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Total and Percent Atropisomers of Gossypol and Gossypol-6-methyl Ether in Seeds from Pima Cottons and Accessions of *Gossypium barbadense* L

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Gossypol occurs naturally in the seed, foliage, and roots of the cotton plant (*Gossypium*) as atropisomers due to restricted rotation around the binaphthyl bond. The atropisomers differ in their biological activities. (−)-(*R*)-Gossypol is more toxic and exhibits significantly greater anticancer activity than the (+)-(*S*)-atropisomer. Most commercial Upland (*Gossypium hirsutum*) cottonseeds have an (*R*)- to (*S*)-gossypol ratio of ~2:3, but some Pima (*Gossypium barbadense*) seeds have an excess of (*R*)-gossypol. There is no known source of cottonseed with an (*R*)- to (*S*)-gossypol ratio of greater than ~70:30. Cottonseed with a high percentage of (*R*)-gossypol would be of value to the pharmaceutical industry. It was theorized that *G. barbadense* cotton might be a source of this desirable high (*R*)-gossypol seed trait. There are 671 different accessions of *G. barbadense* in the U.S. Cotton Germplasm Collection, few of which had been characterized with respect to their (*R*)- to (*S*)-gossypol ratio. This work completed that analysis and found considerable variation in the atropisomer ratio. Approximately half of the accessions have an excess of (*R*)-gossypol, and 52 accessions have essentially a 1:1 ratio. The highest percentage of (*R*)-gossypol was found in accessions GB26 (68.2%) and GB283 (67.3%). Surprisingly, five accessions had 5% or less of (*R*)-gossypol: GB516 (5.0%), GB761 (4.5%), GB577 (4.3%), GB719 (3.7%), and GB476 (2.3%). These accessions may be useful in a breeding program to reduce (*R*)-gossypol in Pima seed, which is a concern to the dairy industry because of the toxicity and male antifertility activity of this atropisomer. Also, GB710 was devoid of gossypol.

KEYWORDS: Cotton; (−)-gossypol; (+)-gossypol; cottonseed; *Gossypium barbadense*; gossypol-6-methyl ether

INTRODUCTION

Gossypol, a dimeric sesquiterpenoid, occurs naturally in the seed, leaves, and roots of the cotton plant (*Gossypium*). Gossypol occurs as two different atropisomers due to restricted rotation around the binaphthyl bond [i.e., axial dissymmetry (*1*)]. The atropisomers are referred to as (*R*)-gossypol and (*S*)-gossypol. The absolute configuration of the (−)-(*R*)-atropisomer of gossypol is shown in **Figure 1**. The atropisomers differ in their biological activities. For example, (*R*)-gossypol exhibited significantly greater anticancer activity than (*S*)-gossypol (*2*) and is a useful adjuvant to other anticancer drugs such as cisplatin (*3*). It is currently undergoing phase II clinical trials for treatment of various types of cancers (*4*). (*R*)-Gossypol is toxic to nonruminants and, if fed in large amounts, to ruminants as well (*5*). Additionally, (*R*)-gossypol exhibits male antifertility activity (*6*, *7*), whereas the (*S*)-atropisomer does not (*8*).

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Most commercial Upland (*Gossypium hirsutum*) cottonseed has a 20% enantiomeric excess (ee) in the (*S*)-atropisomer of gossypol. As indicated above, (*R*)-gossypol is being utilized by the pharmaceutical industry in phase II clinical trials. To obtain pure (*R*)-gossypol, pharmaceutical companies derivatize the mixture of atropisomers obtained from Upland cottonseed with a chiral reagent, separate the resulting diastereomers by chromatography, and then remove the derivatizing agent by hydrolysis. It would greatly facilitate the purification of (*R*)-gossypol if a source were identified that produced a large excess of (*R*)-gossypol in the seed.

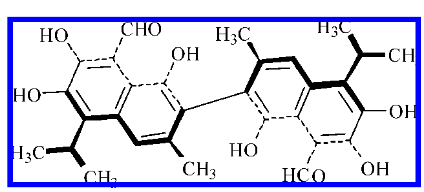

Figure 1. Absolute configuration of (*R*)-gossypol.

