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Enhancement of Volatile Aglycone Recovery Facilitated by Acid Hydrolysis of Glucosides from *Nicotiana* Flower Species

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ABSTRACT: Four different *Nicotiana* flowers (*Nicotiana alata (alata), Nicotiana sylvestris* (Sy), *Nicotiana suaveolens* (Su), and *Nicotiana tabacum* cv. Flue-Cured (FC)) from farms in Virginia and North Carolina were harvested and promptly quenched with liquid nitrogen and hand-ground prior to analysis. Each *Nicotiana* flower was pre-extracted with hexane to remove unbound volatiles. Fifteen standard compounds that were thought to be in the pre-extract were employed to aid in GC-MS identification and quantification. Glucosides were then chromatographically isolated and next hydrolyzed via 2 M sulfuric acid for 24 h at 75 °C. For each flower, the products of hydrolysis were extracted in tandem with hexane and dichloromethane (DCM) prior to analysis by GC-MS. The mixture of hexane and DCM extracts of the flowers after hydrolysis were then analyzed for each of 15 external standards via GC-MS to determine the concentration of any isolated flower-derived aglycone. Quantitative results for each of the possible 15 free volatile compounds extracted before and after hydrolysis were compared. Benzyl alcohol, phenethyl alcohol, and *cis*-3-hexenol were found in all *Nicotiana* both before and after acid hydrolysis. Enormous increases in the mass of benzyl alcohol and phenethyl alcohol were obtained with all flowers as a result of acid hydrolysis. With selected *Nicotiana* flowers, significant increases were observed for eugenol and cinnamaldehyde. The significant increases observed in cinnamaldehyde and eugenol upon mild acid hydrolysis strongly indicate that this approach could be a viable alternative process for the production scale isolation of these important natural flavor compounds.

KEYWORDS: *Nicotiana flowers, greenhouse grown, field grown, glycoside hydrolysis, volatiles*

INTRODUCTION

During the past 25 years, an exceedingly large number of glucosidically bound volatiles have been identified in the plant kingdom. Many interesting results concerning their occurrence, distribution, and role in different plant species have appeared.[1] Some of the most common aglycones occurring as glucosidically bound volatiles are aliphatic alcohols, alkylphenols including phenylpropanes, ionone-related compounds, monoterpenes, and sesquiterpenes. It is interesting, however, to note that some aglycones that are found lack a hydroxyl group for the glucosidic bond. For example, β-damascenone[2] seems to be formed from an acetylenic C_{13}-norisoprenoid, megastigma-5-en-7-yne-3,9-diol. Phenols probably result from decarboxylation of acids such as coumaric acid.[3] Glucosidically bound volatiles occur not only in the aerial green parts of the plant but also in the roots, petals, fruits, and seeds. *Hyssopus officinalis* has been investigated. The highest content of glucosides was found in the roots (145 mg/kg) followed by flowers (114 mg/kg), leaves (69 mg/kg), and stems (21 mg/kg).[4]

Much effort has been directed toward the identification of nonvolatile precursors of aroma compounds in plants.[5] In particular, bound aroma compounds in tobacco have been isolated and described. Evidence exists that such glucosides are unstable under boiling water conditions and yield the expected aglycones as hydrolysis products. It is reasonable to assume that the same phenomenon occurs in a burning cigarette, where glucosides are cracked under pyrolysis conditions. Depending on the type, quality, and part of the plant, smoke condensates of tobaccos such as Virginia and Burley[6] have been shown to contain considerable amounts of bound aroma compounds, which positively influence the aroma of tobacco smoke. Thus, the general experimental procedures used for smoke condensates are the same as those used for tobacco samples. For Virginia/Burley tobaccos, aromatic alcohols, norcembranoids, and norcarotinoids were detected. Similar results were obtained with flue-cured tobacco when it was extracted with methylene chloride and steam-distilled or extracted with 60% aqueous alcohol overnight in the presence of β-glucosidase.

Originally, a large number of volatile norisoprenoid compounds were found in tobacco, whereas very few studies concerning nonvolatile terpene glucosides in tobacco have appeared. More recently, this situation has changed. Kodama et al. have isolated from the nonvolatile constituents in flue-cured tobacco leaves 3-hydroxy-5,6-epoxy-β-ionyl-β-D-glucopyranoside.[7] 3-Hydroxy-5,6-epoxy-β-ionol obtained by enzymatic hydrolysis of the glucoside had the same relative structure as the naturally occurring *trans*-epoxide. The isolation of rishitin-β-sophoroside from flue-cured tobacco leaves has been reported.[8] The absolute configuration of the aglycone, rishitin, was identical with that obtained from potato

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tuber tissue infected by pathogens. In another study, the β-glucosides of 3-oxo-α-ionol and 5,6-epoxy-5,6-dihydro-3-hydroxy-β-ionol were isolated from fresh leaves of *Nicotiana* species.[9] Studies have confirmed that they are present widely in plant species such as tobacco and that they are hydrolyzed by β-glucosidase during the aging and curing process to liberate monoterpene alcohols as perfumes.

