APPENDICES
AN ISOFlavone GLYCOSIDE FROM THE SEEDS OF TRICHOSANTHES ANGUINA

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Key Word Index—Trichosanthes anguina; Cucurbitaceae; seeds; isoflavone glycocide; 5,6,6'-trimethoxy-3',4'-methylenedioxyisoflavone 7-O-β-D-(2"-O-p-coumaroarylglucopyranoside).

Abstract—A novel isoflavone glycocide; 5,6,6'-trimethoxy 3',4'-methylenedioxyisoflavone 7-O-β-D-(2"-O-p-coumaroarylglucopyranoside), has been characterized from the seeds of Trichosanthes anguina.

INTRODUCTION

Trichosanthes anguina Linn. [1] (Cucurbitaceae) is found throughout the hotter parts of India and China. It is used as a tonic and to cure coughs and bilious attacks. The seeds are purgative, anthelmintic and used in the treatment of syphilis [2]. Earlier workers [3-5] have reported a number of bioactive constituents from the leaves of this plant. The present paper deals with the isolation and identification of a novel, isoflavone glycocide (I) from the seeds of T. anguina.

RESULTS AND DISCUSSION

The acetone soluble part of the ethanolic extract of the defatted seeds of T. anguina showed one spot on TLC, which was subjected to column chromatography over silica gel G. Elution with chloroform–methanol (2:1) afforded a brown crystalline compound [1]; C_{28}H_{32}O_{15}, [M] + 680, which gave a positive Molisch test and the characteristic colour reactions of an isoflavonoid [6, 7]. The IR spectrum of I showed absorption bands at 3310 (OH), 2870 (OCH_{3}), 1650 (>C=O), 932 (–OCH_{2}O) and 1600 (aromatic ring system). The molecular weight & its acetyl derivative C_{42}H_{46}O_{19}, [M] + 848, suggested the presence of four acetylated hydroxyl groups. Alkaline hydrolysis of I with 2% NaOH yielded p-methyl coumarate (mmp, co-TLC and superimposable IR and NMR spectra). The ether insoluble part obtained from alkaline hydrolysis gave the isoflavone glycocide (2); C_{23}H_{26}O_{13}, [M] + 534. Compound 2 on acid hydrolysis (7% H_{2}SO_{4}) gave the isoflavone 3 C_{19}H_{15}O_{9}, [M] + 372, and glucose (1 mol). The UV spectrum of 3 exhibited a bathochromic shift of 21 nm in band II on addition of NaOAc, suggesting a free hydroxyl group at the 7 position of ring A [8]. Compound 3 formed a monoaacetate C_{21}H_{15}O_{9}, [M] + 414.

The 1H NMR spectrum of the acetyl derivative of 3 showed a singlet at δ 8.07, a characteristic feature of isoflavonoids [9]. The three sharp singlets appeared at δ 6.65, 3.80 and 3.93 indicating the presence of three methoxy groups. A sharp singlet at δ 6.19 (2H) confirmed the presence of a methylenedioxy group. Alkaline cleavage of 3 with 10% NaOH gave the corresponding deoxybenzoin (4), C_{19}H_{15}O_{8}, [M] + 362, which was identified by spectral data and confirmed that 3 is an isoflavone. The 13C NMR spectrum (See Experimental) of 1 revealed the presence of 34 carbon atoms and confirmed the structure as I. The EI-mass spectrum of I gave a molecular ion peak at 680 with a fragment of m/z 534, which corresponded to the loss of p-coumaric acid. A fragment obtained at m/z 372. Corresponded to the further loss of a monosaccharide sugar. The RDA fragments at m/z 197 and 191 were due to [A_{1} + H] + and [B_{1} - H] + fragments. Permethylation of I and 2 followed by acid hydrolysis led to the conclusion that the attachments of the isoflavone and p-coumaric acid were at C-1" and C-2" of d-glucose respectively. The 7-O-β linkages and pyranose form of the sugar were confirmed by enzymic hydrolysis with almond emulsion and periodic oxidation of 2. From the combined evidence I was assigned the structure, 5,6,6'-trimethoxy-3',4'-methylenedioxyisoflavone 7-O-β-D-(2"-O-p-coumaroarylglucopyranoside).

EXPERIMENTAL

Plant material. The seeds of T. anguina Linn. were collected from M/s United Chemicals and Allied Products, Calcutta and identified by staff of the Botany Department, Dr H. S. Gour University, Sagar (M.P.) India.

General. Mps: uncorr. NMR spectra were measured using TMS as an int. standard and CDCl_{3} as solvent. IR spectra were measured in KBr discs.

Extraction and isolation of compound 1. Dried and powdered seeds (2.5 kg) of T. anguina were extracted
with hot aq. EtOH. The extract was coned to a viscous mass, which was then dissolved in hot H₂O and partitioned with CHCl₃, Et₂O, EtOAc, HOAc and n-BuOH. The Me₂CO fraction on TLC examination (CHCl₃-C₆H₆-Me₂CO, 10:10:1) showed two spots, indicating the presence of two compounds, which were separated by CC over silica gel G, eluted with CHCl₃-MeOH (2:1). The second compound was not obtained in sufficient amount for further identification and was rejected.

Compound 1, crystallized from MeOH as brown needles, mp 165°, which ran as a single spot on TLC in CHCl₃-MeOH-H₂O (9:2:1), [M]+ 680 (Found: C 60.2; H 4.9; Me 13.4; calculated C 60.0, H 4.7; OMe 13.6). IR ν max cm⁻¹: 3310 (OH), 2870 (OCH₃), 1650 (>C=O), 932 (-OCH₃), 1600 (aromatic ring system), 1636, 1265, 1185, 822, UV λ max nm 255, 260, 305; + NaOMe 257, 261, 305sh; + AlCl₃ 257sh, 260, 306sh; + AlCl₃ + HCl 253sh, 258, 257sh; + NaOAc 262, 313sh; + NaOAc + H₂BO₃, 263, 310; ¹H NMR of tetracetate: C₆H₄O₄Na₂ [M]+ 848, mp 145° (90 MHz, CDCl₃, δppm): 8.07 (1H, s, H-2), 3.65 (3H, s, OMe), 3.80 (3H, s, OMe), 4.91 (3H, s, OMe), 6.19 (2H, s, -OCH₂), 7.86 (1H, s, H-2'), 7.60 (1H, s, H-5'), 6.67 (1H, s, H-8), 5.55 (1H, d, J = 7 Hz, H-1' anomeric proton) 4.33–4.85 (5H, m, protons of sugar), 2.13 (3H, s, OAc-3').

