Evaluation of the Effect of Phytol on the Formation of PAHs in Cigarette Smoke*

by

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SUMMARY

Phytol or (2E,7R,11R)-3,7,11,15-tetramethyl-2-hexadecen-1-ol ($MW = 296.53$ a.u.), is naturally present in tobacco at levels around 100–150 $\mu\text{g/g}$ dry leaf, where it is bound in the form of an ester to a porphyrine type group (known as chlorin) with several substituents and chelated to Mg^{2+} ion, to form chlorophyll. This study evaluated the formation of PAHs when several levels of phytol up to ten times higher than typical phytol level were added on 3R4F cigarettes. The cigarettes were smoked under two different smoking conditions. One regimen (indicated as 35/60) used 35 mL puff volume, 2 s puff duration, each puff taken every 60 s, and in the other used 60 mL puff volume, 2 s puff duration, each puff taken every 30 s (indicated as 60/30). The increase in the PAHs level upon phytol addition was not significant. A statistical evaluation of the dependence of total PAHs and the added level of phytol showed that the hypothesis of a zero slope for the dependence line phytol/total PAHs cannot be rejected (with a $p = 0.101$ for 35/60 smoking and $p = 0.626$ for 60/30 smoking). Still, for 35/60 smoking conditions, an increase of about 14% in total levels of PAHs was noticed when the added phytol level was 1.5 mg phytol per cigarette. However, this PAHs increased level was not necessarily determined by the phytol addition, and can be caused by random procedural/analytical errors (as indicated by the statistical analysis) or by the modification of cigarettes during phytol addition. Also, the observed level is much lower than the expected 100% increase in the PAHs level, based on literature prediction. Intensive smoking did not show an increase in PAHs level at all. Flash pyrolysis of free phytol and of chlorophyll *a* provided results that indicated that phytol bound in chlorophyll is not likely to generate different PAHs level compared to free phytol. Thus, phytol has been shown not to be a significant contributor/precursor to the PAHs formation in cigarette smoke. [Beitr. Tabakforsch. Int. 24 (2010) 10–23]

ZUSAMMENFASSUNG

Phytol oder (2E,7R,11R)-3,7,11,15-tetramethyl-2-hexadecen-1-ol ($MW = 296.53$ a.u.) kommt natürlicherweise in einer Menge zwischen 100–150 mg/g Trockenblätter vor, wo es in Form eines Esters an eine Porphyringruppe (bekannt als Chlorin) gebunden vorliegt. Es bildet mit verschiedenen Substituenten und an Mg^{2+} cheliert das Chlorophyll. Diese Arbeit untersucht den Einfluss unterschiedlicher zugesetzter Phytolmengen zu 3R4F Zigaretten (bis zu 10mal höhere Mengen als normale Phytolmengen) auf die Bildung von polyzyklischen aromatischen Kohlenwasserstoffen (PAK).

Die Zigaretten wurden nach zwei verschiedenen Verfahren abgeraucht. Bei dem einen Abrauchverfahren (im weiteren als 35/60 bezeichnet) wurde alle 60 s ein Zug mit 35 ml Zugvolumen und 2 s Zugdauer genommen, während bei dem anderen Abrauchverfahren alle 30 s ein Zug mit 60 ml Zugvolumen und 2 s Zugdauer (im weiteren als 60/30 bezeichnet) genommen wurde. Durch die Zugabe von Phytol kam es zu keinem signifikanten Anstieg der Menge an PAK. Eine statistische Auswertung der Abhängigkeit der Gesamtmenge an PAK und dem zugegebenen Phytol zeigte, dass die Hypothese eines Nullanstieges für die Abhängigkeitslinie Phytol/Gesamt PAK nicht ausgeschlossen werden kann (mit einem $p = 0.101$ für das 35/60- und $p = 0.626$ für das 60/30-Verfahren). Weiterhin konnte ein Anstieg von 14% der Gesamtmenge an PAK bei der Zugabe von 1.5 mg Phytol pro Zigarette bei der Bedingung 35/60 beobachtet werden. Allerdings muss dieser Anstieg der Menge an PAK nicht notwendigerweise durch die Phytolzugabe bedingt sein, sondern kann durch zufällige verfahrens- bzw. analytisch-bedingte Fehler (wie die statistische Auswertung zeigt) oder durch die Modifikation der Zigaretten während der Phytolzugabe verursacht worden sein. Außerdem war die beobachtete Menge der PAK sehr viel geringer als der 100%ige Anstieg, der in der Literatur prognostiziert wird. Ergebnisse einer Flash-Pyrolysestudie von freiem Phytol und von Chlorophyll *a* weisen darauf hin, dass Phytol, wenn es an Chlorophyll

gebunden vorliegt, mit einer geringeren Wahrscheinlichkeit verschiedene PAK bildet als freies Phytol. Demzufolge konnte gezeigt werden, dass Phytol kein charakteristischer Vorläufer für PAK im Zigarettenrauch ist. [Beitr. Tabakforsch. Int. 24 (2010) 10–23]

RESUME

Le phytol ou (2E,7R,11R)-3,7,11,15-tétraméthyl-2-héxadécène-1-ol ($pm = 296,53$ unité arbitraire) est naturellement présent dans le tabac à des niveaux d'environ 100 à 150 mg/g dans les feuilles sèches où il est lié, sous forme estérifiée, à un groupe de type porphyrine (connu sous le nom de chlorin) avec plusieurs substituant et chélaté à un ion Mg^{2+} pour former de la chlorophylle. Cette étude a évalué la formation d'HAP (hydrocarbures aromatiques polycycliques) quand plusieurs niveaux de phytol jusqu'à dix fois supérieurs au niveau de phytol typique ont été ajoutés à des cigarettes 3R4F. Les cigarettes ont été fumées dans deux conditions de fumage différentes. Une dose (indiquée comme 35/60) utilisait 35 ml du volume de la bouffée, lors d'une bouffée de 2 s, chaque bouffée étant aspirée toutes les 60 s, et l'autre utilisait 60 ml du volume de la bouffée, lors d'une bouffée de 2 s, chaque bouffée étant aspirée toutes les 30 s (indiquée comme 60/30). L'augmentation du niveau d'HAP après l'ajout de phytol n'a pas été significative. Une évaluation statistique de la dépendance des HAP totaux et du niveau ajouté de phytol ont montré que l'hypothèse d'une boucle zéro de la ligne de dépendance phytol/HAP totaux ne peut être exclue (avec $p = 0,101$ pour un fumage de 35/60 et $p = 0,626$ pour un fumage de 60/30). Par ailleurs, pour des conditions de fumage de 35/60, une augmentation de près de 14% des niveaux totaux d'HAP a été constatée lorsque le niveau de phytol ajouté était de 1,5 mg de phytol par cigarette. Toutefois, ce niveau accru d'HAP n'a pas forcément été déterminé par l'ajout de phytol et peut être causé par des erreurs aléatoires de procédure / d'analyse (comme indiqué par l'analyse statistique) ou par la modification des cigarettes pendant l'ajout de phytol. Le niveau constaté est également nettement inférieur aux 100% d'augmentation attendus dans le niveau des HAP sur la base des prévisions mentionnées dans la littérature. Un fumage intense n'a révélé aucune augmentation du niveau d'HAP. Une pyrolyse flash de phytol libre et de chlorophylle *a* a fourni des résultats indiquant qu'il est peu probable que le phytol lié à la chlorophylle génère des niveaux d'HAP différents comparés à ceux du phytol libre. Ainsi, il a été démontré que le phytol n'est pas un contributeur/précurseur significatif de la formation d'HAP dans la fumée de cigarette. [Beitr. Tabakforsch. Int. 24 (2010) 10–23]

