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Free and Conjugated Phytosterols in Cured Tobacco Leaves: Influence of Genotype, Growing Region, and Stalk Position

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Although phytosterols in tobacco leaves have specific effects on tobacco quality, there is little research on the distribution of free and conjugated phytosterols in various tobacco leaves. In this paper, we explored the content and composition of phytosterols in representative cured tobacco leaves by gas chromatography of TMS-ether derivatives. We found that phytosterol contents in tobacco leaves ranged from 1.0 to 2.5 mg/g of dried leaf tissue, depending on different types of tobacco leaves. The majority of phytosterols (75–85%) were conjugated as ester and glycosides, with only about 15–25% existing in the free form. Furthermore, the genetic variability gives rise to the significant differences among different tobacco types with phytosterol levels: the contents of phytosterols in tobacco leaves decreased in the order of flue-cured tobacco, Oriental tobacco, Burley tobacco, cigar tobacco, and Maryland tobacco. At the same time, the tobacco curing process leads to a difference in phytosterol existing-form distribution in some variation laws.

KEYWORDS: Phytosterols; cured tobacco leaves; TMS-ether derivatives; conjugates; esters; glycosides

INTRODUCTION

As an important class of isoprenoids, phytosterols consisting of a tetracyclic cyclopenta[*a*]phenanthrene ring and a long flexible side chain at the C-17 carbon atom (*1*) are widely distributed in plants. The components and contents of phytosterols in plant, which are enzymatically synthesized from acetyl-CoA, are different among plant species. While research on the nutritional aspects of phytosterols is currently a hot topic, carcinogens of cigarette smoke resulting from phytosterol pyrolysis are also noticeable, for the reason that cigarette smoking leads to the formation of carcinogenic polynuclear aromatic hydrocarbons (PAHs) through pyrolysis of the native cyclic ring structure of steroids (*2–5*). Therefore, study on the content of tobacco phytosterols will help to comprehend the potential risks of cancer resulted from cigarette smoking.

Tobacco phytosterols consist mainly of campesterol, stigmasterol, and β-sitosterol (*6*); ergosterol in the mildewy tobacco was also found (*7*), as shown in **Figure 1**. Apart from free sterols (FSs), tobacco phytosterols also exist in three conjugated forms: phytosterol fatty acid esters (steryl esters, SEs), hexose glycosylated phytosterols (steryl glycosides, SGs), and acylated steryl glycosides (ASGs) (*8, 9*). The content and composition of phytosterols in tobacco leaves were first studied in 1959 by Stedman and Rusaniwskyi (*10*), when they determined the total phytosterol contents of various tobacco types using the precipitation method, but they failed to report the content of each phytosterol separately. Since then, researchers kept working on the analysis of phytosterols in tobacco leaves (*11–14*), but few of them have given comprehensive information of the distributions and contents of different phytosterols, especially SGs and ASGs, in tobacco leaves. It was not until 1997 that the free phytosterols were first measured by J. Ai using short-column GC/MS/MS (*15*). Furthermore, very recently, an improved, simple, and routine method using GC-FID for quantitative analysis of free and conjugated phytosterols in tobacco leaves was also proposed (*16*).

It is reported that the content and distributions of different phytosterol forms in tobacco vary with tobacco cultivar and cultural practices (*17, 18*). Our study focuses on the analysis of both the free and conjugated phytosterols in various tobacco leaves and the relationship between levels of phytosterols and genetic variability and planting location, which is useful for research on decreasing hazardous substances in cigarette smoke.

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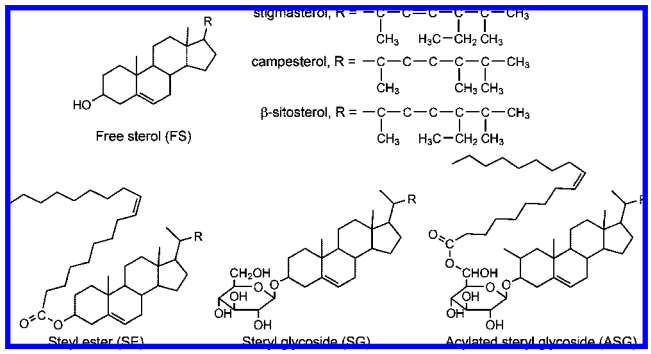

Figure 1. The three major phytosterol structures in different existing forms in tobacco (R varies between different phytosterols).

