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AN ISOFLAVONE GLYCOSIDE FROM THE SEEDS OF *TRICHOSANTHES ANGUINA*

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

Abstract—A novel isoflavone glycoside; 5,6,6'-trimethoxy-3',4'-methylenedioxyisoflavone 7-*O*-β-D-(2''-*O*-*p*-coumaroylglucopyranoside), 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 glycoside (**1**) 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**); $C_{34}H_{32}O_{15}$, $[M]^+$ 680, which gave a positive Molicsh test and the characteristic colour reactions of an isoflavonoid [6, 7]. The IR spectrum of **1** showed absorption bands at 3310 (OH), 2870 (OMe), 1650 (>C=O), 932 ($-OCH_2O$) and 1600 (aromatic ring system). The molecular weight and its acetyl derivative $C_{42}H_{40}O_{19}$, $[M]^+$ 848, suggested the presence of four acetylated hydroxyl groups. Alkaline hydrolysis of **1** with 2% NaOMe yielded *p*-methyl coumarate (mmp, co-TLC and superimposable IR and NMR spectra). The ether insoluble part obtained from alkaline hydrolysis gave the isoflavone glycoside (**2**), $C_{25}H_{26}O_{13}$, $[M]^+$ 534. Compound **2** on acid hydrolysis (7% H_2SO_4) gave the isoflavone (**3**), $C_{19}H_{16}O_8$, $[M]^+$ 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 monoacetate $C_{21}H_{18}O_9$ $[M]^+$ 414.

The ^1H NMR spectrum of the acetyl derivative of **3** showed a singlet at δ8.07, a characteristic feature of isoflavones [9]. The three sharp singlets appeared at δ3.65, 3.80 and 3.93 indicating the presence of three methoxy groups. A sharp singlet at δ6.19 (2H) confirmed the presence of a methylenedioxy group. Alkaline cleavage of **3** with 10% NaOH gave the corresponding deoxybenzoin (**4**), $C_{18}H_{18}O_8$, $[M]^+$ 362, which was identified by spectral data and confirmed that **3** is an isoflavone. The ^{13}C NMR spectrum (see Experimental) of **1** revealed the presence of 34 carbon atoms and confirmed the structure as **1**. The EI-mass spectrum of **1** 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 **1** 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 linkage and pyranose form of the sugar were confirmed by enzymic hydrolysis with almond emulsin and periodic oxidation of **2**. From the combined evidence **1** was assigned the structure, 5,6,6'-trimethoxy-3',4'-methylenedioxyisoflavone 7-*O*-β-D-(2''-*O*-*p*-coumaroyl)glucopyranoside.

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

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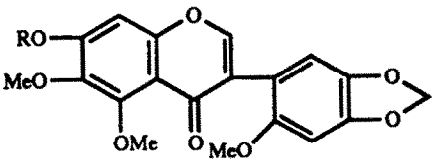
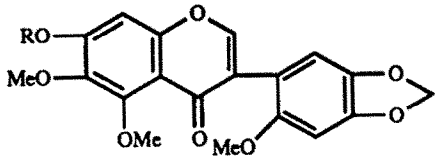
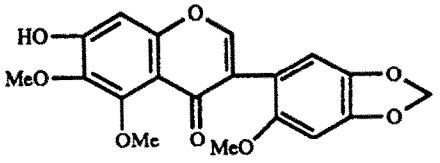


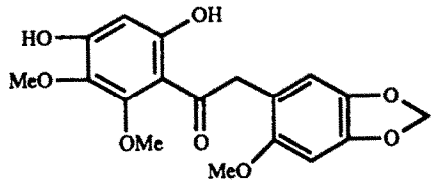
(1) R-glucose-(2″-*O*-*p*-coumaroyl)



(2) R-glucose



(3)



(4)

with hot aq. EtOH. The extract was concd to a viscous mass, which was then dissolved in hot H_2O and partitioned with $CHCl_3$, Et_2O, EtOAc, HOAc and n-BuOH. The Me_2CO fraction on TLC examination ($CHCl_3$–C_6H_6–Me_2CO, 10:10:1) showed two spots, indicating the presence of two compounds, which were separated by CC over silica gel G, eluted with $CHCl_3$–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 $CHCl_3$–MeOH–H_2O (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}^{KBr} cm^{-1}: 3310 (OH), 2870 (OMe), 1650 (>C=O), 932 ($-OCH_2O$), 1600 (aromatic ring system), 1636, 1265, 1185, 822, UV λ_{max}^{MeOH} 255, 260, 305; +NaOMe 257, 261, 305sh; +$AlCl_3$ 257sh, 260, 306sh; +$AlCl_3$+HCl; 253sh, 258, 307sh; +NaOAc; 262, 313sh; +NaOAc+H_3BO_3, 263, 310; ^{1}H NMR of tetracetate; $C_{42}H_{40}O_{19}$ $[M]^+$ 848, mp 145° (90 MHz, $CDCl_3$, δppm): δ8.07 (1H, *s*, H-2), 3.65 (3H, *s*, OMe), 3.80 (3H, *s*, OMe), 3.93 (3H, *s*, OMe), 6.19 (2H, *s*, $-OCH_2O$), 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-α), 7.73 (1H, *d*, J=8 Hz, H-β), 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 **1**: *m/z* 680 $[M]^+$, 534, 372, 197 and 191. ^{13}C NMR (400 MHz, DMSO-d_6): δ154.4 (C-2), 120.5 (C-3), 175.6 (C-4), 155.2 (C-5), 153.2 (C-6), 120.6 (C-7), 112.4 (C-8), 113.2 (C-1′), 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 (OCH_2O).