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Table 1. Percent (*R*)-Gossypol in Pima Embryos

accession or cultivar	mean embryo wt (mg)	mean (SE)[a] total gossypol (mg/g of dry wt)	mean (SE) total methyl goss[b] (mg/g of dry wt)	mean (SE) (*R*)-gossypol (%)	mean (SE) (*R*)-methyl goss (%)
PimaS1	85.6	8.5 (2.6)	1.8 (0.3)	52.3 (0.6)	49.7 (2.0)
PimaS2	79.1	6.2 (0.7)	1.2 (0.1)	53.7 (0.5)	50.8 (0.4)
PimaS3	47.2	2.2 (0.4)	1.7 (0.4)	43.2 (2.4)	41.8 (1.6)
PimaS4	68.6	4.0 (1.0)	2.2 (0.8)	45.7 (2.0)	33.5 (1.1)
PimaS5	73.2	5.8 (1.2)	2.7 (0.3)	49.0 (4.8)	40.1 (3.0)
PimaS6	61.4	6.5 (0.7)	6.6 (1.7)	35.2 (1.7)	39.3 (1.3)
PimaS7	75.4	9.0 (1.2)	3.1 (0.6)	55.2 (0.9)	51.4 (0.6)

[a] SE, standard error of three values. [b] Methyl goss, gossypol-6-methyl ether.

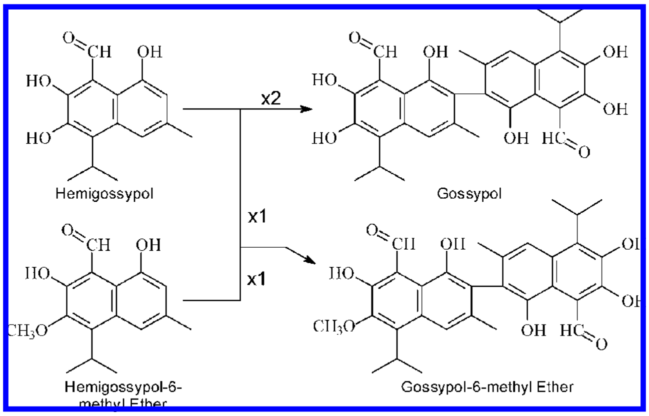

Figure 2. Proposed biosynthesis of gossypol and gossypol-6-methyl ether from hemigossypol and hemigossypol-6-methyl ether.

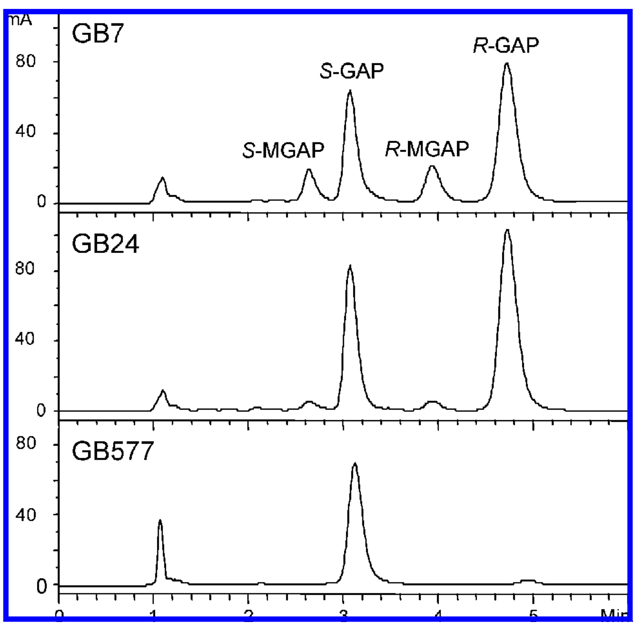

Figure 3. HPLC chromatograms of (*R*)-(−)-2-amino-1-propanol derivatized extract of seeds from three *G. barbadense* accessions with differing total levels and ratios of (*R*)- to (*S*)-gossypol and (*R*)- to (*S*)-gossypol-6-methyl ether [(*R*)- and (*S*)-gossypol (*R*)-(−)-2-amino-1-propanol derivative, *R*-GAP and *S*-GAP; (*R*)- and (*S*)-gossypol-6-methyl ether (*R*)-(−)-2-amino-1-propanol derivative, *R*-MGAP and *S*-MGAP]. The values for these accessions are in **Tables 2** (GB24 and GB577) and **4** (GB7).