The novelty is that we find the genotype is a major determinant factor of the contents of phytosterols. Furthermore, different tobacco types need different tobacco curing methods and growing environments, which may affect both the content and form distribution of phytosterols.

MATERIALS AND METHODS

Tobacco Materials and Chemical Reagents. We collected the tobacco samples from the Technology Center of HongYun Group (Kunming, China). 5α-Cholestane (97%), campesterol (98%, GC), stigmasterol (95%), β-sitosterol (95%), and bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) were from Sigma (St. Louis, MO); absolute ethanol, hexane, dry pyridine, dichloromethane, potassium hydroxide, hydrochloric acid (37%), potassium chloride, anhydrous potassium sulfate, and all other chemicals in analytical grade were from Shanghai Chemical Reagents Co. (Shanghai, China).

Preparation of Tissue Sample. All tobacco samples were dried for 3 h at 40 °C and milled for 60 s using a commercial coffee grinder. Milled tobacco leaves were screened by a 32 mesh sieve, and materials passing through the sieve were collected for compositional determinations. Without loss of generality, we assumed that chemical fractionation did not occur during the sieving step. We determined the moisture contents of those tissue samples at the time of analysis. The levels of the phytosterols shown in this paper referred to the phytosterol contents in 1 g dry weight of tobacco samples ($m_{\text{tobacco}} - m_{\text{moisture content}}$).

Analyses of Phytosterols in Tobacco Leaves. The phytosterol analysis assay in tobacco leaves was carried out as described previously by Liu et al. (*16*). For the total phytosterol analysis, the ground tobacco leaves were directly hydrolyzed by 4 mol/L HCl and 4 mol/L KOH ethanolic solutions, followed by extraction of nonsaponifiables, derivatization to trimethylsilyl ether derivatives, and then GC (Agilent Technologies series 6890 N equipment, Wilmington, DE). The SEs were analyzed by alkaline saponification, extraction, derivatization, and then GC analysis. In this way, the FSs were determined by direct derivatization of tobacco extracts with BSTFA. The compounds were separated on a 30 m × 320 μm i.d., 0.25 μm film thickness HP-5 fused silica capillary column coated with 5% phenymethylsiloxane (J&W Scientific, Folsom, CA). Further parameters were as follows: nitrogen as carrier gas; split ratio, 15:1; injection port, 270 °C; detector, 300 °C; temperature program starting from 240 °C for 10 min, then raising to 260 °C at 2 °C/min, and maintaining for 30 min. Each phytosterol was identified by their relative retention times and GC-MS (*16*). Quantitative analysis of phytosterols was carried out by the internal and external standard combined method, and the total phytosterol contents were calculated by summing up the contents of each individual phytosterol. The relative standard deviations (RSD) ranged from 2.00% to 3.37%, and the recovery ranged from 87% to 99%. All analytical experiments were performed in duplicate. The results were averaged for analytical data of two samples.

RESULTS AND DISCUSSION

Free and Conjugated Phytosterols in Different Tobacco Types. The phytosterols in five different types of tobacco leaves (flue-cured tobacco, oriental tobacco, burley tobacco, Maryland tobacco, and cigar tobacco) were determined by GC-FID. **Table 1** illustrates the levels of three major phytosterols and the distribution of total phytosterols in terms of free and conjugated forms in different tobacco types.

The total phytosterol contents in different types of tobacco leaves ranged from 1.0 to 2.5 mg/g. Most of the phytosterols existed in conjugated forms, with only an approximate 15–25% phytosterols in free forms. The contents of total phytosterols of various tobacco types present significant difference from each other: the highest level in flue-cured tobacco, followed by oriental tobacco, burley tobacco, cigar tobacco, and Maryland tobacco in the lowest level. Despite this, the components of each phytosterol in different kinds of tobacco leaves showed some similarities: the major phytosterol was stigmasterol, followed by β-sitosterol and campesterol in tobacco leaves. This result suggests that tobacco type, i.e., the genetic variability, has an important effect on the phytosterol levels of tobacco leaves. Additionally, multiple factors such as the different agricultural practices, soil environment, nutrients, and weather conditions as well as harvesting and curing procedures can also affect the phytosterol contents in tobacco leaves of different types. Furthermore, it is interesting to notice that the flue-cured tobacco has the highest level of SGs and ASGs, while oriental, burley and Maryland tobacco have more SEs compared to other forms. According to Moreau et al. (*19*) and Duperon et al. (*20*), glycosylation and fatty acylation of phytosterols in *Nicotiana tabacum* cells increased dramatically in response to stress and a fungal elicitor. Besides, the level of conjugated phytosterols, such as SEs in solanum and other plant species, increased with aging and senescence, as reported by Duperon et al. (*21*). To our knowledge, different types of tobaccos need different curing methods. When flue-curing tobacco, heating could promote glycosylation of leaf phytosterols, which is likely to be the reason for the highest proportion of conjugated phytosterols in the cured tobacco leaves. Also, the physiological stress and senescence during the curing process could help to increase the proportion of conjugated phytosterols.

