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SHORT COMMUNICATION

CHRYSOERIOL GLYCOSIDES AND OTHER FLAVONOIDS OF *RUNGIA REPENS* FLOWERS

T. R. SESHADRI and S. VYDEESWARAN
Department of Chemistry, University of Delhi, Delhi-7, India

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Abstract—Condensation of chrysoeriol with tetraacetyl glucosyl bromide has yielded chrysoeriol 7-monoglucoside earlier isolated from *Luffa echinata* and two new glucosides whose structures are established as chrysoeriol 4′-mono and 7,4′-diglucosides. In the flowers of *Rungia repens*, besides luteolin 7-glucoside already reported, there occurs apigenin 7-glucoside and a new glycoside whose constitution has been established as kaempferol 3-*O*-α-L-rhamnopyranosyl (1 → 3)β-D-glucopyranoside. The occurrence of this new disaccharide (rhamnose 1 → 3 glucose) and a flavonol glycoside in the Acanthaceae is of biogenetic interest.

IN A previous publication,[1] the chemical components of *Luffa echinata* were discussed. The 7-monoglucoside and apioglucoside of chrysoeriol were reported along with other components. For purpose of comparison the synthesis of chrysoeriol monoglucoside has been carried out by the direct combination of chrysoeriol with tetra-*O*-acetyl α-glucosyl bromide. The product was a mixture which could be separated into chrysoeriol 7- and 4′-monoglucosides and 7,4′-diglucoside. Their constitutions have been established by analytical and spectral data.

In a recent publication[3] on the chemical components of *Rungia repens*, a chrysoeriol glycoside was reported. The m.p. of this glycoside was close to that of the 4′-glucoside, now made synthetically. For purpose of comparison the natural sample was obtained but it was found to be a mixture containing luteolin[2] and apigenin 7-glucosides. In order to confirm this, we collected a sample of the flowers from Hyderabad and carried out the extraction as given below.

The fresh violet flowers were extracted exhaustively with boiling alcohol. From the concentrate, three flavonoids, designated as A, B and C were separated by preparative paper and TLC techniques. In addition, free glucose and galactose were detected by paper chromatography.

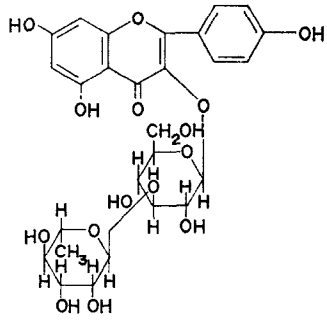
Compound A gave, on acid hydrolysis, luteolin[4] and glucose. Absence of shift with NaOAc in the UV spectrum showed that the sugar was in the 7-position. This was confirmed by permethylation followed by hydrolysis, whereby 2,3,4,6-tetra-*O*-methyl-D-glucose and luteolin 5,3′,4′-trimethyl ether were obtained. Its properties agreed with those of luteolin 7-glucoside.[4]

[1] T. R. SESHADRI and S. VYDEESWARAN, *Phytochem.* **10**, 667 (1971).
[2] S. SANKARA SUBRAMANIAN and A. G. R. NAIR, *Indian J. Chem.* **2**, 338 (1964).
[3] S. SANKARA SUBRAMANIAN and A. G. R. NAIR, *Indian J. Chem.* **4**, 461 (1966).
[4] M. O. FAROOQ, S. R. GUPTA, M. KIAMUDDIN, W. RAHMAN and T. R. SESHADRI, *J. Sci. Ind. Res. India* **12B**, 400 (1953).

Compound B, on acid hydrolysis, yielded apigenin[1] and glucose. The attachment of the sugar at the 7-position was infered from the absence of shift with NaOAc in the UV spectrum and confirmed by permethylation followed by hydrolysis giving 2,3,4,6-tetra-*O*-methyl-D-glucose and 5, 4′-di-*O*-methylapigenin. Its properties agreed with those of apigenin 7-glucoside reported in the literature.[5]

The colour reactions and UV spectrum of compound C indicated that it belonged to the flavonol group. Positive ferric reaction and Molisch's test and a band at 3500 cm^{-1} in the IR spectrum (alcoholic OH) indicated that it was a flavonol glycoside. Analytical data agreed with the formula $C_{27}H_{30}O_{15}$; acetylation gave a nonacetate, as indicated by the NMR spectrum. Acid hydrolysis gave kaempferol[6] and glucose and rhamnose, identified by comparison with authentic samples.