INTRODUCTION

Phytol or (2E,7R,11R)-3,7,11,15-tetramethyl-2-hexadecen-1-ol ($MW = 296.53$ a.u.), is present in tobacco at levels around 100-150 $\mu\text{g/g}$ dry leaf. The compound is bound in

the form of an ester to a porphyrine type group (known as chlorin) with several substituents and chelated to Mg^{2+} ion, to form chlorophyll. Pyrolysis of phytol has been studied with various purposes such as the elucidation of the role of chlorophyll in the formation of petroleum and of oil shale kerogens (1, 2), understanding of the degradation of lignocellulosic biomass containing chlorophyll (3), understanding of the formation in time of humic substances (4, 5), as well as specifically for the potential formation of polycyclic aromatic hydrocarbon (PAHs) (6–8). When pyrolyzed in a quartz tube at 860 ± 5 °C in a flow of nitrogen (30 mL/min) phytol was reported to generate benzo[*a*]pyrene at a level of about 3.9 mg/g from the initial phytol (8). In a flash pyrolysis experiment performed at 900 °C for 10 s the pyrolysis products were found more similar to those of a long chain hydrocarbon than those of a typical alcohol (6). This is common for a large molecule where the functional group is only a small part of a much larger structure. The formation of (2E)-3,7,11,15-tetramethylhexadec-2-enal does take place as expected from an alcohol, but the compound is present in the pyrolyzate at only 0.2 % (mol) from the total pyrolyzate composition. The reaction is shown below:

Elimination of water is unlikely without rearrangement of the double bond from the 2-position. In the case of phytol, most pyrolysis products are various fragments of the phytol molecule from along the hydrocarbon chain. Since long chain hydrocarbon pyrolysis does not generate PAHs with high yield by pyrolysis, preliminary results on phytol flash pyrolysis are in disagreement with the reported results from the study showing a level of about 3.9 mg of benzo[*a*]pyrene per 1g of pyrolyzed phytol (8). The main purpose of the present study is to determine the potential increase in the level of PAHs when phytol is added at several levels on cigarettes.

Since phytol is present in tobacco mainly as an ester bound to chlorophyll, the contribution of phytol moiety to the composition of the total pyrolyzate of chlorophyll was another aspect of interest. To clarify this subject, the pyrolyzates of phytol and of chlorophyll performed under similar conditions were compared and this report describes the findings.

EXPERIMENTAL

Cigarette treatment

The first part of this study was focused on the evaluation of phytol potential to increase the level of PAHs in cigarette smoke. The cigarettes used for the study were 3R4F Kentucky reference cigarettes. Several levels of phytol were added to the cigarettes. For this purpose, phytol (Sigma-Aldrich, Milwaukee, WI 53201, USA) was used to make a 10% (w/v) solution in ethanol. Then 1 μL , 5 μL , 10 μL and 15 μL , respectively, of this solution were

Table 1. Level of added phytol on 3R4F cigarettes

Level	µg/cig
Level 0	initial
Level 1	initial + 100 µg/cig
Level 5	initial + 500 µg/cig
Level 10	initial + 1000 µg/cig
Level 15	initial + 1500 µg/cig

Table 2. Deuterated I.S. solution (Concentration in iso-propanol, µg/mL)

No	Compound	MW	Sol I.S.
1	Naphthalene-d8	136	29.72
2	2-Methylnaphthalene-d10	152	29.92
3	Acenaphthene-d10	164	8.00
4	Fluorene-d10	176	30.40
5	Phenanthrene-d10	188	15.68
6	Anthracene-d10	188	10.44
7	Pyrene-d10	212	7.68
8	Chrysene-d12	240	2.84
9	Benzo[a]pyrene-d12	264	1.60
10	Indeno[1,2,3-cd]pyrene-d12	288	0.84
11	Dibenz[a,h]anthracene-d14	292	0.40
12	Benzo[ghi]perylene-d12	288	0.48

Table 3. Conditions for sample cleanup on SPE

No	Step	Source	Volume mL	Approx. rate mL/min
1	Condition	Methanol	2.0	10
2	Condition	Water/methanol 65/35	2.0	10
3	Load	Sample	12.0	5
4	Rinse	Water	1.6	10
5	Rinse	Water	1.6	10
6	Rinse	Water	1.6	10
7	Rinse	Water/methanol 65/35	1.6	10
8	Dry	Vacuum for 30 min		
9	Collect	Cyclohexane	0.8	5

injected manually in 3R4F cigarettes. The cigarettes were allowed to equilibrate for two weeks. After two weeks it was noticed that some cigarettes developed some spotting. The cigarettes with no spots and also those with spots smaller than 2–3 mm in diameter were used in the study. The added levels of phytol on the 3R4F cigarettes are shown in Table 1.

The cigarettes were smoked on a Borgwaldt rotary machine RM20/CSR (Schnackenburgallee 15, D-22525 Hamburg, Germany) using two smoking regimens. One regimen (indicated as 35/60) used 35 mL puff volume, 2 s puff duration, each puff taken every 60 s (9), and the other used 60 mL puff volume, 2 s puff duration, each puff taken every 30 s (indicated as 60/30). The total particulate matter (TPM) from 5 cigarettes was collected on 44 mm Cambridge pads. For control, 2R4F cigarettes (with no added phytol) were also smoked in the same two conditions.

