

Cigarette Smoke Formation Studies

III. The Contribution of Dotriacontane to the Benzo(a)pyrene Content of Smoke*

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INTRODUCTION

A most popular research topic of recent years has been the implication of the tobacco-leaf paraffinic hydrocarbons in the pyrosynthesis of the polynuclear aromatic hydrocarbon (PAH) content of cigarette smoke. More specifically, the role of the straight-chain hydrocarbon dotriacontane ($n\text{-C}_{32}\text{H}_{66}$) has received considerable attention in the formation of benzo(a)pyrene [B(a)P]. Badger, Donnelly and Spottswood (1) pyrolyzed dotriacontane at 700°C and found over forty compounds, including B(a)P and several other polynuclear aromatic hydrocarbons. Schlotzhauer and Schmeltz (2) have studied the pyrolysis of representative constituents of the hexane-soluble fraction of tobacco in a stream of dry nitrogen at 860°C . In later work, Schlotzhauer, Schmeltz and Osman (3) reported the pyrolysis of dotriacontane at 860°C and at 650°C . From their results, they extrapolated that at least three classes of smoke constituents were likely to form from the decomposition of dotriacontane in the burning cigarette. These classes were the aromatic hydrocarbons at 860°C , a homologous series of alk-1-enes (plus benzene and alkylbenzenes) at 650°C and, mainly, dotriacontane at 450°C .

Rayburn, Wartman and Pedersen (4) were the first to look at the tobacco paraffins in an actual smoking situation. The lack of exact chemical specificity of the added ^{14}C -materials and the smoke constituents has been used to repudiate their findings (5).

In a previous report (6), we discussed the smoke distribution and major mainstream pyrolysis products from cigarettes labeled with dotriacontane-16, $17\text{-}^{14}\text{C}$. We showed that 95% of the total radioactivity in the mainstream smoke was unchanged dotriacontane, with the remaining percentage appearing mostly as total combustion products. By design, our earlier study was aimed at determining only the major pyrolysis and/or distillation products of dotriacontane in mainstream smoke and not to answer whether or not any of the dotriacontane was converted at the trace level to other smoke constituents. This paper describes a detailed

radiotracer experiment designed to determine if dotriacontane is, in any measurable degree, a contributor to the B(a)P content of cigarette smoke.

EXPERIMENTAL

Radiochemicals

Dotriacontane-16, $17\text{-}^{14}\text{C}$ was purchased from the New England Nuclear Corporation. A purification procedure was used in which the dotriacontane was washed with cold (0°C) absolute ethanol, and then added to a cold silica gel column. The column was washed with cold acetone until no further activity was eluted. The dotriacontane-16, $17\text{-}^{14}\text{C}$ was then eluted with hexane. Analyses by gas radiochromatography and thin-layer radiochromatography indicated chemical and radiochemical purity to be better than 99%.

Labeled Cigarette Preparation

Thirty-five grams of cased, blended tobacco filler were sprayed during constant tumbling with 0.75 mCi dotriacontane-16, $17\text{-}^{14}\text{C}$ (3.91 mCi/mM) in 15 ml of hexane. After thorough mixing of the filler, the solvent was removed by evaporation and the tobacco was conditioned at 60% R. H. and 24°C . Cigarettes were handmade using Excel 67 mm length paper which had a 40 sec. porosity. Cigarettes were selected for resistance to draw and total weight. They were equilibrated at 24°C and 60% R.H. for one week prior to their use.

Liquid Scintillation Counting

A Packard Tricarb Model 3003 liquid scintillation spectrometer with optimized gain and window settings was employed. Aliquots of the desired samples were counted in a scintillator solution composed of 40.5 ml of Liquifluor (New England Nuclear) made up to one liter with spectroscopic grade toluene.

Counting times were regulated for proper counting statistics. In the cases of determining counts less than three times background, at least 100 minutes of counting time was used. Low potassium, ultrapure borosilicate glass counting vials (Packard) were used. Background counts were obtained on all vials and scintillator

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solutions before aliquoting. As a check, blank controls were always carried through to indicate any unusual effect by the aliquoting solvent. An internal standard (toluene-¹⁴C, New England Nuclear) was added to each sample and these data were used to apply the proper quenching corrections.

Benzo(a)Pyrene Analysis

The labeled cigarettes were smoked on a total recovery machine (7). The TPM filter pads were extracted by shaking for 24 hours in 25 ml of reagent grade hexane. An aliquot was taken to determine the total ¹⁴C-mainstream and sidestream activities. A known amount of B(a)P-³H (New England Nuclear) was added to enable accurate recovery corrections to be applied for the B(a)P losses incurred during the subsequent isolation procedure.

