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STEROLS OF AMARANTHACEAE

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Key Word Index—*Amaranthus*; *Celosia*; Amaranthaceae; cholesterol; 7,22-ergostadienol; campesterol; stigmasterol; 7,24(28)-ergostadienol; 7-ergostenol; spinasterol; stigmastanol; 7,25-stigmastadienol.

Abstract—Nineteen species and varieties of Amaranthaceae were analysed for sterol composition. The desmethyl sterol content of these plants varied from 0.0084% to 0.034% of the total dry weight. Spinasterol and 7-stigmastenol were the dominant sterols in all species, although low levels of 5-unsaturated sterols were detected. Minor sterols identified in one or more species included cholesterol, campesterol, stigmasterol and sitosterol as well as 7,22-ergostadienol, 7,24(28)-ergostadienol, 7-ergostenol, 7,25-stigmastadienol and 7,24(28)-stigmastadienol. Stigmastanol and 24-methylenecycloartanol were also present. Identifications were based on a combination of gas chromatography, high performance liquid chromatography and gas chromatography/mass spectrometry.

INTRODUCTION

Both lower tracheophytes [1–3] and most higher plants investigated contain primarily 24α-alkyl-sterols with 5(6)-unsaturation. In 1932, Hart [4] identified spinasterol, a 24-alkyl-sterol with 7(8)-unsaturation, as the dominant sterol species in *Spinacea oleracea*. Subsequently 7(8)-sterols were reported to be dominant in tea [5], cucurbits [6] and alfalfa [7], plants considered relatively distant phylogenetically. Labelling experiments with yeast and animals suggested that desaturation of the sterol nucleus follows a $\Delta^8 \rightarrow \Delta^7 \rightarrow \Delta^{5,7} \rightarrow \Delta^5$ sequence [8, 9]. It was therefore proposed that high levels of 7(8)-sterols result from a genetic block [10] or from imperfect control of the enzymes catalysing 5(6)-sterol synthesis [11].

Recently, spinasterol and 7-stigmastenol have been identified as major sterols in horse chestnut [12], *Phytolacca esculenta* [13], *Beta vulgaris* [14] and *Chenopodium album* [15]. Salt and Adler have recently examined 13 species in the Chenopodiaceae, order Caryophyllales, many of which were '7(8)-plants' [16]. This concentration of '7(8)-plants' in Caryophyllales led us to survey sterol composition in the closely related family, Amaranthaceae. The distribution of 7(8)-sterols could serve as a chemotaxonomic marker and perhaps provide insight into the nature of 7(8)-sterol synthesis and accumulation.

In this paper, results of sterol analyses of 19 species and varieties of Amaranthaceae, order Caryophyllales are reported.

RESULTS AND DISCUSSION

GC and HPLC retention data used for sterol identification are summarized in Table 1. Identifications based on chromatographic behavior were supplemented by MS of the sterol acetates from *Amaranthus tricolor*, a species containing all major sterol peaks observed on GC in 19 Amaranthaceae species. Relative retention times (RR_t) of known sterols and those tentatively identified on SE-30 were in agreement with published data [17].

The GC tracing of the acetate derivatives of *A. tricolor* sterols gave 13 peaks which were analysed by GC/MS. The identification of peak 1 as cholesterol acetate was based on RR_t data (Table 1) and on its mass spectroscopy pattern, which was characterized by the absence of a molecular ion, and by *m/z* 368 (100), 353 (14), 255 (12) and 247 (21). Three other 5(6)-compounds identified in *A. tricolor* were campesterol acetate (peak 3), stigmasterol acetate (peak 4) and sitosterol acetate (peak 8). Peak 2 was identified as 7,22-ergostadienol acetate by direct comparison with an authentic standard on SE-30 and on GC/MS, although the configuration of compound 2 at C-24 could be a 24*R*-24*S* mixture. The mass fragmentation pattern obtained from peak 2 was characterized by *m/z* 440 (72), 425 (17), 397 (8), 380 (10), 365 (13), 355 (6), 342 (50), 313 (50), 288 (11), 255 (29), 229 (15) and 213 (15).