Cass et al. (*9*) first reported that *Gossypium barbadense* had an atropisomeric excess of *R* in the seed, and Percy et al. (*10*) analyzed additional accessions. A high percentage of (*S*)-gossypol has been identified in the seed from some Brazilian moco cottons (*G. hirsutum* var. *marie galante*); some contain >92% ee, in the *S* atropisomer (% ee = %*S* − %*R*) (*11*). We theorized that one or more of the >600 accessions in the U.S. Cotton Germplasm Collection of *G. barbadense* might provide a source of >90% ee in (*R*)-gossypol. Only a small fraction of the seed in the Collection had been analyzed. An alternative target of the investigation would be the identification of *G. barbadense* seed that had a >90% ee in (*S*)-gossypol. That is, because of the perceived high (*R*)- to (*S*)-gossypol ratio in Pima seed, the dairy industry has been reluctant to use Pima seed as a feed for their herds. Thus, a lower (*R*)- to (*S*)-gossypol ratio in Pima seed and a lower total gossypol level would be of interest to the dairy industry. Therefore, we initiated the work described herein with the hope of finding sources of high ee (*R*)-gossypol and high ee (*S*)-gossypol in the *G. barbadense* Cotton Germplasm Collection.

G. barbadense also differs from Upland cotton in that it can produce significant amounts of gossypol-6-methyl ether in the seed and foliage. In this study, we found a wide variation in the total amount of gossypol-6-methyl ether produced in these accessions. The gene that controls methylation of hemigossypol has been cloned (*12*).

The identification of the genes that control the relative ratio of (*R*)- to (*S*)-gossypol is also of interest. Gene identification would allow utilization of a molecular marker to accelerate breeding programs to increase the high (*S*)- or (*R*)-gossypol seed trait. We recently identified a dirigent protein that controls the formation of (*S*)-gossypol in *G. hirsutum* var. *marie galante* (*13*). Because of the large numbers of entries in the *G. barbadense* Germplasm Collection, we reasoned that we might derive some information on this protein.

MATERIALS AND METHODS

General. Analyses were performed on a Hewlett-Packard 1090 HPLC equipped with a diode array detector and operated under computer control. Solvents were all of HPLC grade. (*R*)-(−)-2-Amino-1-propanol (D-alaninol) was obtained from Synthetech, Inc., Albany, OR. Mean and standard errors (SE) were calculated using Excel.

Seed Source. All seeds were obtained from the U.S. Cotton Germplasm Collection housed at the Southern Plains Agricultural Research Center, College Station, TX. If sufficient seeds were available, three seeds were analyzed separately. In a few cases, the number of seeds was limited and only one or two seeds were analyzed. Most of the seeds from the USDA Germplasm Collection were grown at the winter nursery in Tecoman, Mexico. This should not affect the (*S*)- to (*R*)-gossypol ratios (*14*). However, environment can affect total gossypol (*15*), which is also reported along with the (*S*)- to (*R*)-gossypol ratios.

Total and (*S*)- to (*R*)-Gossypol Analysis. *G. barbadense*, Pima, and Seabrook Sea Island 12B2 seeds were dried under vacuum for 48 h. A total of 679 accessions or varieties were analyzed. Seeds were individually dehulled, ground using an alumina mortar and pestle, and weighed. The tissue from a single seed was reacted at 70 °C for 30 min with 1.00 mL of D-alaninol reagent (i.e., 88% UV-grade acetonitrile, 10% glacial acetic acid, and 2% D-alaninol). After the reacted sample had been mixed and then centrifuged, a portion of the clear extract was transferred to a vial for HPLC analysis. The HPLC method

Table 2. Embryos of *Gossypium barbadense* Accessions and Cultivars with the Highest (63−68%) and Lowest (2.3−10.0%) (*R*)-Gossypol

