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Unusual cytotoxic sulfated cadinene-type sesquiterpene glycosides from cottonseed (*Gossypium hirsutum*)

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ABSTRACT

Two new sulfated cadinene-type sesquiterpene glycosides, 13-hydroxy-7-*O*-(6′-*O*-sulfate-β-D-glucopyranosyl)-desoxyhemigossypol (**1**) and 13,15-dihydroxy-7-*O*-(6′-*O*-sulfate-β-D-glucopyranosyl)-desoxyhemigossypol (**2**), have been isolated from whole cottonseed (*Gossypium hirsutum*). Their structures, which possess an unusual 6-*O*-sulfate-glucopyranosyl moiety, were determined through the interpretation of 2D NMR spectral data and H/D exchange ESI-MS experiments. Compounds **1** and **2** were screened for their toxicity on Jurkat cells. Both compounds inhibited cellular proliferation with IC_{50} values of 8.1 and 4.2 μg, respectively.

1. Introduction

Cotton (*Gossypium* spp.) produces a large group of sesquiterpenes with the cadinane carbon skeleton that includes desoxyhemigossypol (dHG), hemigossypol (HG), gossypol (G), hemigossypolone (HGQ),[1] and the heliocides H_1, H_2, H_3, and H_4 (Fig. 1).[2] Unstressed *Gossypium* plants accumulate many of these compounds in subepidermal glands of all green tissues, which deter herbivores, and in epidermal cells of roots, probably as defense against soilborne pathogens.[3] Various sesquiterpenoid cadinanes are also elicited by fungal and bacterial infection, by toxic chemicals, and by cold stress.[4,5] Of the phytoalexins, which accumulate in stem stele of *Verticillium* wilt-resistant cotton in response to *Verticillium dahliae infection*, Mace et al. have shown that dHG has the highest antifungal activity.[6] Recently, however, it has been reported that gossypol and its metabolite, gossypolone, have anticancer effects in animal models.[7,8]

Whole cottonseed (WCS) is the unprocessed and unadulterated oilseed, which has been separated from the cotton fiber. Cottonseeds are fed to high-producing dairy cows as a source of fat and highly digestible fiber. They are also used as a forage replacer. Secondary metabolites in WCS have been studied because some components have been blamed for anti-nutritive or toxic effects:[9,10] gossypol is the main anti-nutrient limiting the use of cottonseed in monogastric animals and humans, where it acts by reducing the oxygen-carrying capacity of the blood and results in shortness of breath and of edema of the lungs.[11] Also, flavonoids have recently been reported in WCS.[12,13] The occurrence of this type of secondary metabolite in food and feed is highly desirable because they contribute to their nutritional value.

In the course of our continuing search for novel secondary metabolites of biomedical and ecological importance we investigated the terpenoids of whole cottonseed. Two new compounds, **1** and **2**, with an unusual 6-*O*-sulfate-glucopyranosyl group were isolated as minor components (Fig. 2). The structures of these compounds were elucidated by extensive spectroscopic methods including 1D (^{1}H and ^{13}C) and 2D NMR experiments (DQF-COSY, HSQC, HMBC, NOESY) as well as ESI-MS analysis and H/D exchange MS experiments. Compounds **1** and **2** were tested for cytotoxicity against Jurkat cells a human lymphoblastoid T cell line usually utilized for studies of immunotoxicity in vitro.

To the best of our knowledge this is the first report of the occurrence of glycosylated cadinene-type sesquiterpene in *Gossipium*. Compounds **1** and **2** are structurally related to desoxyhemigossypol (dHG), a key intermediate in the biosynthesis of cotton terpenoid,[1] and are probably derived through the same biosynthetic pathway.

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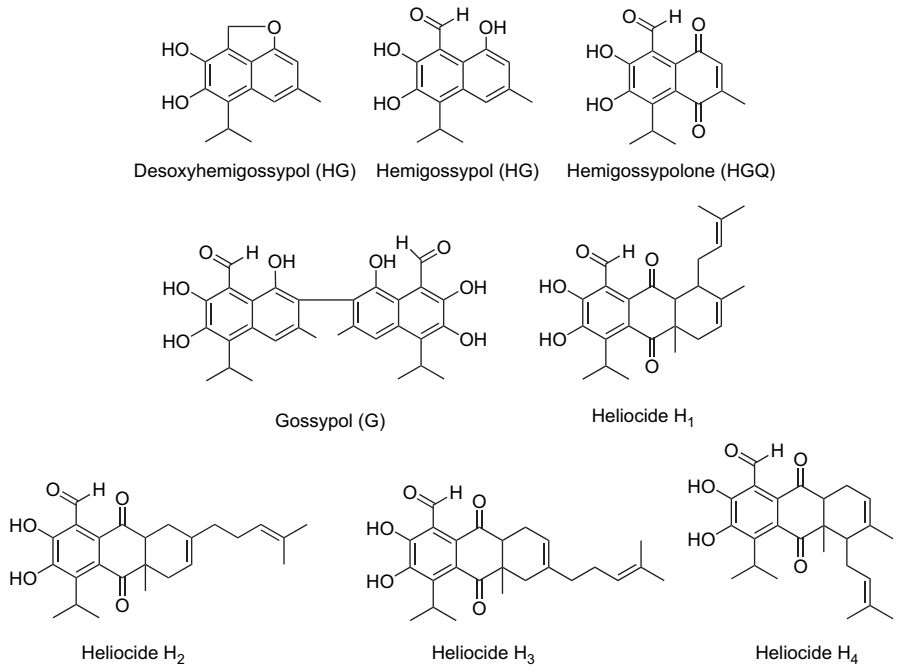

Figure 1. Sesquiterpenes with the cadinane carbon skeleton found in cotton tissue.

