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Phytosterol Composition of Hybrid *Hibiscus* Seed Oils

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The seed oils from fifteen hybrid *Hibiscus* varieties were analyzed for desmethyl sterol content to identify bioactive compounds that could promote the use of these oils for edible applications. *Hibiscus* is being developed as a new crop with edible and nutraceutical applications for the component tissues and tissue extracts. Previously, hybrid varieties were developed for ornamental purposes on the basis of flower morphology and color. Currently, the effects of selective breeding on seed oil components are of interest as these represent potential natural products with bioactive properties. In the present study, sterol structures were identified as the corresponding trimethyl silyl ether derivatives obtained from the unsaponifiable fraction of the seed oils. This material contained an average of 32 wt % sterols and exhibited a relative composition of sitosterol, 76.3%; campesterol, 10.3%; stigmasterol, 7.3%; 5-avenasterol, 4.4%; and cholesterol, 0.6%. The content of 5-avenasterol showed statistically significant variation among the hybrid varieties with a range of 1.2−5.8%.

KEYWORDS: ***Hibiscus*; sitosterol; stigmasterol; campesterol; avenasterol; seed oil**

INTRODUCTION

Members of the genus *Hibiscus* thrive in a variety of climates and produce a diversity of natural compounds with bioactive properties. Several new varieties of *Hibiscus* have been developed through the hybridization of native species to provide ornamental flowers and edible green tissues. Efforts to develop *Hibiscus* as a cultivated crop have attempted to identify edible and nonedible applications for various tissues. For example, the flowers are harvested for ornamental purposes and as an edible garnish. The canes may be harvested to provide a fiber source. Recent attention has focused on the seeds as a source of phospholipids, proteins, and triglycerides. Once a hybrid is established the plants are propagated vegetatively to maintain the desired properties. However, the seeds that are produced contain approximately 15 wt % on a dry weight basis (dwb) of highly unsaturated triglycerides and small amounts of other lipid components. The seed oils are composed predominately of oleic and linoleic fatty acids and have been investigated for potential edible applications (*1*). The recovery and characterization of these seed oils included the isolation of an unsaponifiable fraction that was determined to contain desmethyl sterol compounds. Such phytosterol compounds are known to reduce the absorption of dietary cholesterol when included in the human diet (*2*−*4*). Phytosterols also exhibit other beneficial properties such as antiinflammatory and antitumor activity (*5*, *6*). There is increasing interest in isolating these biologically active components for nutraceutical applications and as ingredients for functional foods (*7*−*9*). Investigation of the *Hibiscus* seed oils was extended to determine the composition and structure of phytosterol compounds present.

MATERIALS AND METHODS

Reagents. Cyclohexane and potassium hydroxide were obtained from Fisher Scientific, Co. (Fairlawn, NJ). Absolute ethanol was obtained from Aaper Alcohol and Chemical Co., (Shelbyville, KY). Campesterol, cholestane, cholesterol, stigmasterol, and sitosterol standards were obtained from Sigma-Aldrich, Inc. (St. Louis, MO). The trimethyl silyl ether (TMS) derivatives of all sterols were prepared using *N*,*O*-bis-[Trimethylsilyl]trifluoroacetamide (BSTFA) reagent (Pierce Chemical Co., Rockford, IL).

***Hibiscus* Seeds.** Seeds were obtained from The Village Botanica, Inc. (Waller, Texas). Seeds were harvested during the summer and fall of 2001. Seeds were removed from the seedpods by hand, ground in a Wiley mill fitted with a 40 mesh screen to produce particles of 0.4-mm diameter or less, and stored at −20 °C prior to extraction.

Extractions. Ground seeds were extracted with supercritical carbon dioxide using a model 3560 supercritical fluid extractor (Isco Corp., Lincoln, NE). Extraction vials were packed with 5-g samples. The instrument was programmed for a 1-min static extraction at 80 °C and 53.70 MPa followed by a 50-min dynamic extraction with carbon dioxide at 2.0 mL/min. The lipid extracts were collected into glass vials and stored under nitrogen at −20 °C. Extractions were run in duplicate.

