Isolation and Identification of 2,6-Dimethyl- and 2,2,6-Trimethyl-4-piperidone, Artifacts Produced by the Reaction of Cigarette Smoke Condensate and Acetone*

by W. N. Einolf, R. N. Ferguson, J. F. Whidby and J. F. DeBardleben

Philip Morris Research Center, Richmond, Virginia, U.S.A.

INTRODUCTION

There is considerable interest in the contribution of secondary reactions of smoke components to the complexity of cigarette smoke condensate (CSC). It is known that nitric oxide can give rise to other oxides of nitrogen as aging artifacts (1). There is an example of one such secondary product, dinitrogen tetroxide, reacting with methanol to give methyl nitrite, another artifact (2). There is other evidence that the apparent level of catechol in experimental cigarettes may be lowered by formation of 4-nitrocatechol in cold traps (3). Crotonaldehyde has been implicated in the formation of norbornene derivatives as artifacts derived from *Diels-Alder* reactions in condensed cigarette smoke (4).

In large scale chemical investigations of CSC, there are additional opportunities for artifact formation during solvent processing and fractionation (5). It has been demonstrated that changes occur in CSC depending on time or temperature of storage (6). The actual incorporation of solvent into an artifact has not been reported, but is a logical possibility due to the high reactivity of smoke components and the abundance of the solvent during processing or fractionation.

We now report the observation of three compounds which have not been reported previously in smoke and which are shown to be artifacts formed by reaction of acetone with CSC. The compounds, *cis*- and *trans*-2,6-dimethyl-4-piperidone, and 2,2,6-trimethyl-4-piperidone, may be indicative of other artifacts present in CSC processed with acetone as the solvent. Since acetone is used routinely by many groups in processing CSC, we wish to stress the importance of possible artifact formation in CSC.

EXPERIMENTAL

Cigarettes were smoked with a Borgwaldt 30-port smoking machine into an *Elmenhorst* trap kept at -79°C with a dry-ice/acetone bath (7). Standard smoking conditions were used: one 35 ml puff per minute of two

seconds duration in a laboratory kept at 75°F and 60% relative humidity. Solvents used in the condensate processing and isolation procedures were acetone and chloroform (reagent grade), methanol, dichloromethane and cyclohexane (all pesticide grade).

Combined gas chromatography / mass spectrometry (gc/ms) was achieved with a DuPont 21-490 instrument equipped with a dual chemical ionization / electron ionization source, a Varian 1440 gc, an all glass jet separator, and a DuPont 21-094B data system. Preparative gc was done with a Bendix Series 2200 gc, splitting the column effluent to a flame ionization detector and a thermal gradient trap (8). ^{13}C and proton (^1H) nuclear magnetic resonance (nmr) spectra were recorded on a Varian XL-100 or Bruker WP-80 instrument. Infrared (ir) spectra were obtained with a Digilab FTS-14 system, while high resolution mass spectra were recorded with an AEI MS-30 instrument at Shrader Analytical Laboratories, Detroit, Michigan.

Acetaldehyde determinations were made by a modification of the method used by *Vilcins et al.* (9). Measurements of pH were made by suspending 1.0 g of CSC in 100 ml distilled water and measuring the pH with a Corning Model 110 meter. Mainstream ammonia determinations were made by smoking into acid and using the *Berthelot* colorimetric method. Nitrate nitrogen was determined by using a modification of *Kamphake's* method (10).

SYNTHESIS AND ANALYTICAL DATA

The physical properties of the synthetic and isolated components were identical in all respects.

2,2,6-Trimethyl-4-piperidone [1]: The procedure of *King et al.* (11) was modified in the following way: To 10 g (0.049 mol) diacetoneamine oxalate was added 15 g (0.13 mol) diethyl acetal and 40 g sec-butyl alcohol. The mixture was refluxed for three hours, cooled to room temperature, and the salt filtered. The filter cake was washed with ethanol and air dried. The dry cake was dissolved in 13 ml H_2O and added to 45 ml 12 N

* Presented, in part, at the 30th Tobacco Chemists' Research Conference, Nashville, Tennessee, U.S.A., in October 1976.