accession or cultivar	mean embryo wt (mg)	mean (SE)[a] total gossypol (mg/g of dry wt)	mean (SE) total methyl goss[b] (mg/g of dry wt)	mean (SE) (*R*)-gossypol (%)	mean (SE) (*R*)-methyl goss (%)
GB26	114.2	4.23 (1.0)	0.59 (0.1)	68.2 (1.2)	51.6 (0.7)
GB283	96.7	1.47 (0.8)	0.25 (0.1)	67.3 (1.5)	48.4 (2.7)
GB289	111.3	0.95 (0.1)	0.37(0.0)	65.4 (0.6)	50.6 (0.8)
GB646	101.3	0.80 (0.2)	0.20 (0.0)	64.1(0.6)	53.5 (0.9)
GB10	77.5	5.01(0.7)	0.45 (0.0)	64.0 (0.5)	51.3 (1.3)
GB11	62.1	4.55 (0.8)	2.66 (0.9)	63.9 (1.7)	51.5 (1.4)
GB29	109.2	4.02 (1.0)	0.55 (0.1)	63.5 (0.3)	52.9 (0.2)
GB279	101.2	3.15 (0.4)	0.26 (0.0)	63.3 (0.3)	51.1 (1.8)
GB24	128.2	5.04 (0.1)	0.88 (0.1)	63.2 (0.5)	53.4 (1.5)
GB240	77.9	2.64 (0.5)	1.69 (0.4)	63.1(1.0)	54.7 (0.5)
SBSI 12B2	79.5	3.51(0.4)	2.49 (0.4)	63.0 (1.5)	51.5 (1.6)
GB475	44.9	1.90 (0.2)		10.0 (5.4)	
GB620	50.5	2.10 (0.4)		7.6 (1.2)	
GB578	40.7	1.86 (0.4)		7.4 (3.1)	
GB612	32.5	2.01(0.6)	0.99 (0.5)	6.6 (0.3)	14.7 (0.3)
GB592	46.4	2.54 (0.5)		6.0 (0.5)	
GB516	35.8	1.09 (0.3)		5.0 (0.5)	
GB761	35.2	1.53 (0.3)		4.5 (0.8)	
GB577	41.9	2.97 (0.3)		4.3 (0.3)	
GB719	45.1	2.37 (0.4)		3.7 (0.1)	
GB476	31.8	0.83 (0.2)		2.3 (0.5)	

[a] SE, standard error of three values. [b] Methyl goss, gossypol-6-methyl ether. The space separates the 11 accessions with the highest percentage of (*R*)-gossypol from the 10 accessions with the lowest percentage.

Table 3. Embryos of *Gossypium barbadense* Accessions and Cultivars with the Highest (66−77%) and Lowest (12−22%) (*R*)-Gossypol-6-methyl Ether

accession or cultivar	mean embryo wt (mg)	mean (SE)[a] total gossypol (mg/g of dry wt)	mean (SE) total methyl goss[b] (mg/g of dry wt)	mean (SE) (*R*)-gossypol (%)	mean (SE) (*R*)-methyl goss (%)
GB95	35.9	7.1 (1.6)	0.2 (0.0)	39.8 (2.5)	76.7 (23.3)
GB647	64.6	1.5 (0.3)	0.4 (0.4)	55.7 (0.7)	74.2 (25.8)
GB349	38.3	4.5 (0.3)	0.1 (0.1)	44.4 (0.2)	74.1 (25.9)
GB473	97.9	1.0 (0.4)	0.1 (0.1)	55.3 (6.4)	70.3 (14.8)
GB394	72.6	1.5 (0.4)	0.2 (0.0)	57.3 (0.7)	69.8 (15.1)
GB364	71.0	2.1 (0.2)	0.2 (0.0)	55.4 (0.9)	69.6 (15.2)
GB713	92.9	2.0 (0.6)	0.2 (0.1)	60.9 (0.9)	68.8 (15.6)
GB443	78.0	1.5 (0.4)	0.2 (0.1)	61.4 (1.0)	68.1 (16.0)
GB554	30.1	9.1 (1.4)	0.4 (0.1)	30.3 (1.7)	68.0 (16.0)
GB219	84.2	1.6 (0.5)	0.1 (0.0)	55.5 (0.2)	67.1 (16.4)
GB686	74.6	2.0 (0.5)	0.2 (0.1)	57.3 (0.7)	67.0 (16.5)
GB398	54.2	1.5 (0.4)	0.2 (0.0)	33.4 (0.7)	66.5 (16.8)
GB782	74.0	1.1 (0.3)	0.2 (0.1)	52.7 (1.4)	66.2 (16.9)
GB636	78.3	1.5 (0.2)	0.2 (0.0)	58.5 (0.3)	66.2 (16.9)
GB613	50.3	1.4 (0.2)	0.3 (0.1)	24.3 (0.2)	21.8 (10.9)
GB442	76.1	1.4 (0.0)	0.1 (0.1)	56.8 (1.0)	21.8 (21.8)
GB326	61.5	0.6 (0.3)	1.0 (0.4)	37.1 (0.6)	21.5 (11.1)
GB178	57.5	1.3 (0.4)	0.1 (0.1)	59.2 (1.4)	20.5 (20.5)
GB676	48.7	1.6 (0.4)	2.5 (0.6)	13.3 (3.2)	18.1 (3.2)
GB155	73.2	1.9 (0.7)	0.2 (0.1)	36.1 (1.6)	16.5 (16.5)
GB612	32.5	2.0 (0.6)	1.0 (0.5)	6.6 (0.3)	14.7 (0.3)
GB158	76.8	1.5 (0.2)	0.4 (0.3)	47.9 (0.9)	13.6 (13.6)
GB529	82.0	0.9 (0.3)	0.1 (0.1)	29.4 (4.3)	11.8 (11.8)

