Contribution of Neutral Volatiles to Flavor Intensity of Tobacco during Smoking

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Gas chromatographic (GC) profiles of tobacco volatiles and analyses of reducing sugars and total alkaloids as well as subjective smoking evaluations were used to test different tobaccos for tobacco flavor and aroma. Twenty of the major peaks from the GC profiles and subjective smoking scores were analyzed by multiple linear regression. Seven compounds of 20 were included in the regression model that was significant with correlation coefficient $R^2 = 0.702$. Total volatile data for the 20 peaks and the subjective scores were transformed to comparable scales and analyzed by linear regression. The regression model was significant with a low correlation coefficient of $R^2 = 0.300$. This study agrees with previously published data that volatiles are positively correlated with tobacco flavor and that some specific volatile compounds might be used as references of tobacco quality.

INTRODUCTION

Smoke flavor is the taste and aroma obtained from a cigarette. The concentration of flavor constituents changes from puff to puff from a cigarette, thereby influencing flavor. This phenomenon is more obvious to some smokers than others because of differences in the way different individuals smoke. Of the more than 4000 compounds identified from cigarette smoke, 1200 are derived directly from tobacco (Dube and Green, 1982). Some constituents can be transferred from tobacco to smoke by volatilization and mass transfer, and the structures of these constituents are unchanged (Jenkins et al., 1979). Flavor from any compound can only be identified if the compound exists in tobacco in sizeable quantities because only approximately one-third of any volatile compound transfers directly from tobacco to the smoke (Wakeham, 1972). This phenomenon reduces the number of volatile compounds that directly influence smoke flavor because of the low concentrations of most volatile compounds. Roberts (1988) indicated of all the volatile compounds produced in tobacco only 41 compounds are found in large enough quantities to enable the smoker to identify specific contributing notes to smoke flavor. Over the past 20 years, volatile compounds from tobacco have been intensively investigated, and the relationship of volatile compounds to smoke flavor is well documented. Kimland et al. (1972) identified specific oxygenated volatile compounds from Greek tobacco that occurred in the smoke and influenced smoke taste. Demole and Berthet (1972) characterized volatile and semivolatiles from burley tobacco by GC/MS and identified these as contributing burley flavor notes to smoke. Wahlberg et al. (1977) identified several hundred acidic, basic, and neutral compounds from the headspace of ageing flue-cured tobacco. They reported that many of the same compounds were found in cigarette smoke. Lloyd et al. (1976) identified 276 volatile acidic, basic, and neutral compounds for the first time from flue-cured tobacco and confirmed that many of the identified compounds contributed to the flavor of blended cigarettes. Sakaki et al. (1986) collected volatiles from the exhaust of tobacco-processing plants for tobacco enrichment studies. The more concentrated compounds identified in that study were also determined from the head space of tobacco and correlated with subjective smoke evaluations from the cigarettes made from individual tobaccos.

Commercial cigarettes are blends of tobacco types and classes by weight to obtain balance, taste, smoke strength and nonirritating smoke. It is also necessary to obtain leaf chemistry, subjective smoke, and physical data of tobaccos to develop new cigarette brands. The tobaccos used in most commercial blends consist of flue-cured, burley, Oriental, and reconstituted tobaccos (Green, 1977). The tobacco blends are cased with sauces, humectants, and flavorants, yet tobacco still remains the primary source of flavor perceived by smokers.

This study was conducted to determine (1) the effect produced by some of the neutral volatile compounds upon smoke flavor; (2) if chromatographic profiles of neutral volatiles obtained from tobacco could be correlated with sensory smoke evaluations obtained from a trained smoke panel; and (3) the use of GC profiles, chemical data, and subjective smoke evaluation of different tobaccos in fabricating blends.

MATERIALS AND METHODS

All of the tobaccos (*Nicotiana tabacum* L.) used in this study were grown in North Carolina, except the Oriental tobacco (Samsun) which was obtained from Philip Morris, USA. Tobacco company leaf experts described these North Carolina grown tobaccos as comparable to those tobaccos obtained from areas where they are normally grown.