Table 4. GC/MS operating parameters

Parameter	Description
GC column	Rxi-5Sil MS ^a
Column dimensions	30 m, 0.25 mm i.d.
Film thickness	0.25 µm
Initial oven temperature	35 °C
Initial time	2.0 min
Oven ramp rate	20 °C/mm
Oven final first ramp	100 °C
Final time first ramp	5.75 min
Oven ramp rate	25 °C/mm
Oven final first ramp	150 °C
Final time first ramp	0 min
Oven ramp rate	4 °C/mm
Oven final temperature	320 °C
Final time	15.5 min
Total run time	71.0 min
Carrier gas	Helium
Flow mode	Constant pressure
Pressure	21.00 psi
Nominal initial flow	2.8 mL/min
GC outlet	MSD
MSD transfer line	280 °C
Ion source temperature	230 °C
Quadrupole temp.	150 °C
MSD EM offset	250 V
MSD solvent delay	12.0 min
MSD acquisition mode	SIM

^a The Rxi-5Sil MS column was purchased from Restek, Bellefonte, PA 16823, USA.

Smoke sample analysis

A number of PAHs were analyzed in the smoke collected from the cigarettes. The analysis was done using a modification of a procedure previously described in the literature (10). A brief description of the used procedure follows. After smoking, each pad was put in an extraction vial (with the volume of about 100 mL). To each vial there were added 100 µL of a solution of deuterated internal standards (CDN Isotopes, Pointe-Claire, Quebec, Canada H9R 1H1, and Supelco, Bellefonte, PA 16823-0048 USA). The list and the concentrations of the deuterated PAHs used as internal standard are given in Table 2.

After this step the pads from cigarettes smoked using 35/60 conditions were extracted with 10 mL methanol for 30 min on a mechanical shaker. The cigarettes smoked using 60/30 conditions were extracted with 20 mL methanol for 30 min on the shaker. From the methanol solution, 5 mL were transferred in a test tube. In the test tubes, to the 5 mL methanol 7 mL of distilled water was added. A slight turbidity was typically seen in the solution. The test tubes with 12 mL solution were used for loading the sample into SPE cartridges. The cartridges were CECYH1 (cyclohexyl endcapped) 500 mg, 3 mL volume (UCT, Bartram Rd., Bristol, PA 19007). The cleanup process was performed manually using the same procedure as previously recommended for a Rapid Trace automatic SPE system (Zymark/Caliper Life Sciences, Hopkinton, MA 01748). The parameters for the SPE procedure are listed in Table 3.

Table 5. PTV operating parameters

Parameter	Description
Inlet temperature	40 °C
Equilib. time	0.5 min
Initial time	0.6 min
Injection volume	3.0 µL
Air volume below	1.0 µL
Eject speed	10.0 µL/s
Rate 1	10 °C/s
End temperature	320 °C
Hold time	10.0 min
Rate 2	10 °C/s
End temperature 2	375 °C
Hold time 2	60.0 min

Table 6. Retention times and ion monitored for quantitation in SIM acquisition for the PAHs analyzed in this study

No	Compound	Ret. time	SIM Ion	Remark
1	Naphthalene-d8	9.11	136	I.S.
2	Naphthalene	9.19	128	
3	2-Methylnaphthalene-d10	12.25	152	I.S.
4	2-Methylnaphthalene	12.33	142	
5	1-Methylnaphthalene	12.60	142	
6	Acenaphthylene	14.59	152	
7	Acenaphthene-d10	15.02	164	I.S.
8	Acenaphthene	15.10	154	
9	Fluorene-d10	16.64	176	I.S.
10	Fluorene	16.74	166	
11	Phenanthrene-d10	20.33	188	I.S.
12	Phenanthrene	20.43	178	
13	Anthracene-d10	20.57	188	I.S.
14	Anthracene	20.65	178	
15	Fluoranthene	26.33	202	
16	Pyrene-d10	27.37	212	I.S.
17	Pyrene	27.46	202	
18	Benz[a]anthracene	34.47	228	
19	Chrysene-d12	34.51	240	I.S.
20	Chrysene	34.66	228	
21	Benzo[b]fluoranthene	40.43	252	
22	Benzo[k]fluoranthene	40.57	252	
23	Benzo[a]pyrene-d12	41.89	264	I.S.
24	Benzo[a]pyrene	42.01	252	
25	Indeno[1,2,3-cd]pyrene-d12	47.20	288	I.S.
26	Indeno[1,2,3-cd]pyrene	47.29	276	
27	Dibenz[a,h]anthracene-d14	47.40	292	I.S.
28	Dibenz[a,h]anthracene	47.53	278	
28	Benzo[ghi]perylene-d12	48.24	288	I.S.
29	Benzo[ghi]perylene	48.35	276	

The final eluates are transferred into sample vials with 100 µL small volume inserts in order to perform the GC/MS analysis.

The analysis of PAHs was performed on a 6890 GC / 5973 MS instrument (Agilent, Wilmington, Delaware 19808), having a Gerstel (Gerstel, GmbH & Co.KG, D-45473 Mülheim an der Ruhr, Germany) programmed temperature vaporizer (PTV) injection port. The parameters for the GC instrument are given in Table 4 and for the PTV injector in Table 5. Using the parameters indicated in Tables 4 and 5,

Table 7. The values for the response factors F used for the quantitation

Factor used for quantitation	Value
Naphthalene/naphthalene-d8	1.033337
2-Methylnaphthalene/2-methylnaphthalene-d10	1.006364
1-Methylnaphthalene/2-methylnaphthalene-d10	1.086625
Acenaphthylene/acenaphthene-d10	0.917304
Acenaphthene/acenaphthene-d10	1.09472
Fluorene/fluorene-d10	0.897411
Phenanthrene/phenanthrene-d10	1.145231
Anthracene/anthracene-d10	1.040747
Fluoranthene/anthracene-d10	0.995174
Pyrene/pyrene-d10	0.869273
Benz[a]anthracene/chrysene-d12	0.914417
Chrysene/chrysene-d12	0.951355
Benzo[b]fluoranthene/benzo[a]pyrene-d12	1.07192
Benzo[k]fluoranthene/benzo[a]pyrene-d12	0.77792
Benzo[a]pyrene/benzo[a]pyrene-d12	0.878973
Indeno[1,2,3-cd]pyrene/indeno[1,2,3-cd]pyrene-d12	1.151286
Dibenz[a,h]anthracene/dibenz[a,h]anthracene-d14	1.009788
Benzo[ghi]perylene/benzo[ghi]perylene-d12	1.158155

PAHs were eluted at the retention times shown in Table 6. The ions used for the SIM detection and quantitation of each PAH are also indicated in Table 6.

A typical chromatogram collected in SIM mode generated from the 500 µg/mL standard of all analyzed PAHs is shown in Figure 1. Deuterated and non deuterated PAHs (Supelco, Bellefonte, PA 16823-0048 USA) were used to prepare the standards. Figure 1 also shows the variation in the retention time of different PAHs as a function of their molecular weight. A chromatogram, generated from a 3R4F cigarette (Level 0 phytol added) smoked under 35/60 type conditions, is shown in Figure 2. As shown in Figure 1, the separation of the analyzed PAHs is very good. Figure 2 shows that the cleanup procedure is efficient and several peaks of PAHs can be clearly seen.

