β-D-glucopyranoside.

1. INTRODUCTION
The plant Albizia procera Benth. (N.O. Leguminosae) is commonly known as ‘Safed Siris’ in Hindi. Its leaves are reported to be useful as an insecticide and in the treatment of ulcers[1-2]. Earlier reports on its chemical constituents include triterpenoidal saponins and other compounds[3-4].

2. RESULTS AND DISCUSSION
Compound 1 was obtained from the acetone soluble fraction of 95% ethanolic extract of the stem of A. procera. It was purified by column chromatography to afford an amorphous pale yellow solid mass, which gave positive Shinoda[5] and Molisch tests indicating 1 to be a flavonoid glycoside.

In the UV spectrum of compound 1 in methanol a weak band I was appearant in the range for flavonols and a bathochromic shift of 35nm with AICl3 which persisted with conc. HCl indicated the presence of 3-0 substituted flavonol with a free hydroxyl at C-5 position[6]. Compound 1 also exhibited a diagnostic shift with NaOMe (relative to MeOH) indicating a free hydroxyl function at C-4'. The 13C-NMR chemical shift of C-2 (δ = 156.2) C-3 (δ = 138.4) and C-4 (δ = 178.2) were indicative of the 3-O-substitution[7].

Acid hydrolysis of compound 1 yielded the aglycone 2, mp 160-162°, [M]+ 360 and D-galactose and D-glucose [Co-TLC and Co-PC]. The aglycone 2 was identified as a new; 5, 2', 4'-trihydroxy-3, 7, 5'-trimethoxyflavonol by various spectral data (see experimental).

The 1H-NMR spectrum of compound 1 showed signals at δ6.54 and 6.7.16 as singlets for the B ring which could only be assigned to isolated protons at C-3' and C-6' confirming the 2', 4', 5'-substitution pattern in the B-ring[8]. Two doublets at δ6.30 and 6.49 with J value of 2.3Hz integrated for one proton each corresponded to protons at C-6 and C-8 position in the A ring[9]. Three singlets at δ3.90, 8.88 and 8.98 integrated for 3 protons each, were assigned to three methoxyl groups in compound 1. The position of one methoxyl group should be at C-3 position due to the fact that it gave a band I in
methanol in the range of flavonol in UV spectrum of compound 1[10]. The presence of C-3 methoxyl was also supported by the fact that no bathochromic shift in the band II with AlCl₃ was observed in UV spectrum of compound 1[11]. The lack of shift of band II with NaOAc indicated that the second methoxyl group at C-7 position in ring A[12].

The 2', 4', 5'-substitution pattern of ring B have already been confirmed by its ¹H-NMR spectrum and have also been determined that C-4' position of ring B have a free hydroxyl group in it. Thus the position of third methoxyl group could only assigned at either 2' or 5' position of ring B. UV spectrum of 1 with NaOAc:H₃BO₃ (relative to MeOH) revealed the absence of ortho-di-hydroxy group in the B ring[6,13], suggested the position of third methoxyl group at C-5' position therefore remaining third hydroxyl group could only be present at C-2' position which was originally involved in the glycosidation in compound 1.

From the above data the structure of aglycone 2 could be assigned as 5,2',4'-trihydroxy-3,7,5'-trimethoxy flavonol which further confirmed by alkaline degradation of compound 2 afforded two products (2a), C₇H₆O₃, [M]+ 131, monomethyl ether of phloroglucinol, mp 78-80⁰ (lit.78⁰)[14] and (2b), C₈H₉O₅, [M]+ 184, 2, 4-dihydroxy-5-methoxy benzoic acid, mp 200⁰[9], which further confirmed the position of hydroxyl and methoxyl groups in compound 1.

Acetylation of compd. 1, gave a brown amorphous octa acetate derivative 3, analysed for molecular formula C₄₆H₅₀O₂₆, mp 125-127⁰, [M]+ 1020. The ¹H NMR of compd. 3 showed the signals of compd. 1 except signals of galactose and glucose sugar moieties (see experimental).