Free and Conjugated Phytosterols in Chinese and Non-Chinese Flue-Cured Tobacco Leaves. Six non-Chinese tobaccos (one from Zimbabwe, one from the United States, and four from Brazil) and three Chinese tobaccos were analyzed to compare the free and conjugated phytosterol levels of Chinese and non-Chinese flue-cured tobacco (**Table 2**).

The total phytosterol contents of all three Chinese flue-cured tobaccos were higher than those of non-Chinese tobaccos, which were often used as fine tobaccos in cigarette manufacture. No significant difference of the relative content of each phytosterol was found between Chinese and non-Chinese flue-cured tobacco leaves. This result might probably be ascribed to the fact that the Chinese, the Zimbabwe, and the US tobaccos were all derived from the same sources in North America within the last 60–70 years; although varietal changes have occurred, some components of tobacco only change a little, including phytosterols.

Free and Conjugated Phytosterols in Different Varieties of Flue-Cured Tobacco Leaves. Six kinds of Chinese flue-cured tobaccos, with the same producing area (Sichuan Province) and stalk position, were analyzed to detect the phytosterol contents and compositions (**Table 3**). They were CB-1, G80, K326, Hongda, Yun85/87, and Yun85.

In general, among the six Chinese flue-cured tobaccos, levels of the total phytosterols were relatively consistent with a range of 2.3–2.5 mg/g of dry leaf. We observed no

Table 1. Levels of Three Major Phytosterols Including Free and Conjugated Forms and Total Phytosterols in Different Tobacco Types[a] (μg/g Dry Weight Tobacco Samples)

		campesterol			stigmasterol			β-sitosterol		
tobacco type	total phytosterols	FS	SE	SG + ASG	FS	SE	SG + ASG	FS	SE	SG + ASG
flue-cured	2492.0	73.6	139.4	168.8	259.6	457.9	520.2	159.0	300.5	413.0
oriental	1884.9	95.2	149.6	102.5	216.0	332.8	237.6	167.6	334.8	248.8
burley	1207.6	79.0	94.4	69.1	204.8	269.5	216.0	83.8	108.6	82.4
Maryland	1008.2	80.0	93.4	77.8	172.2	214.0	167.0	61.6	73.0	69.2
cigar	1084.2	55.0	59.7	35.3	234.1	230.6	138.3	122.5	115.6	93.1

[a] The average values of the phytosterol contents are shown in the table.

Table 2. Levels of Three Major Phytosterols Including Free and Conjugated Forms and Total Phytosterols in Chinese and Non-Chinese Flue-Cured Tobacco Leaves (μg/g Dry Weight Tobacco Samples)

		campesterol			stigmasterol			β-sitosterol		
planting country[a]	total phytosterols	FS	SE	SG + ASG	FS	SE	SG + ASG	FS	SE	SG + ASG
Z	2421.0	97.6	117.6	229.9	323.2	398.0	689.1	131.5	144.7	289.4
U	2260.1	73.4	140.2	216.2	251.5	491.2	687.9	75.0	149.2	175.5
BA	2360.5	129.8	220.2	208.2	199.4	335.0	392.0	180.0	311.2	384.7
BB	1836.2	69.4	167.0	151.7	165.9	406.3	393.9	82.9	182.7	216.4
BC	2034.3	61.5	123.2	145.0	202.0	427.5	416.3	120.2	295.1	243.5
BD	2282.0	73.6	144.7	163.3	285.0	459.4	529.2	122.9	229.6	274.3
CA	2613.4	125.9	209.1	250.1	224.9	456.3	375.3	200.2	376.5	395.1
CB	2422	118.4	185.5	180.2	286.6	505.0	576.5	114.0	200.8	255.0
CC	2440.8	141.7	203.2	231.2	289.0	369.3	354.2	236.4	310.0	314.8

[a] Z and U represent Zimbabwe and USA, respectively; BA, BB, BC, and BD represent four varieties of flue-cured tobacco in Brazil; CA, CB, and CC represent three varieties of flue-cured tobacco in China.