Twelve samples were selected from flue-cured NC-95, NC-2326, DB-101, McNair 944, Speight G-70, and six air-cured tobaccos: Burley 21 and KY 14, Brazilian Galpao and Amarelinho, Oriental Samsun, and American Aromatic (new air-cured type breeding line developed by E. A. Wernsman, NCSU). The tobaccos were cut into rag, conditioned to 11% moisture, and made into 85-mm filtered cigarettes using a hand-making cigarette machine (Central Tobacco Manufacturing, Canada). The cigarettes from the 12 tobaccos were smoked by four NCSU Tobacco Laboratory smokers to evaluate the cigarettes for flavor, irritation, smoke strength, and off-flavor. The tobaccos were ground and analyzed for neutral volatiles by capillary GC (Weeks et al., 1989), total alkaloids, and reducing sugars (Harvey et al., 1969).

On the basis of the above evaluation and data, six of the tobacco types above and the 50:50 blend of flue-cured tobaccos (Speight G-70 and NC-2326) were used to fabricate (by weight, 1 g) 15 blended cigarettes (Table I). Fifteen cigarettes from each of the 7 tobaccos and the 15 blends were delivered to P. Lorillard Tobacco Co. for a smoke evaluation by a trained, four-person smoking panel. The panel evaluations were made using the multiple sample difference method (Abdallah, 1970). Each panelist smoked three test cigarettes against the check cigarette

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Table I. Proportional Composition of 22 Individual Tobaccos and Blends

	% by wt						
sample	NC-2326	Ameri[a]	KY14	Amare[b]	Samsun	Galpao	Sp G-70
1		100					
2			100				
3				100			
4					100		
5						100	
6[c]	50						50
7							100
8			30				70
9		30					70
10						30	70
11					30		70
12		40					60
13		20					80
14		25	15	15	15	15	15
15		40	60				
16		30	70				
17		20	80				
18			25		5		70
19			20		10		70
20	35	30					35
21			30		10		60
22		30	10				60

[a] Ameri, American Aromatic. [b] Amarel, Amarelinho. [c] Check: 50% Speight G-70 and 50% NC-2326.

[Speight G-70/NC-2326 (50:50)]. The check was arbitrarily assigned a value of 10 for flavor intensity. The test cigarettes were evaluated on the basis of the opinion of each panelist's perception of the flavor of the cigarettes and the check. The mean of the four scores for a cigarette was assigned as the score for the test sample.

Twenty-two samples were analyzed for total neutral volatiles using a simultaneous distillation/extraction apparatus (Kontes Scientific Glass Ware Inc.) (Schultz et al., 1977). Ten grams of laminae, ground in a Wiley mill to pass a 40-mesh screen, was slurried in 250 mL of phosphate buffer, pH 6.8, and 5.0 g of sodium sulfate was added. The samples were steam distilled from the slurry into 250 mL of methylene chloride. The methylene chloride was denicotinized by partitioning with 1 M tartaric acid, dried over anhydrous sodium sulfate, and concentrated; and 0.3 μg/μL tetradecane was added as an internal standard. The volume of each sample was adjusted to 1 mL so that a 1-μL injection from the sample was equivalent to a single puff from a cigarette. A response factor of 1 was used to calculate individual peak concentrations from the chromatograms (Weeks et al., 1989).

Gas chromatography was performed with a Varian 3700 capillary GC equipped with a flame ionization detector. The injector and detector temperatures were operated at 250 and 270 °C, respectively. A 0.75 mm i.d. megabore WCOT 60-m Supelcowax (bonded phase) column was used for the chromatography. Helium was used as carrier and makeup gas at rates of 5 and 30 mL/min, respectively. Compressed air and hydrogen flow were calibrated at 375 and 30 mL/min. The oven temperature of the GC was programmed from 60 to 210 °C with multilinear programming, allowing 5-min delays at 140 and 180 °C to enhance separation, for an overall program of 1.5 °C/min. The column was held isothermal at 210 °C for 30 min to conclude the chromatogram. Each chromatogram contained over 170 peaks, of which many were too small to be quantified. The sample was not concentrated because the large peaks would have overloaded the column and resulted in poor resolution of the individual peaks. The 20 largest peaks were identified by GC/MS using a Vg-20-250 Quadrupole GC/MS by Brown and Williamson Tobacco Co. The GC/MS column, temperature program, and gas flow rates were the same as used to obtain the analytical data with the Varian 3700. The 20 compounds from all 22 samples were compared to the flavor ranking data by multiple regression procedure with the Stepwise-Forward option (SAS, 1985).