2.05 (3H, s, OAc-4'), 2.55 (3H, s, OAc-6'), 6.28 (1H, d, J = 7.8 Hz, H-7), 7.73 (1H, d, J = 8 Hz, H-6), 2.44 (3H, s, OAc-4'), 7.59 (2H, d, J = 2.5 Hz, H-2', H-6'), 6.89 (2H, d, J = 2.5 Hz, H-3', H-5'), EI-MS of t. m. = 680 [M]+, 534, 372, 197 and 191, 13C NMR (90 MHz, DMSO-d₆, δ ppm): 154.4 (C-2), 120.5 (C-3), 175.6 (C-4), 155.2 (C-5), 153.2 (C-61), 120.6 (C-7), 112.4 (C-8), 113.2 (C-11), 111.0 (C-2), 142.2 (C-3'), 147.1 (C-4'), 95.1 (C-5'), 152.0 (C-6), 92.6 (C-1'), 72.5 (C-2'), 88.3 (C-3'), 70.2 (C-4'), 81.2 (C-5'), 63.5 (C-6'), 167.1 (C-1'), 118.3 (C-2'), 145.9 (C-3'), 133.5 (C-4'), 124.2 (C-5'), 129.3 (C-6'), 153.8 (C-7'), 130.5 (C-8'), 124.2 (C-9'), 56.1, 55.1, 56.1 (OME), 102.3 (OCH₂O).

Alkaline hydrolysis of compound 1. Compound 1 was dissolved in MeOH and kept overnight after addition of 2% NaOMe. The reaction mixture was neutralized with dilute HOAc and coned under vacuum. The Et₂O soluble part yielded needles of p-methyl coumarate, mp 130°. The Et₂O insoluble part furnished an amorphous compound 2, mp 170°. C₂₅H₂₇O₂₃ [M]+ 534 (found C 56.0, H 4.7, OMe 17.3, calculated 56.1%, H 4.8% OCH₃ 17.4%) TLC homogenous, ν max cm⁻¹: 3312 (OH), 2872 (OME). 1620 (>C=O), 930 (–OCH₂O), 1610 (aromatic ring system). 1634, 1260, 1180, 820, UV λ max nm 256, 261, 305sh; + NaOMe 256, 260, 306sh; + AlCl₃ 257, 261, 306sh; + AlCl₃ + HCl 254sh, 257, 307sh; + NaOAc 263, 314sh; + NaOAc + H₂BO₃ 264, 311. ¹H NMR of acetate derivative: C₂₅H₂₇O₂₄ [M]+ 702, mp 150 (90 MHz, CDCl₃, δ ppm): 8.08 (1H, s, H-2), 3.66 (3H, s, OMe), 3.82 (3H, s, OMe), 3.90 (3H, s, OMe), 6.18 (2H, s, -OCH₂), 7.78 (1H, s, H-2'), 7.61 (1H, s, H-5'), 6.69 (1H, s, H-8), 5.56 (1H, d, J = 7.1 Hz, H-1' anomeric proton) 4.30–4.31 (6H, m, proton of sugar), 2.06 (3H, s, OAc-2'), 2.14 (3H, s, OAc-3'), 2.07 (3H, s, OAc-4'), 2.60 (3H, s, OAc-6').

Acid hydrolysis of compound 2. Compound 2 was hydrolysed (7% H₂SO₄) by refluxing for 10 hr to yield the aglycone 3. The hydrolysate was neutralized with BaCO₃ and BaSO₄ was filtered off. The coned filtrate was run on PC in n-BuOH-HOAc-H₂O (4:1:5 top layer) and gave d-glucose. The quantitative estimation of sugar in the hydrolysate showed the presence of 1 mol of glucose [10].

Identification of the aglycone 3. Needles, mp 177°, C₁₉H₁₆O₄ (found: C 60.1, H 4.5; Me 24.07; calculated C 61.2, H 4.3, OMe 25.0%). [M]+ 372. TLC homogenous; IR ν max cm⁻¹: 3315 (OH), 2875 (OME), 1618 (>C=O), 929 (–OCH₂O), 1600 (aromatic ring system), 1631, 1262, 1183, 824, UV λ max nm 244sh, 257, 270sh, 320; + NaOMe 259, 278sh, 341; + AlCl₃ 248sh, 250, 264sh 305 + NaOAc 272, 318sh, 335; + NaOAc + H₂BO₃, 274sh, 307. ¹H NMR of acetate (90 MHz, CDCl₃, δ ppm): 8.08 (1H, s, H-2), 3.65 (3H, s, OMe), 3.80 (3H, s, OMe), 3.91 (3H, s, OMe), 6.15 (2H, s, OCH₂), 7.80 (1H, s, H-2'), 7.61 (1H, s, H-5'), 6.67 (1H, s, H-8'), 2.38 (3H, s, OAc-7). El-MS of I: [M]+ 372, 197 and 191.