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 concd under vacuum. The Et_2O soluble part yielded needles of *p*-methyl coumarate, mp 130°. The Et_2O insoluble part furnished an amorphous compound (**2**), mp 170°, $C_{25}H_{26}O_{13}$, $[M]^+$ 534 (found C 56.0, H 4.7, OMe 17.3, calculated 56.1%, H 4.8% OMe 17.4%) TLC homogenous; IR ν_{max}^{KBr} cm^{-1}: 3312 (OH), 2872 (OMe), 1620 (>C=O), 930 ($-OCH_2O$), 1610 (aromatic ring system), 1634, 1260, 1180, 820, UV λ_{max}^{MeOH} 256, 261, 305sh; +NaOMe 256, 260, 306sh; +$AlCl_3$ 257, 261, 306sh; +$AlCl_3$+HCl; 254sh, 257, 307sh; +NaOAc 263, 314sh; +NaOAc+H_3BO_3 264, 311; ^{1}H NMR of acetate derivative; $C_{33}H_{34}O_{17}$ $[M]^+$ 702, mp 150° (90 MHz, $CDCl_3$, δppm): δ8.04 (1H, *s*, H-2), 3.66 (3H, *s*, OMe), 3.82 (3H, *s*, OMe), 3.90 (3H, *s*, OMe), 6.18 (2H, *s*, $-OCH_2O$), 7.88 (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% H_2SO_4) by refluxing for 10 hr to yield the aglycone **3**. The hydrolysate was neutralized with $BaCO_3$ and $BaSO_4$ was filtered off. The concd filtrate was run on PC in *n*-BuOH–HOAc–H_2O, 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°, $C_{19}H_{16}O_8$ (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}^{KBr} 3315 (OH), 2875 (OMe), 1618 (>C=O), 929 ($-OCH_2O$), 1600 (aromatic ring system), 1631, 1262, 1183, 824, UV λ_{max}^{MeOH} 244sh, 257, 270sh, 320; +NaOMe 259, 278sh, 341; +$AlCl_3$ 240sh, 250, 264sh 305; +NaOAc 272, 318sh, 335; +NaOAc+H_3BO_3 274sh, 307; ^{1}H NMR of acetate (90 MHz, $CDCl_3$, δppm): δ8.06 (1H, *s*, H-2), 3.65 (3H, *s*, OMe), 3.80 (3H, *s*, OMe), 3.91 (3H, *s*, OMe), 6.15 (2H, *s*, OCH_2O), 7.80 (1H, *s*, H-2′), 7.61 (1H, *s*, H-5′), 6.67 (1H, *s*, H-8), 2.38 (3H, *s*, OAc-7). EI-MS of **1**: $[M]^+$ 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°, $C_{18}H_{18}O_8$ $[M]^+$ 362 (found C 58.1, H 4.5, OMe 24.7; calculated C 59.6, H 4.9, OMe 25.6%). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 2871 (OMe), 1619 (>C=O), 928 (OCH_2O), 1632, 2994. UV λ_{max}^{MeOH}: 216, 233,

257, 310; ^{1}H NMR (90 MHz, $CDCl_3$, δ ppm): δ 5.83 (2H, *s*, $-OCH_2O$), 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*, $-CH_2$), 12.1 (1H, *s*, H-3).

Attachment of the aglycone **3** *and* p-*coumaric acid to glucose.* Compound **1** was treated with MeI and Ag_2O 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_2SO_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_2H and consumed periodate were estimated by the Jones method [11].

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

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REFERENCES

1. *Wealth of India*, A Dictionary of Indian Raw Material and Industrial Products (1950) CSIR p. 247. New Delhi.
2. Kritikar, K. R. and Basu, B. D. (1945) *Indian Medicinal Plants* II, p. 1114. Basu, Allahabad.
3. Venkataramaiah, C., Rao, K. and Narayana (1983) *Z. Pflanzenphysiol.* **111**, 459.
4. Ghosh, B., Dasgupta, B. and Sircar, P. K. (1981) *Plant Biochem. J.* **8**, 66.
5. Lakshminarayana, G., Kaimal, T. N. B., Mani, V. V. S., Sita, K. and Rao, T. (1982) *Phytochemistry* **21**, 301.
6. Narayana, V. and Sheshadri, T. R. (1971) *Indian J. Chem.* **9**, 14.
7. Adinarayan, D. and Rao, J. R. (1972) *Tetrahedron* **28**, 5377.
8. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, p. 160. Springer, New York.
9. Markham, K. R., Rahman, W., Jehan, S. and Mabry, T. J. (1967) *J. Heterocyl. Chem.* **4**, 61.
10. Mishra, S. P. and Mohan Rao, V. K. (1960) *J. Sci. Ind. Res. Sect. C.* **19C**, 170.
11. Hirst, E. L. and Jones, J. K. N. (1949) *J. Chem. Soc.* 1659.