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# VARIETAL SIMILARITIES AND DIFFERENCES IN THE POLYCYCLIC ISOPENTENOID COMPOSITION OF SORGHUM

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**Key Word Index**—*Sorghum bicolor*; Graminae; wax; leaf; $\beta$-amyrin; $\alpha$-amyrin; $\delta$-amyrin; fernenol; isoarborinol; sorghumol; simiarenol; trematol; cholesterol; campesterol; sitosterol; stigmasterol; cycloartenol; phylogenetics.

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**Abstract**—The intraspecific similarities and differences among the various polycyclic isopentenoids (sterols and pentacyclic triterpenes) which occur in leaf tissue and surface wax from three varieties of flowering *Sorghum bicolor*; G 499 GBR, BOK 8 and IS 809, have been determined. The three varieties exhibited differences in phenotypic characters (e.g. shoot height) and pest resistance. While sterol and pentacyclic amyroid compositions were similar in the three varieties, significant differences were evident in the qualitative distribution of the migrated hopanoids. One variety, IS 809, which is phenotypically short and resistant, contained a single migrated hopanoid, sorghumol. Alternatively, the other two varieties, both phenotypically tall and one-G 499 GBR-resistant, the other-BOK 8-susceptible, contained a mixture of four $\Delta^{9(11)}$-migrated hopanoids, i.e. sorghumol and three of its stereoisomers, and the $\Delta^{5(6)}$-migrated hopanoid simiarenol. While ketones corresponding to the $\Delta^{9(11)}$-migrated hopanoids were detected in the three varieties, the ketone of the $\Delta^{5(6)}$-migrated hopanoid was apparently absent. In contrast to other graminaceous plants, the predominant C-3 derivatives did not include the C-3 methyl ethers, but did include esters and polar conjugates. Significant differences apparent in amounts of pentacyclic triterpenes were not apparent in the total amount of sterols extracted from the leaves (including surface wax) of the three varieties. Since *S. bicolor* varietal differences occurred only among the migrated hopanoids (found in mature leaves) it would appear that pentacyclic triterpenes, unlike sterols, have greater applicability as chemotaxonomic indices for intraspecific relationships in sorghum.

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## INTRODUCTION

The species *Sorghum bicolor* (Moench) L. is traditionally divided into three subspecies: *bicolor*, an extremely variable cultivated taxa; *drummondii*, a widely distributed and ecologically variable wild African taxa; and *arundinaceum*, a taxa derived from introgression between domesticated grain sorghums and their closest wild relatives, i.e. between *bicolor* and *drummondii* sorghums [1]. Cultivated grain sorghums have been divided into five basic races (groups of varieties) and ten hybrid races that combine characteristics of any two or more basic races [2]. Modern domesticated 'varieties and hybrids' of this agronomically important crop plant are probably derived from race bicolor sorghums [2] which have been developed for specific agricultural purposes, i.e. harvestability (head height), pest resistance, maturity group, drought resistance, etc. As is true of most plants that have undergone selection by man, intraspecific distinguishing traits within the genus are obscured. Lipid chemotaxonomy has historically been used in several taxons as an aid in clarifying broad taxonomic relationships (c.f. ref. [3] for a key to the literature) and more recently at the species and subspecies levels. For instance, there have been several attempts to delineate the Graminae family and to examine their interspecific relationships based on their triterpenoid content, in particular, the pentacyclic triterpene methyl ether content of the epicuticular wax [4–8]. However, these compounds could not be used as chemotaxonomic indices for intraspecific relationships of *Sorghum bicolor* since in a preliminary investigation of the polycyclic isopentenoid compounds (sterols and pentacyclic triterpenes) of two varieties of *Sorghum bicolor*, G 499 GBR and IS 809, we did not find pentacyclic triterpene methyl ethers as a component of the leaves (plus wax) from flowering plants. We reported the presence of sterols and some pentacyclic triterpenes as free alcohols and other C-3 derivatives [9, 10]. Additionally, Palmer and Bowden [11–13] have reported the presence of esterified pentacyclic triterpenes in grains of *Sorghum bicolor* Pers DC 36 but they did not detect these compounds as the free alcohols or C-3 methyl ethers. In order to further investigate the evolutionary significance of the pentacyclic triterpenes and sterols in the development of sorghum and the use of these compounds to differentiate sorghum at the subspecies level, a study was initiated to more fully examine the sterol and pentacyclic triterpene composition of the surface wax and leaf tissue (plus wax) from three varieties, two of which have similar morphological features (G 499 GBR and BOK 8) while the third (IS 809) is morphologically distinct. The morphological and pest resistance differences [14, 15] between the three varieties grown under the same conditions would suggest the presence of underlying genetic and biochemical differences which may have a phylogenetic component in polycyclic isopentenoids (sterols and pentacyclic triterpenes).

## RESULTS AND DISCUSSION

The qualitative (including free alcohol and C-3 conjugate forms) and quantitative distribution of the sterols and pentacyclic triterpenes (PT) between the leaf tissue (plus wax) and the surface wax of the three varieties were determined (Table 1). The polycyclic isopentenoids listed in Table 1 were initially separated and identified by a combination of chromatographic (HPLC and GLC, Table 2) and spectral constants (GLC-MS, GLC-SIM and $^{1}$H NMR) from a large scale extraction of one of the varieties (G 499 GBR, leaves plus wax) which produces the polycyclic isopentenoids present in the other two plants. In addition to the sterols and PT identified from this large scale extraction, a trace amount of cycloartenol, the first tetracyclic precursor of sterols from 2,3-oxidosqualene [3], was detected. The reverse phase HPLC $\alpha_c$ values in Table 2 were obtained with an Ultrasphere-ODS C18 column. In previous investigations [16] using a Zorbax BP-ODS C18 column, we were not able to separate cholesterol and β-amyrin while cycloartenol co-chromatographed with α-amyrin. Thus the importance of column selection (and perhaps temperature) in reverse phase HPLC to the reported chromatographic constants is apparent.

The distribution of the polycyclic isopentenoids for the leaf tissue (Table 1) was obtained by analysis of the total lipid extract (TLE) from dried leaves (small scale). The TLEs represented, on the average, 5.8 % of the leaf dry weight from each variety. The distribution for the surface wax (Table 1) was obtained by analysis of the hexane wash of the surface of fresh leaves from each variety. Since the surface wax extract represents such a small fraction of the dry weight of the leaf tissue (0.3 % of the leaf dry weight) we can assume that the polycyclic isopentenoid content of the leaf tissue TLE closely represents the intracytoplasmic content of the leaves and that the surface (hexane wash) extract represents the extracytoplasmic polycyclic isopentenoid content. The TLE and surface wash extracts were separated by preparative TLC into free and derivatized fractions, the polar and ester derivatized fractions were hydrolysed, and the polycyclic isopentenoids in each fraction quantified by GLC and identified by GLC-MS comparison to the known compounds from the large scale extraction and available standards.

The sterol composition* of IS 809 leaf tissue (plus wax) was previously characterized [9]. The leaf tissue from the other two varieties grown under the same conditions and examined at the same developmental stage as IS 809 contained essentially the same sterol composition as IS 809 with only minor differences in the quantitative free : ester : hydrolysable polar derivative ratio (Table 1). It should be noted that no sterol C-3 methyl ether or C-3 ketone derivatives were detected. From the distribution data it is also evident that the minor amounts of sterols (free and C-3 conjugates) obtained in the hexane wash of the leaf surface were actually extracted from the intracytoplasmic pool and are not essential components of the surface wax.