Alkaline cleavage of compound 3. Compound 3 reacted with 10% NaOH to give the corresponding deoxybenzoin (4) and formic acid. Compound 4 yielded crystals from MeOH, mp 169°, C₁₆H₁₄O₃ [M]+ 362 (found C 58.1, H 4.5, OCH₁ 24.7; calculated C 59.6, H 4.9, OCH₁ 25.6%). IR ν max cm⁻¹: 3300 (OH), 2871 (OME), 1619 (>C=O), 928 (OCH₂O), 1632, 2994. UV λ max nm 216, 233.
257, 310. \textsuperscript{1}H NMR (90 MHz, CDCl\textsubscript{3}, \(\delta\) ppm): 5.83 (2H, s, -OCH\textsubscript{2}O), 3.87 (3H, s, OMe), 3.85 (3H, s, OMe), 3.82 (3H, s, OMe), 12.45 (1H, s, OH), 12.62 (1H, s, OH), 6.65 (1H, s, H-2'), 6.52 (1H, s, H-5'), 4.07 (1H, s, -CH\textsubscript{2}J), 12.1 (1H, s, H-3).

Attachment of the aglycone 3 and p-coumaric acid to glucose. Compound 1 was treated with Mgl and Ag\textsubscript{3}O in DMF at room temp. for 24 hr and then filtered. The residue was washed with DMF. The filtrate was dried in vacuo and hydrolysed with 20% ethanolic H\textsubscript{2}SO\textsubscript{4} for 8 hr. After the usual work-up, the methylated sugar was identified by co-PC as 3,4,6-tri-O-methyl-D-glucose. Similarly compound 2 gave the methylated sugar 2,3,4,6-tetra-O-methyl-D-glucose.

Periodate oxidation. Compound 2 was dissolved in MeOH and treated with sodium meta-periodate for 2 days. The liberated HCO\textsubscript{3}H and consumed periodate were estimated by the Jones method [11].

Enzymatic hydrolysis. Compound 2 in MeOH was mixed with an equal volume of almond emulsion soln and left at room temp. for 24 hr. Examination of the hydrolysate on PC showed the presence of D-glucose.

Acknowledgements—Thanks are due to the director of the Central Drug Research Institute, Lucknow, for the recording of various spectra.

REFERENCES
Chemical Examination of the Seeds of Trichosanthes anguina Linn

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The present paper deals with the isolation and characterization of kaempferol, quercetin and kaempferol 3-O-β glucoside from the seeds of Trichosanthes anguina.

*Trichosanthes anguina* (family Cucurbitaceae) is commonly known as chachida in Hindi. It is cultivated throughout the hotter parts of India. The seeds are considered cooling. It is used as a purgative and vermifuge. It also lessens thirsts increases appetite, act as a tonic and is good for the stomach. The present paper deals with the isolation and characterization of Kaempferol quercetin and Kaempferol 3-O-β glucoside from the seeds of Trichosanthes anguina which are identified by various chemical degradations and spectroscopic techniques.

The seeds of *T. anguina* Linn. were collected from United Chemicals and Allied, Calcutta. The fresh seeds were then defatted by extraction with pet. ether (40–60°C) and the residue was extracted with MeOH. The combined methanolic extract was concentrated under reduced pressure to give a brown gummy mass which was dissolved in hot H₂O. After cooling it was filtered and the water-insoluble part was dissolved in ether. The water-soluble portion was extracted with Et₂O, EtOAc and MeOH. The methanolic extract of the water-soluble part, on TLC examination over Si-gel (benzene-pyridine-formic acid 36 : 9 : 5) gave three spots. These were separated by CC and assigned TC-1, TC-2 and TC-3.

Compounds TC-1, TC-2 and TC-3 were identified by various chemical and spectral analyses.

TC-1

It was obtained as pale yellow granules. It gave Shinoda test 3, m.pt. 280–281°C. Elemental analysis agreed to the molecular formula C₁₅H₁₀O₆. Found C, 62.80 H, 3.44% whereas C₁₃H₁₀O₆ requires C, 62.93%, H, 3.49%. ν(KBr) cm⁻¹: 3550 (OH), 1675 (C=O), 2945 (C–H), 850 (C=C, aromatic). 1240; UV₅max: *MeOH* 249 sh, 265, 290, 370; + AlCl₃ 259 sh, 266, 300 sh, 315, 430; + NaOMe 277, 300, 398. On acetylation with Ac₂O/py, it gave a tetraacetate derivative. ¹H-NMR (270 MHz, CDCl₃): 8.00 (2H, d, J = 8.5 Hz, H-2',6'), 6.92 (2H, d, J = 8.5 Hz, H-3',5'), 6.18 (1H, d, J = 2.5 Hz, H-6), 6.44 (1H, d, J = 2.5 Hz, H-8), 2.48 (3H, S, OAc, S), 2.39 (9H, S, OAc-3, 4 : 7). MS data: m/z 286.
On the basis of above data and by m.m.p.t. and Co-TLC with authentic sample\(^3\), TC-1 was characterised as kaempferol.

**TC-2**

It was obtained as yellow powder, which on crystallization with CHCl\(_3\)-MeOH gave yellow needle-shaped crystals. It gave all tests of flavonoid, m.p.t. 280-282\(^\circ\)C, molecular formula C\(_{15}\)H\(_{10}\)O\(_{7}\). Found C, 52.60, H, 3.35, whereas C\(_{15}\)H\(_{10}\)O\(_{7}\) requires C, 59.62, H, 3.31%. IR\(_{\text{KBr}}\) cm\(^{-1}\): 3465 (O-H), 1700 (C=O), 2945 (C-H), UV\(_{\text{max}}\): MeOH 258, 302 sh, 370, + AlCl\(_3\) 272, 333, 458, + AlCl\(_3\)-HCl 265, 350, 428, + AlCl\(_3\)-HCl 265, 350, 428, + NaOAc 258 sh, 322, 390 (Dec), NaOAc-H\(_3\)BO\(_3\) 261, 380. On acetylation with Ac\(_2\)O/py, it gave a pentaacetate derivative. \(^1\)H-NMR (270 MHz, CDCl\(_3\)): 7.67 (1H, d, J = 2.5 Hz, H-2'), 7.60 (1H, d, J = 8.5 H and 2.5 Hz, H-6'), 7.16 (1H, d, J = 8.5 Hz, H-5'), 7.34 (1H, d, J = 2.0 Hz, H-8), 6.86 (1H, d, J = 20 Hz, H-6), 2.47 (3H, S, OAc-5), 2.40 (3H, S, OAc-7), 2.35 (9H, 3, OAc-3,4',3'). MS data: m/z M\(^+\) 302. It was characterized as quercetin by comparison of its spectral data and co-chromatography with authentic sample\(^4\).