For the quantitation of PAHs, the peak areas of the compounds to be analyzed were normalized by the area of the internal standards and further multiplied by an average response factor F. The formula to measure concentrations was the following:

$$PAH_{cig} = \left(\frac{Area_{PAH\ analyte}}{F \cdot Area_{d-IS}} \right) \left(\frac{Conc_{d-IS}}{No. of smoked cigarettes} \right) [2]$$

The value of the response factor F was calculated using standards. The first step in the calculation of F was to divide the peak areas of each individual deuterated PAH standards ($Area_{PAH-d-standard}$) by the peak area of a non-deuterated standard ($Area_{PAH-standard}$) with the same concentration as the deuterated one and obtain F_i values by the formula:

$$F_i = \frac{Area_{PAH\ standard}}{Area_{PAH - d\ standard}} [3]$$

The final F values were obtained as an average F_i values generated for four different concentrations. The values for the response factors F are shown in Table 7. As expected, the response factors are close to 1.0, but some variation is still present.

Figure 1. Chromatogram collected in SIM mode from a solution of PAH standards with the concentration of 500 µg/mL

Figure 2. Chromatogram collected in SIM mode for the smoke of 3R4F cigarettes

Table 8. Parameters used for the GC/MS on-line analysis of pyrolyzates

Parameter	Description
GC column	DB-1701 ^a
Column dimensions	60 m long, 0.25 mm id.
Film thickness	1.0 μ m
Initial oven temperature	37 °C
Initial time	4.0 min
Oven ramp rate	2 °C/mm
Oven final first ramp	60 °C
Final time first ramp	0 min
Oven ramp rate	5 °C/mm
Oven final temperature	280 °C
Final time	20 min
Total run time	75.5 min
Inlet temperature	280 °C
Inlet mode	Split
Carrier gas	Helium
Flow mode	Constant flow
Flow rate	1.1 mL/min
Nominal initial pressure	17.5 psi
Split ratio	70:1
Split flow	76.0 mL/min
GC outlet	MSD
Outlet pressure	Vacuum
MSD transfer line temperature	280 °C
Ion source temperature	230 °C
Quadrupole temp.	150 °C
MSD EM offset	250 V
MSD solvent delay	2.0 min
MSD acquisition mode	TIC
Mass range	29-550 a.u.

^a The DB-1701 column was purchased from J&W Scientific, Folsom, CA 95630-4714 USA

Pyrolysis conditions for phytol and chlorophyll a

Pyrolysis results for phytol and chlorophyll *a* were generated for further comparison. For performing the pyrolysis, a filament pyrolyzer Pyroprobe 2000 with an AS 2500 autosampler (CDS Analytical, CDS, Oxford, PA, 19363, USA) on line with an Agilent GC/MS system has been used. Pyrolysis conditions included $T_{eq} = 900$ °C, $\beta = 10$ °C/ms, THT = 10 s, and $T_{hou} = 280$ °C (T_{eq} = equilibrium temperature, β = heating rate, THT = total heating time, and T_{hou} = housing temperature). The analysis of pyrolyzate was done by GC/MS under conditions given in Table 8.

RESULTS AND DISCUSSION

Comparison of PAHs results for 2R4F and 3R4F cigarettes

The first step of the study was the verification of the results for PAHs between 2R4F and 3R4F cigarettes with no addition of phytol, and literature results for 2R4F cigarette (10). These results expressed in ng/cig are given in Table 9. The values from this study were obtained as an average of two analyses. The corresponding relative standard deviations (RSD%) for the averages shown in Table 9 are given in Table 10.

As shown in Table 9, the results for PAHs are not significantly different for 2R4F cigarettes and for 3R4F cigarettes. Also, the results for 2R4F cigarette smoked under 35/60 conditions are in good agreement with the results reported in the literature. The values for measured PAHs obtained when smoking was performed under intensive conditions (60/30) are as expected, 2.5 to 3.0 times higher than the levels generated by 35/60 smoking. Except a few values the RSD % for the measurements are also below 10%, as shown in Table 10.

Table 9. The results for PAHs expressed in ng/cig for 35/60 and for 60/30 smoking conditions on 2R4F and 3R4F cigarettes

Compound	35/60 smoking		60/30 smoking		35/60 smoking
	2R4F	3R4F	2R4F	3R4F	2R4F (Ref. 10)
Naphthalene	355.4	345.4	949.1	1017.2	310.0
2-Me naphthalene	383.3	383.7	1001.8	1029.7	366.4
1-Me naphthalene	323.7	355.9	872.0	891.8	396.5
Acenaphthylene	69.8	68.9	183.5	181.8	69.9
Acenaphthene	53.2	49.8	116.1	107.6	35.9
Fluorene	179.7	173.9	411.1	402.4	169.8
Phenanthrene	136.6	137.5	309.8	293.1	146.8
Anthracene	68.8	55.7	176.2	166.1	52.5
Fluoranthene	64.5	54.3	173.2	178.5	51.0
Pyrene	43.4	43.4	92.7	89.5	45.0
Benz[a]anthracene	13.7	13.8	29.6	26.8	16.6
Chrysene	15.6	15.8	32.6	31.4	16.6
Benzo[b]fluoranthene	5.2	5.2	11.4	10.3	5.4
Benzo[k]fluoranthene	1.7	1.7	3.9	3.3	1.37
Benzo[a]pyrene	6.7	7.2	15.5	14.2	9.2
Indeno[1,2,3-cd]pyrene	2.4	2.4	5.0	4.4	1.5
Dibenz[a,h]anthracene	1.4	1.7	2.9	2.5	0.6
Benzo[ghi]perylene	1.6	1.9	4.7	4.0	2.0

Table 10. Relative standard deviations (RSD %) for the results for PAHs given in Table 9