NaOH. After stirring for 36 hours, the mixture was extracted with 300 ml ether and the ether dried over MgSO_4 . The ether was removed and the product distilled (boiling point 40–41 °C/0.1 torr), yielding 5.1 g (74%).

Spectral data

ir (neat): 3100 cm^{-1} (N-H stretch), 1715 cm^{-1} ($>\text{C}=\text{O}$)

^1H nmr: [CDCl_3] δ 1.10 (3H, s, C2a or C2b), δ 1.21 (3H, d, $J = 6.4$ Hz, C6a), δ 1.28 (3H, s, C2a or C2b), δ 2.12 (4H, m, C3 and C5), δ 3.25 (1H, m, C6)

^{13}C nmr: [CDCl_3 , ppm from TMS] 209.31 (C4), 54.13 (C2), 53.97 (C6), 49.83 (C3), 47.35 (C5), 31.91 (C2a), 25.29 (C2b), 22.87 (C6a) (18)

ms: 141 (M^+ , 22), 126 (40), 98 (17), 85 (13), 84 (100), 83 (13), 70 (10), 58 (12), 57 (13), 56 (15), 45 (24), 44 (21), 42 (51), 41 (16)

Cis- [2] and Trans- [3] 2,6-dimethyl-4-piperidone: Acetaldehyde (44 g, 1.0 mol) and diethyl-1,3-acetone dicarboxylate (101 g, 0.50 mol) were allowed to react with gaseous ammonia at -30 °C as described by Hall (12). The reaction was exothermic and the temperature rose to 57 °C. After saturation was complete the reaction mixture was allowed to cool to room temperature and refrigerated for 25 hours. The residue was refluxed with 700 ml of 10% HCl for 48 hours, with slow evolution of CO_2 . After removing the solvent on a rotary evaporator, 250 ml of 10% Na_2CO_3 was added and the basic solution extracted with CHCl_3 (3×200 ml). The combined organic layers were dried over Na_2SO_4 , and the solvent removed on a rotary evaporator to give 27 g of crude piperidone. Distillation gave 7.9 g (12.5%) of a clear, colorless liquid (boiling point 75–77 °C/15 torr). Combined gc/ms indicated that two isomers were present, although the literature claims that only one isomer is produced (12). Separation of the isomers was completed by preparative gc on a $10' \times 1/4''$ outside diameter copper column packed with 5% SP-1000 on 80/100 mesh Supelcoport operating isothermally at 140 °C. The major isomer was the first to elute and subsequently was found to be the *cis* isomer [2].

Spectral data

Cis isomer [2]:

ir (neat): 3300 cm^{-1} (N-H stretch), 1717 cm^{-1} ($>\text{C}=\text{O}$)

^1H nmr: [acetone- d_6] δ 1.17 (6H, d, $J = 6$ Hz, CH_3), δ 1.85–2.20 (4H, m, C3), δ 2.67–3.11 (2H, m, C2)

^{13}C nmr: [acetone- d_6 , ppm from TMS] 208.3 (C4), 52.72 (C3), 50.25 (C2), 22.70 (CH_3)

ms: 127 (M^+ , 24), 112 (38), 84 (23), 71 (10), 70 (100), 57 (10), 56 (16), 44 (64), 43 (36), 42 (70), 41 (26)

Trans isomer [3]:

ir (neat): 3300 cm^{-1} (N-H stretch), 1714 cm^{-1} ($>\text{C}=\text{O}$)

^1H nmr: [acetone- d_6] δ 1.10 (6H, d, $J = 6.8$ Hz, CH_3), δ 1.79–2.40 (4H, m, C3), δ 3.21–3.59 (2H, m, C2)

