Estimation of Sucrose Esters in Tobacco by Direct Chemical Ionization Mass Spectrometry

W. Noel Einolf* and W. Geoffrey Chan

The sucrose ester content of three tobacco varieties has been determined as a function of plant growth. Quantitation was achieved by using direct chemical ionization (DCI) mass spectrometry with a synthetic sucrose ester as an internal standard. Total sucrose esters in tobacco range from about 218 μg/g of tobacco (218 ppm) in young burley Kentucky 14 tobacco leaves to almost 5000 μg/g of tobacco (5000 ppm) in cured Oriental-Symrna tobacco leaves.

Sucrose esters in tobacco have been reported to be important contributors to tobacco flavor (Severson et al., 1981). The levels of these compounds have been reported to be as high as 1335 ppm in some tobacco varieties (Johnson and Severson, 1982), but there has been no reported study of the effect of growing time on the production of the sucrose esters. In addition, the quantitative results that were reported by Johnson and Severson were obtained by glass capillary gas chromatography after conversion of the sucrose esters to their tetra(trimethylsilyl) ethers. A straightforward procedure involving the analysis of intact, underivatized sucrose esters is the subject of this report. This procedure is based on the technique of direct chemical ionization (DCI) mass spectrometry (Cotter, 1980), using ammonia as the reagent gas since ammonia exhibits satisfactory sensitivity and selectivity toward the sucrose esters.

EXPERIMENTAL SECTION

Plant Growth. Four plants of each tobacco type (flue-cured Coker 319 bright, burley Kentucky 14, and Oriental-Smyrna) were grown under greenhouse conditions. Transplanting was done 25 days after seeding. The Oriental plants and the burley plants were topped 30 days after transplanting, the flue-cured 40 days after transplanting, and the burley plants 40–48 days after transplanting. Suckers were removed during the growth period. The leaves were removed as the plants grew, starting with leaf no. 4 (counting from the bottom stalk position) followed by leaf no. 7, 8, 9, 10, and 12 on days 20, 34, 41, 49, 55, and 62 after transplant, respectively. At day 66 after transplant, leaves were removed from the fifth leaf position from the top of the stalk for the bright and Oriental plants. Curing of these leaves was done in the laboratory in a fashion designed to mimic field curing practices for each of the tobacco types, i.e., with an oven-curing method as a model for flue-curing bright tobacco and a sun-curing method for Oriental. The burley stalks were cut and the stalks were air-cured with the remaining leaves attached to the stalks. After curing was complete, the leaves from the fifth leaf position from the top of the stalk were used for analysis.

At each harvest, three leaves were divided at the midrib, and leaf veins were removed from half of each leaf. These halves were cut into small pieces and freeze-dried. The dry leaf material was then stored in a freezer until used for the extraction procedure.

A separate planting was made for harvesting at the silver dollar size and transplant stage. For both harvests, five plants from each variety were selected, and all leaves were freeze-dried and extracted. Harvest time for the silver dollar size plants corresponded to 27 days from seeding for Oriental and 31 days from seeding for bright and burley. Transplant size corresponded to 46 days from seeding for Oriental and 52 days from seeding for bright and burley plants.

