Cigarette Smoke Formation Studies

Transfer of Added [18-¹⁴C]Octatriacontane *

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SUMMARY

Radiolabeled [18-¹⁴C]octatriacontane ($C_{38}H_{78}$) was used as a model compound to study the transfer of compounds from tobacco into smoke. Non-filter cigarettes were made from Kentucky reference 2R1 cigarette tobacco filler oversprayed with alkane and were smoked on a single-port total recovery smoking machine. Of the recovered activity nearly 60% was found in the mainstream and sidestream particulate phases of smoke, 5% in the gaseous phase and 35% in the butt. Reverse-phase high-performance liquid radiochromatography and thin-layer radiochromatography were used effectively to analyze the cyclohexane extracts of the filler, mainstream and sidestream total particulate matter, and butt. No degradation of the alkane was observed prior to smoking. The radioactivity in the mainstream total particulate matter extracts consisted mostly of intact [¹⁴C]octatriacontane. Sidestream total particulate matter extracts contained both intact octatriacontane and breakdown products. The radioactivity in butt extracts consisted almost entirely of intact octatriacontane, indicating that excess radioactivity in the butt is due to filtration of the intact alkane by the filler

rod. The transfer of octatriacontane into mainstream smoke without pyrolytic degradation parallels that of the tobacco alkane dotriacontane.

ZUSAMMENFASSUNG

Radioaktiv markiertes [18-¹⁴C]Octatriacontan ($C_{38}H_{78}$) diente als Modellschubstanz bei der Untersuchung des Rauchüberganges im Tabak enthaltener Verbindungen. Strangzigaretten aus Fülltabak der Versuchszigarette Kentucky 2R1, der mit dem Alkan besprüht worden war, wurden auf einer Einkanal-Rauchmaschine zur Gesamtbilanzierung abgeraucht. Annähernd 60 % der Radioaktivität fanden sich in der Partikelphase von Haupt- und Nebenstromrauch wieder, weitere 5 % in der Gasphase und 35 % im Stummel. Mittels „reverse-phase“-HPLC mit Radiodetektion und Dünnschicht-Radiochromatographie wurden die Cyclohexanextrakte aus dem Fülltabak, aus der Gesamtpartikelphase von Hauptstrom- und Nebenstromrauch und aus dem Stummel untersucht. Vor dem Abrauchen war kein Abbau des Alkans zu beobachten. Die Radioaktivität im Extrakt der Partikelphase des Hauptstromrauches bestand zum größten Teil aus unverändertem [¹⁴C]Octatriacontan. Die Extrakte der Partikelphase des Nebenstromrauches enthielten neben intaktem Octatriacontan auch Pyrolyseprodukte. In den Stummelextrakten bestand die Radioaktivität fast ausschließlich aus

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unverändertem Octatriacontan. Dies deutet darauf hin, daß die relativ hohe Radioaktivität im Stummel auf die Retention des intakten Alkans im Tabakstrang zurückzuführen ist. Der Übergang von Octatriacontan in den Hauptstromrauch ohne Pyrolyse ist mit dem Rauchübergang des im Tabak enthaltenen Alkans Dotriacontan vergleichbar.

RESUME

De l'octatriacontane ($C_{38}H_{78}$) marqué au carbone radioactif [$18-^{14}C$] a servi de composé modèle pour l'étude du transfert dans la fumée des substances contenues dans le tabac. Des cigarettes sans filtre ont été réalisées avec du tabac de remplissage de référence Kentucky 2R1 humecté avec cet alcane, puis fumées sur une machine monocanal en vue de l'établissement d'un bilan global de la répartition des composés. Près de 60 % de la radioactivité ont été retrouvés dans la phase particulière de la fumée du courant principal et de la fumée secondaire, 5 % dans la phase gazeuse et 35 % dans le mégot. La radiochromatographie en phase liquide à haute performance (HPLC) à phase inversée, avec radiochromatographie en couche mince a permis d'étudier les extraits (cyclohexane) provenant du tabac de remplissage, de la phase particulière totale de la fumée principale et de la fumée secondaire ainsi que du mégot. Avant le fumage, aucune dégradation de l'alcane n'avait été observée. La radioactivité de l'extrait de la phase particulière de la fumée du courant principal s'est révélée en grande partie imputable à l'octatriacontane intact marqué au ^{14}C . Les extraits de la phase particulière de la fumée secondaire contenaient, outre de l'octatriacontane intact, des produits de pyrolyse. Dans les extraits de mégots la radioactivité était due presque exclusivement à l'octatriacontane non dégradé. Ceci semble indiquer que la radioactivité relativement élevée rencontrée dans le mégot est due à la rétention de l'alcane intact dans le boudin de tabac. Le transfert de l'octatriacontane dans la fumée du courant principal sans pyrolyse est comparable à celui du dotriacontane, un autre alcane contenu dans le tabac.

