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SESQUITERPENE GLYCOSIDES FROM COTTON OIL CAKE

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Key Word Index—*Gossypium hirsutum*; Malvaceae; cotton; sesquiterpene glycosides.

Abstract—Two new sesquiterpene glycosides and three known antioxidative phenolic compounds were isolated from cotton oil cake. Their structures were elucidated mainly by NMR spectroscopic analyses. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

In the course of our studies on natural antioxidants in oil seeds, we have focused on cottonseed, an agricultural by-product. Cottonseed contains a relatively large amount of oil (30–35%) which is used as a source of edible oil, as well as other constituents such as cyclopropenoids [1], flavonoids [2] and sesquiterpenoids [3–6]. Separating desired minor compounds from oil (triglycerides), a major constituent, is generally troublesome. However, their isolation from oil cake, the residual waste of the seed oil industry, is much easier. Therefore, we have used oil cake as a source of new compounds [7, 8]. In this paper, we report on the isolation and identification of two new sesquiterpene glycosides (**1**, **2**) together with three known antioxidants, isoquercitrin (**3**), catechin (**4**) and protocatechuic acid (**5**) from cotton (*Gossypium hirsutum* L.) oil cake.

RESULTS AND DISCUSSION

Cotton oil cake was extracted with methanol and the extract partitioned between *iso*-octane and methanol. The methanol-soluble material was partitioned again between *n*-hexane and 80% aqueous methanol, and the 80% aqueous methanol-soluble material was further partitioned with ethyl acetate and water. The ethyl acetate fraction was separated by silica gel column chromatography and preparative HPLC as described in Experimental to yield compounds **1**–**5**.

Compound **1** was isolated as amorphous powder. Its molecular formula was determined as $C_{21}H_{32}O_8$ ($[M+Na]^+$, *m/z* 435.1969) by positive ion HR-FAB mass spectrometry. The IR spectrum contained an absorption band due to hydroxy groups at 3400 cm^{-1}. The ^{13}C NMR spectrum showed 21 carbon signals, including five quaternary carbons, 10 methines, two methylenes, and four methyl groups. The six methines between δ 62–107 each had to have a hydroxy function based on their chemical shift values. Two methines at δ 7.81 (H-5) and δ 7.10 (H-8), and four quaternary carbons suggested the presence of a tetra-substituted benzene ring. One carbon (δ_C 155.1) of the benzene ring was substituted by a hydroxy group. The ^1H NMR spectrum exhibited 28 proton signals. Two of these were exchangeable by D_2O and were assignable to OH protons. The ^1H-^1H COSY spectrum displayed the spin connection: H-2 ↔ H_2-3 ↔ H-4 ↔ H-12 ↔ H-13 and H-14. Thus the presence of an isopropyl group and the partial structure of the saturated ring were determined. The HMBC spectrum (J_{C-H} = 8.3 Hz) showed a long-range correlation from H-4 to C-10, H-5 to C-4, C-7 and C-9, from H_3-11 to C2 and C-9, and from H-8 to C-1 and C-10, which established that the saturated ring was connected to the benzene ring. The correlation between H_3-15 and C-5, C-6 and C-7 indicated that the methyl group was connected to C-6. The remaining methylene and five methine signals were assignable to those of glucose. The presence of glucose was confirmed by GC after acid hydrolysis of **1**. The coupling constant (J = 7.4 mHz) of the anomeric proton signal at δ 5.39 and its correlation with C-2 in the HMBC spectrum established the β configuration for the glucose moiety. Thus the plane structure of compound **1** was established.

The relative configuration of compound **1** was confirmed by NOESY and the ^1H NMR coupling constants. The NOESY spectrum showed the correlation between H_3-11, H_3-13 and H_3-14, and established that the isopropyl group and the methyl group at C-1 must be in the same orientation. The correlation from H-2 to H_α-3 (δ 2.28) and H-4, and from H_3-13

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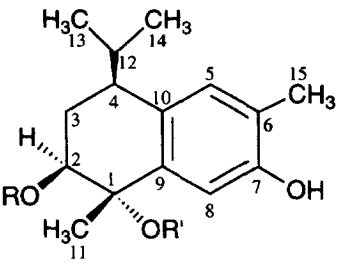
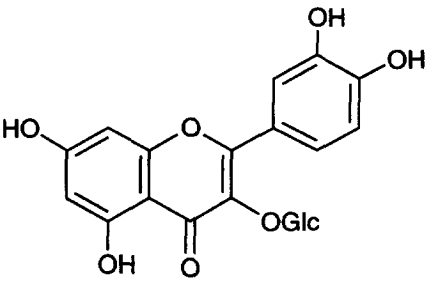