In a more recent paper, HPLC coupled with atmospheric pressure chemical ionization mass spectrometry was used to screen and identify glucosides in tobacco leaf.[10] A total of 12 glucosides were found, and 4 of them were identified on the basis of their abundant $[M + H]^+$ ions, UV spectra, and MS/MS analysis. They were scopolin, rutin, quercetin-3-glucoside, and kaempferol-3-rutinoside. These glucosides were analyzed and compared on the basis of the relative peak area to the internal standard in several kinds of tobacco leaves.

The work reported here concerns tobacco flowers and glucosidically bound volatile components. Two 20-year-old reports are strategically significant as they relate to hydrolysis and sample cleanup. Loughrin et al. extracted glucosidically bound volatile components from flowers of *Nicotiana sylvestris* and *Nicotiana suaveolens* and isolated them using liquid column chromatography.[11] These glucosides, which were precursors of fragrance compounds, were hydrolyzed enzymatically, and a number of the volatiles released were identified by GC-MS. Average yields of volatiles released from glucosides were ca. 230 and 1050 μg/g for *N. sylvestris* and *N. suaveolens* flowers, respectively. Compounds in the glucosidically bound fraction were all phenylpropanoid-derived volatiles such as benzyl alcohol, benzaldehyde, cinnamyl alcohol, and benzyl salicylate. Pronounced differences were obtained in the concentrations of volatiles at different floral maturity stages. In a second related study,[12] ground freeze-dried flowers of 63 *Nicotiana* species were extracted with methanol in an ultrasonic bath and analyzed for their flavonol content. Only quercetin and kaempferol were found in any appreciable quantity. The percentage distribution of these compounds showed that the flavonols of tobacco flowers varied much more than tobacco leaf flavonols.

Mineral acid hydrolysis has been linked with the formation of artifacts when employed in the hydrolysis of relatively high molecular weight glucosides such as those occurring with flavonoids and steroids.[13−17] Most of the artifacts were related to the oxidation of the aglycones, yielding such artifacts as lactones. On the other hand, no aglycone artifacts were noted when mineral acid hydrolysis of relatively low molecular weight glycosides such as those of vanillin was studied. In fact, a regression correlation analysis between the yields obtained employing acid and enzymatic hydrolyses yielded a linear response with an R^2 of 0.91.[17] Nonetheless, artifact formation upon acid hydrolysis of the glycosides of interest in this paper was monitored closely. The goal of our study was to ascertain in a quantitative fashion any changes in yield of volatile products (i.e., aglycones) afforded by acid hydrolysis of various *Nicotiana* flowers.

EXPERIMENTAL PROCEDURES

Materials. Sulfuric acid (H_2SO_4), phosphoric acid (H_3PO_4), phenyl-α-D-glucopyranoside (PADG), *n*-octyl-β-D-glucopyranoside (OBDG), ethyl vanillin glucopyranoside (EVG), benzyl-α-D-glucopyranoside (BADG), and XAD-2 Amberlite resin (all of ACS grade) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All HPLC grade solvents (hexane, CH_2Cl_2, MeOH, and H_2O) were obtained from Fisher Scientific (Pittsburgh, PA, USA). All volatile, tobacco-derived standards (*cis*-3-hexenol, benzaldehyde, *trans*-caryophyllene, isophorone, ketoisophorone, β-damascone, β-damascenone, α-ionone, benzyl alcohol, phenethyl alcohol, β-ionone, *trans*-cinnamaldehyde, nerolidol isomer 1, nerolidol isomer 2, and eugenol) were purchased from Sigma-Aldrich.

Table 1. External Standards and Qualifying Ions for SIM Analysis

analyte	molecular mass	qualifying ion
cis-3-hexenol	100	82
benzaldehyde	106	106
trans-caryophyllene	204	133
isophorone	138	138
ketoisophorone	154	152
β-damascone	192	177
β-damascenone	190	121
α-ionone	192	121
benzyl alcohol	108	108
phenethyl alcohol	122	91
β-ionone	192	177
nerolidol isomers 1 and 2	222	161
trans-cinnamaldehyde	132	131
phenol	94	94
ethyl vanillin	166	137
octanol	130	69
eugenol	202	164

Table 2. Recovery of Alcohol after Optimum Acid and Enzymatic Hydrolyses of Four Glucosides Using Hexane (Hex) and Dichloromethane (DCM) Extraction in Tandem

	av % recovery			
	1-octanol	benzyl OH	phenol	EV
50 mg, 48 h, 45 °C, Hex	78.0	48.5	12.5	84.8
50 mg, 48 h, 45 °C, DCM	3.0	36.5	17.4	34.5
total, enzyme	**81.0**	**85.0**	**30.0**	**119.2**
2 M H_2SO_4 24 h, 75 °C, Hex	77.1	53.7	33.4	76.5
2 M H_2SO_4 24 h, 75 °C, DCM	3.0	42.7	51.2	25.7
total, 2 M H_2SO_4	**80.1**	**96.4**	**84.6**	**102.2**

Table 3. Percent Recovery of Aglycones after Spiking Glucoside Standards onto Flowers Followed by Acid Hydrolysis/Extraction[a]

		% recovery			
t_R, min	internal standard	Alata	FC	Sy	Su
24.71	1-octanol	25 (54)	24 (18)	37 (11)	31 (18)
40.48	benzyl alcohol	1165 (18)	299 (20)	167 (6)	765 (22)
46.67	phenol	27 (27)	25 (19)	31 (18)	17 (35)
64.56	ethyl vanillin	41 (31)	41 (17)	48 (17)	33 (19)

[a]Numbers in parentheses are relative standard deviations ($n = 3$).