^{13}C nmr: [acetone- d_6 , ppm from TMS] 209.85 (C4), 49.5 (C3 or C2), 48.6 (C3 or C2), 21.15 (CH_3)

ms: 127 (M^+ , 23), 112 (46), 84 (21), 82 (22), 71 (11), 70 (100), 56 (14), 44 (51), 43 (36), 42 (58), 41 (25)

RESULTS AND DISCUSSION

The initial step in the study of processed CSC was to partition it between methanol and cyclohexane. Examination of the methanol fraction of CSC from Burley tobacco with added nitrate (Burley + NO_3)* by gc/ms shows the presence of a large peak of unknown composition as well as several polar compounds identified in Figure 1. A striking aspect in this gc profiling technique was the absence of the unknown peak in the gc scan of a comparable CSC fraction from flue-cured Bright cigarettes. Consequently, isolation of the unknown was accomplished by the fractionation procedure shown in Figure 2. We found that a thin layer chromatography (tlc) step was necessary after the preparative gc step when it became evident that two compounds were present within the trapped gc peak. Separation on TLC using silica gel and $\text{CHCl}_3/\text{CH}_3\text{OH}$ (88/12, v/v) as solvent gave compounds with molecular weights (MW) of 141 and 127. These were found to be 2,2,6-trimethyl-

Figure 1. Gas chromatogram of the polar fraction from cigarette smoke condensate produced by Burley cigarettes with added nitrate. Conditions: $6' \times 1/8''$ outside diameter glass column packed with 5% SP-1000 80/100 mesh Supelcoport, temperature program 100 to 250 °C at 4 °/min.

* Burley tobacco with added nitrate corresponds to a total nitrate nitrogen level of 1.10% in filler in all experiments.

Figure 2. Isolation procedure for 2,2,6-trimethyl- and 2,6-dimethyl-4-piperidones.

4-piperidone ([1], MW 141), and *cis*-2,6-dimethyl-4-piperidone ([2], MW 127), based on spectral analysis and synthesis of the authentic compounds. When the structures of [1] and [2] had been established, we examined the base fraction in more detail for related piperidones. Another compound, *trans*-2,6-dimethyl-4-piperidone ([3], MW 127), was found which has a longer gc retention time than the peak containing [1] and [2]. The ratio of [2] to [3] was consistently found to be about 19 to 1, while [1] was always more abundant than [2] + [3] (see Table 1).

Hall reported the isolation of only the *cis*-dimethyl-piperidone [2] from the reaction of diethyl-1,3-acetone dicarboxylate, acetaldehyde and ammonia (12). Harper et al. isolated a single product following Hall's procedure and also showed that it was the *cis* isomer. This assignment was based on the isolation of two epimeric alcohols from reaction of the piperidone with phenyllithium, while the *trans* isomer could give only one alcohol (19).

We found that Hall's procedure gave a mixture of two compounds in about a 9:1 ratio. Based on the spectral data (cf. Experimental) these were shown to be the *cis* and *trans* isomers [2] and [3]. Although stereochemical consideration would indicate that [2] (diequatorial) would

be more stable than [3], spectral data in the literature were inadequate for this distinction. Since both stereo-isomers were available, we were able to apply ^1H and ^{13}C nmr methods to [2] and [3] to show that the major isomer was *cis* as Hall reported, but a small amount of the *trans* isomer is also obtained in this reaction.

In order to confirm the structures of [2] and [3], a comparison was made between model reference compounds (*cis*- and *trans*-1,3-dimethylcyclohexane (13) and *cis*- and *trans*-3,5-dimethylcyclohexanone (14)). In each of the model compounds the ^{13}C nmr resonance of the *cis* methyl groups appeared from 1.5 to 2 ppm further downfield than the resonance of the *trans* methyl groups. This comparison allowed us to assign tentatively the major isomer of the 2,6-dimethyl compound as the *cis* isomer. The alternate method involved the addition of methyl iodide to an acetonitrile- d_3 solution of the suspected *trans* isomer to form the iodide salt of 1,2,6-trimethyl-4-piperidone [4].

