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STEROL CONTENT AND DISTRIBUTION IN TWO *NICOTIANA* SPECIES AND THEIR SPONTANEOUS-TUMORING HYBRID*

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Abstract—Sterol contents of *Nicotiana suaveolens* (Lehm), *N. langsdorffii* (Weinm.) and their spontaneous-tumoring hybrid were compared when plants of both species and the hybrid began flowering, and again after numerous tumours had developed spontaneously on the hybrid plants. In general, the upper plant parts (younger tissues) had higher sterol contents than did the lower plant parts (older tissues) at both sampling dates. β-Sitosterol was the most abundant sterol in the above ground portions while stigmasterol was most abundant in roots. Low cholesterol and high campesterol levels were found in *N. suaveolens* in contrast with high cholesterol and low campesterol in *N. langsdorffii*. Levels of cholesterol and campesterol in the hybrid were intermediate between the parental species. Low cholesterol and high campesterol contents were found in tumours of the hybrid, and in roots of both of the parental species and the hybrid.

INTRODUCTION

BIOCHEMICAL composition of *Nicotiana* species and spontaneous-tumoring interspecific hybrids has received much attention in recent years. Alkaloids, free amino acids, organic acids, soluble carbohydrates, polyphenols, and growth regulators have been studied in several *Nicotiana* species and spontaneous-tumoring hybrids.[1-7]

Sterols have been identified in a number of plant species, including tobacco (*Nicotiana tabacum* L.).[8-10] Several investigators[11-14] have suggested that sterols may function as an integral part of the lipid layer in plant cell membranes. Detailed study of differences in sterol contents between *Nicotiana* species and their interspecific hybrids may aid the understanding of biosynthesis and function of this important group of compounds.

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The objective of our study was to compare the occurrence and distribution of 3-β-hydroxysterols in *Nicotiana suaveolens* (Lehm.), *N. langsdorffii* (Weinm.) and their spontaneous-tumoring interspecific hybrid when plants of both species and the hybrid began flowering, and again after numerous tumors had developed on the hybrid plants. The *N. suaveolens–langsdorffii* hybrid grows normally at a rate comparable to that of the parent species until flowering occurs. Soon after first flowering, tumors develop spontaneously on the stems and roots of the interspecific hybrid plants.

RESULTS AND DISCUSSION

Total 3-β-Hydroxysterol Content

The total 3-β-hydroxysterol contents of various plant portions at time of first flowering, and after numerous tumors developed on the interspecific hybrid are shown in Tables 1 and 2, respectively. Spontaneous tumoring of the hybrid began soon after the first sampling

TABLE 1. TOTAL 3-β-HYDROXYSTEROL CONTENT OF VARIOUS PORTIONS OF *N. suaveolens*, *N. langsdorffii* AND THEIR HYBRID. SAMPLES WERE TAKEN AT FIRST FLOWERING, 3 months AFTER SEEDING

Plant portion	*N. suaveolens* (mg/g)*	*N. langsdorffii* (mg/g)*	Hybrid (mg/g)*
Inflorescence	2·74	2·01	1·88
Upper part			
Stem	—	—	1·60
Mid-vein	1·54	1·27	1·74
Lamina	1·84	1·23	1·25
Lower part			
Stem	1·24	1·14	1·10
Mid-vein	1·24	1·13	1·11
Lamina	1·16	1·07	1·05
Root	2·13	2·47	1·69

* Dry weight basis.

date and continued to the second sampling. In general, the upper parts had higher total sterol concentrations than did the lower parts of the above-ground portions. The same pattern was observed within each sampling date. Also, younger stem tumors had higher sterol concentrations than did the older stem tumors (Table 2). Since sterols may function as an integral part of cell membranes, it is consistent that the less differentiated areas (i.e. those having the highest porportion of young cells per unit of dry matter) of the various plant portions have the highest sterol contents, as was found within each of our sampling dates.

The above-ground portions of *N. suaveolens* had higher total 3-β-hydroxysterol concentrations than did *N. langsdorffii* at each sampling date. Total sterol contents of the various plant portions of the hybrid did not consistently match those of either parent, nor did they consistently fall between those of the parental species.

The concentrations of total sterols in stem and root tumors were higher than those of the tissues on which they were borne (Table 2). Sterol content of stem tumors was higher than that of root tumors even though the sterol content of stems was lower than that of roots.