While the sterol compositions of the three varieties were essentially the same, significant differences were evident in the qualitative distribution of the migrated hopanoids. The pentacyclic triterpene composition of the leaf tissue (plus wax) and surface wax from the three varieties contained the same three amyroids; α-amyrin, β-amyrin and δ-amyrin (Table 1, Fig. 1).† Two of the three varieties, G 499 GBR and BOK 8, contained five migrated hopanoids; fernenol; isoarborinol, sorghumol, trematol and simiarenol (Table 1, Fig. 2).† The third variety, IS 809, contained only one migrated hopanoid, sorghumol, whose structure, along with those of fernenol, isoarborinol and trematol were fully characterized by $^{1}$H NMR and mass spectroscopy [10]. The presence of simiarenol in G 499 GBR and BOK 8 was determined by a comparison of its mass spectrum to that reported for simiarenol (a D:B-friedo-B′:A′-neogammacer-5-en-3β-ol compound) [17] and by a comparison of its full $^{1}$H NMR (200 MHz) spectrum to two other samples of authentic simiarenol isolated from the leaves of *Rhododendron simiarum* by Alpin *et al.* [17] and from the wax of *Syagrus coronata* by Tulloch [18]. The mass spectrum of the simiarenol isolated from the two sorghum varieties indicated the presence of a $\Delta^{5(6)}$-double bond and an isopropyl group in a D:B-friedo-B′:A′-neogammacar-5-en-3β-ol carbon skeleton; characteristic peaks of this carbon skeleton are a weak molecular ion $[M]^+$ at $m/z$ 426, a weak $[M-43]^+$ peak at $m/z$ 383, and strong $m/z$ 274 (100 %), 259 (52 %) and 231 (16 %) peaks which arise from a retro-Diels–Alder cleavage through ring B. The $^{1}$H NMR spectrum of the free alcohol confirmed the presence of a tri-substituted single oliphinic proton (δ5.625, *t*) at C-6, the isopropyl group (0.828, 3H, *d* and 0.887, 3H, *d*) at C-21 (which is not apparent in $\Delta^{5(6)}$-migrated amyroids, e.g., glutinol acetate [19]), the β-oriented hydroxyl group at C-3 (3.475 1H, *m*, $W_{1/2} = 8$ Hz, 3α-H) and six tertiary methyls. Simiarenol has been reported as present in several graminaceous plants by Tulloch *et al.* [19–21] based on a comparison of its mass and $^{1}$H NMR (100 MHz) spectra to published data [17] and to the standard isolated from *Rhododedron simiarum* [17]. Additionally, the data (MS and $^{1}$H NMR) for the simiarenol from the two sorghum varieties is consistent with that for 'simiarenol' isolated from two woody species by other investigators [22, 23]. However, the $^{1}$H NMR (60 MHz) spectrum of 'simiarenol' isolated from an additional woody species [24] exhibited differences both from that isolated from sorghum and that isolated by Tulloch [19] and Alpin *et al.* [17]. In view of the co-occurrence in G 499 GBR and BOK 8 of a complete stereochemical series of compounds in which

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*Sterol nomenclature: cholesterol, 5α-cholest-5-en-3β-ol; campesterol, 24α-methylcholesterol; stigmasterol, 24α-ethylcholesta-5,22-dienol; sitosterol, 24α-ethylcholesterol. Replacement of a hydrogen atom with another group can alter the configurational designation in the *R*, *S* notation; thus we prefer to use the α,β-designation of Platner *et al.* as modified by Nes [W. R. Nes (1977) *Adv. Lipid Res.* **15**, 233]. The α-orientated substitutent (hydrogen, etc.) is in front, when C-22 is to the right (*trans*-orientated with respect to C-13) in the usual view of the molecule. The configuration at C-24 for the ethyl but not the methyl cholesterol was proven by $^{1}$H NMR spectroscopy.

†The IUPAC nomenclature for the PT is as follows: α-amyrin, 5α-urs-12(13)-en-3β-ol; β-amyrin, 5α-olean-12(13)-en-3β-ol; δ-amyrin, 5α-olean-13(18)-en-3β-ol; fernenol, D:C-friedo-B′:A′-neogammacer-9(11)-en-3β-ol (3β,8α,13α,14β,17α,18β,21α); trematol, D:C-friedo-B′:A′-neogammacer-9(11)-en-3β-ol (3β,8α,13α,14β,17α,18β,21β); isoarborinol, D:C-friedo-B′:A′-neogammacer-9(11)-3β-ol (3β,8β,13β,14α,17β,18α,21β); sorghumol, D:C-friedo-B′:A′-neogammacer-9(11)-en-3β-ol (3β,8β,13β,14α,17β,18α,21α); simiarenol, D:B-friedo-B′:A′-neogammacer-5(6)-en-3β-ol (3β,8α,9β,13α,14β,17α,18β,21α). The stereospecificity of simiarenol is that indicated by Alpin *et al.* [17] but as yet it has not been definitively established.

Table 1. Amounts and distribution of free and derivatized pentacyclic triterpene alcohols and sterols from three varieties of *Sorghum bicolor* in μg/g dry wt*

| | G 499 GBR | | | | | | | | BOK 8 | | | | | | | | IS 809 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaf tissue | | | | Surface wax | | | | Leaf tissue | | | | Surface wax | | | | Leaf tissue | | | | Surface wax | | | |
| | F† | E† | P† | Total | F | E | P | Total | F | E | P | Total | F | E | P | Total | F | E | P | Total | F | E | P | Total |
| Pentacyclic triterpenoids | | | | | | | | | | | | | | | | | | | | | | | | |
| β-Amyrin | 97.0 | 21.3 | n.d.‡ | 118.3 | 4.9 | 1.7 | n.d. | 6.6 | 104.3 | 28.0 | n.d. | 132.3 | 8.4 | 1.2 | n.d. | 9.6 | 183.9 | 43.0 | 0.7 | 227.6 | 11.8 | 7.8 | n.d. | 19.6 |
| α-Amyrin | 68.5 | 31.9 | n.d. | 100.4 | 5.3 | 3.1 | n.d. | 8.4 | 114.9 | 26.0 | n.d. | 140.9 | 12.2 | 2.3 | n.d. | 14.5 | 175.5 | 77.0 | 1.3 | 253.8 | 12.9 | 11.5 | n.d. | 24.4 |
| δ-Amyrin | 37.1 | 2.7 | n.d. | 39.8 | 3.6 | 0.7 | n.d. | 4.3 | 20.7 | 14.1 | n.d. | 34.8 | 3.6 | 0.2 | n.d. | 3.8 | 10.5 | 13.0 | 0.4 | 23.9 | 10.4 | 6.1 | n.d. | 16.5 |
| Fernenol | 131.0 | 63.8 | n.d. | 194.8 | 14.8 | 8.6 | n.d. | 23.4 | 133.5 | 77.0 | n.d. | 210.5 | 14.1 | 4.3 | n.d. | 18.4 | n.d. | n.d. | n.d. | — | n.d. | n.d. | n.d. | n.d. |
| Isoarborinol | 117.0 | 40.8 | n.d. | 157.8 | 15.0 | 6.3 | n.d. | 21.3 | 100.0 | 60.0 | n.d. | 160.0 | 18.3 | 2.7 | n.d. | 21.0 | n.d. | n.d. | n.d. | — | n.d. | n.d. | n.d. | n.d. |
| Sorghumol | 108.4 | 16.0 | n.d. | 124.4 | 6.8 | 6.9 | n.d. | 13.7 | 41.5 | 28.0 | n.d. | 69.5 | 9.2 | 1.0 | n.d. | 10.2 | 271.8 | 17.0 | 1.0 | 289.8 | 15.9 | 4.1 | n.d. | 20.0 |
| Trematol | 11.4 | 1.3 | n.d. | 12.7 | 3.8 | trace | n.d. | 3.8 | 4.0 | 2.8 | n.d. | 6.8 | 1.1 | 0.1 | n.d. | 1.2 | n.d. | n.d. | n.d. | — | n.d. | n.d. | n.d. | n.d. |
| Simiarenol | 260.0 | 42.0 | n.d. | 302.0 | 78.3 | 4.7 | n.d. | 83.0 | 204.0 | 62.0 | n.d. | 266.0 | 47.0 | 2.8 | n.d. | 49.8 | n.d. | n.d. | n.d. | — | n.d. | n.d. | n.d. | — |
| Total | 830.4 | 219.8 | — | 1050.2 | 132.5 | 32.0 | — | 164.5 | 722.9 | 297.9 | — | 1020.8 | 113.9 | 14.6 | — | 128.5 | 641.7 | 150.0 | 3.4 | 795.1 | 51.0 | 29.5 | — | 80.5 |
| Sterols | | | | | | | | | | | | | | | | | | | | | | | | |
| Cholesterol | 2.2 | 1.0 | 0.5 | 3.7 | 0.1 | trace | n.d. | 0.1 | 11.3 | 2.8 | 0.04 | 14.1 | 0.2 | 0.2 | n.d. | 0.4 | 7.0 | 0.5 | 0.4 | 7.9 | 0.1 | 0.06 | n.d. | 0.16 |
| Campesterol | 55.5 | 3.6 | 11.8 | 70.9 | 1.2 | 0.1 | n.d. | 1.3 | 61.3 | 17.0 | 1.2 | 79.5 | 3.5 | 0.1 | n.d. | 3.6 | 92.0 | 7.8 | 16.6 | 116.4 | 0.9 | 0.3 | n.d. | 1.2 |
| Stigmasterol | 275.4 | 13.0 | 23.7 | 312.1 | 2.4 | 0.5 | n.d. | 2.9 | 222.6 | 71.7 | 3.2 | 297.5 | 5.9 | 0.6 | n.d. | 6.5 | 282.0 | 18.4 | 24.7 | 325.1 | 3.4 | 1.4 | n.d. | 4.8 |
| Sitosterol | 196.2 | 16.5 | 34.3 | 247.0 | 1.6 | 0.5 | n.d. | 2.1 | 151.0 | 100.0 | 5.2 | 256.2 | 2.2 | 0.8 | n.d. | 3.0 | 161.6 | 24.0 | 36.0 | 221.6 | 3.6 | 1.4 | n.d. | 5.0 |
| Total | 529.3 | 34.7 | 70.2 | 633.7 | 5.3 | 1.1 | — | 6.4 | 446.2 | 191.5 | 9.64 | 647.3 | 11.8 | 1.7 | — | 13.5 | 542.6 | 50.7 | 77.7 | 671.0 | 8.0 | 3.2 | n.d. | 11.2 |