GC-MS Analysis. All GC-MS analyses were performed using an internal standard and 5890 series II GC coupled to a 5972 MSD from Agilent (Wilmington, DE, USA). Separations were obtained using a DB-Waxetr column (30 m × 250 μm, d_f 0.25 μm) from Agilent (catalog no. 1227332). The following operating parameters were used for each analysis: injection port temperature, 250 °C; purge valve, 9 mL/min; purge time, 1 min; total flow, 14 mL/min; constant flow, 1 mL/min; injection volume, 1 μL splitless; column oven initial temperature, 50 °C; column initial time, 1 min; first ramp rate, 2 °C/min; final temperature, 170 °C; second ramp rate, 15 °C/min; final temperature, 220 °C; column oven final time, 5 min; mass spectrometer transfer line temperature, 260 °C; 70 eV; mass spectrometry database, Wiley. Software was Chemstation, version A03.00.

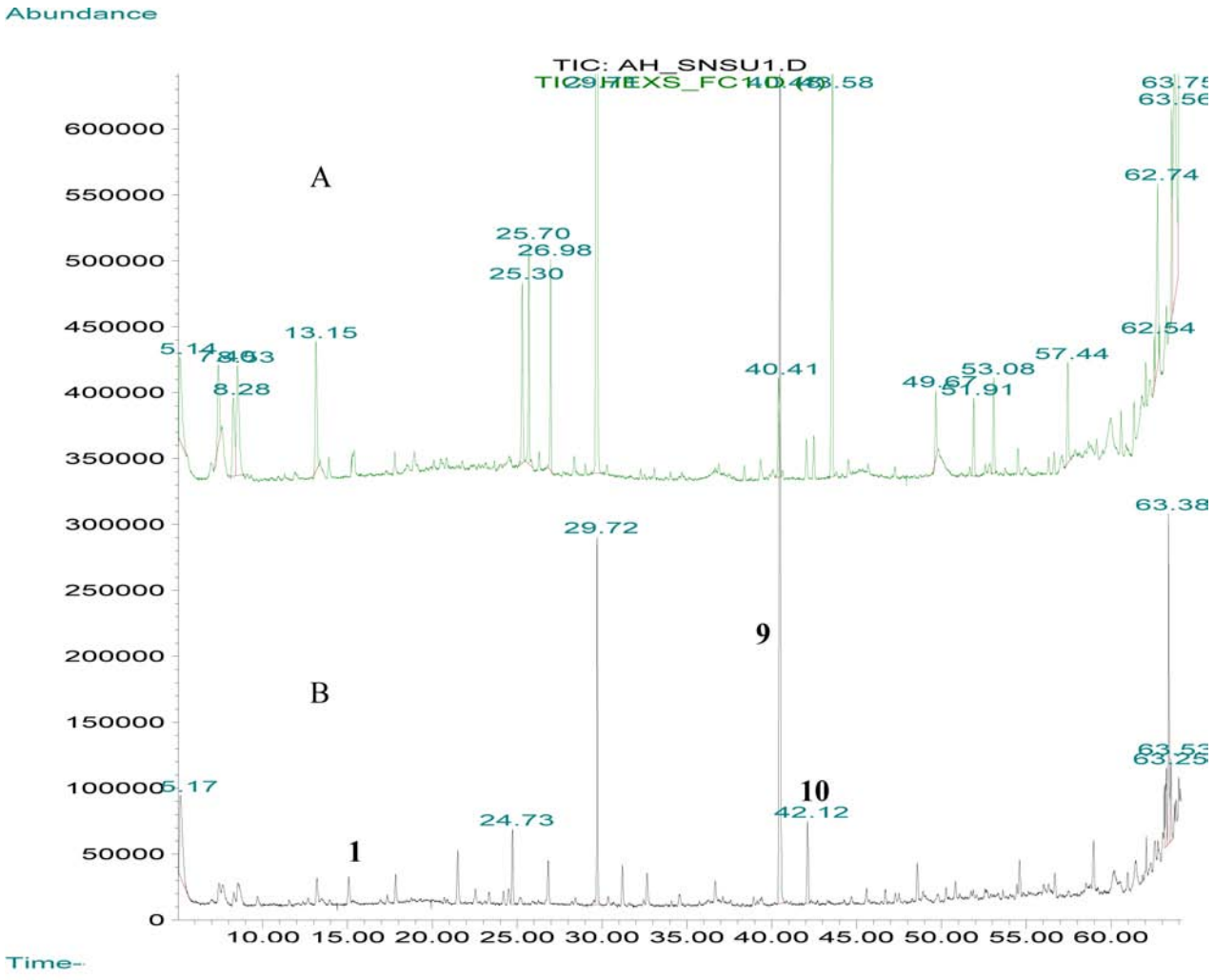

Figure 1. GC-MS of hexane extracts before (A) and after acid hydrolysis (B) for Su (scan mode). Peaks 1, 9, and 10 are assigned to *cis*-3-hexenol, benzyl alcohol, and phenethyl alcohol.