TABLE 2. TOTAL 3-β-HYDROXYSTEROL CONTENT OF VARIOUS PORTIONS OF *N. suaveolens*, *N. langsdorffii* AND THEIR HYBRID. SAMPLES WERE TAKEN AFTER SPONTANEOUS TUMORING OF THE HYBRID HAD OCCURRED, 6 months AFTER SEEDING

Plant portion	*N. suaveolens* (mg/g)*	*N. langsdorffii* (mg/g)*	Hybrid (mg/g)*
Inflorescence	2·45	1·83	1·80
Upper part			
Stem	1·46	1·56	1·08
Mid-vein	1·73	1·39	1·40
Lamina	2·50	1·36	1·36
Lower part			
Stem	1·00	0·93	0·62
Mid-vein	1·58	1·26	1·35
Lamina	2·36	1·85	1·42
Root	1·28	1·43	1·44
Tumor, stem			
Young	—	—	2·69
Old	—	—	2·30
Tumor, root	—	—	1·89

* Dry weight basis.

Individual 3-β-Hydroxysterol content

The individual 3-β-hydroxysterol concentrations of the various plant portions are shown in Tables 3 and 4. Each plant portion contained the four major 3-β-hydroxysterols (cholesterol, campesterol, stigmasterol and β-sitosterol) found in tobacco.[10a] The content of individual sterols, however, differed among the two species and their hybrid. Cholesterol and campesterol levels, averaged over both collection dates, were 7 per cent (6–10 per cent) and 18 per cent (15–21 per cent), respectively, of the total 3-β-hydroxysterols in the above-ground portions of *N. suaveolens*. Higher cholesterol, averaging 24 per cent (15–38 per cent), and lower campesterol, averaging 12 per cent (6–20 per cent), contents were found in these portions of *N. langsdorffii*. Cholesterol and campesterol content of the same portions of the hybrid were generally intermediate between the parental species, averaging 14 per cent (7–18 per cent) and 18 per cent (14–23 per cent), respectively.

Cholesterol accounted for 2–3 per cent of the total sterols in the roots of both species and the hybrid at each sampling date. Also in roots, campesterol accounted for about 30 per cent of the sterols in samples collected at first flowering and in those collected after abundant tumoring had occurred in the hybrid. Neither cholesterol nor campesterol content of the roots varied with species at either sampling date. Our observations with *N. suaveolens* and *N. langsdorffii* are similar to those of Richardson *et al*,[9] who found that cholesterol and campesterol accounted for 2 and 33 per cent, respectively, of the total 3-β-hydroxysterols in roots of *Nicotiana tabacum*.

The marked differences in the cholesterol and campesterol fractions that existed between *N. suaveolens* and *N. langsdorffii* are interpreted to be under genetic control because the plants were grown under the same environment. First sampling was done when all plants were at the same physiological stage of development (first flowering), and the second sampling was done after the plants had aged an additional 3 months in the same growth environment. Since sterol fractions in the above-ground portions did differ widely between these

TABLE 3. INDIVIDUAL 3-β-HYDROXYSTEROLS IN VARIOUS PORTIONS OF *N. suaveolens*, *N. langsdorffii* AND THEIR HYBRID. SAMPLES WERE TAKEN AT FIRST FLOWERING, 3 months AFTER SEEDING

Plant portion	Individual sterols (as % of total sterols)			
	Cholesterol	Campesterol	Stigmasterol	β-Sitosterol
N. suaveolens				
Inflorescence	8	19	20	53
Upper part				
Stem	—	—	—	—
Mid-vein	7	18	33	42
Lamina	10	18	32	40
Lower part				
Stem	7	21	26	46
Mid-vein	6	19	33	42
Lamina	9	20	30	41
Root	2	30	39	29
N. langsdorffii				
Inflorescence	24	12	27	37
Upper part				
Stem	—	—	—	—
Mid-vein	29	6	34	31
Lamina	38	9	28	25
Lower part				
Stem	18	10	28	44
Mid-vein	27	6	39	28
Lamina	26	11	32	31
Root	2	32	55	11
N. suaveolens–langsdorffii				
Inflorescence	13	15	35	37
Upper part				
Stem	15	17	29	39
Mid-vein	15	14	33	38
Lamina	18	16	31	35
Lower part				
Stem	13	17	35	35
Mid-vein	13	13	39	35
Lamina	13	18	35	34
Root	2	32	58	8

species, they appear to be well-suited for continued studies of biosynthesis of 3-β-hydroxysterols, especially of cholesterol.

Cholesterol constituted a low amount (2–4 per cent) of the 3-β-hydroxysterols in both stem and root tumors (Table 4). Campesterol content was similar (22–27 per cent) in stem and root tumors. Proportions of individual sterols were nearly identical in young and old stem tumors. However, root tumors had more stigmasterol and less β-sitosterol than did the stem tumors. Similarly, roots of each species and their hybrid had more stigmasterol and less β-sitosterol than did the stems.