*Amounts, measured by GLC comparison to standard curves, were determined from free fractions isolated by TLC from the TLE; polar fractions and esterified fractions also isolated by TLC, were hydrolysed, TLC and amounts of PT and sterol determined by GLC.

†F = free alcohols, E = esters, P = polar derivative fraction. Trace amounts of PT ketones were detected; absolute amounts are not reported.

‡n.d. = none detected.

Table 2. Chromatographic properties of purified polycyclic isopentenoids isolated from *Sorghum*

| Compound | Source of standard compounds* | | GLC ($RR_{t}c$)† | | | | HPLC ($\alpha_c$)‡ | |
|---|---|---|---|---|---|---|---|---|
| | | | 3% SE-30 | 3% OV-17 | 1% SP-1000 | | I | II |
| *β*-Amyrin | 2 | 1. | 1.64 | 1.83 | 1.55 | 1. | 1.15 | 1.05 |
| | | 2. | 2.17 | 2.25 | 1.29 | 2. | 2.10 | — |
| | | 3. | 1.38 | 1.25 | 0.60 | 3. | 3.19 | — |
| *δ*-Amyrin | 6 | 1. | 1.89 | 2.06 | 1.72 | 1. | 1.15 | 1.09 |
| | | 2. | 2.53 | 2.55 | 1.40 | 2. | 2.09 | — |
| | | 3. | — | — | — | 3. | — | — |
| *α*-Amyrin | 2 | 1. | 1.79 | 2.10 | 1.77 | 1. | 1.28 | 1.12 |
| | | 2. | 2.29 | 2.55 | 1.47 | 2. | 3.19 | — |
| | | 3. | 1.51 | 1.43 | 0.68 | 3. | 3.53 | — |
| Fernenol | 1, 5 | 1. | 2.01 | 2.42 | 2.05 | 1. | 1.80 | 1.84 |
| | | 2. | 2.74 | 2.98 | 1.74 | 2. | 4.32 | — |
| | | 3. | 1.68 | 1.62 | 0.79 | 3. | 5.16 | — |
| Isoarborinol | 1 | 1. | 2.14 | 2.62 | 2.29 | 1. | 1.80 | 1.84 |
| | | 2. | 2.93 | 3.24 | 1.93 | 2. | 4.32 | — |
| | | 3. | 1.76 | 1.75 | 0.86 | 3. | 5.16 | — |
| Simiarenol | 5 | 1. | 2.20 | 2.75 | — | 1. | 1.42 | — |
| | | 2. | — | — | — | 2. | — | — |
| | | 3. | — | — | — | 3. | — | — |
| Sorghumol | 1 | 1. | 2.31 | 2.92 | 2.57 | 1. | 1.92 | — |
| | | 2. | 3.03 | 3.61 | 2.14 | 2. | 3.65 | — |
| | | 3. | 1.92 | 2.13 | 1.0 | 3. | 6.23 | — |
| Trematol | 1 | 1. | 2.44 | 3.17 | 2.60 | 1. | 1.92 | — |
| | | 2. | 3.18 | 3.20 | 2.41 | 2. | 3.65 | — |
| | | 3. | 2.05 | 2.16 | 1.11 | 3. | 6.23 | — |
| Cholesterol | 1 | 1. | 1.00 | 1.00 | 1.00 | 1. | 1.00 | 1.00 |
| | | 2. | 1.43 | 1.39 | 1.15 | 2. | — | — |
| | | 3. | — | — | — | 3. | — | — |
| Campesterol‖ (24-methyl cholesterol) | 1 | 1. | 1.29 | 1.33 | 1.29 | 1. | 1.14 | 1.20 |
| | | 2. | 1.82 | 1.79 | — | 2. | 2.74 | — |
| | | 3. | — | — | — | 3. | — | — |
| Stigmasterol | 1 | 1. | 1.40 | 1.47 | 1.32 | 1. | 1.14 | 1.20 |
| | | 2. | 1.98 | 1.98 | 0.99 | 2. | 2.68 | — |
| | | 3. | — | — | — | 3. | — | — |
| Sitosterol | 1 | 1. | 1.61 | 1.68 | 1.54 | 1. | 1.32 | 1.42 |
| | | 2. | 2.26 | 2.25 | — | 2. | — | — |
| | | 3. | — | — | — | 3. | — | — |
| Cycloartenol | 3, 4 | 1. | 1.92 | 2.06 | 1.63 | 1. | 1.15 | 1.18 |
| | | 2. | 2.35 | 2.61 | 1.46 | 2. | 2.44 | — |
| | | 3. | — | — | — | 3. | — | — |

*The physical constants (chromatographic and spectral) of these compounds isolated from the three varieties were compared to standards obtained from the following sources: 1. This laboratory; cf. refs [9, 10, 16]. 2. Dr. H. W. Kircher. 3. Dr. G. Manners. 4. Dr. W. R. Nes. 5. Dr. A. P. Tulloch. 6. Reference standard not available; identity confirmed through a comparison of $^1$H NMR and mass spectral data obtained to the literature (cf. [31–33]).

†GLC $RR_{t}c$ retention time relative to cholesterol on the indicated column at 235°; 1: free alcohol; 2: acetate; 3: methyl ether.

‡HPLC $\alpha_c$, retention volumes relative to cholesterol. System I: 96% aq. MeOH, $C_{18}$ column (Ultrasphere-ODS, 5 $\mu$, 4.6 mm i.d. × 250 mm). System II: 90% aq. MeOH, $C_{18}$ column 1, Free alcohol; 2; acetate; 3, methyl ether.