A positive Zn/HCl colour reaction suggested that the 3-OH was involved in glycosidation. Methylation with diazomethane followed by hydrolysis gave the 7,4′-dimethyl ether of kaempferol,[7] identified by mixed m.p. and co-chromatography with an authentic sample. Permethylation followed by hydrolysis gave 2,4,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose which showed that the anomeric hydroxyl of rhamnose is condensed with the 3-hydroxyl of glucose. This conclusion has been further substantiated by subjecting the glycoside to HIO_4 treatment, followed by hydrolysis, when only glucose was identified as the unaffected sugar moiety. Finally, in order to establish the exact configuration of the sugar linkages, the molecular rotation of the acetate was determined. It was close to the $[M]_D$ of rutin decaacetate showing thereby that the anomeric hydroxyls in compound C are disposed as in rutin, viz. glucose β and rhamnose α. The complete structure, supported by the NMR spectrum of the acetate, is therefore established as I. The new glycoside has a new disaccharide with new sugar linkage and is therefore named kaempferol 3-rungioside and the disaccharide rungiose.



Kaempferol 3—rungioside (I)

EXPERIMENTAL

M.ps were determined on a Kofler-block and are uncorrected. Paper chromatography was carried out on Whatman No. 1 paper, TLC and column chromatography using NCL grade silica gel. For paper chromatography the following solvent systems were used: (a) 25% aq. HOAc, (b) *n*-BuOH-HOAc-H_2O, 4:1:5 (upper layer), (c) *n*-BuOH–EtOH–H_2O, 5:1:4 (upper layer). NMR spectra were recorded in $CDCl_3$ and the values are expressed in δ units.

[5] M. NAGESE, *J. Agric. Chem. Soc. Japan* **17**, 483 (1941)
[6] T. J. MABRY, K. R. MARKHAM and M. B. THOMAS, *The Systematic Identification of Flavonoids*, p. 119, Springer–Verlag, Berlin (1970).
[7] S. RANGASWAMI and R. THANU IYER, *Indian J. Chem.* **7**, 526 (1969).

Synthesis of chrysoeriol glycosides. Chrysoeriol (0·8 g) was condensed with tetra-*o*-acetyl α-glucosyl bromide (2 g). Use was made of the Ag_2CO_3-py. method.[8] The product was found to be a mixture of three compounds giving positive ferric reaction, designated as X, Y and Z. It was subjected to column chromatography over silica gel. The eluates of 0·5% MeOH in $CHCl_3$ gave compound X. The earlier fractions of 1% MeOH in $CHCl_3$ afforded compound Y, while the later fractions contained a mixture of compounds Y and Z.

Compound X formed colourless needles from MeOH (250 mg), m.p. 262°. (Found: C, 54·8; H, 5·1; $C_{44}H_{48}O_{24}$ requires: C, 55·0; H, 5·0%.) NMR values: 2·06 (*s*, 6H, 2 O–$COCH_3$), 2·10 (*s*, 18H, 6 O–$COCH_3$), 3·96 (*s*, 3H, 1 O–CH_3), 6·52 (*d*, 1H, C_6–H), 6·62 (*s*, 1H, C_3–H), 6·66 (*d*, 1H, C_8–H), 7·35 (*d*, 1H, $C_{5'}$–H), 7·42 (*m*, 2H, $C_{2'}$ and $C_{6'}$–H). Deacetylation was effected by keeping it (100 mg) with 2 N NaOMe soln. for 20 min at 0°. After neutralization with HOAc the product could not be extracted with EtOAc. However, addition of excess of water separated a yellow solid which was filtered and recrystallized from MeOH, yellow needles (40 mg). The new glycoside chrysoeriol 7,4′-diglucoside had m.p. 210° (shrinking 196°). (Found: C, 53·7; H, 5·3; $C_{28}H_{32}O_{16}$ requires: C, 53·9; H, 5·1%.) λ_{max}^{EtOH} 249, 270, 338 nm; + NaOAc no shift; + NaOAc + H_3BO_3 no shift; + $AlCl_3$ 259, 279, 342, 380 nm; + NaOEt 286, 383 nm. R_fs: 0·65 0·60 (solvents *a*, *b* 30°).

