2',4',6'-Trihydroxydihydrochalcone (**2**). Colourless prisms, mp 134–135°, lit. 138–139° [14]. UV λ_{max}^{EtOH} nm (log ε): 212 (4.14), 222 (4.12), 286 (4.20), 326 (sh) (3.57). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 1630. ^1H NMR (Me_2CO-d_6): δ2.96 (2H, *t*, J = 8 Hz, C-β), 3.40 (2H, *t*, J = 8 Hz, C-α), 4.24 (3H, *br s*, 3 × OH), 6.00 (2H, *s*, C-3', C-5'), 7.24 (5H, *s*, ArH). MS *m/z*: 258 $[M]^+$, 240, 241, 214, 153 (100%), 126, 123.

The nitrobenzene soln of phloroglucinol, $AlCl_3$ and dihydrocinnamoyl chloride, freshly prepared from dihydrocinnamic acid and PCl_5, was heated at 60° for 1 hr, to yield **2**, mp 138–139°, as product. The synthetic compound was identical with the natural compound (mmp, ^1H NMR, IR and MS spectra).

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SEXANGULARETIN 3-GLUCOSIDE-7-RHAMNOSIDE FROM *GOSSYPIUM HIRSUTUM*

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Key Word Index—*Gossypium hirsutum*; Malvaceae; cotton; 3,5,7,4'-tetrahydroxy-8-methoxyflavone 3-glucoside-7-rhamnoside; sexangularetin; herbacetin 8-methyl ether.

Abstract—A new diglycosylated flavonol was isolated from immature flower buds of the cotton plant *Gossypium hirsutum*. The structure was determined to be the 3-glucoside-7-rhamnoside of 3,5,7,4'-tetrahydroxy-8-methoxyflavone.

INTRODUCTION

In a study on host plant resistance against insects in cotton, *Gossypium hirsutum* L., many phenolic materials present in plant tissue consumed by lepidopterous larvae have been shown to be significant growth-inhibiting agents towards these pests [1]. Among the several flavonoid glycosides obtained from extracts of immature cotton flower buds (squares) was a diglycosylated derivative of herbacetin 8-methyl ether (sexangularetin), the aglycone of which had previously been obtained from *Sedum sexangulare* [2] in which it occurs as the 7-rhamnoside-3-rutinoside [3]. The 3-glucoside and the 3-rutinoside have been reported as occurring in *Sorbus aucuparis* and *Fagonia arabica*, respectively [4]. Spectroscopic and degradative methods show that the new glycoside is 3,5,7,4'-tetrahydroxy-8-methoxyflavone 3-glucoside-7-rhamnoside (**1**).

RESULTS AND DISCUSSION

The new glycoside (**1**) isolated in *ca* 0.15% yield by Sephadex LH-20 chromatography (methanol) of material adsorbing to Amberlite XAD-2 non-ionic macroreticular resin from aqueous solution gave rhamnose, glucose and 3,5,7,4'-tetrahydroxy-8-methoxyflavone (**2**) upon enzymic hydrolysis using naringinase. The identification of **2** was facilitated by comparison of its UV and ^1H NMR spectra (Tables 1 and 2) with reported values [2, 5], which were in close agreement. Especially significant is the position of the ^1H NMR singlet assigned to H-6 (δ 6.07) which is at higher field than that expected for a H-8 signal (δ 6.3–6.5) [6] thereby confirming oxygenation at positions 5, 7 and 8 of the A-ring. The observed acetate-induced shift in band II of the UV spectrum of **2** indicates a free 7-hydroxyl while the lack of a corresponding borate shift shows that no *ortho*-dihydroxy system is present. Therefore the

Table 1. UV spectral data of 1 and 2

Compound	λ_{max} (nm) in MeOH				
	Alone (log ε)	+ NaOMe	+ $AlCl_3$*	+ NaOAc	+ H_3BO_3
1	225 (4.22), 245, sh (4.14), 272 (4.35), 330 (4.16), 357 (4.16)	258† 272 404	239, 281 309, 353 412	272 400	272 333
2	220, 255, sh, 272, 325, 374	285‡ 338 434	225, sh, 260, sh, 272, 306, 355, 435	281, 310, 318, 401	273, 294, sh, 323, 376

*Acid stable.
†No decomposition in 5 min.
‡*Ca* 25% decomposition in 5 min.