INTRODUCTION

The predominant alkanes in tobacco leaf and smoke range in size from nonacosane to tritriacontane (1, 2). Analyses by gas chromatography/mass spectrometry by CHORTYK et al. (3, 4) have shown that these leaf paraffins are transferred into smoke and that the ratio of these alkanes is the same in leaf and smoke. The use of radiolabeled dotriacontane ($C_{32}H_{66}$) showed that this tobacco alkane is transferred virtually intact into the particulate phase of mainstream smoke (5). At the lower end of the spectrum hexadecane has been reported in both leaf and smoke and the use of radiolabeled hexadecane as a tracer to study its distribution with respect to smoke particle size has been reported

(6). The highest molecular weight alkane reported to date in smoke is hexatriacontane (2).

Our specific objective was to investigate the transfer into smoke of a radiolabeled alkane larger than 32 carbons, as a model to establish relationships between various physical properties and transfer into smoke. In this study we used octatriacontane, $C_{38}H_{78}$, a naturally occurring plant hydrocarbon, but one which has not been reported in tobacco leaf or smoke. Currently the highest molecular weight ^{14}C -labeled alkane that is commercially available is dotriacontane; therefore, a synthetic route to prepare higher molecular weight labeled alkanes was explored and the details for the preparation of [$18-^{14}C$]octatriacontane have been published (7).

A secondary objective of our study was to develop liquid-radiochromatography methods to analyze specific smoke components, to complement or offer alternative methods to gas radiochromatography. Since alkanes are not resolved on silica gel, the use of reverse-phase high-performance liquid chromatography (RP-HPLC) was investigated based on published methods (8, 9).

In this paper the results of smoking cigarettes with added [$18-^{14}C$]octatriacontane and the analyses of radiolabeled smoke components by RP-HPLC radiochromatography are described.

EXPERIMENTAL

Radiochemicals

The radiolabeled alkane [$18-^{14}C$]octatriacontane was prepared in-house (7). [$16,17-^{14}C$]Dotriacontane was purchased from Research Products International Corp., Mount Prospect, Ill. [^{14}C]Hexadecane was purchased from Amersham Corp., Arlington Heights, Ill.

Labeled Cigarette Preparation

25 g of Kentucky reference 2R1 sieved tobacco filler was sprayed with 48 μCi [$18-^{14}C$]octatriacontane (4.3 mCi/mmol) dissolved in 6 ml of cyclohexane. The tobacco was tumbled during spraying to achieve uniform application. Solvent was removed by evaporation and the tobacco was equilibrated at 60% rel. hum. and 75 °F. Cigarettes were handmade using 2R1 85 mm. length paper (non-filter) and were equilibrated for three days at 60% rel. hum. and 75 °F. The average weight of the cigarettes was 1.061 g.

Cigarette Smoking Experiments

In order to measure the uniformity of alkane application, each of two cigarettes was divided into 4 equal sections. Each section was weighed and shaken in cyclohexane (10 ml) as described below.

Cigarettes were smoked on a total recovery single-port smoking machine following procedures similar to those

Table 1.
Axial cigarette distribution of ^{14}C activity.

Cigarette No. 1 (1.1603 g)					Cigarette No. 2 (1.0921 g)			
Section No.								
	I	II	III	IV	I	II	III	IV
Section weight (g)	0.3557	0.3020	0.2534	0.2492	0.3172	0.3314	0.2478	0.1957
Section activity (μCi)	0.5468	0.4269	0.4203	0.4017	0.4523	0.4881	0.3840	0.3260
Specific activity (μCi / g tobacco filler)	1.54	1.41	1.66	1.61	1.42	1.47	1.55	1.67
Average specific activity / g tobacco filler = 1.52 μCi.								

described earlier (10). The cigarettes were smoked to a 23 mm butt. The sidestream and mainstream total particulate matter (TPM) were collected on separate Cambridge filter pads. The total particulate matter weights were recorded and the pads were shaken in cyclohexane (25 ml) in serum bottles for 24 h (water bath, ambient temperature). The butt was extracted with cyclohexane in the same way. The sidestream and mainstream gas phases were absorbed in hyamine hydroxide after oxidation.