[a] SE, standard error of three values. [b] Methyl goss, gossypol-6-methyl ether. The space separates the 14 accessions with the highest percentage of (*R*)-gossypol-6-methyl ether and the 9 accessions with the lowest percentage.

employed to determine the total gossypol and (*S*)- to (*R*)-gossypol ratios in the extract is detailed in Stipanovic et al. (*11*).

RESULTS AND DISCUSSION

Within the U.S. Germplasm Collection at College Station, TX, 671 different accessions of *G. barbadense* L. were available for analysis. The seed from 22 of these accessions were limited, and from these only one or two seeds were analyzed. Seeds from Pima S1 to Pima S7 and Seabrook Sea Island 12B2 were also analyzed. Data on selected accessions are given in **Tables 1−5**. Complete results of the analysis for all available accessions of *G. barbadense* L., as well as Pima accessions and Seabrook Sea Island 12B2. are provided in the Supporting Information. As previously reported, *G. barbadense* produces terpenoids derived from hemigossypol-6-methyl ether including gossypol-6-methyl ether (**Figure 2**) and gossypol-6,6′-dimethyl ether (*16*). In all of the accessions analyzed, only gossypol-6-methyl ether was found; the dimethyl ether was not observed or was present at concentrations that did not allow accurate measurement. Chromatograms for three accessions are shown in **Figure 3**.

(*R*)- and (*S*)-Gossypol and (*R*)- and (*S*)-Gossypol-6-methyl Ether Ratios. The high ratio of (*R*)- to (*S*)-gossypol that is

Table 4. Embryos of *Gossypium barbadense* Accessions and Cultivars with the Lowest (0.0−0.5 mg/g of Dry Wt) and Highest (7.3−12.9 mg/g) Total Gossypol

accession or cultivar	mean embryo wt (mg)	mean (SE)[a] total gossypol (mg/g of dry wt)	mean (SE) total methyl goss[b] (mg/g of dry wt)	mean (SE) (*R*)-gossypol (%)	mean (SE) (*R*)-methyl goss (%)
GB710	81.3	0.0	0.0		
GB432	37.2	0.4 (0.2)	0.1 (0.1)	43.9 (3.6)	0.0
GB510	82.5	0.4	0.3	56.7	43.8
GB254	63.0	0.5 (0.2)	0.8 (0.3)	43.1 (4.0)	42.2 (2.2)
GB750	82.4	0.5 (0.1)	0.0 (0.0)	47.6 (4.8)	
GB503	62.5	0.5 (0.0)	1.1 (0.0)	43.6 (1.0)	39.4 (0.9)
GB467	73.0	0.5 (0.1)	0.1 (0.1)	61.3 (0.7)	50.2 (1.8)
GB44	106.6	0.5 (0.1)	1.4 (0.2)	42.1 (0.4)	47.1 (0.3)
GB230	70.4	0.5 (0.1)	1.0 (0.2)	51.3 (0.4)	44.7 (0.6)
GB7	60.8	7.3 (1.2)	8.0 (1.2)	45.8 (3.0)	48.5 (1.5)
GB25	113.3	7.4 (0.2)	0.9 (0.1)	59.9 (0.6)	49.7 (0.8)
GB35	83.3	7.5 (0.9)	1.8 (0.7)	51.0 (4.0)	48.4 (1.8)
GB557	45.1	7.6 (2.0)	0.6 (0.2)	40.9 (2.3)	50.2 (2.3)
GB32	82.6	8.3 (0.8)	0.7 (0.2)	60.6 (1.0)	48.7 (3.6)
Pima S1	85.6	8.5 (2.6)	1.8 (0.3)	52.3 (0.6)	49.7 (0.2)
GB37	86.6	8.7 (1.5)	1.1 (0.1)	35.4 (0.6)	37.8 (0.5)
GB30	77.8	8.9 (2.1)	0.5 (0.1)	50.8 (1.2)	49.4 (0.6)
Pima S7	75.4	9.0 (1.2)	3.1 (0.6)	55.2 (0.9)	51.4 (0.6)
GB554	30.1	9.1 (1.4)	0.4 (0.1)	30.3 (1.7)	68.0 (16.0
GB378	63.0	9.7 (1.9)	3.9 (0.5)	52.1 (0.6)	44.5 (0.3)
GB377	79.4	10.0 (3.3)	1.2 (0.3)	58.1 (0.3)	52.2 (1.0)
GB34	83.0	10.0 (2.4)	0.7 (0.1)	36.6 (1.5)	41.8 (0.9)
GB39	89.3	12.9 (2.3)	1.6 (0.7)	56.7 (0.6)	47.8 (2.6)