Compound	35/60 smoking		60/30 smoking		35/60 smoking
	2R4F	3R4F	2R4F	3R4F	2R4F (Ref. 10)
Naphthalene	2.2	1.9	13.2	3.6	4.9
2-Me naphthalene	1.8	2.0	3.2	1.2	1.5
1-Me naphthalene	4.4	8.8	5.8	3.1	2.5
Acenaphthylene	3.7	2.0	13.0	15.8	3.9
Acenaphthene	9.6	0.5	3.3	3.2	3.6
Fluorene	7.5	3.0	2.2	1.0	1.8
Phenanthrene	2.4	3.3	3.2	1.3	2.3
Anthracene	13.4	1.0	0.5	1.1	1.4
Fluoranthene	4.0	5.3	8.9	3.9	2.6
Pyrene	4.7	4.8	2.0	1.7	5.6
Benz[a]anthracene	4.3	6.0	0.4	3.1	3.0
Chrysene	5.0	6.6	2.2	1.0	8.1
Benzo[b]fluoranthene	5.1	5.2	6.3	4.8	8.3
Benzo[k]fluoranthene	8.4	12.2	9.1	8.9	8.0
Benzo[a]pyrene	1.6	8.0	6.0	4.1	4.6
Indeno[1,2,3- <i>cd</i>]pyrene	11.6	12.9	7.2	4.1	8.7
Dibenz[a,h]anthracene	9.2	11.7	5.8	7.1	3.8
Benzo[ghi]perylene	9.0	7.7	3.6	4.4	5.2

Comparison of levels of PAHs for cigarettes without and with the addition of phytol

The levels of PAHs obtained from the 3R4F cigarettes with different levels of added phytol when smoking was done under 35/60 conditions are given in Table 11. The corresponding RSD % for individual measurements is given in Table 12. As shown in Table 11, the variation in the level of individual PAHs for the cigarettes with different levels of phytol, shows a certain increase between the cigarettes with Level 0 and Level 15 of phytol. However, this increase is not relevant, particularly for key PAHs such as benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-*cd*]pyrene and dibenzo[a,h]anthracene, where the RSD % between levels is in the same range of values as the RSD % for duplicate measurements. The largest relative increase between Level 0 and Level 15 is seen for anthracene and fluoranthene.

The graph showing the variation in total PAHs level for 35/60 smoking as a function of added phytol is shown in Figure 3. Statistical evaluation of the dependence of total PAHs generated by 35/60 smoking and the added level of phytol showed that the hypothesis of a zero slope for the

dependence line cannot be rejected (with a $p = 0.101$).

The same experiment as previously described for 35/60 smoking was repeated for intensive smoking (60/30). The results for levels of PAHs obtained from the 3R4F cigarettes with different levels of added phytol when smoking was done in 60/30 conditions are given in Table 13. The corresponding RSD % for individual measurements is given in Table 14. As shown in Table 13, the increase in individual PAHs level for 60/30 smoking is less pronounced compared to 35/60 smoking. This increase is in the same range of variation as the RSD % levels between duplicates of the same sample.

The graph showing the variation in total PAHs level for 60/30 smoking as a function of added phytol is shown in Figure 4. Statistical evaluation of the dependence of total PAHs generated by 60/30 smoking and the added level of phytol showed that the hypothesis of a zero slope for the dependence line cannot be rejected (with a $p = 0.626$).

As seen from Tables 11 and 13, as well as from Figures 3 and 4, the addition of phytol to 3R4F cigarette does not increase the level of PAHs in smoke. An increase of about 14% in the total PAHs level for 3R4F cigarette smoked under 35/60 conditions, can be the result of a random

Figure 3. Total PAHs as a function of added phytol on 3R4F cigarettes (35/60 smoking)

Figure 4. Total PAHs as a function of added phytol on 3R4F cigarettes (60/30 smoking)

Table 11. The levels of PAHs in ng/cig in 3R4F cigarettes injected with different levels of phytol (average of two measurements) and the RSD% between cigarettes with different levels of phytol (35/60 smoking)

Compound	Level 0	Level 1	Level 5	Level 10	Level 15 ^a	RSD% between levels
Naphthalene	345.4	306.9	356.8	330.7	399.7	9.9
2-Me naphthalene	383.7	344.8	392.8	370.7	424.8	7.7
1-Me naphthalene	355.9	319.4	381.8	376.2	409.0	9.1
Acenaphthylene	68.9	64.2	68.9	66.6	81.3	9.5
Acenaphthene	49.8	47.0	50.4	47.2	56.2	7.5
Fluorene	173.9	163.4	176.2	169.5	190.1	5.7
Phenanthrene	137.5	128.2	134.5	130.4	159.3	9.0
Anthracene	55.7	69.7	78.5	74.4	85.8	15.4
Fluoranthene	54.3	67.9	76.7	72.3	78.5	13.8
Pyrene	43.4	41.1	42.9	41.3	46.8	5.2
Benz[a]anthracene	13.8	12.8	13.3	13.0	14.6	5.5
Chrysene	15.8	14.8	15.0	14.9	16.2	4.0
Benzo[b]fluoranthene	5.2	4.9	5.1	5.0	5.7	5.8
Benzo[k]fluoranthene	1.7	1.7	1.6	1.8	2.0	7.6
Benzo[a]pyrene	7.2	6.6	6.8	6.8	7.4	4.8
Indeno[1,2,3-cd]pyrene	2.4	2.3	2.4	2.4	2.6	3.9
Dibenz[a,h]anthracene	1.7	1.5	1.4	1.5	1.7	9.5
Benzo[ghi]perylene	1.9	1.7	1.7	1.7	1.9	6.0
TOTAL PAHs	1718.3	1599.0	1806.7	1726.5	1983.6	

^a Only one replicate was generated for Level 15.

Table 12. Relative standard deviations (RSD%) of individual values given in Table 11 for PAHs measurements

Compound	Level 0	Level 1	Level 5	Level 10
Naphthalene	1.9	6.0	8.8	6.4
2-Me naphthalene	2.0	2.9	9.2	4.1
1-Me naphthalene	8.8	8.7	12.4	3.1
Acenaphthylene	2.0	6.5	2.0	4.9
Acenaphthene	0.5	2.0	5.0	0.5
Fluorene	3.0	1.4	5.1	3.7
Phenanthrene	3.3	1.9	2.5	4.3
Anthracene	1.0	3.0	2.3	4.8
Fluoranthene	5.3	1.3	2.0	3.0
Pyrene	4.8	0.3	5.6	3.6
Benz[a]anthracene	6.0	0.1	3.1	3.6
Chrysene	6.6	2.0	5.3	3.2
Benzo[b]fluoranthene	5.2	0.7	5.8	1.3
Benzo[k]fluoranthene	12.2	9.7	4.7	8.5
Benzo[a]pyrene	8.0	3.7	7.1	8.2
Indeno[1,2,3-cd]pyrene	12.9	6.2	5.2	8.5
Dibenz[a,h]anthracene	11.7	12.8	7.5	7.4
Benzo[ghi]perylene	7.7	13.8	10.0	8.5

variation in the measurement of PAHs level, can be caused by the unintentional modifications of the cigarettes when injected with phytol, or can be a real small increase in the PAHs deliveries. However, the 14% increase in the PAHs values between Level 0 addition of phytol and Level 15 addition of phytol is much lower than the expected increase of about 100% based on literature findings (8).