Acid Hydrolysis and Extraction of Flowers. Fresh *Nicotiana* flowers (*Nicotiana alata* (Alata), *Nicotiana sylvestris* (Sy), *Nicotiana suaveolens* (Su), *Nicotiana tabacum* flue-cured cultivar (FC)) from four farms in Virginia and North Carolina were provided by R. J. Reynolds Co. In each experiment, 50 fresh flowers were frozen separately using liquid N_2, then crushed in a motar and pestle while still frozen, and extracted twice with 50 mL of hexane to remove free volatile compounds. The extract residue (i.e., raffinate) was washed with an additional 20 mL of hexane for a total volume of 120 mL. Hexane was then concentrated using a turbo evaporator under stream nitrogen. Next, the volume of the solution was adjusted to exactly 3 mL and later analyzed via GC-MS.

Glucosidically bound volatile components were next analyzed by using a method modeled after that of Loughrin et al.[11] The hexane-extracted flowers were spiked with 50 μL of the four glucoside standard mixture (1.87 mg/mL BADG, 2.54 mg/mL OBDG, 1.69 mg/mL PADG, and 2.55 mg/mL EVG) and then treated twice with 60 mL of cold 0.2 M sodium dihydrogen phosphate buffer, pH 7.1. An additional 10−20 mL of buffer was used to further wash the treated flowers (total = 140 mL). The resulting brown solution was next passed through a bed of Celite 545 to remove suspended particulates. A clear brown solution resulted, which was then passed through a column (1.0 × 25 cm) packed and preconditioned with XAD-2 Amberlite resin at a flow rate of 2−3 mL/min. This procedure was followed by rinsing the column with 50 mL of H_2O to remove sugars and organic acids. To ensure there were no free volatiles in this column, it was washed with 50 mL of hexane, which was discarded. Glucosides were then eluted from the same column using 50 mL of methanol. The volume of methanol was reduced under vacuum on a rotary evaporator at 40 °C. The resulting sample (about 1.5 mL) was then diluted with 25 mL of 2 M H_2SO_4 and hydrolyzed for 24 h at 75 °C. The liberated volatile compounds (i.e., aglycones) were sequentially extracted three times with 15 mL of hexane and three times with 15 mL of DCM. The combined hexane/DCM extracts were dried over Na_2SO_4 and then evaporated via a turbo evaporator ((TurboVap II Caliper Life Sciences, Hopkinton, MA, USA) using a N_2 stream. The final volume was adjusted to exactly 5 mL and analyzed via GC-MS. Table 1 shows the list of external standards and qualifying ions that were used for quantitative determination in SIM mode.

RESULTS AND DISCUSSION

Greenhouse-Grown Flowers. Employing hydrolysis, fractionation, and analytical procedures very similar to those reported herein, a relatively limited number of experiments on greenhouse-grown *Nicotiana* flowers were completed prior to experiments on field-grown *Nicotiana* flowers. Briefly, hexane extractions on *N. alata* flowers before and after hydrolysis using