Saponification and Derivatization. Samples (100-mg) of the extracts were dissolved in ethanol containing 50 μg of cholestane as an internal standard. The dissolved extracts were saponified by adding 1-mL volumes of 2 N ethanolic KOH, heating to 30 °C, and allowing it to stir overnight. The unsaponifiable fraction was recovered by extraction with cyclohexane. The recovered fraction was transferred by pipet into tared glass vials and dried under nitrogen. TMS derivatives

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Table 1. Sterol Distribution of *Hibiscus* Seed Oils (Percent of Total Sterols)[a]

varieties	cholesterol	campesterol	stigmasterol	sitosterol	5-avenasterol[b]	total sterols (mg/g oil)
Georgia Rose	0.3	11.1	7.3	76.3	5.0	6.1
Gov. Anne	0.1	11.6	8.0	74.7	5.6	5.2
Grace Coolidge	0.1	11.2	6.3	77.8	4.6	7.8
Lou Hoover	0.3	11.9	6.6	75.5	5.6	5.9
Martha Washington	1.2	7.6	7.9	77.4	5.7	5.8
New Rosalynn II	0.6	10.2	8.1	76.2	4.9	5.0
Pink Disco Belle	1.0	9.9	9.8	77.4	2.0	4.6
Quatro rojo	1.0	11.2	10.1	72.3	5.4	6.1
Razberri Ruffles II	0.1	10.9	6.1	77.1	5.9	6.1
Raspberry Rhapsody	0.0	11.6	6.4	78.4	3.6	7.2
Raspberry Rhapsody II	0.2	10.4	7.1	81.2	1.2	5.5
Rosalynn	2.6	10.4	7.4	75.5	4.2	5.9
Rosalynn LS	0.0	10.6	6.5	77.2	5.8	4.6
Rosalynn NPHP	1.6	10.6	7.5	75.2	5.0	5.5
The Blues	0.2	7.7	5.1	84.5	2.5	6.1
species						
H. laevis	0.7	11.2	7.4	75.9	4.9	4.9
H. dasycalyx	0.8	12.3	8.4	73.6	4.8	4.9
H. moscheutos	0.7	10.8	9.1	75.3	4.1	4.9
H. mutabilis	0.1	10.5	5.3	82.1	2.0	5.0

[a] Reported values are averages of duplicate analyses, variation < 10% RSD. [b] Values significantly different at 0.05 level by ANOVA.

were prepared by dissolving 5-mg samples of the unsaponifiable material into 1.0 mL of cyclohexane containing 50 μL of BSTFA reagent and heating the mixture for 1 h at 60 °C. The saponification procedure was performed in duplicate or triplicate on aliquots of each lipid extract.

Phytosterol Analysis. Analyses were performed by GC-MS and GC-FID on the corresponding TMS derivatives. Structural identifications and quantitative analyses of phytosterol derivatives were obtained using an Agilent 6890 GC-MS system operated in EI mode. Splitless injections were made with 1-μL sample volumes at an inlet temperature of 250 °C. Helium carrier gas flow was 1.4 mL/min with an average velocity of 43 cm/s. Separations were achieved with an HP-5ms capillary column measuring 30-m × 0.25-mm ID × 0.5-μm film thickness. The oven temperature was programmed to begin at 70 °C, hold for 1 min; increase to 245 °C at 25 °C/min, hold for 1 min; increase to 275 at 3 °C/min; and hold for 20 min. The mass selective detector was operated in scan mode over the range 40−500 *m*/*z* with the source heated to 230 °C and the quadrapole heated to 150 °C. Standards were prepared for reference spectra and used to generate calibration curves. Data were acquired and processed with Chemstation software. The calibration results were based on the integrated areas and adjusted according to the amount of cholestane detected in the samples. Replicate analyses exhibited variation of less than 10% RSD. These results were confirmed by sample analysis performed using an HP 6890 GC-FID operating with an HP-5ms column of the same dimensions and analyzed at the same conditions as used for the GC-MS.