RESULTS AND DISCUSSION

Volatile profiles of individual tobaccos were positively related to scores obtained from the smoke panel. Galpao, Amarelinho, and American Aromatic tobaccos produced the highest quantity of neutral volatiles per cigarette from the 20 peaks compared from the individual tobaccos (profiles A, B, and C, Figure 1). These tobaccos also received the highest scores from the panel (samples 1, 3, and 5, Tables I and II). Samsun, KY 14, and Speight G-70 tobaccos produced the lowest quantities of neutral volatiles per cigarette (profiles D, E, and F, Figure 1), and these tobaccos also received the lowest scores from the panel (samples 2, 4, and 7, Tables I and II). The panel gave Speight G-70 a score of 7.5 compared to 10 that was assigned the check, a 50:50 mixture of NC-2326/Speight G-70. However, preference given to the check in this study was comparable to a previous study conducted by the NCSU Tobacco Laboratory in which the same smoking panel preferred cigarettes fabricated from a 50:50 mixture of Speight G-70 and NC-2326 over cigarettes made from tobacco of either Speight G-70 or NC-2326.

All samples had similar levels of total alkaloids measured as nicotine with the exception of Samsun (sample 4, Table II), but a considerable range in reducing sugar concentrations was obtained (Table II). Four individual air-cured tobacco samples, American Aromatic, KY 14, Amarelinho, and Galpao (samples 1, 2, 3, and 5, Table II), and three air-cured blends (samples 15–17, Table II) were lower in reducing sugars than flue-cured samples 6 and 7 (Table II) and the flue-cured blends 12–14 (Table II). This difference in reducing sugars, however, was expected as a result of the difference in curing between flue-cured and air-cured tobaccos. Flue-curing enhances reducing sugar concentration, while air-curing decreases reducing sugar concentration because sugar is depleted by respiration during air-curing.

Galpao, American Aromatic, and Amarelinho tobaccos produced burley and Oriental character which blended with flue-cured tobacco; therefore, care was taken to avoid overuse of these tobaccos in amounts greater than Oriental and burley tobaccos are used in American brands. For this reason, most of the blends fabricated were predominantly flue-cured. GC profiles of individual tobaccos were carefully examined to select tobaccos that would compliment each other in flavor, and efforts were made to use individual tobaccos in the blends so as to enhance the tobacco with the lowest flavor intensity.

The panel scores of all of the blended cigarettes except blends 11, 18, and 20 (Table II) were greater than the score assigned the check. Although these samples were scored lower than the check (sample 6, Table II), the panel scored these samples higher than Speight G-70 (sample 7, Table II). The panel described sample 22 as the best balanced (but not with the highest flavor intensity) cigarette of the 15 blends. This blend consisted of Speight G-70/KY 14/American Aromatic (60:10:30). Overall, blending the different tobaccos together gave positive results, as shown by the panel scores (Table II).

The 20 peaks chosen from the profiles for statistical analysis and the concentration range of each (Table III) represent the largest peaks in the profiles. This does not mean that the chemical compounds represented by the smaller peaks did not make a contribution to flavor; however, due to the concentration represented by the chemical compounds with larger peaks, we felt that these compounds had the greatest chance of transferring to the smoke as emphasized by Wakeham (1972). The chromatographic data and the panel scores were analyzed using