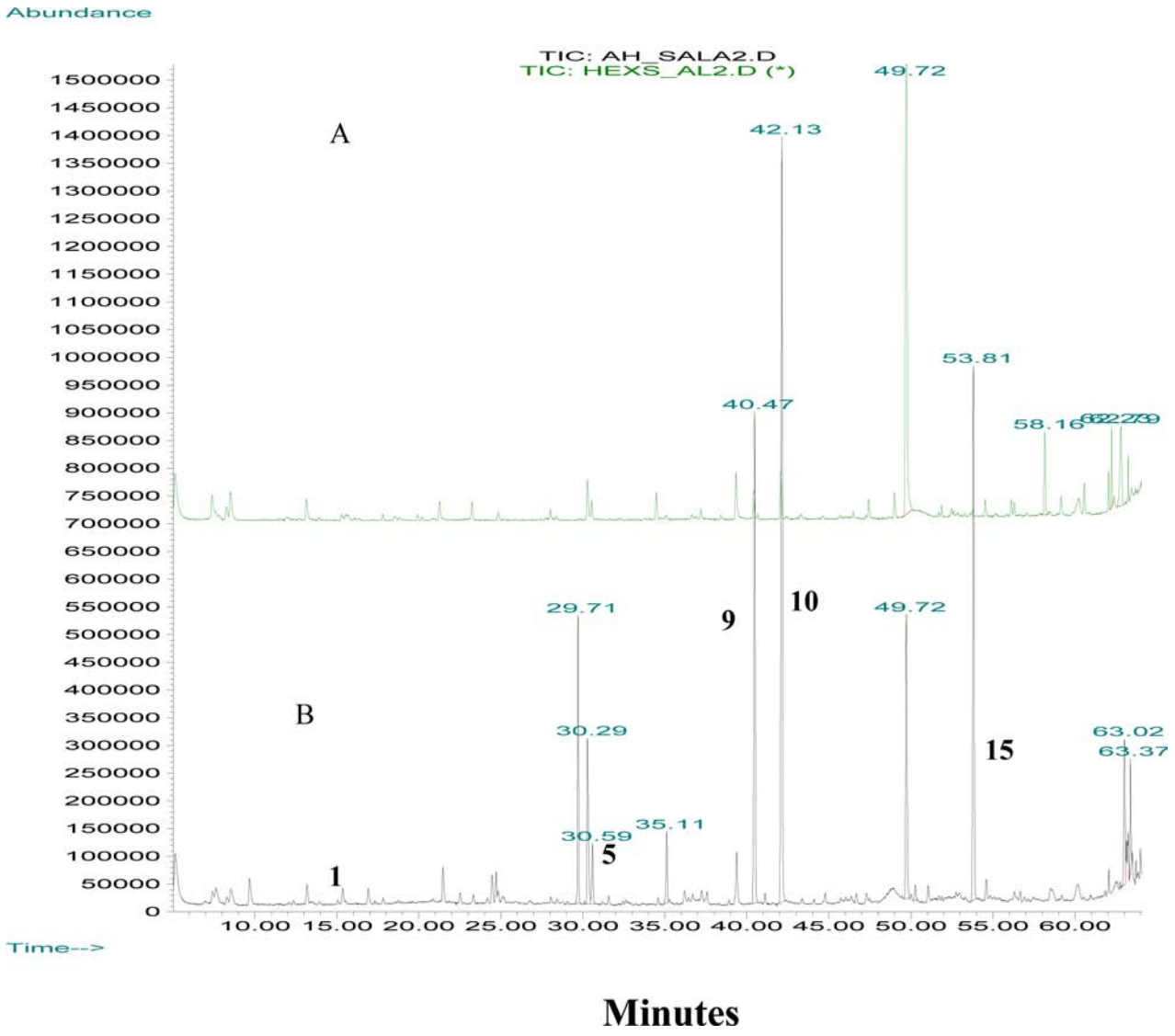

Figure 2. GC-MS of hexane extracts before (A) and after acid hydrolysis (B) for Alata (scan mode). Peaks 1, 5, 9, 10, and 15 are assigned to *cis*-3-hexenol, ketoisophorone, benzyl alcohol, phenethyl alcohol, and eugenol, respectively.

2.5 N HCl revealed notable inceases in the concentration (5−10×) of a number of aroma compounds such as benzyl alcohol, phenethyl alcohol, and eugenol.

These preliminary results from unoptimized hydrolysis conditions prompted a more in-depth investigation into conditions necessary to optimize the hydrolysis procedure(s). Furthermore, the experiments designed to optimize the hydrolysis were performed on freshly harvested field-grown *Nicotiana* flowers versus greenhouse-grown *Nicotiana* flowers.

Optimization of Analytical Procedure. Via selective washing of absorbed compounds from the XAD resin, a mixture of the four glucopyranoside standards outlined under Experimental Procedures was prepared and chromatographically divided into an aqueous and two organic (MeOH and hexane) fractions. After division, none of the four standards were quantitatively found in either the aqueous or hexane fractions via HPLC/ELSD, whereas each of the standards was identified in the MeOH fraction. In addition, no free volatile components of the glucopyranosides were found in the hexane (GC-MS) fraction. Acid hydrolysis of the methanol fraction, which contained the four glucopyranoside standards, after 24 h showed only 25−75% conversion of the bound volatile component to the free unbound volatile component when extracted with hexane (i.e., 1-octanol, phenol, benzyl alcohol, and ethyl vanillin). These poor recoveries prompted a closer examination of the hydrolysis conditions and the extraction process because earlier experiments as outlined above indicated that no glucoside or associated aglycone was seen in the preliminary aqueous or hexane chromatographic factions.

Subsequently, a mixture of the same four glucopyranoside standards was prepared and hydrolyzed via both acidic and enzymatic procedures. The acidic conditions involved either phosphoric acid or sulfuric acid. Enzymatic hydrolysis per a literature recipe[11] was also performed. In each case the products of hydrolysis (i.e., 1-octanol, phenol, ethyl vanillin, and benzyl alcohol) were extracted with hexane prior to analysis via GC-MS. In two cases, percent recovery was again <50% due to poor solubility of the aglycone in hexane. More quantitative recoveries for all four aglycones were, however, achieved when the product extractions were performed in tandem with first hexane and then dichloromethane. The two extracts were combined and analyzed, and the results were compared (Table 2). The preferred method

