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NOTE

A Novel Flavone Glycoside from *Centratherum anthelminticum* Kuntze.

R. N. YADAVA* and DEEPAK BARSAINYA

Natural Products Laboratory, Department of Chemistry, Dr. H. S. Gour University, Sagar-470 003

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The plant *Centratherum anthelminticum* Kuntze. (N.O. Compositae), commonly known as 'Somraj' in Hindi, is distributed throughout India upto 5500 feet[1] and is useful in asthma and kidney troubles.

Here, we report the isolation of a novel flavone glycoside acacetin-7-*O*-β-D-glucopyranosyl(1→4)-α-D-xylopyranoside from the seeds of the plant.

Results and Discussion

Compound **1** responded to Molisch and Shinoda test[2] indicating it to be a flavonoidal glycoside. Its uv-spectral data indicate[3] the presence of free hydroxyl group at C-5 and blocked hydroxyl at C-7 and C-4'.

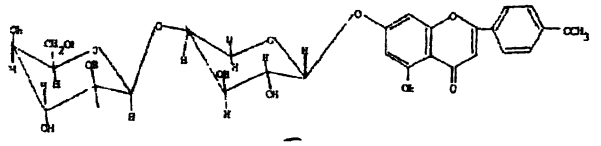
Acid hydrolysis of **1** with 10% H_2SO_4 gave an aglycone (**3**), which gave a bathochromic shift of 12 nm in band-II with NaOAc showing that the sugar was attached at C-7 in the glycoside. The aglycone was identified as acacetin by direct comparison of its m.p. spectral data with the known sample[4]. The sugars were identified as glucose and xylose by co-pc[5].

The acetylation of **1** gave a heptacetate derivative whose ^1H nmr spectrum showed it to be a disaccharide. MS data supported the structure of the new compound as **1**. The molecular ion peak as expected was absent. A fragment ion at *m/z* 326 was due to the loss of acetylated sugar moiety from the molecular ion. The aglycone fragment was obtained at *m/z* 284. The retro-Diels-Alder fragmentation pattern were obtained at *m/z* 153 and132 which were assigned to $[A + H^+]^{+\bullet}$ and $[B_1]^{+\bullet}$ fragment.

Permethylation of glycoside **1** (Me_2CO/K_2CO_3) followed by acid hydrolysis afforded apigenin 4',5-dimethyl ether (**4**) which was confirmed by its m.p. and comparison of its spectral data with known sample[6]. The methylated sugars were identified[7] as 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-xylose showing the intersugar linkage as (1 → 4) and confirmed the attachment of both the sugars moieties at C-7.

The partial hydrolysis of the glycoside with Kiliani mixture (HCl : CH_3COOH : H_2O, 15 : 35 : 50) liberated glucose (co-pc) and a partial glycoside (**2**) which was further hydrolysed with tokadiastase thereby suggesting α-linkage between the aglycone and D-xylose. The hydrolysis of glycoside **1** with almond emulsin yielded the D-glucose and proaglycone confirming the β-linkage between proglycone and D-glucose, thus showing the glucose as the terminal sugar. Quantitative estimation of sugar with Somogyis's method[8] showed the presence of two moles of sugars per mole of aglycone.

On the basis of the above results, compound **1** has been identified as acacetin-7-*O*-β-D-glucopyranosyl(1 →4)-α-D-xylopyranoside.



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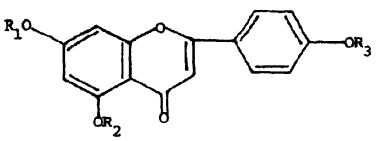

2; R_1 = Xylose, R_2 = H, R_3 = CH_3
3; R_1, R_2 = H, R_3 = CH_3
4; R_1 = H, R_2, R_3 = CH_3

Experimental

M.ps. were determined on a Reichert microscope hot-stage apparatus and are uncorrected. Ir spectra were taken on a Shimadzu IR-408 spectrometer, mass spectra on a JEOL-JMS-300 spectrometer and ^1H nmr spectra on a JEOL-GX instrument (270 MHz).

Air-dried and powdered seeds of *C. anthelminticum* were extracted with 95% EtOH. The combined extract was concentrated under reduced pressure to give a brown viscous mass. The ethyl acetatesoluble fraction was subjected to column chromatography over Si-gel. The fraction collected from EtOAc-acetone (3 : 2) gave compound **1**, crystallised from MeOH : $CHCl_3$ as a yellow needles, m.p. 206–07° (Found : C, 56.08; H, 5.15. $C_{27}H_{30}O_{14}$ calcd. for : C, 56.05; H, 5.19%); ν_{max} (KBr) 3 369–3 460 (OH), 2 875 ($-OCH_3$), 1 660 (α,β-unsat. C=O), 1 510, 1 460, 1 380, 1 375, 1 125, 1 050, 810, 660 cm^{-1}; ν_{max} (MeOH) 260, 320; (+$AlCl_3$) 260 (sh), 285, 300, 385; (+NaOMe) 275, 370; (+NaOAc) 260, 290 (sh), 365 nm.