[a] SE, standard error of three values. [b] Methyl goss, gossypol-6-methyl ether. The space separates the 9 accessions with the lowest amount of total gossypol from the 14 with the highest amount.

Table 5. Embryos of *Gossypium barbadense* Accessions and Cultivars with the Highest (8.0−3.1 mg/g of Dry Wt) Total Gossypol-6-methyl Ether

accession or cultivar	mean embryo wt (mg)	mean (SE)[a] total gossypol (mg/g of dry wt)	mean (SE) total methyl goss[b] (mg/g of dry wt)	mean (SE) (*R*)-gossypol (%)	mean (SE) (*R*)-methyl goss (%)
GB7	60.8	7.3 (1.2)	8.0 (1.2)	45.8 (3.0)	48.5 (1.5)
Pima S6	61.4	6.5 (0.7)	6.6 (1.7)	35.2 (1.7)	39.3 (1.3)
GB14	64.8	3.1 (0.3)	5.2 (0.6)	44.6 (1.3)	46.9 (1.3)
GB9	65.2	4.2 (0.6)	5.0 (0.7)	40.5 (1.9)	44.2 (2.4)
GB15	67.4	7.2 (2.7)	4.8 (1.2)	40.0 (1.3)	41.5 (1.2)
GB50	76.7	2.3 (0.4)	4.7 (0.3)	48.6 (1.3)	44.6 (0.7)
GB5	72.4	4.2 (0.6)	4.0 (0.6)	43.6 (1.0)	44.6 (0.4)
GB378	63.0	9.7 (1.9)	3.9 (0.5)	52.1 (0.6)	44.5 (0.3)
GB192	56.5	4.0 (1.6)	3.7 (1.3)	51.2 (3.6)	46.6 (1.8)
GB506	48.0	1.9 (0.7)	3.7 (1.3)	41.8 (0.7)	43.4 (0.5)
GB619	75.6	1.3 (0.3)	3.6 (0.4)	41.8 (3.2)	43.0 (2.4)
GB8	61.8	6.0 (0.6)	3.6 (0.6)	49.9 (1.6)	43.6 (1.1)
GB314	50.8	1.3 (0.1)	3.6 (0.3)	40.6 (0.4)	44.2 (0.8)
GB96	102.4	4.3 (0.1)	3.5 (0.1)	35.2 (0.9)	37.4 (0.7)
GB382	113.0	2.2 (0.1)	3.5 (0.4)	40.8 (0.3)	32.8 (5.0)
GB40	86.4	3.2 (1.2)	3.4 (1.1)	57.9 (3.2)	50.0 (2.7)
GB55	83.0	2.1 (0.4)	3.4 (0.8)	56.2 (0.6)	54.1 (0.7)
GB43	87.9	4.6 (0.6)	3.3 (0.7)	43.0 (0.7)	45.3 (0.7)
GB103	89.7	2.7 (0.3)	3.1 (0.4)	43.5 (0.5)	42.4 (0.9)
Pima S7	75.4	9.0 (1.2)	3.1 (0.6)	55.2 (0.9)	51.4 (0.6)

[a] SE, standard error of three values. [b] Methyl goss, gossypol-6-methyl ether.

generally thought to prevail among Pima cotton seeds has led the dairy industry to use this seed with caution because feeding cottonseed with a high (*R*)- to (*S*)-gossypol can be toxic to cows if fed in large amounts (*5, 17*). Large variations in the (*R*)- to (*S*)-gossypol ratios among *G. barbadense* accessions and cultivars have been reported (*9, 10*). In the present study, we found ratio variations among Pima S1−Pima S7 (**Table 1**). Significantly, the ratio in the more recent Pima releases shows a shift toward smaller (*R*)- to (*S*)-gossypol ratios except for Pima S7. Thus, the general trend is favorable for dairy farmers, and the ratios in Pima S5 and S6 are in line with that observed in most commercial Upland cottonseed.