**TC-3**

It was obtained as yellow needles on crystallization with MeOH, m.p.t. 176-178\(^\circ\)C. It gave Shinoda test\(^5\), Molish test and showed high solubility in water\(^5\). Elemental analysis agreed to the molecular formula C\(_{21}\)H\(_{20}\)O\(_{11}\). Found C, 56.35, H, 4.40% of whereas C\(_{21}\)H\(_{20}\)O\(_{11}\) requires C, 56.25, H, 4.46%. IR\(_{\text{KBr}}\) cm\(^{-1}\), 3420 (OH), 1650 (C=O), 1110-1020 (C=O, gly), 850 (aromatic C=C). UV\(_{\text{MeOH}}\) MeOH 244, 265, 350. \(\lambda_{\text{max}}\) MeOH + NaOAc 265, 348, 398. \(\lambda_{\text{max}}\) + AlCl\(_3\) 255, 301, 354. \(\lambda_{\text{max}}\) MeOH + AlCl\(_3\)-HCl 274, 298 sh, 398, + NaOMe 246, 249, 350 sh, 380. UV spectral data gave a bathochromic shift 44 nm in band I and 19 nm in band II with AlCl\(_3\)/HCl, in comparison with AlCl\(_3\). It means C-3 position in the above glycoside is blocked. On acetylation it gave a heptaacetate derivative. \(^1\)H-NMR (CDCl\(_3\), 90 MHz): 8.07 (2H, d, J = 9 Hz, H-2',6'), 7.26 (2H, d, J = 9 Hz, H-3',5'), 7.30 (1H, d, J = 2.5 Hz, H-8), 6.79 (1H, d, J = 2.5 Hz, H-6), 5.51 (1H, d, J = 9 Hz, glu, aromatic proton), 2.47 (3H, S, OAc-5), 2.35 (3H, OS, OAc-7), 2.32 (3H, S, OAc, 4'), 1.72, 2.15 (12H, M, OAc, Sugar). MS data: m/z 618 M\(^+\), 286 aglycone moiety\(^*\); 337 acetylated hexopyranoside\(^*,\) 153 A\(_1\) + H\(^++\), 121 B\(_2\). Acid hydrolysis of glycoside with 7% HCl gave an equimolar quantity of aglycone, m.p.t. 280-281\(^\circ\)C, which was characterized as kaempferol by direct comparison with authentic sample\(^3\). The sugar was identified as glucose by Co-PC (R\(_f\) 0.21, 0.31 (EtOAC-Py-H\(_2\)O 12 : 5 : 4) and B : A : W 6 : 1 : 2). Methylation of glycoside (CH\(_3\)I/Ag\(_2\)O/DMF) followed by acid hydrolysis gave 3-OH,5,7,4'-Trimethoxy flavone\(^6\), m.p.t. 135-36\(^\circ\), (Cal. for C\(_{15}\)H\(_{16}\)O\(_{6}\): C, 65.00, H, 4.87%; found: C, 65.90, H, 4.88% and 2,3,4,6-tetra-O-methoxy-D-glucose. This confirmed the location of glucose in the glycoside at C-3 position. The enzymatic hydrolysis with almonds emulsion confirmed the presence of sugar as glucose as well as \(\beta\)-linkage between sugar and aglycone.

Thus it was identified as Kaempferol 3-O-\(\beta\)-glucoside.
REFERENCES


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Dear Sir,

This is to inform you that your paper "A novel flavone glycoside from the seeds of Trichosanthes anguina Linn." has been accepted for the publication.

As per the reprints, we supply free of charge 25 copies to the main Author and 10 copies to each other Author: should you need further copies, please inform us.

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A Novel Flavone Glycoside From The Seeds of 

\textbf{Trichosanthes anguina}

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Plant. \textit{Trichosanthes anguina} Linn\textsuperscript{1} (Cucurbitaceae) commonly known as "Chachinda" in Hindi and distributed in hotter parts of India and China, seeds supplied and identified by United and Allied Chemicals, Calcutta, India. A voucher specimen available in the Natural Products Laboratory of this University.

Uses in traditional medicine. Extract of fruits is used as tonic, purgative and anthelmintic and to cure cough and biliousness. Seeds are antidiarrhoel and used in syphilis\textsuperscript{2}.

Previously isolated constituents. Phenolics\textsuperscript{3}, lectin\textsuperscript{4} and folic acid\textsuperscript{5}.

New isolated constituents. 7 Hydroxy-6-methoxy flavone 5-O-\textalpha-L-rhamnopyranoside : (yield, 0.00.068\%)m.p. 327\textdegree C. UV max (MeOH) : 248, 280 and 317 (sh); (NaOAc + MeOH) 270, 295 and 320; (AlCl\textsubscript{3} + MeOH) 250, 280, 321 (sh) nm; shift of 15 nm in band-II with NaOAc (relative to MeOH) confirmed the presence of \textomega-OH group at C-7, but no markable shift
appear with AlCl₃ (relative to MeOH), indicating the presence of glucosyl residue at C-5. IR bands (KBr): 3451, 1650, 1520, 2920, 1215, 1125, 840, 2872 cm⁻¹; MS data: [M⁺] 430, m/z 284, 183, 102 (found C, 61.38; H, 5.10%; calculated for C₂₂H₂₂O₃: C, 61.39; H, 5.11%); ¹H NMR (90 MHz, CDCl₃) of acetate derivative: δ 3.90 (3H, s, -OME-6); this sharp singlet confirmed the presence of -OME group at C-6. 2.36 (3H, s, 7-OAc), 6.85 (1H, s, H-8), 6.62 (1H, s, H-3), 7.45 (3H, m), 7.85 (2H, m), 1.78-1.98 (9H, 3 s), 4.5-5.50 (5H, m, sugar protons), 1.02 (3H, d, J 6.0 Hz, rham-CH₃), 4.9 (1H, d, J 2.0 Hz, H-1" anomeric proton).