Table 3. Levels of Three Major Phytosterols Including Free and Conjugated Forms and Total Phytosterols in Different Varieties of Tobacco Plants (μg/g Dry Weight Tobacco Samples)

		campesterol			stigmasterol			β-sitosterol		
tobacco variety	total phytosterols	FS	SE	SG + ASG	FS	SE	SG + ASG	FS	SE	SG + ASG
Honda	2449.8	141.7	203.2	231.2	289.0	369.3	354.2	236.4	310.0	314.8
CB-1	2587.8	80.0	143.3	190.1	308.2	546.0	590.5	135.2	245.4	349.1
Yun85/87	2396.0	67.1	135.5	147.5	211.0	369.8	450.0	182.7	355.6	476.8
K326	2529.3	187.1	269.0	250.9	255.9	346.3	444.9	164.0	281.9	329.3
Yun85	2320.2	139.4	143.8	158.0	75.1	562.1	493.5	250.3	300.1	197.9
G80	2541.3	131.5	182.1	143.8	271.9	729.3	345.6	247.7	184.2	305.2

Table 4. Levels of Three Major Phytosterols Including Free and Conjugated Forms and Total Phytosterols in Tobacco Plants from Different Producing Locations (μg/g Dry Weight Tobacco Samples)

		campesterol			stigmasterol			β-sitosterol		
planting location[a]	total phytosterols	FS	SE	SG + ASG	FS	SE	SG + ASG	FS	SE	SG + ASG
PZH	2320.1	139.4	143.8	158.0	75.1	562.1	493.5	250.3	300.1	197.9
CQ	2396.0	67.4	135.5	147.2	190.0	398.0	442.8	192.7	355.6	466.8
LS	2213.4	96.5	177.0	211.2	235.3	444.1	473.3	122.9	221.6	231.5
LH	2613.4	125.9	209.1	250.1	224.9	456.3	375.3	200.2	376.5	395.1
ZMD	2422.0	118.4	185.5	180.2	286.6	505.0	576.5	114.0	200.8	255.0

[a] PZH, CQ, and LS represent Pangzhihua, Chongqing, and Liangshan (Sichuan Province, China), respectively; LH and ZMD represent Luohe and Zhumadian (Henan Province, China), respectively.

statistically significant differences among these tobaccos. However, different types of tobacco exhibited their own characters: K326 had more campesterol; CB-1 contained higher stigmasterol, and phytosterol glycosides were the dominant form of phytosterols; Yun85/87 had similar form distribution of phytosterols as CB-1 with a higher level of β-sitosterol. Although the discrepancy among total phytosterol levels was slight, significant differences existed in the distributions of phytosterols among different tobacco varieties. Hongda had the highest free phytosterols (about 27%); Yun85 and G80 contained about 44% phytosterol ester; CB-1 and Yun85/87 had the highest level of phytosterol glycosides of 44%. These data suggest that the varietal factor makes little difference on the total phytosterol contents but has great influence on the existing-form distribution of phytosterols.

Free and Conjugated Phytosterols in Flue-Cured Tobacco Leaves from Different Chinese Planting Locations. In order to investigate the relationship between phytosterol distribution and planting locations, we analyzed five flue-cured tobaccos with the same variety (Yun85) and stalk position from different Chinese planting locations including Panzhihua (PZH), Chongqing (CQ), and Liangshan (LS) in Sichuan Province and Luohe (LH) and Zhumadian (ZMD) in Henan Province (**Table 4**).