Peak 5 was tentatively identified as 7,24(28)-ergostadienol acetate based on its RR_t data (Table 1) and MS fragmentation pattern: *m/z* 440 (25), 425 (26), 380 (5), 342 (4), 313 (100), 255 (33) and 213 (33). On OV-17 and SP-2330, peak 5 migrated with peak 6 (7-ergostenol acetate). The amounts of these compounds present were determined from GC on DB-1.

Peak 6 was one of the major sterols isolated from *A. tricolor* and was identified as 7-ergostenol acetate. The MS from peak 6 showed the presence of the following signals: *m/z* 442 (72), 427 (27), 382 (7), 367 (8), 315 (11), 255 (100) 229 (3) and 213 (19). The RR_t data (Table 1) also support this identification for peak 6. Peak 7, spinasterol acetate, was the most abundant compound present. The identification was based on its RR_t data (Table 1) and its mass spectrum: *m/z* 452 (71), 439 (14), 411 (14), 394 (11), 379 (13), 342 (50), 313 (69), 255 (38) and 213 (12).

Peak 9 was identified as stigmastanol acetate based on its RR_t data and the following aspects of its mass

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Table 1. GLC and HPLC relative retention data of individual sterols from *Amaranthus tricolor* leaves

Sterol	OV-17*	SP-2330†	SE-30‡	DB-1§	HPLC‖	% of total sterol
1 Cholesterol	1.00	1.00	1.00	1.00	1.00	2.6
2 7,22-Ergostadienol	—	—	1.24	1.22	—	3.0
3 Campesterol	1.30	1.20	1.29	1.25	1.11	1.1
4 Stigmasterol	1.41	1.22	1.39	1.35	1.04	3.8
5 7,24(28)-Ergostadienol	—	—	1.43	1.39	—	4.3
6 7-Ergostenol	1.53	1.44	1.47	1.42	1.06	11.2
7 Spinasterol	1.64	1.50	1.58	1.53	1.07	52.4
8 Sitosterol	1.60	1.41	1.60	1.54	1.24	2.6
9 Stigmastanol	—	—	1.64	1.56	—	2.1
10 7,25-Stigmastadienol	1.87	1.85	1.74	1.65	0.93	2.1
11 7-Stigmastenol	1.87	1.69	1.81	1.72	1.20	8.6
12 7,24(28)-Stigmastadienol	2.08	1.99	1.87	1.77	1.00	4.8
13 24-Methylenecycloartanol	2.15	1.88	2.12		—	1.3

Sterols were analysed as free sterols on OV-17 and HPLC, as TMSi-ethers on SP-2330 and SE-30, and as steryl acetates on DB-1. Retention times are given relative to the appropriate cholesterol derivative or free cholesterol. % of total sterol data was obtained from DB-1.

*3% OV-17 column, 1.8 m × 3.4 mm i.d., column temp. 260°, helium carrier gas flow rate 20 ml/min. Retention times are reported relative to cholesterol.

†Capillary SP-2330 column, 60 m × 0.25 mm i.d., column temp. 220°, helium flow rate 7.4 ml/min.

‡Capillary SE-30 column, 60 m × 0.25 mm i.d., column temp. 240°, helium flow rate 7.0 ml/min.

§Capillary DB-1 column, 30 m × 0.32 mm i.d., column temp. 265°, helium as carrier gas.