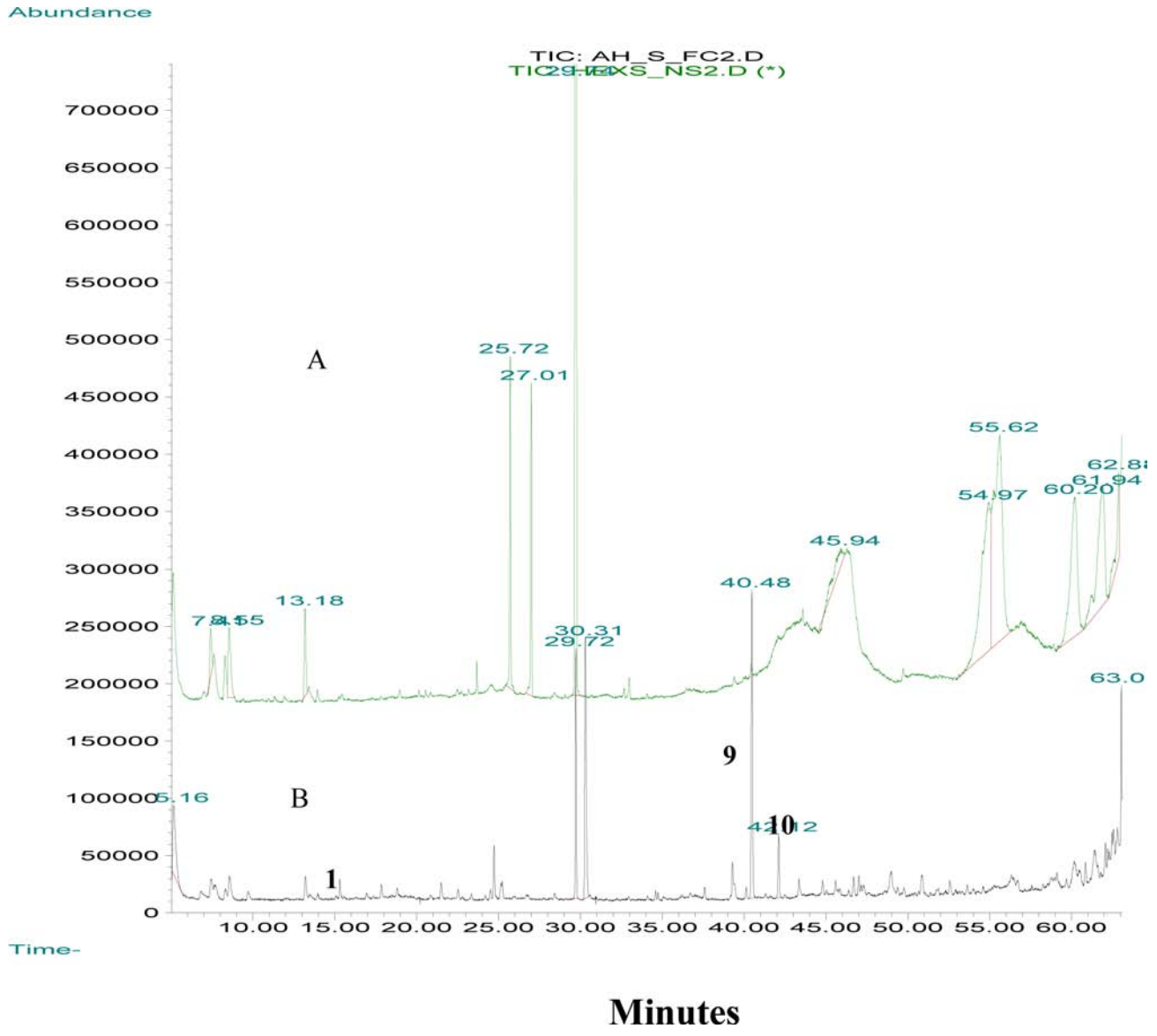

Figure 3. GC-MS of hexane extracts before (A) and after acid hydrolysis (B) for flue-cured (scan mode). Peaks 1, 9, and 10 are assigned to *cis*-3-hexenol, benzyl alcohol, and phenethyl alcohol, respectively.

of hydrolysis proved to be acid hydrolysis with sulfuric acid, and the preferred aglycone extraction process was hexane and dichloromethane in tandem. As with the results of Schneider,[17] no aglycone artifact formation was noted in any of the chromatograms.

Recovery of Spiked Glucoside Standards. GC-MS calibration curves (i.e., SIM mode) were constructed for the selected 15 volatile standards to determine their individual concentrations in each extract ($R^2 > 0.995$). Because the initial phase of our study concerned the recovery of glucoside standards, GC-MS calibration plots in SCAN mode for the aglycones of these four spiked standards were next developed. 1-Octanol and phenol exhibited low spiked recoveries near 25% (Table 3). Ethyl vanillin recovery was approximately 40%. 1-Octanol, phenol, and ethyl vanillin are not expected to be found in any of the flowers. Benzyl alcohol, on the other hand, was found in higher concentration than the spike, which is due to the fact that benzyl alcohol is indigenous to the flowers. In this study, it is therefore difficult to determine the recovery of benzyl alcohol, yet recovery of ethyl vanillin did not exceed 50%. This lower recovery of ethyl vanillin as well as 1-octanol and phenol could be due to not having optimized conditions for extraction of larger samples. As previously noted, 50 flowers of each *Nicotiana* species were treated, resulting in a recovery of approximately 50% for ethyl vanillin, whereas when 25 flowers were treated, recovery was near 100%. Furthermore, the extracted flowers were not freeze-dried; thus, 100% of the extracts was never analyzed, which may account for the lower recovery. Thus, once again, optimization of hydrolysis for naturally occurring flavor precursors may not be the same as that observed for pure glucosides.