Table 5. Levels of Three Major Phytosterols Including Free and Conjugated Forms and Total Phytosterols in Different Stalk Positions (μg/g Dry Weight Tobacco Samples)

		campesterol			stigmasterol			β-sitosterol		
stalk position[a]	total phytosterols	FS	SE	SG + ASG	FS	SE	SG + ASG	FS	SE	SG + ASG
TA	2573.2	119.9	200.8	223.7	280.0	573.1	611.7	124.5	200.9	238.6
CA	2639.8	123.3	242.7	320.9	202.4	504.2	680.0	101.2	239.8	225.3
PB	2525.4	97.3	209.1	190.8	293.5	551.6	522.4	145.4	266.7	248.6
TB	2450.9	107.8	190.8	201.8	228.2	486.2	485.6	185.5	283.5	281.5
CB	2582.4	90.1	279.1	205.2	207.4	520.7	496.7	154.9	305.2	323.1
PB	2185.5	177.9	200.1	318.9	204.8	363.4	348.7	129.3	230.7	207.7

[a] TA, CA, and PA represent tips, cutters, and primings of A tobacco, respectively; TB, CB, and PB represent tips, cutters, and primings of B tobacco, respectively.

The total phytosterol contents in two Henan tobaccos appeared a little higher than those in all three Sichuan tobaccos, which were possibly related to differences in growing environment and weather condition. As for each phytosterol level, Henan tobaccos contained more campesterol and stigmasterol, while the β-sitosterol level in Sichuan tobaccos was higher. Since total phytosterols were different between Chinese and non-Chinese flue-cured tobaccos as described above, growing environment and weather condition were more important to phytosterol biological synthesis and accumulation. As for phytosterol forms in Henan and Sichuan tobaccos, the free phytosterols were higher in Henan tobaccos, while Sichuan tobaccos had more phytosterol esters; the relative levels of glycosides in Henan and Sichuan tobaccos were similar. In conclusion, growing environment and weather condition contribute to the difference of phytosterol levels and composition distributions.

Free and Conjugated Phytosterols in Different Stalk Positions of Flue-Cured Tobacco Leaves. We chose three stalk positions (tips, cutters, and primings) of Yun85/87 tobacco (Chongqing, China, named as A) and Yun85 (Liangshan, China, named as B), with tips on the top of stalks and primings at the bottom, to analyze and discuss characters of phytosterol distribution (**Table 5**).

The total phytosterol contents in cutters were higher than in tips and primings, with tips in a little higher level of total phytosterols than primings. Possible reasons for that result might be as follows: the leaves lying on cutters undergo a relatively prosperous stage of tobacco growth with abundant nutrient supply, which results in an increase in dry weight accumulation such as phytosterols. On the other hand, compared with leaves lying on the primings, leaves on the tips exhibit higher phytosterol levels due to longer photoperiod and plenty of water. As for the phytosterol form, cutter accumulated more glycosides. Despite the same soil and weather conditions, leaves lying in different stalk positions have different conditions in sunshine, temperature, and nutrient, which results in the discrepancy in their phytosterol levels.

In conclusion, the contents of free and conjugated phytosterols in tobacco leaves of various types, planting location, varieties, and stalk positions were analyzed, and some relationships were discovered between that and various factors. Phytosterol content variation is mostly related to tobacco type genetic factor, agricultural practices, weather condition, and soil types. At the same time, the tobacco curing process leads to the difference in phytosterol existing-form distribution in some variation laws, and further research is necessary. Basically, we present useful and comprehensive information about the phytosterol distribution of various cured tobacco leaves. These results are important in the comprehensive evaluation of the relationship between chemical composition and tobacco quality and safety, as well as the manufacture of commercial cigarettes. Further studies will involve deeper understanding about the mechanism of how the tobacco curing process, such as temperature, curing technology, and other factors, can affect the phytosterol conjugation.

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LITERATURE CITED

(1) Piironen, V.; Lindsay, D. G.; Miettinen, T. A.; Toivo, J.; Lampi, A. M. Plant sterols: biosynthesis, biological function and their importance to human nutrition. *J. Sci. Food Agric.* **2006**, *80*, 939–966.

(2) Britt, P. F.; Buchanan, A. C., III; Kidder, M. M.; Owens, C.; Ammann, J. R.; Skeem, J. T.; Luo, L. Mechanistic investigation into the formation of polycyclic aromatic hydrocarbons form the pyrolysis of plant steroids. *Fuel* **2001**, *80*, 1727–1746.

(3) Britt, P. F.; Buchanan, A. C.; Kidder, M. K.; Owens, C. V. Influence of steroid structure on the pyrolytic formation of polycyclic aromatic hydrocarbons. *J. Anal. Appl. Pyrol.* **2003**, *66*, 71–95.