1 R=β-Glc, R'=H
2 R=H, R'=β-Glc



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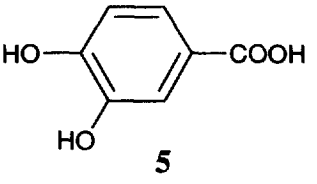

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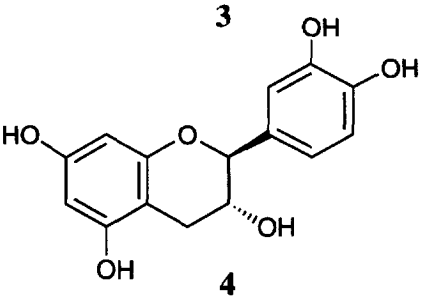

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to H-4 and H-2 showed that H-2, H_α-3 and H-4 had the same orientation. The coupling constants of the ring protons ($J_{2\text{-}3\beta}$ = 12.8 Hz, $J_{3\beta\text{-}4}$ = 12.2 Hz), which were determined by means of the ^{1}H-decoupling spectra, showed that H-2, H-3$_\beta$ and H-4 were axial, and that the substituents at C-2 and C-4 were equatorial. Thus, compound **1** was identified as 1,2,3,4-tetrahydro-1α,2β,7-trihydroxy-1β,6-dimethyl-4β-isopropylnaphthalene-2-*O*-β-D-glucoside.

Compound **2** was also isolated as an amorphous powder, and its molecular formula was determined as $C_{21}H_{32}O_8$ (HR-FAB, $[M+Na]^+$, *m/z* 435.1969). The ^{13}C NMR spectrum of **2** was similar to that of compound **1** and contained 21 carbon signals. However, two of the carbon signals of **2** were different from those of **1**, i.e. C-2 was observed at higher field (δ_C 72.2), while C-1 had moved to lower field (δ_C 83.1). In addition, the HMBC spectrum showed the correlation of the anomeric proton at δ 5.28 (J = 7.6 Hz) to C-1, which suggested that β-D-glucose was connected to C-1. The relative stereochemistry of **2** was shown to be the same as that of compound **1** by NOESY of **2** and was thus identified as 1,2,3,4-tetrahydro-1α,2β,7-trihydroxy-1β,6-dimethyl-4β-isopropylnaphthalene-1-*O*-β-D-glucoside.

Compounds **3**–**5** were identified as isoquercitrin (**3**), (−)-catechin (**4**) and protocatechuic acid (**5**) by MS, ^{1}H NMR and ^{13}C NMR, and by comparison of these data with those in the Refs [9, 10] and/or of the authentic samples. The optical rotation of **4** (−10.1°) was less than the reported one [11]. These data indicated that **4** was partly racemized.

EXPERIMENTAL

NMR: ^{1}H, 500 MHz; ^{13}C, 125 MHz. Chemical shifts were given in δ with TMS as an int. standard. Spots on TLC were detected by spraying 5% vanillin–conc. H_2SO_4 followed by heating. HPLC: reversed phase [YMC AQ-314 ODS] column (30 cm × 6.0 mm I.D.) with detection by a 830-RI differential refractometer.

Extraction and isolation

Dried cotton oil cake (500 g) was extracted (×3) with MeOH for 24 h. The MeOH soln were concentrated under red. pres. to give the MeOH extract (25 g). The MeOH extract was partitioned between *iso*-octane and MeOH to obtain the *iso*-octane extract (3.3 g). The MeOH layer was further partitioned between *n*-hexane and 80% MeOH to give the hexane extract (3.6 g). The 80% aq. MeOH was subsequently partitioned between EtOAc and H_2O to yield the H_2O extract (15.6 g) and the EtOAc extract (2.1 g). The EtOAc extract was subjected to silica gel CC, and eluted with $CHCl_3$–MeOH (10:1 → 5:1) and $CHCl_3$–MeOH–H_2O (10:3:1) to give seven fractions (frs A–G). Fraction E was chromatographed repeatedly on silica gel using $CHCl_3$–EtOAc–MeOH–H_2O (2:8:2:1) and $CHCl_3$–MeOH–H_2O (10:3:1) to give five fractions (frs E1–5). Fraction E4 was further purified by HPLC with MeOH–H_2O (1:1) to yield **1** (5.0 mg) and **2** (0.6 mg). Fraction E3 was also purified by HPLC to give **3** (5.7 mg). Fraction D was subjected to repeated chromatography on a silica gel column (*n*-hexane–EtOAc–MeOH 4:8:1) followed by HPLC to afford **4** (6.3 mg). Fraction C was chromatographed on a silica gel column [*n*-hexane–$CHCl_3$–MeOH (4:8:1) and $CHCl_3$–MeOH (10:1)] to give three fractions (frs C21, C22, C23). Fraction C21 was purified on HPLC with MeOH–H_2O (1:1) to give **5** (7.0 mg).