Quantitation of Selected Analytes. From an aroma and flavor perspective, it was important to determine if there were any other volatile compounds present in the flower both before and as a result of the extraction/isolation/hydrolysis process. For this purpose, all hexane extracts of flowers before acid hydrolysis and all hexane/DCM extracts after acid hydrolysis were analyzed via GC-MS using both SCAN and SIM modes. Figures 1−4 show GC-MS traces of each flower extract before and after acid hydrolysis. The bold numbers appearing in Figures 1−4 refer to

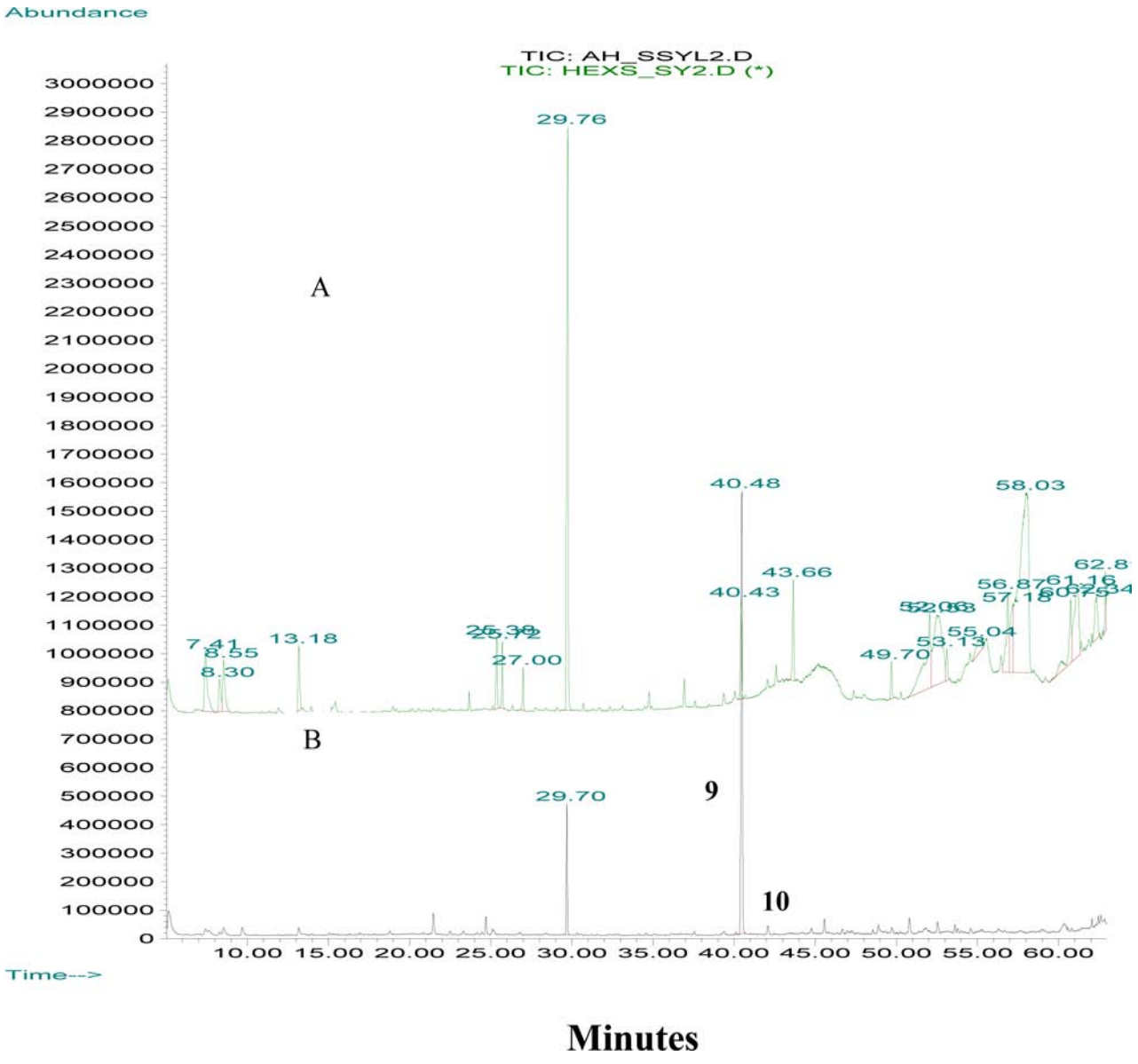

Figure 4. GC-MS of hexane extracts before (A) and after acid hydrolysis (B) for Sy (scan mode). Peaks 9 and 10 are assigned to benzyl alcohol and phenethyl alcohol, respectively.

the compound numbers listed in Table 4, which shows the calculated concentrations of the 15 compounds that were monitored. The concentration of each compound was determined using external calibration (see Table 1). Benzyl alcohol, phenethyl alcohol, eugenol, and *cis*-3-hexenol were quantitatively found in most extracts both before and after acid hydrolysis. Several of the standards were not found in either extract. Our initial premise that acid hydrolysis would increase the yield of aglycones was borne out. With specific flowers, enormous increases in yield of the aforementioned aglycones were observed. The magnitude of enhanced yield of benzyl alcohol, phenethyl alcohol, *trans*-cinnamaldehyde, and eugenol by acid hydrolysis with 2 M sulfuric acid was impressive in selected cases. For example, all *Nicotiana* flowers yielded after hydrolysis 9.8−94.6-fold additional benzyl alcohol. The situation for phenethyl alcohol was not as general. There was little or no increase in yield with all *Nicotiana* flowers except Alata and FC, for which increases of 4.9- and 46.3-fold were noted for Alata and FC, respectively (Table 5). A similar situation described eugenol in that only Alata exhibited a large increase in yield (i.e., 160-fold). Finally, it is noteworthy that Su uniquely gave a great increase in *trans*-cinnamaldehyde upon hydrolysis (i.e., 10-fold). Each extract was also examined in SCAN mode for general compound identification. Tables 6 and 7 list those compounds that yielded strong signals and were tentatively assigned on the basis of a comparison of library-searchable spectra. The quality of match exceeded 75% in most cases. In no case were artifacts detected in the chromatograms of the aglycones.