(4) Schmeltz, I.; Hoffmann, D. Polynuclear aromatic hydrocarbons: Chemistry, Metabolism and Carcinogenesis. In *Carcinogenesis*; Freudenthal, R. I., Jones, P. W., Eds.; Raven Press: New York, 1973; pp 225–239.

(5) Schlotzhauer, W. S.; Severson, R. F.; Chortyk, O. T.; Arrendale, R. F.; Higman, H. C. Pyrolytic formation of polynuclear aromatic hydrocarbons from petroleum ether extractable constituents of flue-cured tobacco leaf. *J. Agric. Food. Chem.* **1976**, *24*, 992–997.

(6) Stedman, R. L. The chemical composition of tobacco and tobacco smoke. *Chem. Rev.* **1968**, *68*, 153–207.

(7) Bindler, G. N.; Piadé, J. J.; Schultbess, D. Evaluation of Selected Steroids as Chemical Markers of Past or Present Occurring Fungal Infections on Tobacco. *Beitr. Tabakforsch. Int.* **1988**, *14*, 127–134.

(8) Breinholder, P.; Mosca, L.; Lindner, W. Concept of sequential analysis of free and conjugated phytosterols in different plant matrices. *J. Chromatogr. B* **2002**, *777*, 67–82.

(9) Lagarda, M. J.; Garcia-Llatas, G.; Farre, R. Analysis of phytosterols in foods. *J. Pharm. Biomed. Anal.* **2006**, *41*, 1486–1496.

(10) Stedman, R. L.; Rusaniwskyj, W. Composition studies of tobacco V. Free and combined 3-β-sterols of freshly harvested, aged or fermented tobacco. *Tob. Sci.* **1959**, *3*, 44–47.

(11) Keller, C. J.; Bush, L. P.; Grunwald, C. Changes in content of sterols, alkaloids, and phenols in flue-cured tobacco during conditions favoring infestation by molds. *J. Agric. Food Chem.* **1969**, *17*, 331–334.

(12) Ellington, J. J.; Schlotzhauer, P. F.; Schepartz, A. I. Quantitation of tobacco lipids. *J. Chromatogr. Sci.* **1977**, *15*, 295–300.

(13) Ellington, J. J.; Schlotzhauer, P. F.; Schepartz, A. I. Lipid Distribution in Flue-Cured Tobacco Plants. *J. Agric. Food Chem.* **1978**, *26*, 407–410.

(14) Severson, R. F.; Ellington, J. J.; Arrendale, R. F.; Snook, M. E. Quantitative gas chromatographic method for the analysis of aliphatic hydrocarbons, terpenes, fatty alcohols, fatty acids and sterols in tobacco. *J. Chromatogr.* **1978**, *160*, 155–168.
(15) Ai, J. Rapid measurement of free phytosterols in tobacco by short-column GC/MS/MS. *J. Agric. Food Chem.* **1997**, *45*, 3932–3935.
(16) Liu, W. H.; Ding, B.; Ruan, X. M.; Xu, H. T.; Yang, J.; Liu, Sh. M. Analysis of free and conjugated phytosterols in tobacco by an improved method using gas chromatography-flame ionization detection. *J. Chromatogr. A* **2007**, *1163*, 304–311.
(17) Grunwald, C.; Bush, L. P.; Keller, C. J. Variation in sterols, Variation in sterols, alkaloids, and polyphenols of two nicotiana varieties under different nitrogen fertilization and drying processes. *J. Agric. Food Chem.* **1971**, *19*, 216–221.
(18) Cheng, A. L. S.; Chaplin, J. F.; Tso, T. C. Sterol variation in flue-cured tobacco varieties. *Tob. Sci.* **1968**, *12*, 33–34.
(19) Moreau, R. A.; Preisig, C. L. Lipid changes in tobacco cell suspensions following treatment with cellulose elicitor. *Physiol. Plant* **1993**, *87*, 7–13.
(20) Moreau, R. A.; Powell, M. J.; Whitaker, B. D.; Bailey, B. A.; Anderson, J. D. Xylanase treatment of plant cells induces glycosylation and fatty acylation of phytosterols. *Physiol. Plant* **1994**, *91*, 575–580.
(21) Duperon, R.; Thiersault, M.; Duperon, P. High level of glycosylated sterols in species of solanum and sterol changes during the development of the tomato. *Phytochemistry* **1984**, *23*, 743–746.

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