Compound **1**. Amorphous powder, mp 138–140° $[\alpha]_D^{25}$ −58.9° (MeOH; *c* 0.55). UV λ_{max}^{MeOH} nm (log ε): 282 (3.41); IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 1050; HR-FAB-

MS *m/z*: Calcd for $C_{21}H_{32}O_8Na$: 435.1995 $[M+Na]^+$, Found: 435.1969; ^1H NMR (C_5D_5N): δ 0.65 (3H, *d*, $J = 7.3$ Hz, H-14), 0.88 (3H, *d*, $J = 6.7$ Hz, H-13), 1.81 (3H, *s*, H-11), 1.88 (1H, *dd*, $J = 12.8$, 11.6 Hz, H-3_{ax}), 2.28 (1H, *m*, H-3_{eq}), 2.36 (1H, *m*, H-12), 2.47 (3H, *s*, H-15), 2.96 (1H, *m*, H-4), 3.89 (1H, *m*, G-5), 4.10 (1H, *dd*, $J = 7.9$, 8.5 Hz, G-2), 4.17 (1H, *m*, G-3), 4.24 (1H, *m*, G-4), 4.33 (1H, *dd*, $J = 10.4$, 4.3 Hz, G-6), 4.38 (1H, *dd*, $J = 12.8$, 4.3 Hz, H-2), 4.44 (1H, *dd*, $J = 10.4$, 4.3 Hz, G-6), 5.39 (H, *d*, $J = 7.3$ Hz, G-1), 6.35 (1H, *br*, OH-1), 7.10 (1H, *s*, H-5), 7.81 (1H, *s*, H-8), 11.05 (1H, *br*, OH-7); ^{13}C NMR: Table 1.

Compound **2**. Amorphous powder, mp 149–151°, $[\alpha]_D^{25}$ −66.6° (MeOH; *c* 0.55). UV λ_{max}^{MeOH} nm (log ε): 282 (3.42); IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 1050; HR-FAB-MS *m/z*: Calcd for $C_{21}H_{32}O_8Na$: 435.1995 $[M+Na]^+$, Found: 435.2007; ^1H NMR (C_5D_5N): δ 0.68 (3H, *d*, $J = 6.7$ Hz, H-14), 0.97 (3H, *d*, $J = 7.0$ Hz, H-13), 1.83 (3H, *s*, H-11), 1.85 (1H, *dd*, $J = 12.8$, 11.9 Hz, H-3_{ax}), 2.15 (1H, *m*, H-3_{eq}), 2.44 (1H, *m*, H-12), 2.50 (3H, *s*, H-15), 3.05 (1H, *m*, H-4), 3.89 (1H, *m*, G-5), 4.15 (2H, *m*, G-2, G-3), 4.20 (1H, *m*, G-4), 4.27 (1H, *dd*, $J = 11.6$, 5.5 Hz, G-6), 4.51 (1H, *dd*, $J = 11.6$, 2.4 Hz, G-6), 4.65 (1H, *dd*, $J = 12.5$, 4.0 Hz, H-2), 5.28 (H, *d*, $J = 7.6$ Hz, G-1), 6.31 (1H, *br*, OH-1), 7.18 (1H, *s*, H-5), 8.19 (1H, *s*, H-8), 11.24 (1H, *br*, OH-7); ^{13}C NMR: Table 1.