Optimized relatively mild acid hydrolysis of fresh field-grown *Nicotiana* flowers revealed significant increases in the concentration of a number of flavor compounds such as phenethyl alcohol, eugenol, and cinnamaldehyde. Details of the experimental procedures strongly implicated the hydrolysis of selected glucosides as the main mechanism for the observed concentration increases. Thus, processes designed for optimal recovery of volatile flavor compounds from fresh field-grown *Nicotiana* flowers must account for the meaningful increases in volatile flavor compounds resulting from relatively mild acid hydrolysis

Table 4. Average Concentration (Micrograms/50 Flowers) of Selected Volatile Compounds (n = 3) in *Nicotiana* Flowers as Free and Glucosidically Bound[a]

			free volatiles, initial hexane extraction before acid hydrolysis				glucosidically bound volatiles, after acid hydrolysis			
	t_R, min	analyte	Alata	FC	Sy	Su	Alata	FC	Sy	Su
1	15.36	*cis*-3-hexenol	4.9 (28)	9.6 (32)	1.6 (75)	3.2 (23)	7.2 (37)	2.8 (21)	0.7 (11)	1.4 (21)
2	21.51	benzaldehyde	0	0	0.2 (68)	0	0	0	0	0
3	25.24	*trans*-caryophyllene	0	0	0.6 (60)	0	0	0	0	0
4	25.24	isophorone	0	0.7 (14)	0	0	1.3 (178)	26 (7)	3.3 (13)	1.3 (27)
5	30.59	ketoisophorone	9.12 (24)	0	0	0	14.4 (17)	2.2 (4)	0	0
6	37.05	β-damascone	0	0	0	0	0	0	0	0
7	37.29	β-damascenone	0	0	0	0	0	0	0	0
8	38.82	α-ionone	0	0	0	0	0.2 (31)	0	0.1 (77)	0
9	40.48	benzyl alcohol	12.8 (19)	25.8 (14)	109.4 (16)	4.9 (29)	586.3 (31)	253.1 (9)	1084.2(5)	462.6 (23)
10	42.12	phenethyl alcohol	14.9 (24)	10.5 (64)	12.3 (13)	16.9 (18)	690.6 (26)	41.4 (7)	17.4 (6)	33.6 (26)
11	43.11	β-ionone	0	0.3 (128)	0.4 (77)	0	0	0	0	0
12	47.19	nerolidol iso 2	0	0	0	0	0	0	0	0
13	47.51	*trans*-cinnamaldehyde	0	0	0	0.5 (104)	0	0	0	4.9 (19)
14	49.00	nerolidol iso 1	3.2 (9)	0	0	0	0	0	0	0
15	53.83	eugenol	2.7 (14)	0	12.0 (30)	0.4 (170)	433.5 (32)	0	7.2 (13)	0

[a]Numbers in parentheses are relative standard deviations. Each compound has been identified on the basis of a reference volatile individually purchased from Sigma-Aldrich Co.

Table 5. Approximate Extraction Yield Enhancement (n-fold) of Selected Volatiles: Hexane versus Acid Hydrolysis/ Hexane/DCM (Micrograms/50 Flowers)

Nicotiana	benzyl alcohol	phenethyl alcohol	eugenol	cinnamaldehyde
FC	9.8	46.3	0	0
Alata	46.5	4.9	160	0
Su	94.6	1.4	0	0
Sy	9.9	2.0	0	10.0

Table 6. Compounds Identified in Hexane Extract of *Nicotiana* Flowers As-Received Exclusive of the 15 Standards

t_R, min	analyte	FC	Alata	Su	Sy
13.15	3-methylcyclopentanol[a]	Y		Y	Y
25.69	3-hexanone	Y		Y	Y
26.99	butyl ketone	Y			Y
29.72	norsolanadione[a]	Y		Y	
43.58	neophytadiene	Y			Y
45.36−45.93	duvatrienols	Y			
51.91	heneicosane	Y			
53.08	norsolanadiene	Y			

[a]Tentatively identified.

Table 7. Compounds Identified in Hexane/DCM Extracts after Acid Hydrolysis Exclusive of the 15 Standards

t_R, min	analyte	FC	Alata	Su	Sy
29.72	4,4-dimethyl-2-pentene	Y	Y	Y	Y
30.29	2-methylbutyric acid	Y	Y		
63.36	benzoic acid	Y	Y	Y	Y

of the flowers prior to conventional solvent extraction. These findings on field-grown flowers were in concert with results from experiments performed on greenhouse-grown flowers. The dramatic increases in concentration of eugenol and cinnamaldehyde due to mild acid hydrolysis strongly indicate that this relatively simple hydrolysis could be elevated to a production scale level yielding marketable quantities of these important natural flavors.

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Notes

The authors declare no competing financial interest.

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