A plausible explanation for this result is the difference between the conditions of pyrolysis of phytol in a cigarette and pyrolysis in a flow through or a static system.

Comparison of pyrolysis results between phytol and chlorophyll a

Since phytol is not present in large level as a free compound in tobacco, but it is connected as an ester to form chlorophyll, individual pyrolysis experiments were performed on both phytol and on chlorophyll *a* to compare the results between the two compounds and find if the pyrolyzates show significant differences. Pyrolysis of phytol was performed on 0.60 mg compound as described in the experimental part. The resulting pyrogram is given in Figure 5 and the peak identification based on the mass spectra of the compounds is given in Table 15. The calculation of the mol % was obtained by dividing the individual peak areas in the chromatogram by total area and by the compound molecular weight. As shown in Table 15, almost 50% (mol) of the phytol does not decompose in the experimental conditions selected for the pyrolysis. This is probably caused by the evaporation of phytol before it reaches the decomposition temperature and its rapid transfer into the GC/MS system. For phytol the reaction occurring with hydrogen elimination and formation of (2E)-3,7,11,15-tetramethylhexadec-2-enal as expected for an alcohol does take place, but the compound is present in the pyrolyzate at only 0.2% (mol) from the total pyrolyzate composition. Elimination of water is unlikely without rearrangement of the double bond from the 2-position. In the case of phytol, most pyrolysis products are various fragments of phytol molecule from along the hydrocarbon chain. Pyrolysis products are more similar to those of a long-chain hydrocarbon than those of a typical alcohol. This is common for a large molecule where the functional group is only a small part of a much larger structure.

Table 13. The levels of PAHs in ng/cig in 3R4F cigarettes injected with different levels of phytol (average of two measurements) and the RSD% between cigarettes with different levels of phytol (60/30 smoking)

Compound	Level 0	Level 1	Level 5	Level 10	Level 15	RSD% between levels
Naphthalene	1017.2	1036.7	1081.4	1109.2	1068.7	3.5
2-Me naphthalene	1029.7	1009.2	1088.2	1039.8	1031.3	2.9
1-Me naphthalene	891.8	1007.9	973.6	980.3	919.9	8.4
Acenaphthylene	181.8	174.8	177.8	185.0	174.5	3.1
Acenaphthene	107.6	120.2	114.4	115.9	107.5	2.1
Fluorene	402.4	390.3	421.3	410.3	410.4	3.2
Phenanthrene	293.1	301.2	317.2	314.4	309.1	4.1
Anthracene	166.1	173.8	178.0	173.8	170.9	2.5
Fluoranthene	178.5	174.9	185.0	161.2	165.8	7.2
Pyrene	89.5	94.0	93.6	95.4	94.4	3.7
Benz[a]anthracene	26.8	29.1	28.7	31.0	31.0	5.6
Chrysene	31.4	33.7	33.9	34.3	34.0	5.4
Benzo[b]fluoranthene	10.3	9.1	10.1	10.4	10.7	9.2
Benzo[k]fluoranthene	3.3	3.2	3.1	3.9	3.8	3.6
Benzo[a]pyrene	14.2	15.3	14.8	16.1	16.1	8.1
Indeno[1,2,3- <i>cd</i>]pyrene	4.4	4.4	4.3	4.9	4.9	8.5
Dibenz[a,h]anthracene	2.5	2.0	2.2	2.5	2.5	7.1
Benzo[ghi]perylene	4.0	4.2	3.9	4.9	4.7	9.3
Total PAHs	4454.7	4584.0	4731.7	4693.2	4560.2	

Table 14. Relative standard deviations (RSD%) of individual values given in Table 13 for PAHs measurements

Compound	Level 0	Level 1	Level 5	Level 10	Level 15
Naphthalene	3.6	1.8	2.4	3.4	2.1
2-Me naphthalene	1.2	5.1	1.7	0.8	1.8
1-Me naphthalene	3.1	5.7	5.2	4.2	4.1
Acenaphthylene	15.8	2.9	3.0	7.3	1.5
Acenaphthene	3.2	5.1	4.9	11.5	1.4
Fluorene	1.0	3.6	1.9	1.8	1.9
Phenanthrene	1.3	5.4	5.0	2.2	4.8
Anthracene	1.1	8.4	4.8	1.5	3.6
Fluoranthene	3.9	9.5	1.1	2.2	4.0
Pyrene	1.7	7.8	3.3	2.4	4.3
Benz[a]anthracene	3.1	7.8	3.7	5.4	5.4
Chrysene	1.0	7.1	5.8	2.1	3.2
Benzo[b]fluoranthene	4.8	0.6	3.0	9.2	3.5
Benzo[k]fluoranthene	8.9	3.1	2.9	5.1	8.1
Benzo[a]pyrene	4.1	4.2	0.2	5.8	5.5
Indeno[1,2,3- <i>cd</i>]pyrene	4.1	2.8	0.8	4.5	4.6
Dibenz[a,h]anthracene	7.1	9.5	4.0	9.8	13.8
Benzo[ghi]perylene	4.4	3.8	3.4	1.3	4.2

The resulting pyrogram obtained at 900 °C from 0.2 mg chlorophyll *a* is shown in Figure 6, and the peak identification is given in Table 16. The calculation of the mol % was obtained by dividing the individual peak areas in the chromatogram by total area and by the compound molecular weight. As shown in Table 16, fragment molecules are generated from both the chlorin ring and from the phytol side chain. Neophytadiene (the alkene generated from phytol dehydration) is one important component of the pyrolyzate. Several substituted pyrroles are also present in the pyrolyzate. Due to the high molecular weight and lack of volatility, chlorophyll pyrolysis at 900 °C generated a relatively large proportion

of small molecules such as CO₂, propene, and 2-methylpropene. However, the main pyrolysis products generated from the phytol moiety from chlorophyll are very similar to those generated from pure phytol.

CONCLUSIONS

This study evaluated the formation of PAHs when several levels of phytol were added on 3R4F cigarettes. The cigarettes were smoked under two different smoking conditions, 35/60 and intensive 60/30. The increase in the PAHs level upon phytol addition was not significant. A

Figure 5. Pyrogram of phytol at 900 °C

Figure 6. Pyrogram obtained at 900 °C for chlorophyll a (MW = 823)

statistical evaluation of the dependence of total PAHs and the added level of phytol showed that the hypothesis of a zero slope for the dependence line cannot be rejected (with a $p = 0.101$ for 35/60 smoking and $p = 0.626$ for 60/30 smoking). Still, for 35/60 smoking conditions, an increase of about 14% in total levels of PAHs was noticed when the added phytol level was 1.5 mg phytol per cigarette (ten times typical level in tobacco leaf). However, this increase was not necessarily determined by the phytol addition, and can be caused by random errors (as the statistical analysis

would indicate) or by the modification of cigarettes during phytol addition. Also, the level is much lower than the expected 100% increase in the PAHs level, based on literature prediction (8). Intensive smoking did not show an increase in PAHs level. Flash pyrolysis of free phytol and of chlorophyll *a* provided results that would indicate that phytol bound in chlorophyll is not likely to generate different PAHs level compared to free phytol. The study showed that phytol is not a significant contributor/precursor to the PAHs formation in cigarette smoke.