In the *G. barbadense* Collection, we found that the (*R*)- to (*S*)-gossypol ratios in seed from ∼50% of the accessions have an excess of (*R*)-gossypol and approximately ∼8% (52 accessions) had between 49 and 51% (*R*)-gossypol (see Supporting Information). The seeds with the highest percentage of (*R*)-gossypol were from accessions GB26 and GB283, with 68.2 and 67.3% (*R*)-gossypol, respectively (**Table 2**). We did not identify any accessions with extremely high (>90%) ee in (*R*)-gossypol; however, five accessions had 5% or less (*R*)-gossypol (**Table 2**). These were GB516 (5.0%), GB761 (4.5%), GB577 (4.3%), GB719 (3.7%), and GB476 (2.3%). These percentages rival those of the Brazilian moco cottons (*G. hirsutum* var. *marie galante*). Accessions from moco cottons that exhibit very low percentages of (*R*)-gossypol in the seed are currently being used in breeding programs to introduce the low (*R*)-gossypol seed trait into commercial Upland cultivars (*G. hirsutum*) to provide

Table 6. Embryos of *Gossypium barbadense* Accessions and Cultivars with the Highest Total (15.2−8.5 mg/g of Dry Wt) Gossypol plus Gossypol-6-methyl Ether

accession or cultivar	mean (SE) total G[a]	mean (SE) total MG[b]	mean total G + MG
GB7	7.3 (1.2)	8.0 (1.2)	15.2[c]
GB39	12.9 (2.3)	1.6 (0.7)	14.4
Pima S6	6.5 (0.7)	6.6 (1.7)	13.2
Pima S7	9.0 (1.2)	3.1 (0.6)	12.0
GB15	7.2 (2.7)	4.8 (1.2)	12.0
GB34	10.0 (2.4)	0.7 (0.1)	10.7
Pima S1	8.5 (2.6)	1.8 (0.3)	10.3
GB37	8.7 (1.5)	1.1 (0.1)	9.8
GB8	6.0 (0.6)	3.6 (0.6)	9.6
GB536	6.8 (0.7)	2.8 (0.5)	9.6
GB554	9.1 (1.4)	0.4 (0.1	9.4
GB30	8.9 (2.1)	0.5 (0.1)	9.4
GB35	7.5 (0.9)	1.8 (0.7)	9.3
GB9	4.2 (0.6)	5.0 (0.7)	9.2
GB32	8.3 (0.8)	0.7 (0.2)	8.9
Pima S5	5.8 (1.2)	2.7 (0.3)	8.5

[a] SE, standard error of three values; G, gossypol. [b] MG, gossypol-6-methyl ether. [c] Differences in totals are due to rounding.

cottonseed meal that can be safely fed to poultry (*18*, *19*). Following a similar strategy, the *G. barbadense* accessions listed above with very low levels of (*R*)-gossypol could also be used for breeding the low (*R*)-gossypol seed trait into commercial Pima cottons. These low (*R*)-gossypol seeds also had fairly low total gossypol content (**Table 2**), but they are small seeds. It is interesting to note that all of the accessions exhibiting the low (*R*)- to (*S*)-gossypol seed trait listed in **Table 2** are so-called "kidney cottons" (*20*). Kidney cottons originate from the same general areas in the Caribbean, northeastern South America, and Central America. Specific locations for the accessions examined in this study are as follows: GB516, Guiana; GB761, Venezuela; GB577, French West Indies; GB719, St. Croix (probable); GB476, French West Indies.

The accessions with the highest and lowest percentages of (*R*)-gossypol-6-methyl ether in the seed are given in **Table 3**. The percent for accessions with very low levels of total gossypol-6-methyl ether are misleading because, when the quantity of the compound is near the limit of detection (i.e., 0.1 mg/g of dry weight), the integration is prone to significant uncertainties. Given this qualification and their total levels of gossypol-6-methyl ether between 0.4 and 2.5 mg/g of dry weight, GB158, GB612, GB676, and GB326 have the lowest ratios of (*R*)- to (*S*)-gossypol-6-methyl ether, with ∼14:86 *R* to *S*. The accessions showing the highest (*R*)- to (*S*)-gossypol-6-methyl ether ratios were GB95 and GB647, with ratios of ∼77:23 and 74:26, respectively (**Table 3**). Interestingly, these accessions had a higher (*R*)- to (*S*)-gossypol-6-methyl ether ratio than the accession showing the highest (*R*)- to (*S*)-gossypol ratio (i.e., GB26, ∼68:32).