‖Octadecyl (C_{18}) reverse phase column, 250 mm × 4.5 mm i.d., MeOH–H_2O (98:2) (v/v), flow rate 1.0 ml/min, 60 atm. UV detector at 200 nm (α_c relative to cholesterol).

spectrum: *m/z* 458 (33), 398 (75), 383 (33), 370 (38), 215 (75), 288 (6), 257 (4) and 356 (7). Peak 10 was identified as 7,25-stigmastadienol acetate. Both *RR*$_t$ data (Table 1) and MS supported this identification. The mass fragmentation pattern of peak 10 was as follows: *m/z* 454 (72), 439 (43), 394 (15), 379 (20), 356 (12), 341 (21), 313 (85), 288 (4), 255 (25) and 213 (25). Peak 11 was identified as 7-stigmastenol acetate based on its *RR*$_t$ data (Table 1). This was confirmed by fragments at *m/z* 456 (72), 441 (12), 396 (8), 381 (9), 315 (18), 288 (8), 255 (100) and 213 (50). Peak 12 was identified as 7,24(28)-stigmastadienol acetate based on its GC, HPLC and GC/MS data. The GC/MS of peak 12 gave the molecular ion at *m/z* 454 (30) and other ions at *m/z* 439 (12), 411 (4), 356 (32), 313 (100), 255 (8) and 213 (18). Peak 13 was tentatively identified as 24-methylenecycloartanol acetate based on OV-17 (*RR*$_t$ 2.15), SP-2330 (*RR*$_t$ 1.88), and SE-30 (*RR*$_t$ 2.12) gas chromatographic data.

Table 2 shows the relative proportions of seven sterols in species and varieties of Amaranthaceae. The predominance of 7(8)-sterols in this family is immediately apparent. A single 7(8)-sterol, spinasterol (7), makes up 46–76% of the total sterols, while 7-stigmastenol (11) contributes 11–24%. However, the 7(8)-sterols of these species are accompanied by low levels of 5(6)-sterols. Fernando and Bean have also reported 7(8)-sterols in *Amaranthus* [18, 19].

In all systems of classification, Amaranthaceae is closely allied to Chenopodiaceae [20, 21]. Amaranthaceae is more advanced than Chenopodiaceae in most respects in which the two families differ. However, several authorities indicate that Amaranthaceae cannot be regarded as directly derived from Chenopodiaceae [20]. Although the information presented here cannot resolve the relationship between these families, the predominance of 7(8)-sterols in these families is consistent with their common ancestry. This work, along with several other recent reports [11–14], demonstrate that higher plant species with predominant 7(8)-sterol composition are more common than previously thought.

The presence of 5(6)-sterols with the 7(8)-sterols makes it unlikely that 7(8)-sterols accumulate as the result of a complete genetic block of 5(6)-sterol production. Coexistence of 5(6)- and 7(8)-sterols has been recently reported in beet [14], cucurbits [22] and spinach [23], and methods of sterol analysis now available may show it to be the usual pattern for plants containing predominantly 7(8)-sterols. If so, an important but as yet undefined role for 5(6)-sterols may be implied.

EXPERIMENTAL

Amaranthus tricolor and *A. caudatus* seeds were obtained from Park Seed Co., U.S.A. *Amaranthus tricolor* 'splendor', *Celosia plumosa* 'fancy plumes' *C. plumosa* 'fire dragon', *C. cristata* 'red velvet' and *C. cristata* 'semidwarf' seeds were obtained from W. Atlee Burpee Co., U.S.A. *Amaranthus caudatus* 'kiss me' seeds were obtained from Ferry-Morse Seed Col., U.S.A. and *A. tricolor* 'Hong Kong', *A. tricolor* 'chin', *A. tricolor* 'coz', *A. hypochondriacus* '79-R101', *A. hypochondriacus* and *A. cruentus* '79-R123' seeds were kindly donated by Dr. Austin Campbell, New Crop Lab., Agricultural Research Center, Beltsville, MD. *Amaranthus retroflexus*, *A. gangeticus* and *A. leucocarpus* seeds were provided by Ms. Linda Sonnen, National Seed Storage Lab., Fort Collins, CO. All seeds were grown in the greenhouse of the University of Maryland during the spring and summer of 1983. The plants were grown under natural light and harvested at maturity.