Me - CH₃

R - rhamnopyranoside

Acknowledgements. Authors thank CDRI, Lucknow, for spectral analysis.

REFERENCES.


To,

Prabir K. Gupta,
Executive Editor,
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92, Acharya Prafulla Chandra Road,
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Dear Sir,

Kindly find enclosed herewith three copies of our manuscript entitled "A Novel Flavone Glycoside From the seeds of Trichosanthes anguina Linn." for favour of publication in your esteemed journal.

Hope you will do the needful.

Thanking you,

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Yours sincerely,

(Dr. R. N. Yadava)
A Novel Flavone Glycoside From The Seeds of Trichosanthes anguina Linn.

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Key Words Index: Trichosanthes anguina Linn., cucurbitaceae, a novel flavone glycoside-5,6-dihydroxy 7,8,3'-trimethoxy flavone-4'-O-α-D-xylofuranosyl (1→4)-O-β-D-glucopyranoside.

ABSTRACT: A novel flavone glycoside was isolated from the seeds of Trichosanthes anguina. Its structure has been determined by spectroscopic and degradative methods as 5,6-dihydroxy 7,8,3'-trimethoxy flavone-4'-O-β-D-xylofuranosyl (1→4)-O-β-D-glucopyranoside.

INTRODUCTION: Trichosanthes anguina\(^1\) (N.O. Cucurbitaceae) which is commonly known as chachinda in Hindi. It is distributed throughout the hotter parts of India and China. It is reported to be used as tonic, to cure cough, biliousness. The seeds are purgative, anthelmintic and used in the treatment of syphilis\(^2\). Earlier workers\(^3-5\) have been already reported the occurrence of many bioactive constituents from the leaves of this plant.

The present paper deals with the isolation and identification of a novel flavone glycoside [1] from the seeds of Trichosanthes anguina.
RESULTS AND DISCUSSION

The EtOAc soluble part of the ethanolic extract obtained from powdered seeds of plant showed two spots on TLC examination, which was subjected to column chromatography over si-gel. Elution with CHCl₃ : MeOH (6:2) afforded a yellow crystalline substance [1] which responded Molisch test and all positive tests for flavonoidal glycoside⁶,⁷, molecular formula C₂₉H₳₄O₁₇ (Found C, 53.20%; H, 5.0%; calculated C, 53.21%; H, 5.1%), m.p. 336°C. Its IR spectrum showed absorption maxima at 3345 cm⁻¹ (free OH), 1110 (O-gly), 2872 (OCH₃), 2950 (C-H), 825 (aromatic C=C). The UV spectrum showed bands at 250, 287 and 362 nm and its changes in the presence of diagnostic shift reagents⁸, indicated the presence of free OH groups at 5,6-positions and blocked 7,8,3'-hydroxyl groups.

The glycoside [1] on acid hydrolysis with 7% H₂SO₄ gave an aglycone [2] and sugars which were identified as xylose and glucose (Co-PC and Co-TLC and GLC). The aglycone [2], molecular formula C₁₈H₁₆O₈, m.p. 235°C on alkaline hydrolysis gave two compounds—p-hydroxy-3-methoxy benzoic acid and 2,5,6-tri-hydroxy-3,4-dimethoxy acetophenone (mmp and co-TLC). On the basis of alkaline hydrolysis the aglycone [2] was identified as 5,6,4'-tri-hydroxy 7,8,3'-trimethoxy flavone. Which was further confirmed by comparison of m.p., mmp, UV, IR with authentic sample⁹. The glycoside [1] on acetylation with
Ac₂O/Py gave an octacetate derivative, molecular formula C₄₅H₅₀O₂₅, m.p. 198°C. The ¹H NMR spectrum of the acetate derivative of [1] showed that the compound [1] is a diglycoside as it showed eight singlet at δ 2.48, 2.36, 2.02, 2.08, 3.09, 2.05, 2.10 and 2.15 showing the presence of eight acetyl groups in the compound [1]. The three sharp singlets at δ 3.75 (3H, s, -OMe), 4.00 (3H, s, OMe); 3.82 (3H, s, -OMe), showed the presence of three methoxyl groups in the glycoside [1]. The ¹H NMR spectrum also exhibited A₂B₂ pattern of B ring as it showed two double doublets at δ 7.20 (2H, dd, J = 8.5 Hz and 2.5 Hz, H-2'6') and δ 6.65 (2H, dd, J = 8.5 Hz and 2.5 Hz H-3'5'). The H-1 proton of xylose appeared as doublet at 5.69 (1H, d, J = 9 Hz) and H-1 protons of glucose also appeared as a doublet at 5.45 (1H, d, J = 8.8 Hz), from the J value of doublets, it is confirmed that both the sugars are in B configuration. The remaining sugar protons were appeared at δ 3.95-4.42 as a multiplet for eleven protons. ¹³C nmr spectrum of the [1] revealed the presence of 29 carbon atoms in the compound and confirmed the structure of glycoside as [1], data given in table-I. In mass spectrum of the glycoside, the molecular ion peak is absent as expected. The mass spectrum showed a fragments at m/e 521 and m/e 522 due to removal of disaccharide. The RDA fragments were appeared at m/e 213, and 164 due to [A₁ + H⁺] and B₁ fragment. The formation of [A₁ + H⁺]⁺
and \( [B_1]^+ \) fragment confirmed the presence of two methoxyl and two hydroxyl groups in A ring and one methoxyl group in B ring.