Liquid Scintillation Counting

A liquid scintillation counter (Beckman* model LS 7500) was used to measure the radioactivity of samples. Radioactivity of cyclohexane extracts was counted in Ready-SolvTM NA (Beckman) cocktail. Hyamine aliquots were counted in Insta-Gel[®] cocktail (Packard**).

Instrumentation

The radiochromatographic analyses were performed with a Waters chromatograph (U6K injector) coupled to a Radiomatic Instruments HPLC radioflow monitor (Flo-One Beta model 1C). The analytical column was an Ultrasphere ODS 5 μm column (150 mm $\times$ 4.6 mm, Altex, Anspec Co.) fitted with a Brownlee Labs MPLC NewGuard cartridge (RP18, 7 μm , 15 mm $\times$ 3.2 mm).

HPLC and TLC Sample Preparation and Analysis

Cyclohexane extracts (3–6 ml) of radioactive tobacco filler, mainstream smoke TPM, sidestream smoke TPM, and butt were concentrated to near dryness under nitrogen gas at ambient temperature. The residues were redissolved in 0.3 ml cyclohexane and filtered through a disposable filter assembly (Gelman Acrodiscs, 0.45 μm) into microvials. Aliquots containing 2–25 nCi were

analyzed by HPLC. Identification of octatriacontane in smoke samples was based on retention time and co-injection with radiolabeled octatriacontane. The elution solvent was acetone (100%) at a flow rate of 1 ml/min. The electronic variable ratio splitter of the Flo-One was set at 50% (cycle time: 1 s) and the column effluent was counted in a 0.5 ml flow cell using Flo Scint I (Radiomatic) cocktail at a flow rate of 1 ml/min. Samples (1–4 nCi) were applied to pre-coated silica gel plates (E. Merck, 0.25 mm thickness, silica gel 60 F–254) and chromatograms were developed in cyclohexane. After their development the distribution of radioactivity was determined using a TLC radioanalyzer (Berthold TLC scanner). Alkanes and other lipids were visualized as blue spots with phosphomolybdic acid reagent spray.

RESULTS AND DISCUSSION

Determinations of cigarette sections (Table 1) showed radioactivity to be uniformly distributed along the axis of the cigarette rod within experimental error. Based on the average weight of the cigarettes used for smoking (1.061 g) the average radioactivity per cigarette was calculated to be 1.61 μCi .

In Table 2 the distribution of radioactivity in various smoke fractions is summarized. The results are the average of 8 determinations. The total activity recovered ranged from 1.42 to 1.70 μCi , presumably a result of individual cigarette differences. The total radioactivity recovered was 96% of the initial amount indicating that virtually all the activity is recovered after smoking. Nearly 60% of the activity was associated with the particulate phase of the smoke whereas less than 6% was in the gaseous phase. A similar preponderance of radioactivity in the particulate phase was noted for cigarettes labeled with [^{14}C]dotriacontane (5). Analysis of the data based on activity consumed during smoking indicated that about 9% of the consumed activity was filtered into the butt.

* Beckman Instruments, Inc., Fullerton, Calif.

** Packard Instrument Co., Meriden, Conn.

Figure 1.
Reverse-phase HPLC radiochromatogram of [18-¹⁴C]octatriacontane.

Sample: 11.2 nCi, 10 μ l; column: Ultrasphere ODS (5 μ m), mobile phase: acetone (100%), flow rate: 1 ml/min.

Figure 2.
Reverse-phase HPLC radiochromatogram of
mainstream smoke ^{14}C total particulate matter (TPM) extract.

Sample: 12.8 nCi, 10 μl ; column: Ultrasphere ODS (5 μm), mobile phase: acetone (100%), flow rate: 1 ml/min.

Figure 3.
Reverse-phase HPLC radiochromatogram of
sidestream smoke ^{14}C total particulate matter (TPM) extract.

Sample: 15 nCi, 20 μl ; column: Ultrasphere ODS (5 μm), mobile phase: acetone (100%), flow rate: 1 ml/min.