‖The configuration at C-24 for 24-methylcholesterol has not been determined.

the molecules that comprise the series possess both an epimeric and partially antipodal steric arrangement of the ring systems and thus have similar mass spectra, e.g. fernenol, isoarborinol, sorghumol and trematol ([10] and refs cited therein), it is not clear whether the simiarenol from sorghum, or that isolated by the other investigators, has the steric arrangement specified by Alpin *et al.* [17] based on mass spectral analysis alone. Limited $^1$H NMR analysis, e.g. a comparison of the vinyl absorption, may not give enough information to rule out the presence of one stereoisomer versus another. For example, in the ferneol, isoarborinol, sorghumol, trematol series only

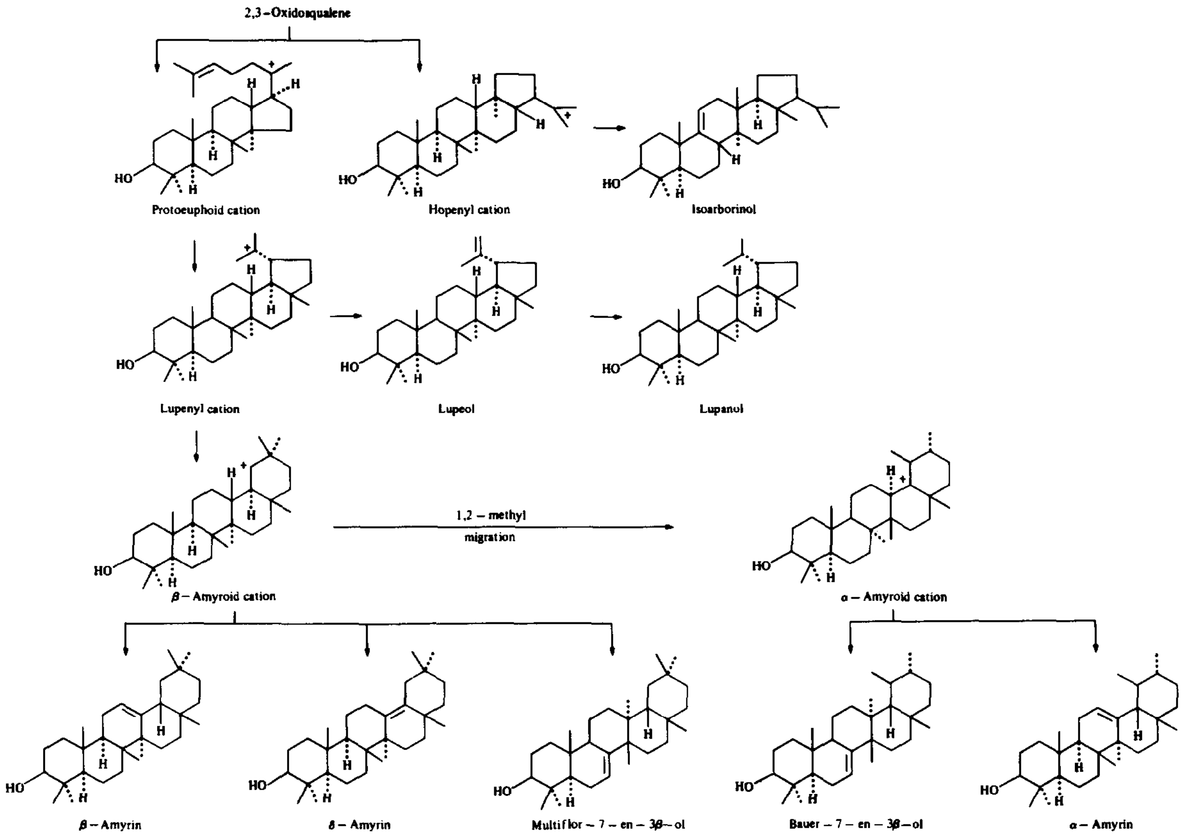

Fig. 1. Biosynthesis of triterpenoids in sorghum.

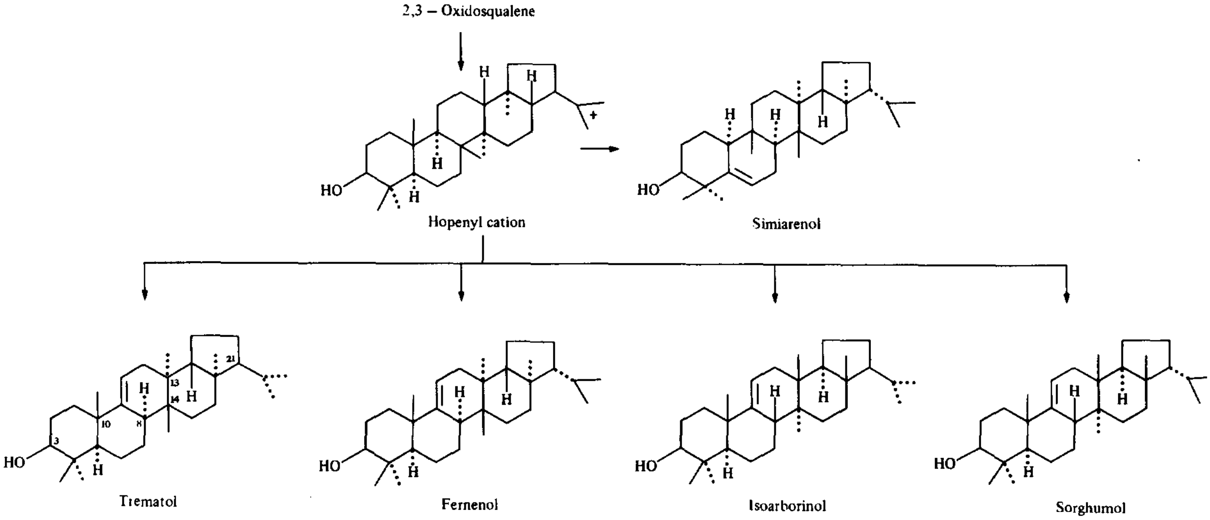

Fig. 2. Structures of the five migrated hopanoids isolated from sorghum. All five of these compounds arise from 2,3-oxidosqualene via the hopenyl cation; only isaorborinol is shown in Fig. 1 for brevity.

three of the four compounds have separable vinyl absorptions [10]. It is probable that in nature a series of D:B-friedo-B′:A′-neogammacer-5-en-3β-ol compounds may also exist. Two of the three varieties of sorghum investigated here contain only one compound of this series at the developmental stage examined. Further investigation into the stereochemistry of the simiarenol isolated from sorghum is in progress.

The PT of G 499 GBR and BOK 8 were detected as free alcohols and esters in both the leaf tissue and surface wax extracts; those of IS 809 were detected as free alcohols, esters and hydrolysable polar derivatives in leaf tissue extracts and as free alcohols and esters in surface wax extracts (Table 1). In addition, traces of C-3 ketone derivatives of each varieties' PT, except that of simiarenol, were found equally distributed between both the leaf tissue and surface wax extracts (data not presented). The ketones were identified through a comparison of their chromatographic and spectral properties (GLC-MS) to those of the corresponding alcohols. In general the $RR_t$s (GLC) of the ketones on 3% OV-17 were 0.94 that of the corresponding alcohols (Table 2). However, the $\alpha_c$s in HPLC system I for the ketones were not as straightforward, e.g. α-amyrone (1.25), β-amyrone (1.15), fernenone (1.75), isoarborinone (1.75), sorghumone (2.05) and trematone (2.05); the trace levels of δ-amyrone precluded the determination of its $\alpha_c$. The mass spectra of α- and β-amyrone were characterized by an $[M]^+$ peak at $m/z$ 424 and a base peak at $m/z$ 218; a lack of $[M-H_2O]^+$ and $[M-H_2O-Me]^+$ peaks in spectra that were otherwise similar to those of the free alcohols further characterized these molecules. δ-Amyrone was identified by the presence of a strong $[M]^+$ peak at $m/z$ 424, a base peak at $m/z$ 205 and the lack of $[M-H_2O]^+$ and $[M-H_2O-Me]^+$ peaks in its spectrum. Fernenone, isoarborinone, sorghumone and trematone were identified from spectra containing $[M]^+$ peaks at $m/z$ 424, strong $m/z$ 409 $[M-Me]^+$ and 271 peaks and base peaks at $m/z$ 257 while lacking $[M-H_2O]^+$ and $[M-H_2O-Me]^+$ peaks in spectra that were otherwise similar to those of the free alcohols. Endogenous C-3 methyl ether derivatives of the PT were not detected in the leaf tissue or surface wax of the three varieties. The amounts and distribution of the PT (both free alcohol and derivatives) in the leaf tissue and surface wax of G 499 GBR and BOK 8 were similar with only minor differences in the free : ester ratios (Table 1). The PT content of IS 809 not only differed qualitatively but also quantitatively from that of G 499 GBR and BOK 8; the total PT content (free and derivatized forms) for IS 809 leaf tissue was *ca* 79% and the surface wax *ca* 55% that of the other two varieties (Table 1). The inability to detect four of the five migrated hopanoids in IS 809 was further investigated through a 7-day-feed of $[2\text{-}^{14}C]$acetate to leaves from a plant at the same developmental stage as those used in the identification study [25]. Only the $[^{14}C]$-labelled PT α-amyrin, β-amyrin, δ-amyrin and sorghumol were recovered from the nonsaponifiable lipid fraction (NLF) of the TLE from the treated leaves. No mass or radioactivity could be associated with fernenol, isoarborinol or simiarenol during the purification and separation of the PT fraction. From the data we can conclude that IS 809, at the developmental stage investigated, is not actively biosynthesizing four of the five migrated hopanoids present in G 499 GBR and BOK 8. Further investigation of the three varieties at different developmental stages are in progress to fully assess their ability to synthesize the migrated hopanoids.