Enzymatic hydrolysis of the glycoside with almond emulsin liberated D-xylose and D-glucose, indicating that the D-xylose was attached to D-glucose through a \( \beta \)-linkage and further confirmed that D-glucose and attached to the aglycone by \( \beta \)-linkage.

Pyranose form of sugars were confirmed by periodate oxidation\(^{10}\). Acid hydrolysis of the permethylated glycoside gave 2,3,5-tri-O-methyl xylose and 2,3,4,6-tetra-O-methyl glucose which indicated that xylose was attached to glucose by (1\( \rightarrow \)4) linkage and that the glucose moiety was attached to the aglycone [2] by \( C_1-OH \). Thus the compound [1] was identified as 5,6-dihydroxy-7,8,3'-tri-methoxy-4'-O-\( \beta \)-D-xylopyranosyl (1\( \rightarrow \)4)-O-\( \beta \)-D-glucopyranoside.

**EXPERIMENTAL**

**GENERAL EXPERIMENTAL METHODS**

Melting points were determined, are uncorrected. The uv spectra was taken on a Hitachi - 320 spectrophotometer and MS were obtained on a Jeol-D-300 (EI/CI) operating at 65 eV. The \(^1\)H-nmr spectra were taken on a Perkin Elmer R-32 (90 MHz) instrument and \(^{13}\)C-nmr spectra
were obtained on a Bruker WM-400 (400 MHz) were given in value (PPM) with TMS as an internal standard and CDCl₃ as solvent. IR spectra were recorded for KBr discs on a Perkin Elmer 881 spectrophotometer.

PLANT MATERIAL

The plant material was supplied by M/s United Chemicals and Allied Products, Calcutta, and authenticated by Botany Department of this University, a voucher specimen has been deposited in room no. 36 of Chemistry Department.

ISOLATION

Air dried seeds (2 kg) were extracted with 95% EtOH and the extract was concentrated under reduced pressure to a yellow viscous mass. The EtOAc soluble part of EtOH extract showed one spot on tlc (EtOAc-Acetone-H₂O 10:3:1:1), and subjected to CC over si-gel. On elution with CHCl₃-MeOH (8:2), compound [1] was obtained as yellow crystals (0.065%) molecular formula, C₂₉H₃₄O₁₇; M.P. 336°C, [M]⁺ 654, (found C, 53.20%, H 5.0%, calculated C, 53.21%, H 5.1%). UV spectrum of [1] showed characteristic peaks at λ max (MeOH) 250, 287 and 362, λ max (NaOME) 272, 391, λ max (AlCl₃-HCl) 245 sh, 260 sh, 380; λ max (NaOAc) 299, 309, 342, 395; λ max (NaOAc-H₃BO₃) 297 sh and IR) KBr cm⁻¹ 3345, 1110, 2872, 2950, 825, 1660, 1600, 1585. Compound 1 (75 mg) was mixed with Ac₂O/py (10 ml). It formed
octacetate derivative, molecular formula C_{45}H_{50}O_{25}, M.P. 98°C, acetyl (34.7%) [M]^+ 990, suggested eight acetylisable -OH groups.

The $^1$H NMR data (90 MHz, CDCl$_3$, PPM): 8 3.75 (3H, s, -OME), 4.00 (3H, s, OMe); 3.82 (3H, s, -OMe); 7.20 (2H, dd, J = 8.5 Hz and 2.5 Hz H-2'-6'); 6.65 (2H, dd, J = 8.5 Hz and 2.5 Hz H-3-5'); 2.48 (s, 3H-5-OAc), 2.36 (s, 3H, 6-OAc), 5.45 (1H, d, J = 8.8 Hz, 1"-anomeric proton), 2.02 (3H, s, 2"-OAc), 2.08 (3H, s, -3"-OAc), 3.09 (3H, s, 6"-OAc), 5.69 (1H, d, J = 9.0 Hz, 1"'-anomeric proton) 2.05 (3H, s, 2"'-OAc), 2.10 (3H, s, -3"'-OAc), 2.15 (3H, s, 5"'-OAc), 3.95 - 4.42 (11H, m, protons of sugar residue. EIMS data of [1]: [M]^+ absent, m/z 521, 522, 360, 332, 331, 213, 212, 184, 164.

ACID HYDROLYSIS

Compound [1] (400 mg) was hydrolysed with 7% H$_2$SO$_4$ by refluxing for about (8 hrs) yielded alycone [2]. The hydrolysate was neutralized with BaCO$_3$ and BaSO$_4$ was filtered off and the filtrate was concentrated to a yellow viscous mass and was found to contain two sugars xylose (Rf 0.29) and glucose (Rf 0.17) (Co-PC and Co-TLC).

IDENTIFICATION OF THE AGLYCONE [2]

Yellow crystalline solid, (0.22%) m.p. 235°C, [M]^+ 360 molecular formula C$_{18}$H$_{16}$O$_8$ (found C-60.0%, H 4.4%,
calculated C 60.02%, H 4.3%, UV λ_{max} \text{nm} 249 \text{sh}, 293,
344; λ(NaOMe) 260, λ(AlCl}_3 \text{) 242 \text{sh}, 262, 307, 381; λ(AlCl}_3-HCl) 241 \text{sh}, 260 \text{sh}, 389; λ(NaOAc), 295, 309, 342, 393;
λ(NaOAc - H}_3\text{BO}_3) 297 \text{sh}, IR ν_{max} \text{cm}^{-1} 3421, 2844, 1661,
1650, 1586, 822. \_1^1H \text{ NMR of the acetylated derivative of}
[2] (90 MHz, DMSO-d}_6) \delta 3.90 (3H, s, -OMe), 4.05 (3H, s,
-OMe), 3.65 (3H, s, -OMe), 7.53 (2H, dd, J = 8.6, 2.5,
H-3'-5'), 6.56 (2H, dd, J = 8.6, 2.5; H-2'-6'), 2.47 (3H,
s, 5'-OAc), 2.32 (3H, s, 6'-OAc).
EIMS data [M]_1^1: 360, 332, 331, 213, 212, 184, 164.