In method development, the separation of homologous alkanes by RP-HPLC was investigated using a liquid chromatography—flame ionization detector (9). Reverse-phase columns such as Waters Resolve C₁₈ (10 μ m) Radial-Pak cartridges and Altex Ultrasphere ODS (3 μ m and 5 μ m) columns gave satisfactory resolution of alkanes up to 40 carbons at ambient temperature, with acetone as mobile phase. Improved separation was accomplished by adding acetonitrile as a co-solvent up to 10% to acetone. Under these conditions some tailing was observed presumably due to the hydrophobicity of the compounds. The mobile phase used for the HPLC analyses described in this paper was acetone (100%).

Radiochromatographic analysis of the radiolabeled alkane showed the compound to have a radiochemical purity in excess of 98% (Figure 1). The compound eluted with an average retention time of 12.9 min with some tailing. A minor radiochemical impurity was observed at 10.2–10.5 min accounting for less than 1% of the net activity. RP-HPLC of a mixture of [¹⁴C]C₃₂H₆₄ and [¹⁴C]C₃₈H₇₈ showed two well separated peaks eluting at 8.6 min and 12.9 min, respectively.

No degradation of the radiolabeled alkane prior to smoking was observed, as indicated by radiochromatography of the tobacco filler extract. HPLC radiochromatograms of mainstream smoke TPM extracts (Figure 2) showed one peak which co-eluted with C₃₈H₇₈ and accounted for more than 97% of the net activity. This result indicates that octatriacontane is transferred intact into mainstream smoke TPM. The same observation was made in the case of dotriacontane wherein gas radiochromatography of the mainstream smoke TPM extract showed that more than 95% of the radioactivity was associated with the alkane (5).

HPLC radiochromatograms of sidestream smoke TPM extracts showed [¹⁴C]C₃₈H₇₈ to be the major component (Figure 3). In addition, earlier eluting radioactive components were also observed. The presence of breakdown products in sidestream smoke TPM suggests that between puffs when the gas flow is toward the high-temperature zone some of the alkane passing through this zone undergoes pyrolysis. Radiochromatograms of the butt extracts showed virtually all of the radioactivity to be [¹⁴C]C₃₈H₇₈ and therefore it can be concluded that the excess radioactivity in the butt is due to filtration or distillation of the intact alkane along the rod.

Thin-layer radiochromatography of the above smoke extracts confirmed observations based on HPLC analyses. Chromatography on silica gel of mainstream smoke TPM extracts showed more than 98% of the radioactivity to be associated with the alkane region (R_f 0.85) and less than 2% of the activity remained at the origin. The radioactive region was visualized as a light blue color overlapping in part a more intense blue region with a lower R_f value, presumed to represent other tobacco lipids. The light blue spot was observed

Table 2.

Total smoke distribution of ¹⁴C activity.

Smoke phase	Average (μ Cl)	Distribution based on recovered activity (%)	Distribution based on consumed activity (%)
Sidestream gas	0.07	4.46	6.19
Sidestream TPM	0.44	28.02	38.94
Mainstream gas	0.01	0.64	0.88
Mainstream TPM	0.50	31.84	44.25
Butt	0.55	35.03	9.73**
Ash	0.001	0.01	0.09
Total recovery	1.57	100.00	
Total consumed activity*	1.13		100.00

* Total recovered activity — butt activity present before smoking (0.44 μ Cl).

** Total butt activity — butt activity present before smoking.

to co-chromatograph with standard alkanes. TLC of sidestream smoke TPM extracts showed about 95% of the total activity to migrate with the alkane region and the rest remained at the origin. This suggests that some of the early eluting components detected by RP-HPLC in sidestream smoke TPM may be long-chain compounds derived from C₃₈H₇₈.

These chromatographic analyses showed RP-HPLC to be an effective method for separation and radioanalysis of the ¹⁴C-labeled products in smoke, and TLC was found to be a good complementary method.

The intact transfer of octatriacontane into mainstream smoke TPM is striking. It is evident that increasing the chain length from 32 carbons to 38 carbons does not affect the intact transfer of these alkanes into mainstream smoke TPM. The mechanism(s) of transfer of these compounds as well as other non-volatile tobacco components such as solanesol, neophytadiene and various sterols is not a well understood process. It is probable that more transfer studies and smoke aerosol formation studies will be needed to determine the factors which lead to intact transfer vs. pyrolysis of these compounds.

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