The techniques employed for the isolation and purification of the B(a)P were essentially those of Robb et al. (8) and later refined by Oakley et al. (9). This technique consisted of the hexane solution being chromatographed through an activated silica gel column. A solution of 5% benzene in hexane was used to elute the B(a)P and other polynuclear hydrocarbons from the column. After solvent evaporation, the residue was dissolved in 1 ml hexane and chromatographed on an acetylated cellulose thin-layer plate using methanol-ethyl ether-water 4:4:1 (V/V/V). The B(a)P spot was removed and dissolved in hexane and the concentration of B(a)P determined by scanning spectrofluorometry.

RESULTS AND DISCUSSION

The distribution of activity in the various portions of smoke from the dotriacontane-16, 17-¹⁴C containing cigarettes has been reported previously (6, 7). The total particulate matter (TPM) was collected on standard Cambridge pads on a total smoke recovery machine. After extraction, the activities in the TPM were determined, (the mainstream activity has been shown to be composed of 95% unchanged dotriacontane). The extract was condensed and the B(a)P separated as already described. The B(a)P content was determined and the amount of carbon-14 assayed. Special care had to be exerted in the radioactivity measurements because of the extremely small amounts of activity present. This

included low background vials, predetermined backgrounds, long and repetitive counting times and internal standardization for the determination of quenching effects. In order to report the maximum radiochemical yields in the B(a)P fraction, all numbers were rounded to give maximum results. On several determinations, no counts above background were detected, but in each case, a minimum of 1 count per minute above background was assumed for the purpose of calculating maximum B(a)P formation.

The maximum B(a)P conversions are shown in Table 1, breaking down each run into the contribution to the mainstream and the sidestream B(a)P fractions. Data are presented on a per cigarette basis and have been converted to 100% B(a)P delivery for each cigarette. The amount of B(a)P recovered was determined by assaying the TLC spot by fluorometry. The recovery was monitored by the use of B(a)P-³H and the proper corrections applied to calculate the corrected total delivery.

The data in Table 1 show a mainstream maximum conversion of dotriacontane-16, 17-¹⁴C of 2.6×10^{-7} and a sidestream conversion of 4.2×10^{-7} . In a control run where dotriacontane-16, 17-¹⁴C was added to the TPM on the Cambridge pads collected from "cold" cigarettes, a "maximum delivery" of 5.6×10^{-12} Ci was isolated in the B(a)P spot. This, in turn, would give an apparent B(a)P conversion of 2.5×10^{-7} . The appearance of radioactivity in the B(a)P fraction could only have come from a trace amount of the dotriacontane-16, 17-¹⁴C that was not totally separated by the isolation scheme. Based on these data, it is entirely feasible that the traces of activity present in the B(a)P from the labeled cigarettes also are due to unseparated dotriacontane-16, 17-¹⁴C. It must be remembered that it has been shown that 95% of the mainstream radioactivity is due to unchanged dotriacontane-16, 17-¹⁴C.

In this paper, for the purpose of calculating the maximum possible conversions of dotriacontane to B(a)P, all radioactivity in the B(a)P fraction has been treated as being "pure" B(a)P.

In order to evaluate the results in Table 1 in proper perspective, one need only apply already published tobacco data and some simple logic. Mold et al. (10) have reported that the total paraffin fraction of a commercial blended cigarette constituted 0.20 to 0.28% of the dry weight of tobacco. Carruthers and Johnstone

Table 1.

	Mainstream	Sidestream	Control
Max. radioactivity in B(a)P (100% delivery)	4.7×10^{-12} Ci ^a	7.8×10^{-12} Ci ^a	5.6×10^{-12} Ci ^b
Max. conversion of dotriacontane to B(a)P	2.6×10^{-7}	4.2×10^{-7}	2.5×10^{-7}

a) Total radioactivity of 1.83×10^{-9} Ci per cigarette.

b) Dotriacontane-16, 17-¹⁴C (2.27×10^{-9} Ci) added to TPM pads after smoking.

(11) have reported that the dotriacontane content of the total paraffin fraction is 5.1%. It is common tobacco knowledge that a cigarette has about 0.9 g dry weight of tobacco. It follows from simple arithmetic that the dotriacontane content can be easily calculated as follows: $0.28\% \times 5.1\% \times 0.9 \text{ g} = 0.13 \text{ mg}$ of dotriacontane naturally present in an average cigarette. The maximum amount of B(a)P that could, therefore, come from the dotriacontane present is the concentration of dotriacontane (moles) times the maximum conversion rate from Table 1, followed by conversion to grams:

Maximum B(a)P from $n\text{-C}_{32} =$

$$\frac{\text{C}_{32} \text{ Conc.} \times \text{Maximum Conversion} \times \text{Mol. Wt. B(a)P}}{\text{Mol. Wt. } n\text{-C}_{32}}$$

Substitution of the data from Table 1 produces a maximum mainstream contribution from dotriacontane of $0.02 \times 10^{-9} \text{ g B(a)P}$. Analytical data on this type of cigarettes have shown a normal B(a)P delivery of $29 \times 10^{-9} \text{ g}$. The $0.02 \times 10^{-9} \text{ g}$ maximum yield from dotriacontane therefore represents a mainstream maximum contribution of about 1/1,500 of the total cigarette B(a)P produced.