PERMETHYLATION OF [1] FOLLOWED BY ACID HYDROLYSIS

(1) On permethylation followed by acid hydrolysis
gave methylated sugars which were identified as 2,3,5-tri-
O-methyl xylose and 2,3,4,6-tetra-O-methyl glucose, thereby
confirming that C\_4-OH of D-glucose was linked with C\_1-OH
of D-xylose.

QUANTITATIVE ESTIMATION OF THE SUGARS

Quantitative estimation of the sugars in the
glycoside was carried out by the procedure of Mishra and
Rao\_11 which revealed that two sugars were present in
equimolar ratio 1:1.

PERIODATE OXIDATION OF THE GLYCOSIDE

The sodium meta periodate oxidation of the
glycoside (100 mg) consumed 3.01 moles of periodate and
liberated 1.15 moles of formic acid there by confirming
the presence of two moles of sugar attached to the aglycone and also confirmed that D-glucose was present in pyranose form and D-xylene was in furanose form.

ACKNOWLEDGEMENT

Thanks are due to the Director, Central Drug Research Institute (CDRI), Lucknow, for spectral analysis.

LITERATURE CITED


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To,

Prabir K. Gupta,
Executive Editor,
Journal of Indian Chemical Society,
92, Acharya Prafulla Chandra Road,
Calcutta - 700 009

Dear Sir,

Kindly find enclosed herewith three copies of our manuscript entitled "A Novel Flavanone Glycoside From the Aerial Parts of Coccinia indica W&A" for favour of publication in your esteemed journal.

Hope you will do the needful.

Thanking you,

Encl : Three copies of the manuscript

Yours sincerely,

(Dr. R. N. Yadava)
A NOVEL FLAVANONE GLYCOSIDE FROM THE AERIAL PARTS OF
COCCINIA INDICA W&A

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KEY WORDS INDEX: Coccinia indica W&A cucurbitaceae, a
novel flavanone glycoside 6-methyl 7-methoxy flavanone
5-O-α-L-rhamnopyranoside (1→4)-O-β-D-glucopyranoside.

ABSTRACT: A novel flavanone glycoside has been isolated
from the aerial parts of Coccinia indica W&A, and its
structure was determined by spectroscopic methods and
chemical transformation.

INTRODUCTION: Coccinia indica W&A (N.O. cucurbitaceae)
occur throughout India, ceylon and tropical Africa
1,2. The aerial parts are used in the treatment of jaundice,
leprosy, bronchitis, asthma and blood diseases3. A number
of bio-active constituents4 have been isolated by earlier
workers. The present paper deals with the isolation and
structure elucidation of a novel flavanone glycoside.

RESULTS AND DISCUSSION

The compound 1 was isolated as yellow crystals,
m.p. 300°C, [M]+ 592 and analysed for C29H36O13; it gave
dark blue colour in Gibb's test, positive Molisch test,
colour reactions of flavanone\textsuperscript{5,6}, reduce tollen's reagent showing that it to be a flavanone glycoside. The IR spectrum of the glycoside revealed the presence of 3452 cm\textsuperscript{-1} (OH), 1655 (\(\alpha\),\(\beta\)-unsaturated C=O), 2873 (OCH\textsubscript{3}), 2920 (CH\textsubscript{3}) and a complex aromatic substitution pattern 1525, 1210, 1140, 820 cm\textsuperscript{-1}. The UV spectrum showed maximum absorption with MeOH at 259 and 316 (sh); and no changes in the presence of diagnostic shift reagents\textsuperscript{7}, indicated the absence of free OH groups.

Total hydrolysis of the glycoside with 7\% HCl gave two sugars rhamnose and glucose (Co-Pc and GLC) and a aglycone 2, molecular formula \(C_{17}H_{16}O_{4}\), m.p. 278\textdegree C. The aglycone gave a bathochromic shift of 25 nm with AlCl\textsubscript{3} (band I), indicating that sugar was linked to C-5 position of the aglycone. The aglycone 2, was identified as 5-hydroxy, 6-methyl, 7-methoxy flavanone by direct comparison with authentic sample\textsuperscript{8}. The \textsuperscript{1}HNMR spectrum of the acetyl derivative of 1 (in DMSO-\textsubscript{d}\textsubscript{6}) showed signals at \(\delta\) 5.67 (1H, dd, \(J = 11.9\) Hz and 3.6 Hz, H-2), \(\delta\) 2.62 (1H, dd, \(J = 17\) Hz and 3.6 Hz H-3), \(\delta\) 3.52 (1H, dd, \(J = 17\) Hz and 4.2 Hz, H-3) attributed to the C-ring protons of the flavanone\textsuperscript{9}. Two sharp singlet at \(\delta\) 3.92 (3H, s) and \(\delta\) 1.55 (3H, s) for one methoxy group at C-7 and one methyl group at C-6. The presence of two multiplets, one integrating for three protons \(\delta\) 7.88-8.0, whereas other for two protons at \(\delta\) 7.50-7.70 showed the presence of an unsubstituted B-ring.
One singlet at δ 6.68 was assigned for H-8 proton. The rhamnose unit's protons were appeared in the range of δ 4.65-4.87, the glucose unit's protons appeared in the range of δ 5.35-5.50. The anomeric protons appeared at δ 4.25 (1H, d, 1"-H) (J=2.0 Hz) and δ 4.41 (1H, d, 1"'-H) (J=8.5 Hz). The rhamnosyl methyl appeared as a doublet for three protons at δ 1.03 (J=6.0 Hz). EIMS of the glycoside showed fragment ions at m/e 444, 445, 284 due to removal of sugar moieties. The RDA fragments were appeared at 181 [A₁ + H⁺]⁺ and 102 [B₁]⁺ fragments, confirming the presence of one methyl, one methoxy and one hydroxy group in ring A of the aglycone 2.