On addition of acid to [4], the nitrogen inversion process was stopped and two resonances of equal areas could be observed for the 2,6-methyl groups; one for the methyl *cis* to the N-methyl and one for the methyl *trans* to the N-methyl. Only one N-methyl resonance was observed (presence of the *cis* isomer would require two N-methyl resonances if two 2,6 resonances were observed).

After the structures of the unknowns were determined, we returned to the problem of the origin of the large amount of [1] in CSC from Burley + NO_3 cigarettes, contrasted with its absence in CSC from flue-cured Bright cigarettes. To determine whether [1] was present in the tobacco, filler from Burley + NO_3 cigarettes was extracted with methanol. No evidence of [1] was found. Consequently, [1] must have been formed either in the smoking process or as an artifact of trapping or processing the smoke. We next determined whether acetone was involved in the formation of [1], based on the following observations: (a) diacetone amine was used in the synthesis of [1], (b) acetone is used in the processing step, and (c) acetone was implicated as a reactant to produce 2,2,6,6-tetramethyl-4-piperidone as an artifact in a fungal extract (15).

The cigarette selected for studying the effect of acetone on CSC was again the Burley with added nitrate, since a high level of [1] was found in the processed CSC. After smoking the cigarettes and trapping the CSC as usual, the condensate was removed from the trap with methanol and cyclohexane, instead of acetone. The sample was subsequently processed by heating to 40°C , followed by removal of solvent under reduced pressure (about 10 torr). A methanol/cyclohexane partition (see Figure 2) followed by gc analysis showed no evidence

of [1] (<0.1 µg/cigarette) in the polar fraction. These results suggest that a large excess of acetone is required to form significant amounts of [1] since there is some acetone present in fresh smoke from both Burley and Bright cigarettes (16).

The effect of other parameters on the yield of [1] was also investigated. Reagent grade acetone from several suppliers was evaluated; none was contaminated with acetaldehyde, and only the one found lowest in diacetone alcohol was used for further work. All sources of acetone resulted in considerable amounts of [1] in the processed Burley + NO₃⁻ CSC. Reaction temperature and time were found to be important, giving good yields at 40 °C and two hours. Under these conditions reproducibly high yields were obtained, and these conditions were used for all further work.

Further proof that acetone was involved in the formation of [1] was provided by washing the CSC (Burley + NO₃⁻) from the trap with acetone-d₆. After fractionating the sample, the mass spectrum showed that up to ten deuterium atoms had been incorporated in the isolated [1]. This supported the hypothesis that two molecules of acetone were incorporated into each molecule of [1] formed during processing. It is difficult to fully interpret the ¹H nmr results because deuterium scrambling could occur in the reaction process leading to [1].

Recognizing at this point that [1] was an artifact of CSC processed with acetone, we sought to identify the components in CSC which were involved in the reactions. We examined four other cigarette types in addition to

the flue-cured and the Burley + NO₃⁻ to quantitate [1] — [3] in the processed CSC. These four tobacco types included the Kentucky Reference 1R1 blended cigarette, a flue-cured Bright cigarette with sodium nitrate added to the filler, a Burley cigarette (the control for the Burley with added nitrate), and a model cigarette which was known to deliver high levels of ammonia to the smoke. All types, with the exception of 1R1, were 85 mm cigarettes with a cellulose acetate filter. These choices were made to evaluate the importance of some obvious differences in flue-cured and Burley cigarettes, e.g. higher nitrate in Burley filler, higher ammonia delivery in Burley smoke, and higher pH of Burley smoke condensate.

Quantitation of the piperidones was done by gc/ms of the polar fraction from processed CSC. Using the selected ion monitoring technique, isobutane was used as the reagent gas in the chemical ionization mode, and the mass spectrometer focused on the M + 1 ion (m/e 142 for [1] and m/e 128 for [2] and [3]). Results of [2] and [3] are reported as a summation [2 + 3], since the ratio of *cis* to *trans* was consistently in the range of 95:5 by this technique. The lower detection limit for [1] in these experiments was determined to be 2 ng. The calibration curves were linear in the sample range studied. The quantitations were done in this manner because of the coincidence of retention times for [1] and [2]. By using chemical ionization, with isobutane as reagent gas, the mass spectrum in the region of interest is virtually a one-peak spectrum, thus eliminating any interfering ions (17).