Table 2. ^{1}H NMR spectral data (δ, ppm) of 1- and 2-per-TMSi ethers* (90 MHz, CCl_4, TMS as internal standard)

1	2
1.20, complex, 3H, rha H_6	3.83, *s*, 3H, 8-OMe
3.2–4.0 complex, 10H, sugar protons	6.07, *s*, 1H, H-6
3.83, *s*, 3H, 8-OMe	6.84, *d*, 2H, J = 9 Hz, H-3′ and 5′
5.17, *br s*, rha H-1″	8.05, *d*, 2H, J = 9 Hz, H-2′ and 6′
5.82, *d*, J = 7 Hz, glu H-1″	
6.47, *s*, 1H, H-6	
6.84, *d*, 2H, J = 9 Hz, H-3′ and 5′	
8.03, *d*, 2H, J = 9 Hz, H-2′ and 6′	

*^{1}H NMR spectra of underivatized 1 and 2 are presented in Experimental.

methoxyl must be attached at position 8. It is of interest to compare the UV spectrum of pollenitin (3,5,8,4′-tetrahydroxy-7-methoxyflavone) [7] with that of 2 to which it is very similar except for not exhibiting an acetate shift of band II (273 nm). It may be added that similar flavones possessing oxygenation on positions 5, 6 and 7 show significantly different UV spectra. Thus, 3,5,7,4′-tetrahydroxy-6-methoxyflavone [8] in methanol and with the various diagnostic reagents gave band I maxima displaced to shorter wavelengths (from 7 to 23 nm) than those of the corresponding positions in sexangularetin. From 1 was obtained a nona-acetate exhibiting two phenolic acetate signals in the ^{1}H NMR spectrum (δ 2.30, 2.40) distinct from the sugar acetate signals, thereby indicating glycosylation of the two remaining flavonol hydroxyls. The UV spectra (Table 1) of 1 were indicative of a flavonol having a free hydroxyl group at position 4′ which was confirmed by shift of the ^{1}H NMR signal of H-3′ and H-5′ to lower field for the acetate by *ca* 0.4 ppm compared to that of either 1 or of its trimethylsilyl ether [6]. Also, from the lack of shift of band II in the UV spectrum of 1 in the presence of sodium acetate, it may be inferred that position 7 is glycosylated (the aglycone 2 shows a bathochromic shift of 9 nm in the sodium acetate spectrum). Examination of the ^{1}H NMR spectrum of the tetramethylsilyl ether of 1 permits assignment of the point of attachment of glucose to position 3 since its H-1″ signal (δ 5.82) is well downfield from those of 5- and 7-glucosides, which occur around 5 ppm [6]. The observed position of the rhamnose H-1″ signal (δ 5.17) is consistent with attachment at the 7-position. The anomeric protons of glucose and rhamnose are recognizable by the magnitude of their coupling constants to the respective adjacent ring protons. Thus glucose shows a broad doublet of $J_{a,a}$ *ca* 6–7 Hz for H-1″ while the anomeric proton of rhamnose appears as a broad singlet ($J_{e,e}$ *ca* 2 Hz) [6].

EXPERIMENTAL

All mps are corr. UV spectra (Table 1) were determined in MeOH according to standard procedures [5]. ^{1}H NMR spectra were taken at 90 MHz using TMS as internal standard.

Extraction and separation. Cotton square powder, 220 g, was extracted with two 2000 ml portions of hexane using a large Waring blender. The solid material after filtration was extracted similarly with MeOH. The methanol filtrate was taken to dryness and then partitioned between 1000 ml each of Et_2O and H_2O. The resulting aq. soln was treated with 10 g gelatin to remove the major portion of tannins. After standing overnight, the gelatin suspension was centrifuged to separate precipitated sludge, and the supernatant was then stirred for 2 hr with 350 g Amberlite XAD-2 resin after which time the aq. soln gave a negative $FeCl_3$ test. The resin beads were collected by suction and washed with H_2O. Treatment with MeOH freed the adsorbed phenolic substances (2.1 g after removal of solvent *in vacuo*). Chromatography on Sephadex LH-20 (950 × 50 mm column