From the distribution data for the three varieties (Table 1), we predict that the sterol profile is the same for all members of the subspecies *bicolor*. Since the subspecies *bicolor* is the cultivated taxa, it is likely that the sterol profile is the same for the other two subspecies of *Sorghum bicolor*. Unlike other chemotaxonomic lipid characters (e.g. carotenoids), sterols may not vary due to their inherent functional roles [26–28]. Therefore their biosynthesis appears to be more tightly regulated than that of the PT; the genetic information (structural genes and regulatory genes) for key enzymes involved in their synthesis is probably ubiquitous. Ohmoto *et al.* [4] examined 56 species of Graminae and found that all the species, including two of sorghum, contained campesterol, sitosterol and stigmasterol as the major sterol end products. The variance in the PT profiles at the varietal level (Table 1) [11–13] may be due to less stringent regulation of their biosynthesis, e.g. the cyclization of 2,3-oxidosqualene to the individual PTs (Figs 1 and 2). To date the only major speculative function for PT in higher plants is as a membrane component [26]. Additionally, there appears to be no clear relationship between sterol and PT composition and parasite resistance; G 499 GBR (aphid-resistant) and BOK 8 (aphid-susceptible) have essentially the same sterol and PT profile. Although the PT profile of the two tall varieties G 499 GBR and Bok 8 were very similar and significantly different from that of IS 809 (short variety), a correlation of PT composition and shoot height must await further analysis of the interaction of sorghum PT and endogenous growth regulators, e.g. gibberellins, during plant development.

In conclusion, we believe that pentacyclic triterpenes, unlike sterols, have greater applicability as chemotaxonomic indices for *intraspecific* rather than *interspecific* relationships in sorghum.

## EXPERIMENTAL

For reverse phase HPLC, an Altex model 110A pump (Altex, Berkeley, CA) was connected through a model 7125 (Rheodyne) injector to a new column of Ultrasphere-ODS (5 μm dp, 4.6 mm × 250 mm). The eluent was 96% (system I) or 90% (system II) aq. MeOH. The flow rate was 1.6 ml/min; the detector was a Hitachi variable-wavelength spectrometer (Altex model 155) set at 205 nm. HPLC retention times were expressed relative to cholesterol, $\alpha_c$ [29]. For GLC, samples were dissolved in EtOH (sterols) or $C_6H_6$ (PT) and aliquots (2 μl vol.) injected onto the packed columns (injection port temp. 240°). Samples were analysed isothermally (235°). Helium was the carrier gas (flow rate 30 ml/min). The flame ionization detector (FID) was held at 310° ($H_2$ flow, 30 ml/min; air flow, 400 ml/min). Three different packed columns were used representing a significant range of polarity and McReynolds constants: 3% SE-30 (on gas chrom Q, 100/120 mesh, 1.8 m × 2 mm i.d.), 3% OV-17 (on gas chrom Q, 100/120 mesh, 1.8 m × 2 mm i.d.) and 1% SP-1000 (on Supelcoport, 100/120 mesh, 1.8 m × 2 mm i.d.). GLC retention times were expressed relative to that of cholesterol. Plots of increasing amounts of cholesterol (GLC) and $[^{14}C]$cholesterol (HPLC, system I) verses detector response were linear between 64 ng and 4 μg (GLC) and 1.0 μg and 70 μg (HPLC). GLC plots were used to quantitate the triterpene alcohols in each sample. Recovery of $[^{14}C]$cholesterol from the HPLC column was > 97%. EI/MS (70 eV) were taken with a GLC-MS or with probe injection. GLC-MS conditions were as follows: samples, dissolved in EtOH (sterols) or $C_6H_6$ (PT), were injected (Grog splitless injection, injector temp. 280°) onto a fused silica capillary column (WCOT, 007 methylsilicone, film thickness 0.25 μ, 13.5 m × 0.32 mm i.d., Quadrex, New Haven, CT). The column was held at 90° for 0.5 min, programmed to 225° at 30°/min and then taken to 280° at 3°/min. The flow rate was 2 ml/min. The electron emission current was 200 μA, the source temp. 140° and the separator temp. 270°. MS scanning was begun immediately after injection and the spectra recorded from 15 to 600 amu at 1.5 sec/decade. $^1H$ NMR spectra were obtained with a Nicolet

200 operating in the Fourier transform (FT) mode at 200 MHz. Samples were dissolved in $CDCl_3$ and TMS added as an internal standard; signal location is expressed in $\delta$ units (ppm) relative to TMS. Spectra were obtained at room temp. (22–23°). Assignments, when given, were confirmed through shift reagent studies.

Seeds from the commercially available variety of *Sorghum biocolor* (L.) Moench, cv G 499 GBR, were obtained from Funk Seeds International (Bloomington, IL). Seeds of the breeding line IS 809 and BOK 8 were obtained from Dr. D. Dreyer, USDA/ARS, WRRC Albany, CA. These varieties were originally developed for parasite resistance (Aphid); G 499 GBR and IS 809 are classified as resistant and BOK 8 as susceptible. Plants were grown for 66 days (25° ± 3°, 70 ± 10% relative humidity, 16 hr light per 24 hr at 15 000 lux) in pots. Under these conditions G 499 GBR and BOK 8 were approximately the same in height (63 cm), whereas IS 809 was considerably shorter (36 cm) with thinner leaves. The inflorescences of each were just emerging from the sheaths with their anthers not extruded from their florets. We have operationally defined 'leaf tissue' (LT) as sheath, ligule, blade and surface wax.