Table 1a,b. Levels of acetaldehyde, ammonia, pH, 2,2,6-trimethyl-4-piperidone [1], and *cis*- and *trans*-2,6-dimethyl-4-piperidone [2 and 3] in cigarette smoke condensate as a function of filler type and conditions.

Cigarette type	Nitrate in filler (% NO ₃ ⁻ N)	Ammonia (µg/cigarette)	Acet-aldehyde (µg/cigarette)	[1] (µg/cigarette)	[2 + 3] (µg/cigarette)	pH of CSC
Flue-cured Bright	0.03	24	840	ND	ND	6.0
Flue-cured Bright + NO ₃ ⁻ *	1.10	22	1010	ND	ND	4.5
Flue-cured Bright + NO ₃ ⁻ * (NaHCO ₃ added to CSC)	1.10	—	—	0.19	—	6.3
Burley	0.42	370	810	31	15	7.9
Burley + NO ₃ ⁻ *	1.10	570	860	176	41.4	8.0
1R1	0.23	29	930	ND	0.34	5.6
1R1 (NH ₄ OH added to CSC)	0.23	—	—	76	—	6.9
Cigarette A	0.55	818	520	91	9.7	5.7
Cigarette A (NaHCO ₃ added to CSC)	0.55	—	—	146	—	7.1
Cigarette A (¹³ CH ₃ ¹³ CHO added to CSC)	0.55	—	—	256	—	—
Burley + NO ₃ ⁻ **	1.10	370	810	ND	0.2	—

CSC: cigarette smoke condensate

a: ND = not detected

b: all values uncorrected for recoveries (see text)

*: adjusted to final nitrate level with NaNO₃

**: no acetone used in processing

Additional experiments were run to evaluate recoveries for [1] and [2 + 3]. In the case of [1], the acetone-processed Kentucky Reference 1R1 CSC was spiked with the synthetic material giving an 80% recovery in the polar fraction. For [2 + 3], a mixture of the synthetic isomers was added to the acetone-processed Kentucky Reference 1R1 CSC. The recovery determined in the base fraction (Figure 2) for the [2 + 3] mixture was found to be 84%.

The results of the quantitation of [1] and [2 + 3] are given in Table 1 for the polar fraction. These results are uncorrected for recovery, since in the absence of ^{14}C labeling studies, we cannot be certain that recoveries would be the same for each cigarette type studied. For simplicity, values for smoke components are given in micrograms per cigarette.

The data in Table 1 are important in considering the origin of the piperidones during processing. It is apparent that large amounts of the piperidones can be produced from some CSC samples, in the range of 100 to 200 μg per cigarette. We think that these quantities would exclude many trace components of smoke as the potential species reacting with acetone to form the piperidones. Two abundant components of cigarette smoke which could play a role in the piperidone formation are acetaldehyde and ammonia. The reactivity of ammonia and the fact that the piperidone level shown in Table 1 increases as ammonia levels increase suggest that ammonia could be the source of the piperidone nitrogen. That acetaldehyde plays a part in the formation of [1] is suggested by the unexpectedly low value of [1] in CSC from cigarette A. The importance of ammonia in the formation of [1] is stressed by the results for the flue-cured, flue-cured with added nitrate, and 1R1 results, where no [1] is detected, and the ammonia values in the smoke are low.

The value of 0.2 μg /cigarette for [2 + 3] in the Burley + NO_3 CSC sample processed in the absence of acetone indicates that endogenous acetone may be involved in the formation of [2 + 3] in this case. Both flue-cured Bright and Burley cigarettes give about the same amount of acetone in their smoke (630–680 μg /cigarette) (16); however, there is no detectable [2 + 3] in the flue-cured Bright results shown in Table 1, suggesting that the higher ammonia level of the Burley + NO_3 sample may be responsible for the formation of the small amount of [2 + 3].