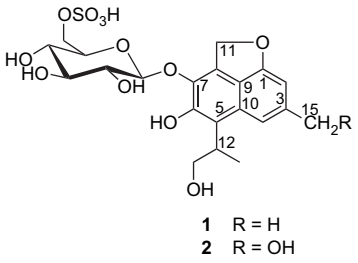

Figure 2. Cadinene-type sesquiterpene **1** and **2** from cottonseed.

There has been only two publications with reference to the structural characterization of dHG and these two studies did not report ^{13}C NMR data for dHG.[14,15] Compounds **1** and **2**, which, respectively, bear one and two oxygenated methylene substituent on naphthalene ring, instead of methyl groups present in dHG, were fully characterized by NMR and spectral data reported herein could be useful for the future NMR assessment of cotton terpenoids.

2. Results and discussion

2.1. NMR structure elucidation of 1 and 2

Compound **1** showed absorption bands for hydroxyl (3390 cm^{-1}), aromatic (1681 cm^{-1}), and sulfate (1211 cm^{-1}) groups in its IR spectrum. The (−)-ESI-MS spectrum of **1** showed as base peak the pseudomolecular ion $[M-H]^-$ at *m/z* 501 and its MS/MS spectrum gave ions at *m/z* 421 $[M-H-80]^-$ and *m/z* 259 $[M-H-80-162]^-$, which indicated the presence of a sulfate ($-OSO_3H$) or phosphate ($-OPO_3H_2$) group and a hexose. The HRESI-MS of compound **1** showed an $[M-H]^-$ ion peak at *m/z* 501.1100, consistent with the molecular formula $C_{21}H_{26}O_{12}S$.

The ^{13}C NMR spectrum of **1** showed 21 carbon signals (Table 1), 6 of which could be assigned to a hexose moiety. Aglycon signals including 10 carbons in the range δ_C 101–163 ppm, ascribable to a naphthalene ring with two directly attached hydrogen atoms, according to HSQC and HMBC spectra, an oxygenated methylene (δ_C 77.19 ppm), a methyne (δ_C 36.87 ppm), and a tertiary and a secondary methyl group. All the information mentioned above was in support of compound **1** being a sesquiterpene glycoside. Inspection of the ^1H NMR spectrum of **1** led to the identification of the following representative signals: a methyl doublet signal protons at δ 1.46 (d, J=6.9 Hz), a methyl singlet protons at δ 2.84, a methyne proton at δ 3.73 (m), two oxymethylene protons at δ 4.03 (m), two protons of a further oxymethylene groups linked directly at the naphthalene ring (δ 5.82 and 5.90; J=15.3 Hz), and two *meta*-related doublets (δ 6.34 and 7.06; J=1.5 Hz). The ^1H NMR spectrum

Table 1
^1H and ^{13}C NMR (600 MHz) data for compounds **1** and **2** in CD_3OD[a]

Position	**1**		**2**	
	δ ^1H (J_{HH} in Hz)	δ ^{13}C	δ ^1H (J_{HH} in Hz)	δ ^{13}C
1		163.29		163.30
2	6.34 (d, 1.5)	101.20	6.52 (d, 1.5)	98.70
3		139.96		143.6
4	7.06 (d, 1.5)	112.44	7.28 (d, 1.5)	111.30
5		138.12		130.10
6		138.13		138.74
7		150.10		150.11
8		126.09		125.90
9		123.21		124.39
10		121.83		122.64
11a	5.90 (d, 15.3)	77.19	5.86 (d, 15.3)	77.28
11b	5.82 (d, 15.3)		5.94 (d, 15.3)	
12	3.73 (br s)	36.87	3.75 (br s)	36.92
13a	4.03 (m)	66.47	4.05 (m)	66.46
13b	3.98 (m)		4.00 (m)	
14	1.46 (d, 6.9)	15.77	1.53 (d, 6.9)	15.78
15	2.84 (s)	23.15	4.69 (s)	66.49
1′	4.71 (d, 7.5)	105.90	4.74 (d, 7.5)	105.85
2′	3.61 (dd, 9.5, 7.5)	74.80	3.61 (dd, 9.5, 7.5)	74.80
3′	3.59 (t, 9.5)	77.32	3.58 (t, 9.5)	77.32
4′	3.54 (t 9.5)	71.13	3.53 (t, 9.5)	71.13
5′	3.70 (m)	76.61	3.71 (m)	76.61
6″a	4.43 (dd, 12.2, 4.5)	68.18	4.44 (dd, 12.2, 4.5)	68.12
6″b	4.21 (dd, 12.2, 4.5)		4.21 (dd, 12.2, 4.5)	

[a] Chemical shift values are in parts per million from TMS, and values in hertz are presented in parentheses. All signals were assigned by DQF-COSY, HSQC, and HMBC experiments.