RESULTS AND DISCUSSION

The extraction of ground hybrid *Hibiscus* seeds with supercritical carbon dioxide produced lipid fractions ranging from 12 wt % for Grace Coolidge to 22 wt % for Rosalynn with an average value of 15 wt % (dwb). The unsaponifiable fractions obtained from this group of hybrid *Hibiscus* seed oil extracts were subsequently determined to contain between 280 and 340 μg of sterol/mg unsaponifiable material. This represents an average 32 wt % of the unsaponifiable material. This was comparable to the sterol content of 260−400 μg of sterol/mg unsaponifiable material determined for a group of related native *Hibiscus* species that included *laevis*, *dasycalyx*, *mutabilis*, and *moscheutos*. The native species were grown in the same location as the hybrid varieties, under the same conditions, and harvested at the same time. Variation in sterol content between the group of hybrid varieties and the group of native species was determined not to be significant by ANOVA for an alpha level of 0.05. In accessions of crops such as canola and corn, the phytosterol profiles can show significant variation due to genetic and agronomic factors (e. g., planting location and temperature) (*10*−*13*).

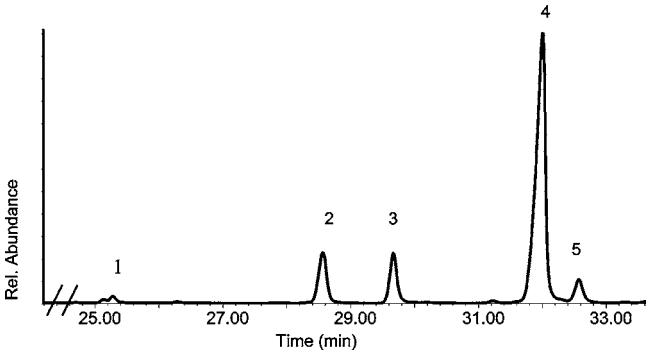

Figure 1. Total ion chromatogram of TMS sterol derivatives obtained from the unsaponifiable fraction of a *Hibiscus* seed extract. Peaks were identified from the retention times of standard compounds and by spectra: 1, cholesterol; 2, campesterol; 3, stigmasterol; 4, sitosterol; 5, 5-avenasterol.

Analysis of the corresponding TMS derivatives by GC-MS revealed a pattern of one minor peak and four prominent peaks tentatively identified as cholesterol, campesterol, stigmasterol, sitosterol, and 5-avenasterol in the total ion chromatogram (**Figure 1**). Structural identification was confirmed by comparison of the retention times and mass spectra obtained for these *Hibiscus* samples with those of derivatized sterol standards **(Figure 2**). The collected spectra were also in agreement with reported values for the molecular ions and the main fragmentation ions for these phytosterols (*14*, *15*). None of the structurally related saturated sterols were detected (*16*).

The distribution of the phytosterol compounds is presented in **Table 1** for the hybrid varieties. The most abundant sterol in all of the varieties tested was sitosterol, followed by campesterol, stigmasterol, and 5-avenasterol. Cholesterol was detected in most varieties but at very low levels. The variation in sterol distribution was tested by ANOVA and determined to be significant only for 5-avenasterol. The sterol distribution for

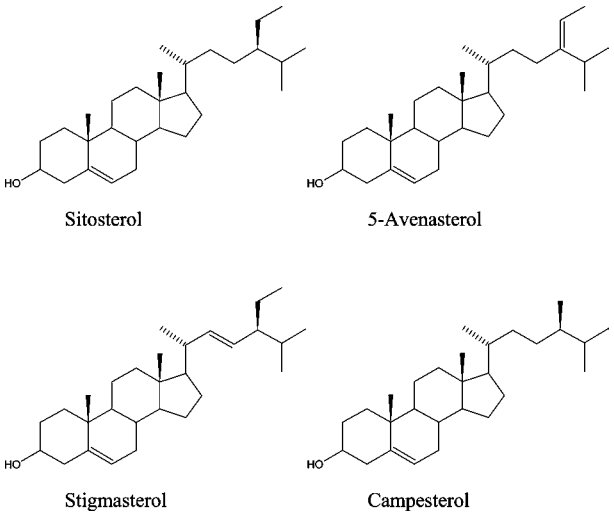

Figure 2. Steroid structures identified in *Hibiscus* seed extracts.

the native species exhibited significant variation with an alpha level of 0.05. The most abundant sterol detected in all of the native seed oils tested was sitosterol followed by campesterol, stigmasterol, and 5-avenasterol (**Table 1**). Trace amounts of cholesterol were also detected. These results were tested for between group variation by ANOVA and it was determined that the variation in the sterol distribution between the hybrids and the native species was not significant.

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