The fact that the yields of piperidones from the various CSC types do not correlate well with the ammonia, acetaldehyde and nitrate levels shown in Table 1 suggests that the differences may also be due to the pH of the smoke condensate, since the pH could easily play a role in the extent of a reaction sensitive to acid or base involvement. The pH of the processed smoke condensate of the six test cigarettes was measured, and these values are also given in Table 1. The results from two additional experiments are shown in Table 1 as well. In the first experiment, aqueous sodium bicarbonate was added to the freshly trapped

smoke condensate from cigarette A. This was done to shift this sample to a basic pH during processing with acetone. A considerable increase in [1] was observed for this cigarette, implying an effect of the pH on the formation of [1] in the CSC. The second experiment involved addition of ammonia, as aqueous ammonia, to freshly prepared 1R1 smoke condensate. This condensate is low in ammonia but contains considerable acetaldehyde. The dramatic increase in the amount of [1] formed during this experiment is shown in Table 1; however, the experiment must be interpreted cautiously because the ammonia addition also altered the pH of the condensate. Other factors may be important in the formation of [1] and [2 + 3] during condensate processing, but the data in Table 1 and the necessity of acetone imply that the solvent, acetone, and the ammonia and acetaldehyde in the smoke are the piperidone precursors.

In order to demonstrate that acetaldehyde would be incorporated into [1] – [3] during processing, a sample of freshly trapped CSC was treated with $^{13}\text{CH}_3^{13}\text{CHO}$. The double labeling would show the position of the label, by ^{13}C nmr spectra, and the ratio of the labeled to unlabeled component by mass spectra. Only the large gc peak corresponding to the 2,2,6-trimethyl-4-piperidone and *cis*-2,6-dimethyl-4-piperidone was trapped and analyzed. The ^{13}C nmr confirmed that acetaldehyde units had been incorporated into each of the two products in the expected positions. The ms analysis showed approximately equal amounts of unlabeled ($M^+ = 141$ or 127) and labeled ($M^+ = 143$ or 131) product. The unlabeled material results from incorporation of endogenous acetaldehyde from the smoke into the artifacts. This experiment strongly supports the proposed origin of the artifacts isolated from CSC.

In conclusion, the formation of 2,2,6-trimethyl-4-piperidone and *cis*- and *trans*-2,6-dimethyl-4-piperidone has been shown to be due to the solvent, acetone, reacting with ammonia and acetaldehyde in the CSC. A number of routes are available for the reaction to occur, and some of these are shown in Figure 3 for the 2,2,6-trimethyl-4-piperidone. Even though these reactants are involved, others (e.g. crotonaldehyde) cannot be excluded on the basis of our experiments, since cigarette smoke and smoke condensate are complex mixtures which contain many reactive components. These results emphasize that in addition to the unavoidable reactions be-

Figure 3. Some possible routes for formation of 2,2,6-trimethyl-4-piperidone in cigarette smoke condensate from acetone, ammonia and acetaldehyde.

tween smoke components in the absence of solvent, the solvent itself can participate in artifact formation during processing of the smoke condensate. The identification of other components produced by acetone is our goal in the extension of the present study.

SUMMARY

Cigarette smoke condensate (CSC) processed with acetone was found to contain 2,2,6-trimethyl-4-piperidone and the *cis* and *trans* isomers of 2,6-dimethyl-4-piperidone. The compounds were found to be artifacts formed by the reaction of the smoke components ammonia and acetaldehyde with the solvent acetone. Conditions which affected the yield of the artifacts from the processed CSC include pH, temperature, reaction time, and levels of ammonia and acetaldehyde present in CSC.