diameter) with MeOH gave nearly pure **1**, 346 mg, elution vol. 1900–2250 ml, mp 184–186° (aq MeOH). (Found: C, 50.8; H, 5.52. $C_{28}H_{32}O_{16} \cdot 2H_2O$ requires: C, 50.91; H, 5.49.) ^{1}H NMR: (90 MHz, CD_3OD): δ 1.27 (3H, *d*, J = 6 Hz, rha-Me), 3.3–4.2 (10H, complex, sugar), 3.83 (3H, *s*, 8-OMe), 5.27 (1H, *br d*, gluc H-1″), 5.56 (1H, *br s*, rha H-1″), 6.61 (1H, *s*, H-6), 6.89 (2H, *d*, J = 9 Hz, H-3′ and 5′), 8.10 (2H, *d*, J = 9 Hz, H-2′ and 6′). The ^{1}H NMR spectrum of **1**-per-TMSi ether is presented in Table 2.

Acetate of **1**. Colorless crystals from EtOH, mp 209–210°. ^{1}H NMR: (90 MHz, $CDCl_3$): δ 1.22 (3H, *d*, J = 6 Hz, rha-Me), singlets at 1.88, 1.95, 1.98, 2.01, 2.03, 2.08 and 2.17 (3H each, sugar-OAc's), singlets at 2.30 and 2.40 (3H each, phenolic-OAc's), 3.5–4.1 (2H, complex, rha and gluc H-5‴s), 4.00 (3H, *s*, 8-OMe), 4.9–5.7 (10H, complex, sugar), 6.83 (1H, *s*, H-6), 7.22 (2H, *d*, J = 9 Hz, H-3′ and 5′), 8.12 (2H, *d*, J = 9 Hz, H-2′ and 6′).

Enzymic hydrolysis of **1**. Naringinase (Sigma Chemical Co), 200 mg, was suspended in 10 ml H_2O and then filtered. To this solution was added 100 mg **1**, and the pH was adjusted to 3 with HOAc. After 16 hr incubation at 25° the mixture was extracted with EtOAc. After removal of solvent the amount of crude **2** was 40 mg. After crystallization from HOAc–H_2O (1:1) the mp was 272–273° (dec.), lit. [2] 272°. From MeOH were obtained crystals of mp 281–283°, dec. ^{1}H NMR (90 MHz, CD_3OD): δ 3.87 (3H, *s*, 8-OMe), 6.20 (1H, *s*, H-6), 6.90 (2H, *d*, J = 9 Hz, H-3′ and 5′), 8.15 (2H, *d*, J = 9 Hz, H-2′ and 6′). The ^{1}H NMR spectrum of **2**-per-TMSi ether is presented in Table 2. The latter spectrum is in good agreement with literature values [2].

Acetate of **2**. Colorless crystals from EtOH. Mp 161–163°. ^{1}H NMR (90 MHz, $CDCl_3$): δ 2.30 (6H, *s*), 2.33 (3H, *s*), 2.36 (3H, *s*), phenolic OAc's, 3.93 (3H, *s*, 8-OMe), 6.80 (1H, *s*, H-6), 7.23 (2H, *d*, J = 9 Hz, H-3′ and 5′), 7.86 (2H, *d*, J = 9 Hz, H-2′ and 6′). ^{1}H NMR (90 MHz, C_6D_6): δ 1.76 (3H, *s*), 1.83 (3H, *s*), 1.90 (3H, *s*), 2.20 (3H, *s*), phenolic OAc's, 3.50 (3H, *s*, 8-OMe), 6.66 (1H, *s*, H-6), 7.06 (2H, *d*, J = 9 Hz, H-3′ and 5′), 7.68 (2H, *d*, J = 9 Hz, H-2′ and 6′).

Sugar analysis of **1**. Refluxing 1M HCl for 1 hr effected complete hydrolysis. After removal of aglycone by EtOAc extraction the aq. soln was taken to dryness, and the residual sugars were converted to TMSi ethers [9]. Analysis by GLC (18′ × 1/8″, Silar-10C, 180°, 35 ml/min N_2 showed both glucose and rhamnose in about equal amounts.

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