Acetylation of compound **1** : Compound **1** on acetyla-

tion with Ac_2O/Py (1 : 2) for 48 h at 25° room temperature and on usual work up afforded a heptacetate derivative (**1a**), $C_{41}H_{44}O_{21}$, m.p. 132–33°; δ (270 MHz; $CDCl_3$) 6.72 (1H, d, *J* 2.0 Hz, H-6), 6.79 (1H, d, *J* 2.0 Hz, H-8), 7.35 (2H, d, *J* 8.0 Hz, H-3′,5′), 7.82 (2H, d, *J* 8.0 Hz H-2′,6′), 3.89 (3H, s, OCH_3-4′), 2.45 (3H, s, OAc-5), 6.37 (1H, s, H-3), 1.89–2.10 (18H, m, sugar acetoxyls), 4.42–5.58 (12H, m, sugar protons), 4.58 (1H, d, *J* 2.0 Hz, H-1″), 5.49 (1H, d, *J* 8.5 Hz, H-1‴); *m/z* 578, $[M^+]$ absent; 326 [M^+-acetylated sugar moiety]$^+$; 284 [326-Ac$^•$]$^{+•}$; [aglycone]$^{+•}$; 283 [aglycone-H^+]$^{+•}$; 256 [aglycone-Co]$^+$; 153 $[A_1+H^+]^{+•}$; 132 $[B_1]^{+•}$; 135 $[B_2]^{+•}$.

Acid hydrolysis of compound **1** : Glycoside **1** was refluxed with 10% H_2SO_4 (10 ml) for 2 h, at 100°, acidified and then the mixture was extracted with EtOAc. The EtOAc fraction contained aglycone which was crystallised from $CHCl_3$: MeOH as yellow needles (**3**), m.p. 261° (Found : C, 67.58; H, 4.20. $C_{16}H_{12}O_5$ calcd. for : C, 67.60; H, 4.22%); λ_{max} (MeOH) 260, 340; (+NaOAc) 272, 370 nm. It was identified as acacetin.

The aqueous hydrolysate after neutralisation with Na_2CO_3 was subjected to co-pc using butanol : acetic acid : water (4 : 1 : 5) with authentic sugars as checks. The R_f values of sugars were identified with those glucose (R_f, 0.18) and xylose (R_f, 0.26).

Enzymatic hydrolysis of compound **1** : A mixture of **1** (15 ml) and almond emulsin (10 ml) were kept at 25° for 30 h and then after addition of water it was extracted with n-BuOH. The n-BuOH extract was chromatographed over silica-gel column to give a partial glucoside (**2**), $[M]^+$ 416, m.p. 278–79° (Found : C, 60.30; H, 4.65. $C_{21}H_{20}O_9$ calcd. for : C, 60.47; H, 4.77%). It was identified as acacetin-7-*O*-α-D-xylopyranoside by its spectral studies.

Permethylation of compound **1** : Permethylation of **1** was followed by acid hydrolysis. A mixture of CH_3I (1 ml), Ag_2O (30 mg) and a solution of **1** (20 mg) in DMF (5 ml), was stirred in dark at room temperature for 48 h. It was then filtered and the residue treated with ethanol (25 ml). The syrupy residue was hydrolysed with 10% HCl and after usual work-up gave apigenin 4′,5-dimethyl ether (**4**), $[M]^+$ 298, m.p. 264° (Found : C, 68.48; H, 4.72. $C_{17}H_{14}O_5$ calcd. for : C, 68.45; H, 4.69%). The methylated sugars were identified as 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-xylose separated by preparative tlc on Si-gel (toluene : MeOH, 4 : 1).

Quantitative estimation of sugar : The anhydrous glycoside (25 g) was hydrolysed with 10% HCl. After cooling overnight, the aglycone was filtered and dried (16.1 mg). The ratio of aglycone to glycoside was found to be 46.2%, indicating the presence of two moles of sugar/mole of aglycone.

References

1. R. N. CHOPRA, I. C. CHOPRA and B. S. VERMA, "Glossary of Indian Medicinal Plants", Publication and Information Directorate, New Delhi, 1956; "Wealth of India : A Dictionary of Raw Materials and Industrial Product", C.S.I.R., New Delhi, 1950, Vol. II; K. R. KIRTIKAR and B. D. BASU, "Indian Medicinal Plants", Lalit Mohan Basu and Co., Allahabad, 1935, Vol. II.
2. J. SHINODA, *J. Chem. Pharm. Soc. Jpn.*, 1928. 48, 214.
3. T. J. MABRY, K. R. MARKHAM and M. B. THOMAS, "The Systematic Identification of Flavonoids", New York, 1970; K. R. MARKHAM and T. J. MABRY in "The Flavonoids", eds. J. B. HARBORNE, T. J. MABRY and H. MABRY, Chapmann and Hall, London, 1975.
4. E. WOLLENWEBER, *Biochem. Syst. Ecol.*, 1975, 3, 47.
5. E. LEDERER and M. LEDERER, "Chromatography", Elsevier, London, 1957, Vols. 28–29, P. 247.
6. S. D. SRIVASTAVA, "Drugs of Future", 1983, pp. 8, 669.
7. F. PETEK, *Bull. Soc. Chim. Fr.*, 1965, 263.
8. M. SOMOGYIS, *J. Biol. Chem.*, 1952, 19, 195.