Table 15. Identification of the main peaks in the chromatogram shown in Figure 5 for the pyrolysis of phytol. Hydrogen, CO, CO₂, methane, ethane, and water are not included ('?' indicates a tentative/possible compound assignment).

No	Compound	Retention time (min)	MW	CAS-No.	Mol % pyrolyzate
1	Propene	4.88	42	115-07-1	7.17
2	Formaldehyde	5.10	30	50-00-0	0.09
3	Isobutane	5.20	58	75-28-5	0.02
4	1-Butene	5.51	56	106-98-9	5.12
5	1,3-Butadiene	5.74	54	106-99-0	0.97
6	2-Butene	5.93	56	107-01-7	0.30
7	3-Methyl-2-butene	6.38	70	563-45-1	0.72
8	1-Pentene	7.08	70	109-67-1	0.50
9	3-Methyl-1-butene	7.27	70	563-45-1	1.19
10	1,2-Dimethylcyclopropane	7.5	70	930-18-7	0.17
11	2-Methyl-1,3-butadiene (isoprene)	7.99	68	78-79-5	3.05
12	1,3-Pentadiene	8.72	68	504-60-9	0.31
13	4-Methyl-1-pentene	9.16	84	691-37-2	1.64
14	Cyclopentadiene	9.44	66	542-92-7	0.18
15	2-Methyl-1,4-pentadiene	10.23	82	763-30-4	0.22
16	1-Hexene	10.62	84	592-41-6	0.38
17	4-Methyl-2-pentene	11.40	84	691-38-3	0.24
18	3-Methyl-1,3-pentadiene (E)	12.47	82	2787-43-1	0.14
19	2,3-Dimethyl-1-pentene	13.80	98	2213-32-3	0.11
20	3-Methyl-1-hexene	13.98	98	3404-61-3	0.20
21	4-Methyl-1,3-pentadiene	14.25	82	926-56-7	0.22
22	1-Methylcyclopentene	14.57	82	693-89-0	0.24
23	1-Methyl-1,3-cyclopentadiene	14.78	80	96-39-9	0.57
24	4-Methyl-1-hexene	15.08	98	3769-23-1	1.18
25	3-Methylenecyclopentene	15.33	80	930-26-7	0.33
26	5-Methyl-1,3-cyclopentadiene	15.47	80	96-38-8	0.17
27	2-Methyl-1,5-hexadiene	16.62	96	4049-81-4	0.10
28	1,3-Cyclohexadiene	17.09	80	592-57-4	0.11
29	Benzene	17.75	78	71-43-2	0.58
30	4-Methyl-1,4-hexadiene	17.88	96	1116-90-1	0.12
31	5-Methyl-1,4-hexadiene	20.73	96	763-88-2	0.13
32	4-Methyl-1-heptene	21.07	112	13151-05-8	0.37
33	3-Methyl-1,3,5-hexatriene (Z)	21.51	94	24587-27-7	0.15
34	6-Methyl-2-heptene	21.93	112	73548-72-8	0.67
35	2-Methyl-3-heptene	22.42	112	17618-76-7	0.33
36	3-Methylcyclohexene	22.55	96	591-48-0	0.12
37	1,3-Cycloheptadiene ?	22.73	94	4054-38-0	0.14
38	2-Methyl-1-heptene	22.87	112	15870-10-7	0.69
39	1-Methyl-1,4-cyclohexadiene	23.24	94	4313-57-9	0.14
40	Toluene	24.32	92	108-88-3	0.88
41	1,3-Dimethylcyclohexene	25.4	110	2808-76-6	0.38
42	3-Methyl-1-octene	26.02	126	13151-08-1	0.12
43	2,6-Dimethyl-1-heptene	26.23	126	3074-78-0	1.56
44	4-Nonene ?	27.58	126	2198-23-4	0.15
45	1,3-Dimethyl-2-methylene- <i>cis</i> -cyclohexane	27.84	124	19781-47-6	0.07
46	1,2,3-Trimethylcyclohexane	28.01	126	1678-97-3	0.11
47	2-Methyl-3-butenol	28.54	84	107-86-8	0.56
48	3,7-Dimethyl-1-octene	28.93	140	4984-01-0	0.53
49	Ethylbenzene	29.09	106	100-41-4	0.17
50	1,3-Dimethylbenzene (<i>m</i> -xylene)	29.48	106	108-38-3	0.41
51	2-Undecene ?	30.35	152	60212-29-5	0.11
52	1,4-Dimethylbenzene (<i>p</i> -xylene)	30.8	106	106-42-3	0.11
53	2,6-Dimethyl-1-octene	31.07	140	6874-29-9	0.19
54	4-Methyl-1-decene	33.33	154	13151-29-6	1.67
55	4-Methyl-1-undecene	37.14	168	74630-39-0	0.25
56	2,5-Dimethyl-2-undecene	40.23	182	49622-16-4	0.32
57	7-Methyl-6-tridecene	42.58	196	24949-42-6	1.12
58	1-Pentadecene ?	44.18	210	13360-61-7	1.71
59	3-Tetradecene ?	44.6	196	41446-68-8	0.04
60	2,6,10-Trimethyldodecane	47	212	3891-98-3	0.15

Table 15. (cont.)

No	Compound	Retention time (min)	MW	CAS-No.	Mol % pyrolyzate
61	3,7,11,15-Tetramethyl-2-pentene	53.66	266	N/A	2.50
62	Phytol fragment	54.4	278	N/A	0.13
63	3,7,11,15-Tetramethyl-2-hexadecene	54.83	280	14237-73-1	0.16
64	Phytol fragment	55.74	278	N/A	0.21
65	3,7,11,15-Tetramethyl-2-hexadecene isomer	55.87	280	N/A	0.12
66	3,7,11,15-Tetramethyl-2-hexadecene isomer	56.09	280	N/A	0.20
67	2-(4,8,12-Trimethyltridecyl)-1,3-butadiene neophytadiene	56.34	278	504-96-1	2.65
68	3,7,11,15-Tetramethyl-2-hexadecen-1-ol ?	56.85	296	102608-53-7	0.51
69	Eicosadiene	57.14	278	N/A	0.54
70	1,4-Eicosadiene	57.36	278	1000131-16-3	1.17
71	(2E)-3,7,11,15-Tetramethylhexadec-2-enal	57.5	294	N/A	0.20
72	6,10,14-Trimethyl-2-pentadecanone	58.32	268	502-69-2	0.37
73	6,10,14-Trimethyl-2-pentadecanone isomer	59.56	268	N/A	0.50
74	Isophytol ?	59.77	296	505-32-8	0.47
75	Phytol type ?	61.63	252	N/A	0.28
76	3,7,11,15-Tetramethyl-1-hexadecanol	63.14	298	645-72-7	0.95
77	(2E ?)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol (phytol)	63.68	296	150-86-7	16.84
78	(2Z ?)-Phytol	64.49	296	150-86-7	30.72
79	Unknown	65.21	296	N/A	0.64
80	Unknown	66.32	296	N/A	1.15