Compound Y formed colourless needles from MeOH, 100 mg, m.p. 204°. (Found: C, 56·8; H, 5·0. $C_{30}H_{30}O_{15}$ requires: C, 57·1; H, 4·8%.) NMR values: 2·06 (*s*, 3H, 1-OCOCH$_3$), 2·12 (*s*, 9H, 3-OCOCH$_3$), 3·98 (*s*, 3H, 1-OCH$_3$), 6·54 (*d*, 1H, C_6–H), 6·64 (*s*, 1H, C_3–H), 6·68 (*d*, 1H, C_8–H), 7·35 (*d*, 1H, $C_{5'}$–H), 7·42 (m, 2H, $C_{2'}$ and $C_{6'}$–H). 50 mg was deacetylated as described above and the product chrysoeriol 4′-glucoside was extracted with EtOAc and recrystallized from MeOH, yellow needles (20 mg), m.p. 283–4° (Found: C, 57·2; H, 4·6, $C_{22}H_{22}O_{11}$ requires: C, 57·1; H, 4·8%.) λ_{max}^{EtOH} 242, 270, 337 nm; + NaOAc 251 (inf.), 275, 316 (inf.), 343 nm; + NaOAc + H_3BO_3 no shift; + $AlCl_3$ 252, 283, 343, 376 nm; + NaOEt 280, 376 nm. R_fs: 0·78, 0·73 (solvents *a*, *b*, 30°).

Mixture of Y and Z. Yield, 80 mg. It was deacetylated as described above and the synthetic chrysoeriol 7-glucoside was separated from 4′-monoglucoside by preparative paper chromatography using solvent *b*. The 7-glucoside appeared as reddish brown spot in UV light but on exposure to ammonia it turned to olive green whereas the 4′-glucoside remained as reddish brown when viewed in UV light after exposure to ammonia. Yellow needles of 7-glucoside from aq. MeOH, 20 mg. R_fs: 0·70, 0·64 (solvents *a*, *b*, 30°). It agreed in all respects with the natural sample[1] (mixed m.p., co-chromatography and superimposable IR spectra).

Isolation of the components from flowers of Rungia repens. The fresh flowers (200 g) were extracted with boiling EtOH and the concentrate dissolved in MeOH. Precipitation with excess Et_2O removed waxy materials and the precipitated solid was redissolved in MeOH. This solution was applied on paper (3 MM) and was irrigated with solvent a. The different bands, detected by ammonia vapour, were cut and eluted with MeOH. The earlier two bands (R_f, 0·73 and 0·81) afforded luteolin and apigenin 7-glucosides in a pure state while the last band (R_f, 0·86) was found to contain kaempferol 3-rungioside admixed with glucose and galactose. Hence, it was passed through a column of silica gel and from the MeOH–$CHCl_3$ (8:92) eluates containing still the free sugars, the glycoside was separated by preparative TLC using 25% MeOH in $CHCl_3$ as the solvent system (R_f, 0·6).

Luteolin 7-glucoside (25 mg). It had m.p. 258°. λ_{max}^{EtOH} 256, 352 nm. Hydrolysis (10 mg) with 7% aq. H_2SO_4 gave luteolin, m.p. >310°. Glucose was identified by paper chromatography. Permethylation of the glycoside (10 mg) using Hakomori's[9] procedure followed by Kiliani[10] hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-glucose and 5,3′,4′-tri-*O*-methylluteolin, identified by comparison with authentic samples on paper and TLC respectively. It may be noted here that the aglycone methyl ether could be detected only when permethylation was carried out in the cold.

Apigenin 7-glucoside (20 mg). It had m.p. 217–219°. λ_{max}^{EtOH} 269, 338 nm. Hydrolysis gave apigenin, identified by mixed m.p., co-TLC and superimposable IR spectrum with an authentic sample. Glucose was identified by paper chromatography. Permethylation (8 mg) followed by hydrolysis, carried out in the same way as before, gave 2,3,4,6-tetra-*O*-methyl-D-glucose and 5 4′-di-*O*-methyl apigenin, identified by comparison with authentic samples on paper and TLC respectively.