Gossypol and Gossypol-6-methyl Ether Concentrations in Seed. The 12 accessions with the highest total seed gossypol per gram of dry embryo when grown in the greenhouse are listed at the bottom of **Table 4**. Of these, GB39 (12.9 mg/g), GB34 (10.0 mg/g), and GB554 (9.1 mg/g) had the highest levels. Surprisingly, one accession (GB710) was glandless (no gossypol) and eight other accessions had ≤0.5 mg/g total gossypol (top of **Table 4**).

G. barbadense differs from *G. hirsutum* in that the former can produce significant amounts of gossypol-6-methyl ether in the seed and foliage. The 17 accessions with the highest gossypol-6-methyl ether are shown in **Table 5**. Eleven of these accessions and Pima S6 had more gossypol-6-methyl ether than gossypol. Sixteen cultivars or accessions had a combined total of gossypol and gossypol-6-methyl ether of ≥8.5 mg/g of dry weight (**Table 6**). Prominent among these are GB7, which had the highest total (15.2 mg/g of dry tissue), and the cultivars Pima S6, Pima S7, and Pima S1, which were ranked third, fourth, and seventh, respectively. Thirty-nine accessions had no gossypol-6-methyl ether in the seed (data not shown, see Supporting Information).

Gossypol-6-methyl ether is formed by the dimerization of hemigossypol and hemigossypol-6-methyl ether (**Figure 2**). Bell et al. (*21*) showed that most cottons, including *G. hirsutum*, contain a structural gene that controls methylation at the 6-position, but *G. hirsutum* and other species have evolved dominant regulatory genes that restrict or prevent methylation, especially in pigment glands and differentiated tissue (*22*, *23*). Methylation is inherited as a recessive trait except in the case of *G. sturtianum*, where it is expressed as a completely dominant trait. Insecticidal and fungicidal studies conducted on the methylated cotton terpenoids related to gossypol show that these compounds are less toxic to these pests than the unmethylated analogue (*24*, *25*). Thus, down-regulation of methylation in *G. hirsutum* may be an adaptation to increase resistance to insects and pathogens. If so, selecting plants with lower levels of methylated terpenoids in foliage in *G. barbadense* may increase resistance to insects. Similar to the insect toxicity findings, in preliminary unpublished work, we found that gossypol-6,6′-dimethyl ether has low toxicity to nonruminants and that gossypol-6-methyl ether may exhibit similar characteristics. Thus, the ideal *G. barbadense* cotton would have increased levels of gossypol-6-methyl ether in seed with a concomitant reduction in gossypol, but with reduced levels of methylated terpenoids in foliage. Such plants may be a worthwhile breeding target that would increase insect resistance and make cottonseed safe as a feed for dairy cattle. The possibility of breeding such plants will be the subject of future studies.

Dirigent Protein. In other research, we found that a dirigent protein controls the dimerization of hemigossypol to give (*S*)-gossypol in moco cottonseed (*13*). The variation in ratios we now report among the *G. barbadense* accessions ranged from a high of 68:32 to a low of 2:98. This indicates that two distinct dirigent proteins control the dimerization that yield either (*R*)- or (*S*)-gossypol. However, these results do not unequivocally demonstrate whether the observed atropisomer ratio is controlled by operation of both dirigent proteins within an accession or by operation of a single dirigent protein expressed in quantities insufficient to completely overcome random dimerization. Of particular interest are the 193 accessions with seed containing 45−55% (*R*)-gossypol. It is unclear if the two dirigent proteins are almost equally expressed or if only one is weakly expressed with most of the gossypol formed by random dimerization.

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Supporting Information Available: Cultivar or accession number, mean embryo weight, range embryo weight, mean total gossypol, mean total gossypol-6-methyl ether, range total gossypol-6-methyl ether, mean (*R*)-gossypol percent, range (*R*)-gossypol percent, mean (*R*)-gossypol-6-methyl ether percent, and range (*R*)-gossypol-6-methyl ether percent for all available accessions, Pima S1−Pima S7 and SBSI-12B2. This material is available free of charge via the Internet at http://pubs.acs.org.

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