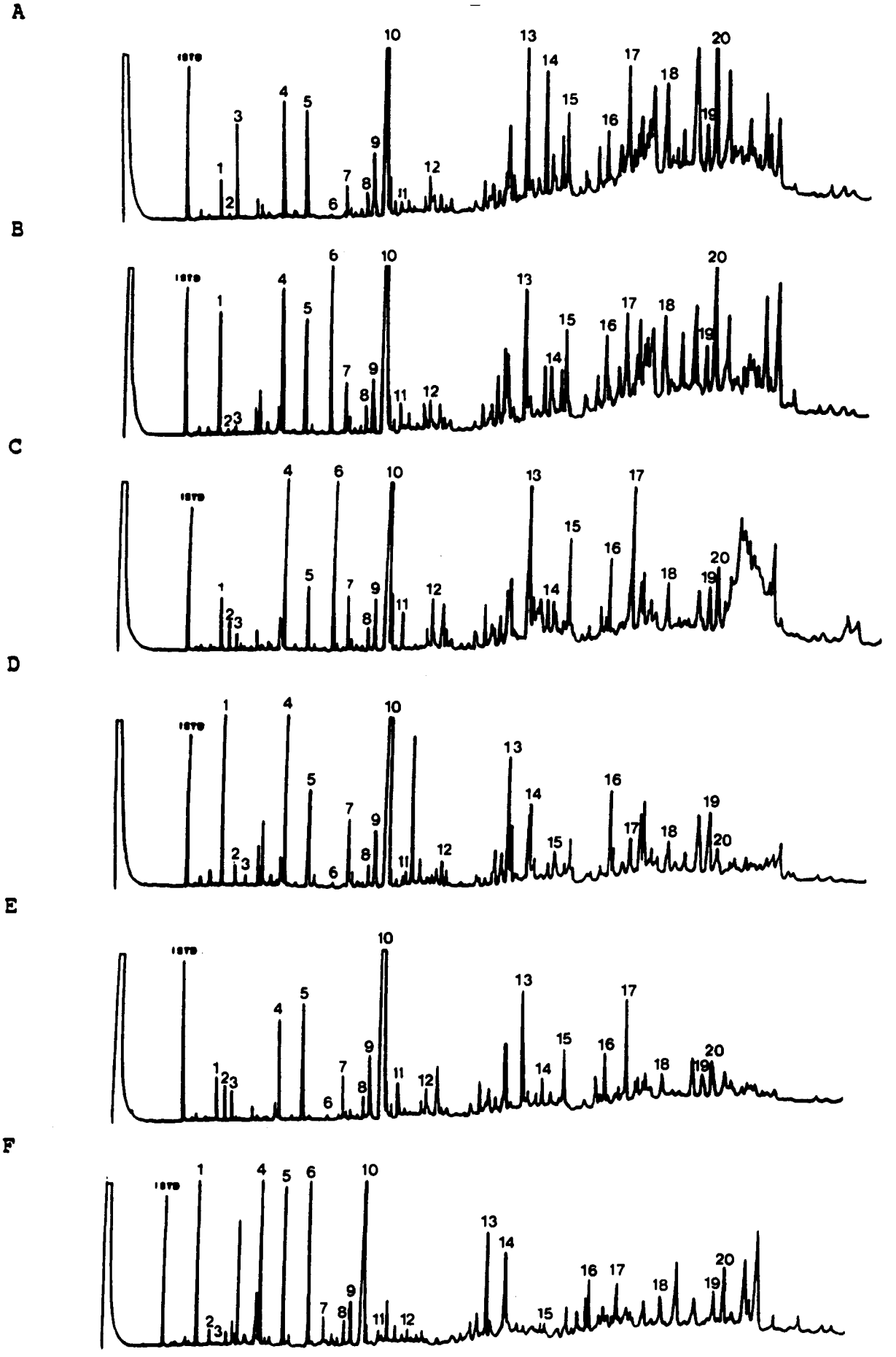

Figure 1. Profiles of neutral volatiles of individual tobaccos: (A) Galpao; (B) Amarelinho; (C) American Aromatic; (D) Speight G-70; (E) KY 14; (F) Samsun.

Table II. Panel Score and Chemical Data of Individual Tobaccos and Blends

sample	total alkaloids, %	reducing sugars, %	total volatiles of 20 peaks, ppm	panel score
1	3.02	0.35	831	13.62
2	3.25	0.30	700	6.50
3	3.12	2.60	876	13.00
4	0.96	15.80	601	7.50
5	3.02	0.55	900	14.00
6	2.65	24.70	754	10.00[a]
7	3.07	22.60	818	7.50
8	3.05	16.00	1178	12.00
9	3.08	16.00	725	12.25
10	3.05	16.60	900	11.50
11	2.43	20.56	631	8.75
12	3.04	15.00	992	13.25
13	3.05	18.70	813	14.00
14	2.77	8.40	988	12.25
15	3.11	0.33	1064	12.25
16	3.08	0.30	1347	12.75
17	3.06	3.00	1048	11.00
18	3.01	16.60	670	8.25
19	2.89	17.40	623	10.50
20	2.75	18.81	948	9.50
21	2.91	15.14	797	10.75
22	3.07	13.56	850	12.50

[a] Panel score assigned arbitrarily.