*Large scale extraction, purification and identification of polycyclic isopentenoids from the leaf tissue of G 499 GBR.* Leaves from G 499 GBR (1.6 kg fr. wt, 229.6 g dry wt) were oven-dried (80°, 24 hr) and homogenized with $Me_2CO$ in a high speed polytron. After filtering, the homogenized leaf tissue was exhaustively extracted with $Me_2CO$ in a Soxhlet apparatus (18 hr). The filtrate and extract were taken to dryness to give the total lipid extract (TLE) (10.3 g). A portion of this fraction (TLE) (6.66 g) was saponified with 10% methanolic KOH (w/v, 100 ml) under reflux for 1 hr [30]. An equal vol. of $H_2O$ was added to the methanolic mixture and the mixture was extracted with three equal portions of $Et_2O$. The pooled $Et_2O$ extract was dried over $Na_2SO_4$ and then concd under red. pres. to give the non-saponifiable lipid fraction (NLF). The NLF (1.03 g) was fractionated by adsorption chromatography on a column (38 cm × 3 cm i.d.) of 3% deactivated $Al_2O_3$ (3 g $H_2O$ and 97 g alumina W200 neutral, activity grade super 1) eluted with increasing amounts (10% increments of 100 ml each) of $Et_2O$ in $C_6H_{14}$ [30]. Fractions (100 ml) were collected, dried *in vacuo* and aliquots analysed by GLC. Pentacyclic triterpene (PT) (free alcohols) and long chain fatty alcohols (LCFA) eluted in the 30% $Et_2O$ in $C_6H_{14}$ fraction (300 mg). The PT were purified from this fraction by reversed-phase chromatography on LH-20 Sephadex. The column was pre-eluted isocratically with 5% MeOH in $C_6H_{14}$ (1 l. at 0.625 ml/min, ambient temp.). A portion of the fraction (150 mg) dissolved in minimal mobile phase, was added to the the column and the column eluted isocratically with 5% MeOH in $C_6H_{14}$ (initial flow rate 0.625 ml/min, *ca* 22° and 1 atm). PT eluted in 420–490 ml, while the long chain fatty alcohol mixture ($C_{22}$–$C_{30}$) eluted in 220–280 ml. The purified PT mixture (120 mg) was acetylated and fractionated over activated ( × 3 at 70° for 10 min) $AgNO_3$–Anacil B (20 g $AgNO_3$ in 10 ml $H_2O$ added to 100 g Anacil B) eluted with the following gradient: 100 ml each of 0%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30% $C_6H_6$ in $C_6H_{14}$. Fractions (20 ml) were collected and analysed by GLC. The isolated PT were deacetylated and further separated and purified by reverse phase HPLC using system I and where necessary system II (cf. Table 2). The isolated PT eluted from the $AgNO_3$–Anacil B column were identified by GLC-MS and $^1H$ NMR as follows. The major components of each fraction are indicated by an asterisk (*).

10% $C_6H_6$ in $C_6H_{14}$ (40–60 ml). $\delta$-Amyrin, MS free alcohol $m/z$ (rel. int.): 426 $[M]^+$ (100), 411 (12), 408 (5), 393 (11), 229 (42), 221 (20), 218 (52), 207 (16), 206 (16), 205 (57), 204 (61), 203 (52), 202 (17), 191 (29), 189 (48), 175 (39), 161 (46), 147 (37), 135 (41), 133 (12), 123 (38), 121 (35), 119 (44), 109 (43), 107 (37). MS acetate $m/z$ (rel. int.): 468 $[M]^+$ (100), 453 (2), 425 (4), 409 (8), 408 (18), 393 (5), 365 (6), 275 (5), 249 (5), 229 (33), 218 (49), 207 (2), 206 (12), 205 (59), 204 (56), 203 (77), 202 (14), 191 (29), 189 (63), 175 (40), 161 (53), 149 (32), 147 (29), 135 (32), 133 (27), 123 (22), 121 (28), 119 (31), 109 (39), 107 (25). $^1H$ NMR free alcohol: $\delta$0.783 (3H, *s*), 0.873 (3H, *s*), 0.905 (3H, *s*), 0.923 (3H, *s*), 0.928 (3H, *s*), 0.978 (3H, *s*), 0.992 (6H, *s*), 1.066 (3H, *s*), 3.95 (1H, *m*, $W_{1/2} = 12$ Hz), 5.35 (1H, *m*, $W_{1/2} = 6$ Hz).

$\alpha$-Amyrin, MS free alcohol $m/z$ (rel. int.): 426 $[M]^+$ (9), 411 (2), 408 (0.5), 218 (100), 207 (7), 205 (2), 204 (2), 203 (11), 191 (1.44), 189 (10), 175 (4), 161 (5), 147 (8), 135 (13), 133 (13), 123 (10), 121 (10), 109 (12), 107 (12). MS acetate $m/z$ (rel. int.): 468 $[M]^+$ (16), 453 (3), 408 (3), 294 (4), 218 (100), 205 (5), 204 (7), 203 (16), 189 (16), 175 (6), 161 (11), 147 (10), 133 (12), 123 (10), 121 (11), 119 (11), 109 (12), 107 (13). $^1H$ NMR free alcohol: $\delta$0.790 (3H, *d*, $J = 6$ Hz, H-29), 0.793 (3H, *s*, H-24), 0.799 (3H, *s*, H-28), 0.910 (3H, *br s*, $J < 5$ Hz, H-30), 0.954 (3H, *s*, H-25), 0.998 (3H, *s*, H-23), 1.008 (3H, *s*, H-26), 1.070 (3H, *s*, H-27), 3.320 (1H, *q*, $J_{2\alpha,3\alpha} = 10$ Hz, $J_{3\alpha,2\beta} = 6$ Hz, H-3$\alpha$), 5.130 (1H, *t*, $J = 4$ Hz, H-12). $^1H$ NMR acetate: $\delta$0.795 (3H, *d*, $J = 6$ Hz, H-29), 0.797 (3H, *s*, H-28), 0.867 (3H, *s*, H-23), 0.874 (3H, *s*, H-24), 0.911 (3H, *br s*, H-30), 0.978 (3H, *s*, H-25), 1.008 (3H, *s*, H-26), 1.064 (3H, *s*, H-27), 2.050 (3H, *s*, H-3$\beta$, Ac), 4.510 (1H, *m*, $W_{1/2} = 12$ Hz, H-3$\alpha$), 5.130 (1H, *t*, $J = 4$ Hz, H-12). $^1H$ NMR methyl ether: 0.776 (3H, *s*, H-24), 0.794 (3H, *d*, $J = 6$ Hz, H-29), 0.800 (3H, *s*, H-28), 0.911 (3H, *br s*, H-30), 0.954 (3H, *s*, H-25), 0.981 (3H, *s*, H-23), 1.006 (3H, *s*, H-26), 1.068 (3H, *s*, H-27), 2.675 (1H, *dd*, $J_{2\alpha,3\alpha} = 12$ Hz, $J_{3\alpha,2\beta} = 4$ Hz, H-3$\alpha$), 3.362 (3H, *s*, H–Me–O–R), 5.130 (1H, *t*, $J = 4$ Hz, H-12).

Fernenol*, MS free alcohol $m/z$ (rel. int.): 426 $[M]^+$ (71), 411 (100), 408 (3), 393 (20), 356 (1), 341 (1), 323 (1), 286 (1), 273 (13), 259 (95), 255 (12), 247 (11), 241 (42), 229 (10), 220 (1), 218 (2), 215 (6), 205 (11), 202 (1), 201 (7), 191 (3), 163 (9), 152 (2), 133 (15), 121 (8), 109 (31), 107 (28), MS acetate $m/z$ (rel. int.): 468 $[M]^+$ (47), 453 (90), 439 (2), 409 (4), 408 (9), 393 (44), 365 (2), 315 (9), 301 (100), 289 (10), 273 (6), 271 (6), 257 (10), 255 (23), 243 (23), 241 (72), 229 (18), 221 (1), 219 (1), 215 (9), 205 (13), 201 (10), 191 (3), 189 (15), 187 (13), 179 (3), 173 (13), 165 (6), 163 (11), 161 (16), 149 (18), 147 (20), 137 (55), 135 (33), 133 (26), 121 (41), 119 (46), 109 (51), 107 (40), MS methyl ether $m/z$ (rel. int.): 440 $[M]^+$ (63), 425 (100), 411 (1), 409 (1), 408 (1), 393 (42), 368 (1), 341 (1), 339 (2), 323 (2), 311 (2), 297 (3), 287 (8), 281 (7), 274 (17), 273 (76), 271 (3), 259 (2), 257 (6), 255 (23), 247 (1), 243 (20), 241 (64), 229 (14), 221 (1), 217 (6), 215 (8), 207 (6), 206 (2), 205 (10), 202 (3), 201 (8), 189 (13), 187 (11), 173 (11), 161 (12), 159 (17), 149 (14), 147 (17), 145 (14), 137 (23), 135 (20), 131 (15), 123 (15), 121 (21), 119 (22), 109 (31), 107 (25). $^1H$ NMR free alcohol, acetate, methyl ether: see ref. [10].