EXPERIMENTAL

Nicotiana suaveolens (Lehm.), *N. langsdorffii* (Weinm.) and their interspecific hybrid were grown in a greenhouse during the winter and spring of 1969. The plants received natural daylight plus supplemental low-intensity light to give 16-hr photoperiods. Under these conditions, all plants reached the flowering stage at the same time. Five plants of each group were harvested at first flowering, but before spontaneous tumoring

TABLE 4. INDIVIDUAL 3-β-HYDROXYSTEROLS IN VARIOUS PORTIONS OF *N. suaveolens*, *N. langsdorffii* AND THEIR HYBRID. SAMPLES WERE TAKEN AFTER SPONTANEOUS TUMORING OF THE HYBRID HAD OCCURRED, 6 months AFTER SEEDING

Plant portion	Individual sterols (as % of total sterols)			
	Cholesterol	Campesterol	Stigmasterol	β-Sitosterol
N. suaveolens				
Inflorescence	7	19	19	55
Upper part				
Stem	8	18	26	48
Mid-vein	6	15	29	50
Lamina	8	16	26	50
Lower part				
Stem	6	19	23	52
Mid-vein	7	17	30	46
Lamina	7	17	26	50
Root	3	27	57	13
N. langsdorffii				
Inflorescence	20	13	31	36
Upper part				
Stem	31	12	21	36
Mid-vein	27	15	25	33
Lamina	18	20	29	33
Lower part				
Stem	24	10	26	40
Mid-vein	22	12	30	36
Lamina	15	18	28	39
Root	3	29	56	12
N. suaveolens–langsdorffii				
Inflorescence	13	17	35	35
Upper part				
Stem	14	18	30	38
Mid-vein	13	15	34	38
Lamina	10	21	39	30
Lower part				
Stem	14	17	33	36
Mid-vein	12	16	38	34
Lamina	7	23	37	33
Root	3	27	49	21
Tumor, stem				
Young	2	22	21	51
Old	4	23	22	51
Tumor, root	2	27	35	36

of the hybrid (3 months after seeding). Another five plants from each group were harvested after spontaneous tumoring of the hybrid had occurred in abundance (6 months after seeding). At each sampling date, plants were separated into inflorescence, upper and lower stem, and root portions. Buds and flowers were combined as the inflorescence. Lamina and midveins were separated from the stems. Greenish tumors from stems were designated young tumors, while purplish tumors were designated old tumors. Root tumors were removed and washed. Within each group the various portions from the five plants were composited and freeze-dried. The dry samples were pulverized and stored in an evacuated desiccator prior to extraction.

Extraction and Isolation

5-g samples of the freeze-dried materials were extracted with acetone and evaporated to dryness. The residues were saponified with alcoholic H_2SO_4 followed by alcoholic KOH. The non-saponifiable fraction, which contained free sterols, was extracted with light petroleum and washed with 90% MeOH. The extracts were evaporated to dryness and dissolved in 95% EtOH. Sterols were precipitated from the solution with

2% digitonin in 80% EtOH. The digitonin-precipitable sterols were determined by the gravimetric method.[15] Sterol content was determined by multiplying the weight of digitonides by 0·253.[16]

Gas–Liquid Chromatography

Free sterols were regenerated from the sterol-digitonides by pyridine and the digitonin precipitated with Et_2O. The free sterols were acetylated by using pyridine–Ac_2O (1:1, v/v) and were left standing overnight at room temp. The sterol acetates were precipitated with H_2O and filtered. The sterol acetates were weighed, dissolved in tetrahydrofuran and analyzed by gas chromatography (F & M Model 402).* The column was a 4-ft length of ¼ in. (i.d.) silanized glass tubing containing 3·8% UCW98, Gas Chrom 80–100S (Hewlett-Packard Company).* Injection port temperature was 300° and the columns were operated at 240° with a helium carrier gas flow rate of 60 ml/min. The detector temperature was 250°. Cholestane was used as an internal standard.

For identification purposes, a reference mixture of cholesterol acetate, stigmasterol acetate (Sigma Chemical Company),* campesterol acetate and β-sitosterol acetate (campesterol and β-sitosterol were purchased from Applied Science Laboratories* and their acetates were prepared in our laboratory) was run before and after each two or three plant samples. Retention times were measured relative to cholestane. The quantity of each sterol in chromatography was determined by peak area measurement. Corrections were made for relative detector responses for different sterol acetates.

* Mention of a trade name does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply their approval to the exclusion of other products that may also be suitable.

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