Permethylated of 1 followed by acid hydrolysis yielded permethylated aglycone 3 identified as; 3,5,7,4',5'-pentamethoxy-2'-hydroxy flavonol suggested that glycosidation occurred at C-2' position and methylated sugars were identified as; 2,3,6-tri-O-methyl-D-glucose and 2,3,4,6-tetra-O-methyl-D-galactose as according to Petek[15] suggested that C₁⁰-OH of the glucose was linked to the C₂-OH of the aglycone 2 and C₄'-OH of glucose linked to the C₁'-OH of the galactose. In the ¹³C-NMR spectra, C-4" signal of glucose showed a downfield shift of 5.4 ppm revealing 1→4 intersugar glycosidic linkage[16].

Enzymatic hydrolysis with β-glucosidase enzyme revealed the presence of β-linkage between the glucose and the aglycone and also between glucose and galactose. The ¹H-NMR data of compound 1a, showed two doublets at δ 4.55 (J = 7.5 Hz) and δ 6.07 (J = 8 Hz) for glucose and galactose respectively confirmed that both the glycosidic linkages were β. Thus structure of 1 was elucidated as a novel flavonol glycoside; 5, 2', 4'-trihydroxy-3, 7, 5'-trimethoxyflavonol-2':O-β-D-galactopyranosyl-(1→4)-O-β-D-glucopyranoside.

The acetone soluble fraction of the Ethanolic extract of the plant has found to show moderate anti-inflammatory activity an albino rats. The % inhibition calculated for the acetone solubles was found 37.08% as compared to standard drug ASA.

3. EXPERIMENTAL
3.1 General : All the mps were determined on a thermo-electrical mp apparatus and are uncorr. UV spectra were measured with Perkin-Elmer Lambda 3B UV-VISIBLE spectrophotometer. IR spectra were recorded with Shinadzu 8201PC (4000 - 350 cm⁻¹) FTIR KBr pallets. ¹H and ¹³C-NMR spectra were recorded with Bruker WM-300 and Bruker DRX 300 respectively using Me₂CO-d₆ and DMSO-d₆ as solvents and TMS as internal standard. MS were recorded on JEOL D-300 (EI source) mass spectrometer. TLC was carried out by using Merck Si-gel.

3.2 Plant Material : The stems of Albizzia procera were collected from the Bandari forest in Sagar Distt. (M.P.) and identified by Taxonomist of Botany Department of Dr. H. S. Gour University, Sagar (M.P.).
3.3 Extraction and Isolation: Air dried and powdered stems of plant (2 Kg) were extracted with 95% ethanol. Extract obtained was concentrated under reduced pressure and further extracted with C₆H₆, EtOAc, Ac₂O and MeOH. The acetone soluble part was chromatographed on Si-gel G column using Ac₂O-MeOH mixts of increasing polarity and collecting 200ml fractions. Fraction 15-35, on evaporation of solvent gave an amorphous compound. This crude compound was again subjected to CC. Compound 1 obtained was about 0.240gm and subjected to prep. TLC (EtOAc-Me₂CO-AcOH-H₂O, 5:3:1:1) to ensure its purity.