Table 2. Sterol composition of 19 species and varieties of *Amaranthus* and *Celosia*

Species name	Individual sterol* (as % of total sterol)						
	1	3	4	6	7	11	13
A. tricolor	2.2	1.7	4.4	6.1	63.8	17.5	4.5
A. tricolor Hong Kong	0.9	0.8	4.3	5.4	66.7	13.1	8.7
A. tricolor 9 Ind.		tr	3.7	5.3	73.8	12.1	5.0
A. tricolor 'chin'		tr	1.8	4.7	76.1	15.0	2.5
A. tricolor P.R.C.	tr	4.0	5.1	6.1	68.2	15.0	2.0
A. tricolor 'coz'	tr	0.5	0.2	4.4	66.1	15.7	8.0
A. tricolor (Splendor)	2.0	tr	4.0	7.1	67.2	16.2	0.4
A. gangeticus	1.5	1.9	2.2	6.3	68.7	17.1	1.7
A. hypochondriacus (79-R101)		1.5	1.6	6.6	69.3	15.7	5.9
A. cruentus (79-R123)		7.6	6.4	6.1	55.4	15.6	9.0
A. caudatus (Kiss me)	0.6	1.1	3.4	7.1	55.1	22.1	4.7
A. caudatus	8.7	3.2	5.6	4.9	55.7	16.0	3.6
A. retroflexus		0.6	6.1	4.1	69.5	19.6	0.2
A. leucocarpus		tr	1.8	4.7	76.1	15.0	
Celosia plumosa (Fancy plumes)		2.8	5.0	10.6	65.9	11.2	4.5
C. plumosa (Fire dragon)	3.3	tr	tr	6.6	49.1	33.4	6.3
C. cristata (Red velvet)				1.9	61.3	27.0	3.0
C. cristata (Semidwarf)	1.09		2.7	4.6	68.6	18.1	4.1
A. hypochondriacus (seed)	0.5	0.2	1.5	22.5	46.7	24.3	3.6
A. hypochondriacus (callus)		tr	8.8	2.3	70.5	11.3	4.2

*1, Cholesterol; 3, campesterol; 4, stigmasterol; 6, 7-ergostenol; 7, spinasterol; 11, 7-stigmastenol; 13, 24-methylenecycloartanol. From GLC OV-17 data.

Freshly harvested plant material was washed with tap water, cut into pieces and oven-dried for 48 hr at 85°. Dry material was ground to pass a 20-mesh screen in a knife mill.

Duplicate 20 g samples were extracted with 150 ml of $CHCl_3$–MeOH (2:1) using a Soxhlet extractor for 24 hr. After evaporation, the residue was redissolved in $CHCl_3$ and filtered. The $CHCl_3$ was evaporated under vacuum and the residue saponified for 1 hr at reflux temp. using 20% KOH in 60% EtOH. The nonsaponifiable lipids were removed by partitioning against Et_2O for 24 hr in a liquid–liquid separator.

The free sterols were recovered from the Et_2O phase and purified by alumina column chromatography [24] and digitonin precipitation [25].

Sterol identification. Sterols were identified by a combination of GC, HPLC and GLC/MS. GLC was performed on a Varian Model 3700 Gas Chromatograph equipped with a Varian CDS 401 data system. HPLC was performed on a Varian Model 5000 Liquid Chromatograph. For preparation of TMSi-sterol derivatives, 100 μg sterol, 100 μl pyridine and 50 μl Sylon BFT (Supelco, Bellefonte, PA) were combined in a microreaction vial with a Teflon lined cap and warmed for 20 min. Afterwards 100 μl light petroleum was added and the solvent was evaporated under N_2. Samples were resuspended in iso-octane for GC.

MS were determined with a Finnigan-MAT model 4500 spectrometer. GC was carried out on a 30 m × 0.32 mm i.d. glass capillary column coated with 0.25 mm film of DB-1, split ratio at 150:1. The column temp. was 265° and the pressure was 16 psi with helium as carrier gas. The source voltage was 70 eV for ionization.

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