Isoarborinol*, MS free alcohol $m/z$ (rel. int.): 426 $[M]^+$ (68), 411 (100), 408 (3), 393 (18), 356 (0), 341 (1), 323 (3), 286 (1), 273 (14), 259 (88), 255 (10), 247 (10), 241 (8), 229 (9), 220 (1), 218 (2), 215 (6), 205 (11), 202 (2), 201 (8), 191 (8), 163 (17), 152 (2), 133 (19), 121 (31), 109 (33), 107 (31). MS acetate $m/z$ (rel. int.): 468 $[M]^+$ (50), 453 (100), 439 (2), 409 (5), 408 (8), 393 (48), 365 (4), 315 (12), 301 (100), 289 (10), 273 (6), 271 (6), 255 (21), 243 (20), 241 (58), 229 (16), 221 (1), 219 (3), 215 (10), 205 (16), 201 (11), 191 (9), 189 (16), 187 (14), 179 (6), 173 (14), 165 (8), 163 (21), 161 (16), 149 (19), 147 (22), 135 (34), 137 (54), 133 (28), 121 (42), 119 (47), 109 (49), 107 (46), MS methyl ether $m/z$ (rel. int.): 440 $[M]^+$ (64), 425 (100), 411 (2), 409 (1), 408 (2), 393 (49), 368 (1), 341 (2), 339 (1), 323 (3), 311 (2), 297 (3), 287 (11), 281 (7), 274 (17), 273 (73), 271 (6), 259 (2), 257 (8), 255 (20), 247 (1), 243 (17), 241 (56), 229 (13), 221 (2), 217 (4), 215 (8), 207 (18), 206 (4), 205 (10), 202 (7), 201 (9), 189 (12), 187 (12), 173 (13), 161 (12), 159 (17), 149 (4), 147 (17), 145 (15), 137 (28), 135 (22), 133 (12), 131 (14), 123 (18), 121 (25), 119 (25), 109 (34), 107 (25). $^1H$ NMR free alcohol, acetate, methyl ether: see ref. [10].

10% $C_6H_6$ in $C_6H_{14}$ (60–80 ml). $\beta$-Amyrin, MS free alcohol $m/z$ (rel. int.): 426 $[M]^+$ (7), 411 (2.1), 408 (0.5), 393 (0.5), 218 (100), 207 (2), 205 (5), 204 (6), 203 (24), 191 (0.5), 189 (5), 175 (5), 161 (4), 147 (5), 135 (8), 133 (4), 123 (16), 121 (19), 119 (9), 109 (7), 107 (8). MS acetate $m/z$ (rel. int.): 468 $[M]^+$ (6), 453 (1), 426 (2), 408 (2), 393 (2), 249 (3), 218 (100), 205 (4), 204 (8), 203 (24), 191 (2), 189 (9), 175 (6), 161 (6), 147 (6), 133 (6), 123 (5), 121 (8), 109 (10), 107 (10). MS methyl ether $m/z$ (rel. int.): 440 $[M]^+$ (7), 425 (1), 272 (1), 271 (1), 257 (1), 255 (1), 224 (4), 221 (7), 220 (2), 219 (17), 218 (100), 207 (1), 205 (2), 204 (4), 203 (19), 201 (1), 190 (9), 189 (8), 187 (1), 175 (4), 161 (3), 147 (4), 135 (6), 131 (2), 121 (5), 119 (4), 109 (5), 107 (5). $^1$H NMR free alcohol: $\delta$0.788 (3H, *s*, H-24), 0.826 (3H, *s*, H-28), 0.866 (6H, *s*, H-29, H-30), 0.932 (3H, *s*, H-25), 0.960 (3H, *s*, H-23), 0.992 (3H, *s*, H-26), 1.128 (3H, *s*, H-27), 3.220 (1H, *m*, $W_{1/2} = 12$ Hz, H-32), 5.185 (1H, *t*, $J = 4$ Hz, H-12). $^1$H NMR acetate: $\delta$0.829 (3H, *s*, H-28), 0.872 (12H, *s*, H-23, 24, 29, 30), 0.965 (6H, *s*, H-25, 26), 1.128 (3H, *s*, H-27), 2.050 (3H, *s*, H-3$\beta$ Ac), 4.500 (1H, *m*, 3$\alpha$-H), 5.180 (1H, *t*, $J = 4$ Hz, H-12). $^1$H NMR methyl ether: $\delta$0.774 (3H, *s*, H-24), 0.832 (3H, *s*, H-28), 0.872 (6H, *s*, H-29, 30), 0.939 (3H, *s*, H-25), 0.965 (3H, *s*, H-23), 0.981 (3H, *s*, H-26), 1.134 (3H, *s*, H-27), 2.67 (1H, *dd*, $J_{2\alpha,3\alpha} = 12$ Hz, $J_{3\alpha,2\beta} = 4$ Hz, H-3$\alpha$), 3.365 (3H, *s*, H-3$\beta$ OMe), 5.185 (1H, *t*, $J = 4$ Hz, H-12). $\alpha$-Amyrin*, fernenol*, isoarborinol*; spectral data as above.

10% $C_6H_6$ in $C_6H_{14}$ (80–100 ml). $\beta$-Amyrin*, $\alpha$-amyrin, fernenol, isoarborinol; spectral data as above.

11% $C_6H_6$ in $C_6H_{14}$ (20–40 ml). Trematol*, MS free alcohol $m/z$ (rel. int.): 426 $[M]^+$ (49), 411 (100), 408 (4), 393 (20), 356 (0), 341 (0), 323 (2), 286 (6), 273 (14), 271 (13), 259 (86), 255 (13), 247 (8), 241 (28), 229 (11), 220 (1), 218 (2), 215 (5), 205 (12), 202 (2), 201 (8), 191 (4), 163 (12), 152 (3), 133 (18), 121 (30), 109 (33), 107 (33). MS acetate $m/z$ (rel. int.): 468 $[M]^+$ (64), 453 (100), 439 (2), 409 (5), 408 (13), 393 (40), 365 (6), 339 (2), 315 (9), 301 (83), 289 (8), 273 (6), 271 (10), 257 (10), 255 (20), 243 (19), 241 (42), 229 (13), 221 (1), 219 (2), 215 (6), 205 (14), 201 (9), 191 (4), 189 (11), 187 (12), 179 (2), 173 (13), 165 (5), 163 (10), 161 (14), 159 (17), 151 (14), 149 (20), 147 (19), 137 (46), 135 (35), 133 (27), 123 (25), 121 (35), 119 (36), 109 (42), 107 (49). MS methyl ether $m/z$ (rel. int.): 440 $[M]^+$ (85), 425 (100), 411 (2), 409 (0), 408 (5), 393 (38), 368 (0), 341 (0), 339 (1), 323 (2), 311 (1), 297 (2), 287 (10), 281 (1), 274 (15), 273 (69), 271 (11), 259 (4), 257 (8), 255 (17), 247 (1), 243 (18), 241 (2), 229 (13), 221 (1), 217 (5), 215 (7), 207 (3), 206 (8), 205 (12), 203 (8), 201 (9), 189 (12), 187 (12), 173 (12), 161 (13), 159 (15), 151 (12), 149 (18), 147 (19), 145 (16), 137 (36), 135 (30), 133 (23), 131 (14), 123 (23), 121 (28), 119 (34), 109 (38), 107 (38). $^1$H NMR free alcohol, acetate, methyl ether: see ref. [10].