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Table 16. Peak identification as a function of retention time for the pyrogram of chlorophyll a shown in Figure 6. H₂, H₂O, HCN, CO, NH₃, CH₄, N₂, not included due to the MS settings.

No.	Compound	Retention time min ^a	MW	CAS-No.	Mol %
1	Carbon dioxide	4.98	44	124-38-9	17.53
2	Propene	5.26	42	115-07-1	11.31
3	2-Methylpropene	5.91	56	115-11-7	5.36
4	1,3-butadiene	6.15	54	106-99-0	1.18
5	3-Methyl-1-butene	6.81	70	563-45-1	0.87
6	1-pentene	7.54	70	109-67-1	1.15
7	2-Methyl-1-butene	7.73	70	563-46-2	1.10
8	2-Methyl-1,3-butadiene (isoprene)	8.47	68	78-79-5	3.04
9	4-Methyl-1-pentene	9.66	84	691-37-2	1.93
10	1-Methylcyclopentadiene	15.35	80	96-39-9	0.76
11	4-Methyl-1-hexene	15.65	98	3769-23-1	1.06
12	Benzene	18.27	78	71-43-2	0.78
13	2-Methylheptane	21.43	114	592-27-8	0.30
14	6-Methyl-2-heptene	22.38	112	73548-72-8	0.70
15	5-Methyl-2-heptene	22.87	112	22487-87-2	0.36
16	2-Methyl-1-heptene	23.30	112	15870-10-7	0.93
17	Toluene	24.75	92	108-88-3	1.08
18	2,6-Dimethyl-1-heptene	26.61	126	3074-78-0	2.01
19	3,7-Dimethyl-1-octene	29.30	140	4984-01-4	0.64
20	1,3-Dimethylbenzene	29.87	106	108-38-3	0.47
21	3,6-Dimethyloctane	30.06	142	15869-94-0	0.28
22	2,6-Dimethyl-1-octene	31.42	140	6874-29-9	0.21
23	2,6-Dimethylnonane	33.60	156	17302-28-2	0.20
24	4-Methyldecene	33.68	154	13151-29-6	1.77
25	2,3-Dimethyl-1H-pyrrole	36.14	95	600-28-2	0.36
26	2,3,7-Trimethyldecane	37.47	184	62238-13-5	0.18
27	2,3,4-Trimethyl-1H-pyrrole	38.72	109	3855-78-5	0.54
28	4-Ethyl-2-methyl-1H-pyrrole	39.32	109	5690-96-0	1.11
29	2,6-Dimethylundecane	40.14	184	17301-23-4	0.89
30	1-Cyclopropyloctane ? ^b	40.25	154	1472-09-9	0.31
31	3,7-Dimethyl-1-undecene	40.59	182	N/A	0.69
32	2-Butyl-1,1,3-trimethylcyclohexane	40.97	182	54676-39-0	0.27
33	3-Ethyl-2,4-dimethyl-1H-pyrrole	41.06	123	517-22-6	0.24
34	4-Ethyl-2,3-dimethyl-1H-pyrrole	41.22	123	491-18-9	0.73
35	3-Ethyl-2,5-dimethyl-1H-pyrrole	41.33	123	69687-78-1	0.61
36	7-Methyltridecane	41.92	198	26730-14-3	0.50
37	4-Ethyl-2,5-dimethyl-1H-pyrrole	42.02	123	N/A	0.90
38	7-Methyl-6-tridecene	42.94	196	24949-42-6	1.88
39	3-Ethyl-2,4,5-trimethyl-1H-pyrrole	43.70	137	520-69-4	0.88
40	3,7,11-Trimethyl-1-dodecene	44.53	210	1189-38-2	2.17
41	2,6,10-Trimethyldodecane	44.96	212	3891-98-3	1.03
42	2,6,10-Trimethyltridecane	47.22	226	3891-99-4	0.89
43	4,8,12-Trimethyl-1-tridecene	47.36	224	N/A	0.47
44	2,3,5-Trimethyl-4-prop-2-enyl-1H-pyrrole	49.38	149	N/A	0.45
45	2,6,10-Trimethylpentadecane	51.84	254	3892-00-0	0.69
46	2,6,10,14-Tetramethylpentadecane	53.13	268	1921-70-6	0.17
47	2,6,10,14-Tetramethylpentadec-1-ene	53.99	266	N/A	1.14
48	2,6,10,14-Tetramethylpentadec-1-ene (stereoisomer)	54.13	266	N/A	0.40
49	2,6,10,14-Tetramethylhexadec-2-ene	55.18	280	56554-34-8	0.68
50	2,6,10,14-Tetramethylpentadec-1-ene (stereoisomer)	55.48	266	N/A	0.16

Table 16 (cont.)

No.	Compound	Retention time min ^a	MW	CAS-No.	Mol %
51	7,11-15-Trimethyl-heptadecadiene	56.09	278	N/A	0.92
52	[?-[?,?-(?)]-3,7,11,15-Tetramethyl-2-hexadecene	280	280	N/A	2.29
53	[R-[R,R-(E)]]-3,7,11,15-Tetramethyl-2-hexadecene	280	280	14237-73-1	4.02
54	Neophytadiene	278	278	504-96-1	10.02
55	An eicosadiene	278	278	N/A	0.87
56	(E)-2,4-Phytadiene	278	278	N/A	2.42
57	Phytol	296	296	150-86-7	4.20
58	Heneicosene	294	294	N/A	0.29
59	An eicosadiene	278	278	N/A	0.87
60	A heneicosadiene	292	292	N/A	0.17
61	A heneicosadiene	292	292	N/A	0.15
62	A heneicosadiene	292	292	N/A	0.16
63	Trimethyltridecylbenzene	302	302	N/A	0.26

^a Small differences are seen in the retention time of identical compounds in phytol and chlorophyll pyrolyzate as a result of random variability in experimental conditions.

^b A ? indicates a tentative/possible compound assignment