ZUSAMMENFASSUNG

Es wurde gefunden, daß das Kondensat des Cigarettenrauches (CSC) nach Behandlung mit Aceton 2,2,6-Tri-methyl-4-piperidon sowie die *cis*- und *trans*-Isomere von 2,6-Dimethyl-4-piperidon enthält. Es zeigte sich, daß diese Verbindungen als Artefakte bei der Reaktion der Rauchbestandteile Ammoniak und Acetaldehyd mit dem Lösungsmittel Aceton entstehen. Die Ausbeute erwies sich als abhängig u. a. von folgenden Faktoren: pH-Wert, Temperatur, Reaktionszeit und Menge des im Rauchkondensat enthaltenen Ammoniaks und Acetaldehyds.

RÉSUMÉ

Il a été constaté que le condensat de fumée de cigarette (CSC) traité avec de l'acétone contient de la 2,2,6-triméthyl-4-pipéridone et les isomères *cis* et *trans* de la 2,6-diméthyl-4-pipéridone. On a trouvé que ces composés sont des artefacts formés par la réaction des constituants de la fumée l'ammoniac et l'acétaldéhyde avec l'acétone utilisée comme solvant. Les conditions influençant le rendement en artefacts à partir du CSC traité comprennent le pH, la température, le temps de réaction et les niveaux d'ammoniac et d'acétaldéhyde présents dans le condensat de fumée de cigarette.

REFERENCES

1. Greig, J. D., P. G. Hall: *Trans. Faraday Society* 63 (1967) 655.
2. Vilcins, G., J. O. Lephardt: *Chem. and Ind.* 1975, 974.
3. Kallianos, A. G., R. E. Means, J. D. Mold: *Tob. Sci.* 12 (1968) 125.
4. Harke, H.-P., C.-J. Drews, D. Schüller: *Tetrahedron Letters* 43 (1970) 3789.
5. Stedman, R. L., R. C. Benedict, M. Dymicky, D. G. Bailey: *Beitr. Tabakforsch.* 5 (1969) 97.
6. Schönherr, H., H.-J. Klimisch, H.-P. Harke: *Beitr. Tabakforsch.* 7 (1973) 18.
7. Elmenhorst, H.: *Beitr. Tabakforsch.* 3 (1965) 101.
8. Brownlee, R., R. M. Silverstein: *Anal. Chem.* 40 (1968) 2077.
9. Vilcins, G., C. J. Varsel, F. E. Resnik: *Tob. Sci.* 8 (1964) 49.
10. Kamphake, L. J., S. A. Hannah, J. M. Cohen: *Int. J. Air and Water Pollution* 1967, 205.
11. King, A. T., F. A. Mason, S. B. Schryver: *Brit. Pat.* 101, 738, 1916.
12. Hall, H. K., Jr.: *J. Amer. Chem. Soc.* 79 (1957) 5444.
13. Dalling, D. K., D. M. Grant: *J. Amer. Chem. Soc.* 89 (1967) 6612.
14. Weigert, F. J., J. D. Roberts: *ibid.* 92 (1970) 1347.
15. Bjeldanes, L. F., G. W. Chang: *J. Agric. Food Chem.* 23 (1975) 1010.
16. Buyske, D. A., L. H. Owen, P. Wilder, M. E. Hobbs: *Anal. Chem.* 28 (1956) 910.
17. Munson, B.: *Anal. Chem.* 43 (1971) 28A.
18. Carbons C2b and C6a were assigned by a comparison with the ^{13}C nmr spectra of reference compounds. See Kutschan, R., L. Earnst, H. Wolf: *Tetrahedron* 33 (1977) 1833.
19. Harper, N. J., A. H. Beckett, A. D. J. Balon: *J. Chem. Soc.* 1960, 2704.

Acknowledgments

We thank the members of the Analytical Division for their help, especially Drs. Phil Pitner and John Lephardt, and Mr. Gunars Vilcins. We also thank Mr. Henry Secor and Mr. Ralph Hellams for their assistance.

Authors' address:

Philip Morris Research Center, P.O. Box 26583,
Richmond, Va., 23261, U.S.A.