12% $C_6H_6$ in $C_6H_{14}$ (0–60 ml). Sorghumol*, MS free alcohol $m/z$ (rel. int.): 426 $[M]^+$ (94), 411 (100), 408 (1), 393 (21), 356 (3), 341 (10), 323 (6), 286 (5), 273 (21), 259 (75), 255 (13), 247 (10), 241 (25), 229 (12), 220 (2), 218 (3), 215 (9), 205 (10), 202 (3), 201 (9), 191 (30), 163 (25), 152 (2), 133 (27), 121 (38), 109 (49), 107 (43). MS acetate $m/z$ (rel. int.): 468 $[M]^+$ (100), 453 (93), 439 (2), 409 (6), 408 (16), 393 (46), 383 (8), 365 (4), 339 (5), 323 (10), 315 (8), 301 (64), 289 (8), 273 (13), 271 (11), 257 (13), 255 (17), 243 (16), 241 (31), 229 (14), 221 (1), 219 (6), 215 (13), 205 (10), 201 (10). 191 (26), 189 (11), 187 (13), 179 (17), 173 (13), 165 (21), 163 (25), 161 (18), 149 (18), 147 (21), 137 (41), 135 (32), 133 (25), 121 (38), 119 (43), 109 (45), 107 (39). MS methyl ether $m/z$ (rel. int.): 440 $[M]^+$ (100), 425 (69), 411 (2), 409 (3), 408 (5), 393 (41), 368 (1), 365 (4), 341 (1), 339 (3), 323 (6), 311 (4), 297 (2), 287 (8), 281 (1), 274 (6), 273 (43), 271 (5), 259 (3), 257 (9), 255 (14), 247 (1), 243 (10), 241 (26), 229 (10), 221 (1), 217 (4), 215 (6), 207 (1), 206 (5), 205 (5), 202 (3), 201 (8), 191 (14), 189 (8), 187 (11), 173 (13), 161 (11), 159 (14), 149 (13), 147 (14), 145 (15), 137 (24), 135 (25), 131 (13), 123 (18), 121 (25), 119 (27), 109 (31), 107 (28). $^1$H NMR free alcohol, acetate, methyl ether: see ref. [10].

25% $C_6H_6$ in $C_6H_{14}$ (0–100 ml). Cycloartenol (trace), MS free alcohol $m/z$ (rel. int.): 426 $[M]^+$ (17), 411 (13), 408 (23), 393 (20), 365 (11), 339 (8), 313 (3), 297 (3), 286 (19), 274 (4), 271 (6), 259 (6), 243 (3), 231 (4), 217 (6), 205 (11), 203 (11), 189 (8), 187 (10), 175 (17), 161 (13), 159 (10), 149 (17), 147 (14), 135 (16), 121 (22), 109 (37), 107 (33), 69 (100). $^1$H NMR free alcohol: $\delta$0.333 (1H, *d*, $J = 4.5$ Hz, C-19), 0.555 (1H, *d*, $J = 4.5$ Hz, C-19), 0.809 (3H, *s*, C-31), 0.872 (3H, *d*, $J = 6.5$ Hz, C-21), 0.889 (3H, *s*, C-32), 0.965 (6H, *s*, C-30 and C-18), 1.605 (3H, *br s*, C-26), 1.687 (3H, *br s*, C-27); 3.280 (1H, *m*, $W_{1/2} = 12$ Hz, 3$\alpha$-H), 5.105 (1H, *t*, $J = 8$ Hz, C-24).

30% $C_6H_6$ in $C_6H_{14}$ (0–100 ml). Simiarenol*, MS free alcohol $m/z$ (rel. int.): 426 $[M]^+$ (7), 411 (3), 408 (88), 393 (48), 391 (7), 383 (1), 365 (6), 323 (2), 286 (2), 274 (100), 259 (52), 255 (7), 245 (8), 231 (16), 218 (4), 205 (16), 204 (13), 191 (13), 189 (23), 187 (21), 163 (16), 161 (29), 152 (27), 145 (22), 136 (26), 134 (87), 121 (64), 107 (49). $^1$H NMR free alcohol: $\delta$0.780 (3H, *s*), 0.828 (3H, *d*, $J = 6.5$ Hz), 0.887 (3H, *d*, $J = 6.5$ Hz), 0.894 (3H, *s*), 0.925 (3H, *s*), 1.005 (3H, *s*), 1.045 (3H, *s*), 1.141 (3H, *s*), 3.475 (1H, *m*, $W_{1/2} = 8$ Hz, 3$\alpha$-H), 5.625 (1H, *t*, $J = 4$ Hz).

The mass spectral and $^1$H NMR data cited for each PT were identical to that obtained from standard compounds available to the author from several sources (cf Table 2). Identities for each were confirmed independently through decoupling, shift reagent and temperature studies using $^1$H NMR and $^{13}$C NMR [Nes, W. D. and Heupel, R. C., unpublished].

*Sterols.* Sterols from the NLF eluted in the 60% $Et_2O$ in $C_6H_{14}$ fraction of the $Al_2O_3$ column. Quantitation by GLC (Table 1) of this fraction showed four sterols: cholesterol, campesterol, sigmasterol and sitosterol. The fraction was purified by adsorption TLC (silica gel G eluted with $C_6H_6$–$Et_2O$, 9:1). The sterols were identified by GLC-MS; their MS were identical to those reported for the sterols from IS 809 leaves [9].

*Small scale extraction of polycyclic isopentenoids from leaves.* Leaf tissue (plus wax) from G 499 GBR, BOK 8 and IS 809 were oven-dried (43 g dry wt, 10.61 g dry wt and 1.95 g dry wt, respectively) and crushed to a fine powder in mortar and pestle. The powdered tissues were exhaustively extracted with $Me_2CO$ in a Soxhlet apparatus (18 hr). The extracts were taken to dryness to give their respective TLEs (1.15 g, 823.23 mg, 87.07 mg, respectively).

*Extraction of polycyclic isopentenoids from leaf surface wax.* Fresh leaves from G 499 GBR (56.4 g fr. wt, *ca* 8.51 g dry wt), BOK 8 (58.6 g fr. wt. *ca* 10.70 g dry wt) and IS 809 (21.14 g fr wt; *ca* 3.14 g dry wt) were swirled in 800 ml $C_6H_{14}$ for 2 min. The $C_6H_{14}$ extracts were filtered through Whatman paper # 1 and taken to dryness (G 499 GBR, 26.83 mg; BOK 8, 34.47 mg; IS 809, 21.40 mg).

*Analysis of free and derivatized polycyclic isopentenoids.* The leaf tissue TLE and surface wax extracts from G 499 GBR, BOK 8 and IS 809 were separated into six principle fractions by preparative TLC on silica gel G eluted with $C_6H_6$–$Et_2O$ (9:1); polar derivatives ($R_f$ 0.0–0.1), free sterols ($R_f$ 0.3), free PT ($R_f$ 0.5), free simiarenol ($R_f$ 0.6), PT ketones ($R_f$ 0.8) and esters ($R_f$ 0.9–1.0). Note that the polar derivative fraction may contain glycosides and that the ester fraction may contain C-3 methyl ether and/or cinnamate derivatives. Samples were applied as a streak (0.5 mg/cm) to the plates. The polycyclic isopentenoids were visualized using $H_2SO_4$–EtOH (50% w/v) and heat. Samples were eluted from the absorbant by successive extraction with $Me_2CO$, $C_6H_6$ and $Et_2O$. The free sterols, PT and PT ketones were quantitated by GLC (on three packed columns) and identified by GLC-MS.

The polar derivative conjugates were hydrolysed as follows: the fractions were dissolved in 6 N HCl in 40% aq. MeOH and heated at 65° for 2 hr. Equal amounts of $H_2O$ were added to each and the hydrolysate partitioned three times against equal vols of $Et_2O$. The combined $Et_2O$ fractions from each hydrolysate were washed with $H_2O$ and dried *in vacuo*. Ester fractions were hydrolysed under basic conditions; the fractions were dissolved in 0.5 N KOH in 90% aq. MeOH and heated for 1 hr at 65°. The hydrolysates were extracted as described for the polar conjugate hydrolysates. C-3 methyl ether derivatives, if present, would not be hydrolysed under these conditions. These hydrolysed fractions were then chromatographed in the described TLC system to obtain free sterol, PT and PT ketone fractions which were examined by GLC. In the described TLC system C-3 methyl ether derivatives would have eluted near the solvent front. Neither PT nor sterol C-3 methyl ethers were detected by GLC-MS analysis of these fractions from the three varieties. Methyl ethers, and other compounds, could have been quantified, and their identities confirmed by MS, even if they were present at levels as low as 0.01% of the mixture injected onto the GLC columns.

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