3.4 Study of Compound 1: Crystallized from MeOH as pale yellow needles, mp 200-202°, [M]⁺ at m/z 683.6684, [found C, 52.62 H, 5.30; C₃₀H₃₆O₁₉; requires C, 52.64 H, 5.28]. UV λ_max (MeOH) nm: 265, 300, 352; + (AlCl₃ and AlCl₃-HCl): 268, 312, 387; + (NaOMe): 258, 298, 404; + (NaOAc and NaOAc-H₂BO₃): 258, 300, 337. IR ν_max (KBr) cm⁻¹: 3350(OH), 1630(conjugated C=O), 1075-1030(glycosidic C=O) 880 (Ome). ^13C-NMR of Compound 1 (300MHz, DMSO-d₆): 154.6(C-2), 138.4(C-3), 178.2(C-4), 162.8(C-5), 98.2(C-6), 169.1(C-7), 94.5(C-8), 154.2(C-9), 103.4(C-10), 120.2(C-1'), 110.2(C-2'), 158.4(C-3'), 114.9(C-4'), 112.8(C-5'), 132.0(C-6'), 100.5(C-1''), 74.8(C-2''), 76.4(C-3''), 75.5(C-4''), 76.6(C-5''), 61.5(C-6''), 100.1(C-1'''), 73.1(C-2''''), 74.3(C-3''''), 70.1(C-4''''), 63.4(C-5'''). EIMS m/z (rel. Int.): [M⁺] absent, 360 [M⁺-Acetylated sugar moieties-Ac] (15), 343 [M-OH]+ (65), 329 [M-OMe]+ (50), 317 [M-MeCO]+ (12), 167 [C₆H₇O₄]+ (6).

3.5 Acetylation of Compound 1: Compound 1 on acetylation with Ac₂O/pyridine gave an octa acetate derivative of 1 as brown amorphous powder 1a; mp 125-127°, [found C, 54.11; H, 5.14; C₄₆H₅₂O₂₆; calcld C, 54.13; H, 5.15]. ^1H-NMR (300MHz, Me₂CO-d₆): δ 8.32 (1H, d, J = 2.3Hz, H-6), 8.51 (1H, d, J = 2.3Hz, H-8), 8.95 (1H, s, H-3'), 8.10 (1H, s, H-6), 8.39 (3H, s, OMe-7), 8.38 (3H, s, OMe-3), 8.39 (3H, s, OMe-5), 8.32 (3H, s, OMe-4-Ac), 8.55 (1H, d, J = 7.5Hz, H-1''), 8.67 (1H, d, J = 8Hz, H-1''), galactose), 6.85-2.15 (21H, m, Sugar 7 x OAc), 8.40-5.52 (Sugar H's).

3.6 Acid Hydrolysis of Compound 1: A 10% H₂SO₄ soln (10ml) of glycoside (40mg) was heated for two hrs at 100°. After cooling aglycone was deposed as a amorphous product. It was recrystallized by ether as brown amorphous compound 2, identified as: 5,2',4', trihydroxy-3,7,5'-trimethoxyflavonol by various spectral data.

The aqueous hydrolysate was neutralized with BaCO₃ and BaSO₄ filtered off. The filtrate on evaporation gave a residue which was subjected to PC using solvent n-BuOH-OHAc-H₂O (4:1:5) and sugars were identified as glucose and galactose (Rf values: 0.18 and 0.16 respectively).

3.7 Study of Compound 2: Crystallized from ether as brown amorphous powder, mp 160-162°, [M]⁺ at m/z 360, [found C, 59.98; H, 4.5; C₁₈H₁₆O₆; calcld C, 60.00; H, 4.4]. UV λ_max (MeOH) nm: 266, 355; + (AlCl₃ and AlCl₃-HCl): 268, 300, 388; + (NaOMe): 260, 398; + (NaOAc): 265, 358; + (NaOAc-H₂BO₃): 267, 350. IR ν_max (KBr) cm⁻¹: 3435, 1638, 1650, 1600, 1360, 900 and 810. ^1H-NMR (300MHz, Me₂CO-d₆): δ 6.53 (1H, s, H-3'), δ 7.12 (1H, s, H-6), δ 6.31 (1H, d, J = 2.3Hz, H-6), δ 6.48 (1H, d, J = 2.3Hz, H-8), δ 3.92 (3H, s, OMe-7), δ 3.88 (3H, s, OMe-3), δ 3.96 (3H, s, OMe-5). EIMS m/z (rel. Int.): 360 [M⁺] (85), 343 [M-OH]+ (60), 329 [M-OMe]+ (42), 317 [M-MeCO]+ (15), 167 [C₆H₇O₄]+ (10), 166 [C₆H₆O₄]+ (12).
3.8 Alkaline Degradation of Compound 2: Alkaline degradation was done by refluxing 2 with 50% KOH (50ml) in MeOH (10ml). The reaction mixture was cooled, acidified with HCl and extracted with Et₂O. The Et₂O layer was washed with water and dried over Na₂SO₄. The Et₂O distilled in vacuo afforded (2a), monomethyl ether of phloroglucinol, C₇H₈O₃, mp 78-80°, [M]+ 131. The aqueous phase was acidified with HCl and extracted with Et₂O, washed with water to give (2b), 2, 4-dihydroxy-5-methoxy benzoic acid, C₈H₆O₅, mp 200°, [M]+ 184°.