A similar treatment of the sidestream data results in a maximum delivery of $0.03 \times 10^{-9} \text{ g}$, or about 1/4,200 of the total sidestream B(a)P delivery.

Badger et al. (1) have reported the pyrolysis of dotriacontane at 700°C through a silica tube packed with porcelain chips and found small amounts of B(a)P in the pyrolysis tar. This is not surprising, as they also state, "the high-temperature pyrolysis of almost all organic compounds would be expected to give rise to tars containing polycyclic aromatic hydrocarbons . . .". Apparently, under the proper laboratory conditions, almost any organic compound can be regulated to produce a spectrum of desired pyrosynthesis products. Schlotzhauer et al. (2), under chosen laboratory conditions, pyrolyzed a number of compounds present in tobacco. Among these compounds was dotriacontane, which produced approximately 33 mg of B(a)P per gram of dotriacontane pyrolyzed or a conversion yield of about 1/30. This led them to the same ultimate conclusion as Badger et al. (1) regarding their own pyrolysis experiments: "That is, at high temperatures, anything is bound to occur or is at least energetically possible".

Rayburn and co-workers (4) studied the contribution of ^{14}C -labeled tobacco paraffins and found only 1/180 of the total polycyclic hydrocarbon contribution came from this source. Our results, which indicate that only 1/1,500 of the mainstream B(a)P could have come from the dotriacontane in the cigarette, substantiate and extend to a lower level Rayburn's conclusion.

The results presented in this paper have shown that dotriacontane is not, in any normal definition of the word, a meaningful precursor to the B(a)P content of cigarette smoke and, therefore, it is recommended that in further research, tobacco constituents other than

dotriacontane and its chemical neighbors be chosen as likely B(a)P sources. Tobacco constituents which cannot distil intact into the smoke stream, and must be exposed to the high temperatures necessary for the B(a)P pyrosynthesis, would make better laboratory choices in any study of the precursors of the polycyclic aromatic hydrocarbons.

SUMMARY

Cigarettes labeled with dotriacontane-16, 17- ^{14}C were smoked and the maximum conversions of dotriacontane to benzo(a)pyrene calculated. The maximum conversions are discussed in light of the known tobacco information showing that naturally occurring dotriacontane can contribute only 1/1,500 of the total benzo(a)pyrene content of mainstream smoke and 1/4,200 of the total sidestream smoke benzo(a)pyrene.

The results of this study reveal that the dotriacontane naturally present in an average cigarette cannot be, in any normal definition of the word, a meaningful precursor to the B(a)P content of cigarette smoke.

ZUSAMMENFASSUNG

Nach dem Verrauchen von mit Dotriacontan-16, 17- ^{14}C markierten Zigaretten wurde berechnet, in welchem Maße sich Dotriacontan maximal zu Benz(a)pyren umsetzt. Die Diskussion der erhaltenen Werte entsprechend dem Stand der gegenwärtigen Kenntnisse über Tabak zeigt, daß natürlich vorkommendes Dotriacontan zur Bildung des gesamten Benz(a)pyren-Gehaltes des Hauptstromrauches nur im Verhältnis 1/1500 und zur Bildung der Verbindung im Nebstromrauch nur im Verhältnis 1/4200 beitragen kann.

Die Ergebnisse der Untersuchung zeigen, daß das in einer durchschnittlichen Zigarette nativ vorkommende Dotriacontan keinesfalls eine Vorstufe des im Zigarettenrauch befindlichen Benz(a)pyrens sein kann.

RESUME

Des cigarettes marquées au dotriacontane-16, 17- ^{14}C ont été fumées, et les conversions maximales du dotriacontane en benzo-a-pyrène ont été calculées. Les conversions maximales sont discutées en tenant compte des informations connues sur les tabacs, prouvant que le dotriacontane présent naturellement ne contribue que pour 1/1500 à la teneur totale en benzo-a-pyrène dans le flux principal de fumée, et pour 1/4200 dans le flux secondaire.

Les résultats de cette étude démontrent que le dotriacontane naturellement présent dans une cigarette moyenne ne peut, dans toute acceptation normale du terme, être un précurseur important du benzo-a-pyrène présent dans la fumée de cigarette.

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