Enzymatic hydrolysis of the glycoside 1 with almond emulsin liberated L-rhamnose and D-glucose, indicating that the L-rhamnose was attached to D-glucose through β-linkage and hydrolysis with takadiastases, indicating the presence of α-linkage between D-glucose and the aglycone 2.

Pyranose form of sugars were confirmed by periodate oxidation. Acid hydrolysis of permethylated glycoside gave 2,3,4-tri-O-methyl rhamnose and 2,3,6-tri-O-methylglucose, which confirmed that rhamnose was attached to glucose by (1→4) linkage and glucose with aglycone 2 by C-1. Thus the compound 1 was identified.
as 6-methoxy, 7-methoxy flavanone 5-O-α-L-rhamnopyranosyl (1→4)-O-β-D-glucopyranoside.

**EXPERIMENTAL**

The aerial parts of Coccinia indica supplied by M/s United and Allied Products, Calcutta, India. A herbarium specimen has been deposited in natural products laboratory.

**Isolation of compound 1**

The dried and powdered aerial parts of Coccinia indica were extracted with hot aq. MeOH. The aq. MeOH extract was concentrated to a yellow viscous mass which was then dissolved in hot distilled H₂O and partitioned with CHCl₃, ether, EtOAc and n-BuOH. The EtOAc fraction on column chromatography over si-gel gave one compound with EtOAc : Acetone (6:4) as eluants, which was found to be homogenous on TLC examination using EtOAc-MeOH-H₂O (10:7:3).

Compound 1, crystallised from MeOH as yellowish needles mf. C₂₉H₃₆O₁₃ [M]+ 592, m.p. 300°C (Calculated C; 58.78%, H; 6.08%; found C; 58.75%; H, 6.03%) IR (KBr)

\[ \nu_{\text{max}} \text{ cm}^{-1} : 3452 (\text{OH}), 2873 (\text{OCH}_3), 2920 (\text{CH}_3), 1655 (\alpha,\beta-\text{unsaturated C} = \text{O}), 1525 (\text{C} = \text{C}), 1210, 1140, 820 \]

UV \[ \lambda_{\text{max}} \text{ EtOH} (\text{MeOH}) 259, 316 (sh); \]

¹H NMR of the acetyl derivative:

\[ \delta 5.67 (1\text{H}, \text{dd}, J=11.9 \text{ Hz and } 3.6 \text{ Hz } H-2), 2.62 (1\text{H}, \text{dd}, \]
J=17 Hz and 3.6 Hz H-3), 3.52 (1H, dd, J=17 Hz and 4.2 Hz H-3), 3.92 (3H, s, 7-OME); 1.55 (3H, s, 6-Me); 7.80-8.0 (2H, m, H-2',6'); 7.50-7.70 (3H, m, H-3',4',5'); 6.68 (1H, s, H-8); 4.25 (1H, d, 1'-anomic proton) (J=2.0 Hz) and 4.41 (1H, d, 1''-anomic proton) (J=8.5 Hz); 4.65-4.87 (4H, m, protons of rhamnose unit). 5.35-5.50 (6H, m, protons of glucose unit), 1.07-1.40 (18H, m, OAc of disaccharide), 1.03 (3H, d, rham-CH3), (J=6.0 Hz), MS data: [M]+ 592; (absent), m/z 444, 445, 284, 255, 181, 180, 152, 103, 102.

**Acid hydrolysis of 1**

Acid hydrolysis of 1 (50 mg) was performed by refluxing compound 1 with 7% HCl for 6 hours and then the reaction mixture was poured into iced H2O and filtered. The filtrate was neutralized with BaCO3 and BaSO4 filtered off. The solution was evaporated to dryness under reduced pressure, the sugars were identified by TLC using percoated cellulose plates developed in pyridine - EtOAc-CH3CO-H2O (36 : 36 : 7 : 21). The precipitate was identified as aglycone 2.

**Identification of aglycone 2**

Aglycone 2, crystallised from MeOH as yellow crystals mf. C17H16O4, m.p. 278°C [M]+ 284 (found C; 71.6%; H; 5.3%, calculated C, 71.8%; 5.6%). IR (KBr) max cm⁻¹: 3450 (OH), 1652 (α, β-unsaturated C=O), 2874 (OCH3), 2925
(CH₃), 1594, 1136, 1211, 823. UV λ<sub>max</sub> (MeOH) 260 and 314 (sh), (AlCl₃) 262 and 349 (sh); <sup>1</sup>HNMR (DMSO-d₆) = 90 MHz δ 5.68 (H, dd, J = 11.8 Hz and 3.5 Hz, H-2), 2.64 (1H, dd, J = 17 Hz and 3.6 Hz, H-3), 3.21 (1H, dd, J=17 Hz and 4.2 Hz H-3), 3.93 (3H, s, O-Me), 1.56 (3H, s, -Me), 6.69 (1H, s, H-8), 7.90-8.12 (2H, m, H-2'-6'), 7.60-7.77 (3H, m, H-3',4',5'). MS data: [M]<sup>+</sup> 284; m/e 256, 255, 181, 180, 152, 103, 102.

*Permethyltion of 1 followed by acid hydrolysis*

1 on permethylation followed by acid hydrolysis gave methylated sugars which were identified as 2,3,4-tri-O-methyl rhamnose and 2,3,6-tri-O-methyl glucose, thereby confirming that C-1 position of L-rhamnose was linked with C-4 position of D-glucose.

*Quantitative estimation of the sugars*

Quantitative estimation of the sugars in the glycoside was carried out by the procedure of Mishra and Rao<sup>11</sup> which revealed that two sugars were present in equimolar ratio 1:1.

**ACKNOWLEDGEMENT**

Thanks are due to the Director, Central Drug Research Institute (CDRI), Lucknow, for spectral analysis.
REFERENCES


(I)

(II)