Table III. Twenty Volatile Peaks Chosen for Statistical Analysis

peak	compound	range, μg/g
1	furfural	8.7–161.0
2	furfuryl acetone	0.0–10.9
3	linalool	2.2–18.2
4	furfuryl alcohol	8.6–122.0
5	solanone	20.0–51.0
6	damascenone	1.0–123.0
7	geranyl acetone	12.0–33.0
8	solanol	3.0–47.0
9	phenylethanol	9.0–39.0
10	neophytadiene	169.0–940.0
11	1,3,7,7-tetramethyl-9-oxo-2-oxabicyclo[4.4.0]dec-5-ene	2.0–8.0
12	2,4,6,8-megastigmatrien-3-one	3.0–24.0
13	oxysolanone	9.0–48.0
14	methylethylmaleimide	3.0–23.0
15	paravinylphenol	3.0–29.0
16	4,6,8-megastigmatrienone	6.0–26.0
17	dihydroactinidiolide	4.0–58.0
18	indole	10.0–62.0
19	5,8-oxido-3,13-duvadiene-9-methylen-1-ol	5.0–40.0
20	4-hydroxy-β-damascone	8.0–90.0

Table IV. Multiple Regression Analysis (SAS-Stepwise-Forward)

source	DF	SS	MS	F	$P > F$
reg	7	75.7335	10.8191	4.71	0.0066
error	14	32.1337	2.2953		
total	21	107.8672			
R-SQ		0.702			

the Stepwise-Forward multiple regression procedure (SAS). Seven compounds from the 20 were included in the regression model. The F value was significant ($p < 0.007$) with the correlation coefficient $R^2 = 0.702$ (Table IV). This model is described by the equation $Y = 13.66 + 0.007X_4 - 0.29X_5 + 0.10X_{10} - 0.08X_{15} + 0.06X_{16} - 0.08X_{19} + 0.10X_{20}$. The concentrations of these seven individual compounds from the 22 samples were greater than 5 ppm with three exceptions (peak 15 in sample 19 and peaks 19 and 20 in the check).

The data were subjected to further analysis by correlating the total volatiles from the 20 compounds with the flavor scores of the 22 samples. Total volatiles were obtained by summing the concentrations of 20 compounds from each profile used for statistical analysis. The range of total volatiles of these 20 compounds was from 601 to 1347 ppm by nature, but the sensory scores obtained subjectively ranged from 6.5 to 14 by the scale established for evaluation from 5 to 15. The volatile data and the panel scores could not be correlated proportionally using this data. Thus, it was necessary to transform the data to perform the statistical analysis (Steel and Torrie, 1980). The total volatiles were arranged in ascending order from lowest to highest, and the median was determined; the panel scores were arranged in descending order, and the mean was determined. Total volatiles and panel scores were divided into two categories. The samples with total volatiles from the median and below were classified in group 1, and panel scores below the mean were also assigned to group 1. The samples with total volatiles above the median and the panel scores above the mean were assigned to group 2. The transformed data were analyzed by regression analysis. The correlation between total volatiles and flavor scores was significant with the correlation coefficient $R^2 = 0.300$ (Table V). This result supports the (Stepwise) regression analysis that identified 7 peaks of the 20 that were highly correlated with flavor intensities.

Table V. Linear Regression Analysis

source	DF	SS	MS	F	$P > F$
reg	1	1.6364	1.6364	8.57	0.0083
error	20	3.8182	0.1909		
total	21	5.4545			
R-SQ		0.300			

$Y = 0.7273 + 0.5454X$

CONCLUSIONS

The data from this study indicate that volatiles in cured tobacco are associated with flavor, and chemical data can be readily obtained to assist subjective smoking in selecting different tobacco types for fabricating blends which will produce balanced and flavorful smoking materials. This study also suggests that volatile profiling could be used with subjective smoking to monitor quality control. Volatile profiling can also be used as a tool to troubleshoot and identify sources of off-flavor, irritation, and other subjective notes described by a smoke panel. Volatile profiling of large quantities of tobacco along with other chemical data could help accurately predict smoking quality of tobacco before cigarettes are manufactured. The data also suggested that the air-cured tobacco types used in this study could be partially substituted for Oriental tobacco that is currently imported. Additionally, volatile profiling could be used to assist tobacco breeders in evaluating breeding lines for flavor characteristics.

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Registry No. Furfural, 98-01-1; furfuryl acetone, 699-17-2; linalool, 78-70-6; furfuryl alcohol, 98-00-0; solanone, 1937-54-8; damascenone, 23726-93-4; geranyl acetone, 3796-70-1; solanol, 40525-38-0; phenyl ethanol, 60-12-8; neophyladiene, 504-96-1; 1,3,7,7-tetramethyl-9-oxo-2-oxabicyclo[4.4.0]dec-5-ene, 20194-67-6; oxysolanone, 60619-46-7; methyl ethylmaleimide, 20189-42-8; *p*-vinylphenol, 2628-17-3; dihydroactinidiolide, 17092-92-1; indole, 120-72-9; 4-hydroxy-β-damascone, 35734-61-3.