Compound **3**. Yellow powder, mp 170–172°. IR ν_{max}^{KBr} cm^{-1}: 3200, 2900, 1650, 1600; FAB-MS *m/z*: 465 $[M+H]^+$; ^1H NMR (CD_3OD): δ 3.23 (1H, *m*, G-5), 3.37 (1H, *m*, G-4), 3.43 (1H, *m*, G-3), 3.48 (1H, *m*, G-2), 3.57 (1H, *dd*, $J = 12.0$, 5.5 Hz, G-6), 3.71 *dd*, $J = 12.0$, 2.4 Hz, G-6), 5.24 (1H, *d*, $J = 7.9$, G-1), 6.21 (1H, *d*, $J = 1.8$ Hz, H-6), 6.40 (1H, *d*, $J = 1.8$ Hz, H-8), 6.86 (1H, *d*, $J = 8.5$ Hz, H-15), 7.58 (1H, *dd*, $J = 1.8$, 8.5 Hz, H-16), 7.71 (1H, *d*, $J = 1.8$ Hz, H-12); ^{13}C NMR: Table 1.

Compound **4**. Pale yellow oil, $[\alpha]_D^{25}$ −10.1° (Me_2CO; *c* 0.32). IR ν_{max}^{KBr} cm^{-1}: 3200; FAB-MS *m/z*: 313 $[M+Na]^+$; ^1H NMR (DMSO-d_6): δ 2.50 (1H, *dd*, $J = 16.2$, 8.6 Hz, H-4_{ax}), 2.85 (1H, *dd*, $J = 16.2$, 5.5 Hz, H-4_{eq}), 3.97 (1H, *ddd*, $J = 7.9$, 8.6, 5.5 Hz, H-3), 4.56 (1H, *d*, $J = 7.9$ Hz, H-2), 5.85 (1H, *d*, $J = 1.8$ Hz, H-8), 5.92 (1H, *d*, $J = 2.4$ Hz, H-6), 6.72 (1H, *dd*, $J = 8.5$, 1.8 Hz, H-16), 6.76 (1H, *d*, $J = 8.5$ Hz, H-15), 6.83 (1H, *d*, $J = 1.8$ Hz, H-12); ^{13}C NMR: Table 1.

Compound **5**. Colorless powder, mp 198–200°. IR ν_{max}^{KBr} cm^{-1}: 3400, 1680; EI-MS *m/z*: 154 $[M]^+$ (100), 137 $[M-OH]^+$ (96); ^1H NMR (DMSO-d_6): δ 6.79 (1H, *d*, $J = 8.5$ Hz, H-6), 7.28 (1H, *dd*, $J = 8.5$, 1.8 Hz, H-7), 7.33 (1H, *d*, $J = 1.8$ Hz, H-3); ^{13}C NMR: Table 1.

Table 1. ^{13}C NMR spectra for compounds **1**–**5** (125 MHz)

C	1*	2*	3†	4‡	5‡
1	74.5	83.2			167.2
2	85.7	72.2	159.1	82.9	121.6
3	27.5	27.4	135.7	68.8	116.5
4	42.1	42.2	179.5	28.5	144.8
5	128.9	129.2	163.1	157.6	150.0
6	124.1	124.6	99.9	96.3	115.1
7	155.2	155.1	166.2	157.8	121.9
8	112.1	114.1	94.8	95.5	
9	144.1	141.7	158.5	156.9	
10	127.7	129.7	105.7	100.1	
11	26.5	23.9	123.1	132.2	
12	31.4	31.3	123.2	115.3	
13	20.5	20.6	146.0	146.2	
14	15.8	15.6	150.0	146.2	
15	16.7	16.8	116.0	116.1	
16			117.6	120.0	
glc-1	106.7	99.2	104.4		
glc-2	76.2	75.8	75.8		
glc-3	78.6	78.7	78.2		
glc-4	71.4	72.0	71.3		
glc-5	78.5	78.1	78.4		
glc-6	62.5	62.8	62.6		

* In C_5D_5N.
† In CD_3OD.
‡ In DMSO-d_6.

GC analysis of sugar moiety

Compound **1** (2 mg) in 1 N HCl/MeOH (3 ml) was heated at 60° for 30 min, to give an H_2O fraction (0.8 mg), the sugar constituent of which was identified by a method essentially as described previously [12]. To a soln of the crude sugar fraction (0.8 mg) in pyridine (0.5 ml) was added trimethylchlorosilane (0.05 ml) and 1,1,1,3,3,3-hexamethyldisilazane (0.1 ml). GC analysis of the silylated sugar was carried out on 3% OV-101 (stainless column, 0.3 mm i.d. × 2 m).

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