3.9 Enzymatic Hydrolysis of Compound 1: A mixture of compd 1 and enzyme β-glucosidase (10ml) were kept together in a round bottomed flask (100ml) at 25° for 30 hrs. After addition of water it was extracted with n-butanol and subjected to CC over Si-gel afforded an aglycone, 5,2',4'-trihydroxy-3,7,5'-trimethoxyflavonol (by UV, MS spectral data) and sugars, glucose and galactose were identified by Co-PC and Co-TLC.

3.10 Permethylation of Compound 1: The compound 1 (50mg) was treated with CH₃I (8ml) and Ag₂O (50mg) in dimethylformamide (10ml) in 150ml conical flask and left for 38hrs. at room temp. The contents filtered, washed with dimethylformamide, hydrolysed with 10% HCl to give the permethylated aglycone 3 and methylated sugars; 2, 3, 6-tri-O-methyl-D-glucose and 2, 3, 4, 6-tetra-O-methyl-D-galactose (by Co-PC and Co-TLC).

3.11 Anti-inflammatory Activity of The Acetone Solubles: The anti-inflammatory activity of the acetone soluble fraction of this plant was carried out by non-immunological carrageenan induced hind paw edema method [17].

Adult albino rats of either male or female weighing 150-200gm were used in the investigation. The volume was measured by plethysmograph. Initial volume of right hind paw of albino rats were measured plethysmographically without administration of test drug. Acetyl salicylic acid was used as a standard drug. Activity was determined by measuring the change in the volume of inflamed foot produced by injection of 0.05ml of 1% freshly prepared carrageenan suspension.

Albino rats were divided into three groups, each group consisting of four rats. First group of rats were treated intraperitoneally (i.p.) with 50mg/Kg body weight of the acetone solubles of the ethanolic extract of the plant.

Second group was administered i.p. 30mg/Kg body weight of the aqueous suspension of acetyl salicylic acid and the third control group was fed with the same volume of distilled water. One hour after the drug administration the animals were injected with 0.05ml suspension of carrageenan in the right hind paw.

The measurement of the paw volume were taken using mercury displacement technique with the help of plethysmograph immediately before and after 1, 2 and 3 hrs. the carrageenan injection. The percent inhibition (I) of inflammation after 3 hrs. was calculated by the formula given by Newbould [18].

\[
I = 100 \left[ 1 - \frac{a \cdot x}{b \cdot y} \right]
\]

where:

\( x = \) mean foot volume of rats before the administration of carrageenan injection in the test and the standard drug.

\( a = \) mean foot volume of rats after the administration of carrageenan and the test drug injection in the test and standard group.
\[ y = \text{mean foot volume of rats before the administration of carrageenan injection in the control group.} \]

\[ b = \text{mean foot volume of rats after the administration of carrageenan injection in the control group.} \]

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test solutions applied</th>
<th>Dose mg/Kg i.p.</th>
<th>Volume of paw after drug administration</th>
<th>Total increase in paw volume</th>
<th>% inhibition</th>
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<td>1.</td>
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REFERENCES