Kaempferol 3-rungioside (100 mg). Yellow needles from MeOH–Et_2O, m.p. > 310° (shrinking at 265°). (Found: C, 54·3; H, 5·2; $C_{27}H_{30}O_{15}$ requires C, 54·5; H, 5·1%.) λ_{max}^{MeOH} 267 (4·1), 295 (inf.), 350 (3·9) nm; + NaOAc 274, 305 (inf.), 368 nm; + NaOAc + H_3BO_3 no shift; + $AlCl_3$ 276, 308, 370, 395 nm; + NaOMe 285, 330, 410 nm. Hydrolysis of the glycoside (10 mg) with 7% aq. H_2SO_4 (0·5 ml) for 2 hr gave kaempferol, m.p. 280°. The sugar moieties were found to be glucose and rhamnose by comparison with authentic samples on paper chromatography.

Acetylation of the glycoside (40 mg) was carried out by keeping it with pyr. and Ac_2O in the cold overnight. Acetate, needles from EtOAc–petrol. (30 mg), m.p. 124° (Found: C, 55·4; H, 5·1; $C_{45}H_{48}O_{24}$ requires

[8] S. R. GUPTA, B. RAVINDRANATH and T. R. SESHADRI, *Phytochem.* **9**, 2231 (1970).

[9] S. HAKOMORI, *J. Biochem.* **55**, 205 (1964).

[10] H. KILIANI, *Ber. Chem.* **63**, 2836 (1930).

C, 55·6; H, 5·0%.) $[\alpha]_D^{30}$ −91·3 ($CHCl_3$). NMR values: 1·05 (*d*, 3H, rhamnosyl CH_3), 1·94 (*s*, 6H, 2 O–$COCH_3$), 2·01 (*s*, 6H, 2 O–$COCH_3$), 2·08 (*s*, 6H, 2 O–$COCH_3$), 2·30 (*s*, 6H, 2 O–$COCH_3$), 2·43 (*s*, 3H, 1 O–$COCH_3$), 6·82 (*d*, 1H, C_6–H), 7·12 (*d*, 1H, C_8–H), 7·28 (*m*, 2H, $C_{3'}$ and $C_{5'}$–H), 7·98 (*m*, 2H, $C_{2'}$ and $C_{6'}$–H).

The glycoside (15 mg) was dissolved in excess MeOH and ethereal CH_2N_2 was added. The mixture was kept in the refrigerator overnight and the product was worked up as usual and hydrolysed with 7% aq. H_2SO_4 for 2 hr. The aglycone was extracted with ether and purified through preparative TLC (4 mg). It had m.p. 179°. It agreed in all respects with an authentic sample of kaempferol 7,4′-dimethyl ether. The aq. portion showed glucose and rhamnose on paper chromatography.

Permethylation[9] of the glycoside (10 mg) with NaH, DMSO and CH_3I was carried out. The product obtained as a syrup was hydrolysed using Kiliani mixture. The methylated sugars were identified as 2,4,6-tri-*O*-methyl-D-glucose (solvent C, R_g 0·75, lit. value 0·76) and 2,3,4-tri-*o*-methyl-L-rhamnose (R_g 1·01 lit. value 1·01).

The glycoside (15 mg) was dissolved in MeOH (1 ml) and an aq. soln. of $NaIO_4$ (0·3 ml) was added. The mixture was allowed to stand for 4 days in the dark. The product was then taken up in *n*-BuOH and washed with H_2O to remove the excess periodate and concentrated leaving a yellow residue. This was then hydrolysed by Kiliani mixture and glucose was identified by paper chromatography. After extraction with ether, kaempferol was identified by co-TLC and undepressed mixed m.p.

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Key Word Index—Rungia repens; Acanthaceae; flavonoid glycosides; chrysoeriol; kaempferol-3-*O*-α-L-rhamnopyranosyl(1→3)β-D-glucopyranoside; rungiose.