Preparation of the Internal Standard. Sucrose (3.42 g, 10 mmol) was dissolved in 100 mL of pyridine and cooled to 0 °C. β-Methylvaleryl chloride (4.00 g, 30 mmol) was added dropwise over a period of 30 min. The mixture was stirred at 0 °C for 3 h and then at room temperature for 18 h. Water (10 mL) was then added, and the pyridine was partially removed by evaporation under reduced pressure. The residue was partitioned between dichloromethane and dilute hydrochloric acid. The aqueous fraction was washed twice with dichloromethane, and the combined organic solution was washed with saturated sodium bicarbonate solution and saturated sodium chloride solution and finally dried over magnesium sulfate. Evaporation of solvent gave 2.5 g of colorless syrup. The syrup was subjected to liquid chromatography on silica gel using a Waters Prep LC 500 A instrument with isopropyl alcohol–dichloromethane (5:95) as the eluting solvent. Fractions were collected and subjected to mass spectral examination. Those fractions that showed a peak at m/z 752 [$(M + NH_4)^+$] were pooled and rechromatographed on a Whatman Partisil M9 10/50 ODS-2 column, eluting with methanol–water (2:3). The solvent was removed on a rotary evaporator using a water aspirator and a water bath temperature of 40 °C. The residue was dried overnight at room temperature under vacuum to yield 150 mg of sucrose tetra-β-methylvalerate. The exact substitution pattern of the ester groups on the two saccharide rings has not been determined, but the DCI mass spectrum shows a fragment ion at m/z 359, indicating substitution of two β-methylvaleryl moieties on each ring.

Extraction and Isolation of Sucrose Esters. A 5.0-g portion of each tobacco sample was extracted with dichloromethane in a Soxhlet extractor using 250 mL of solvent and a minimum of 12 cycles, followed by solvent removal under reduced pressure on a rotary evaporator using a water bath at 35 °C. The residue was transferred with methanol–dichloromethane (1:1) to a vial in a quantitative fashion to make a total volume of 5.0 mL, and a 200-μL aliquot was placed on a prewetted (methanol–water, 1:1) C_{18} Sep-PAK cartridge to which a 200-μg sample of the internal standard, sucrose tetra-β-methylvalerate (40 mg/mL in dichloromethane), had been added. The cartridge was eluted, first with methanol–water (1:1) until 2.0 mL was collected, followed by dichloromethane. The next 0.3–0.4 mL of eluate was collected after introduction of dichloromethane on the column. This eluate was the

Philip Morris Research Center, Richmond, Virginia 23261.

0021-8561/84/1432-0785$01.50/0

remaining methanol–water solution and was discarded. The sucrose esters were than eluted with 2.0 mL of dichloromethane, and this fraction was evaporated to dryness with a stream of dry nitrogen. The residue was dissolved in 0.5 mL of dichloromethane and was used for quantitation.

Quantitation of Sucrose Esters. A 1.0-μL aliquot of the solution containing the sucrose esters was applied to the tip of a DCI probe (Einolf, 1983). The probe was then inserted into the ion source of the mass spectrometer (MAT 112S). The ion source temperature was held at 220 °C, and the mass range scanned was 630–860 μ. The source pressure with ammonia as the reagent gas was calculated to be 0.4–0.6 torr (Hancock et al., 1979). The sucrose esters, as well as the internal standard, were desorbed under these conditions within a period of 6 min. During this time the mass spectrometer was scanning magnetically in the normal acquisition mode at 2 s/decade with an interscan time of 0.5 s. Data were acquired with the MAT SS200 data system using Version 4 software and were processed with the standard SS200 routines to determine peak areas. Quantitation was achieved by calculating the ratio of peak area intensities of the unknown sucrose ester isomers (ions at m/z 640, 654, 668, 682, 696, 710, and 724) with that of the sucrose tetra-β-methylvalerate (ion at m/z 752). It is possible that the parent adduct ion stabilities of the internal standard and of the tobacco-derived sucrose ester isomers are different. However, in the absence of authentic sucrose ester standards for calibaration, true quantitation is not possible. Consequently, the values reported for sucrose ester quantitation assume equal response factors for the internal standard and the tobacco-derived sucrose esters.

In order to determine the precision of the DCI method in the quantitative analysis of the sucrose esters, several experiments were done. In the first experiment, all fractions eluting from the Sep-PAK cartridge were analyzed by the DCI technique. More than 99% of the sucrose esters were found in the dichloromethane fraction, with less then 1% eluting in the first methanol–water fraction. No sucrose esters were found in the intermediate fraction. An additional experiment involved four separate Sep-PAK elutions using the same sample (Oriental extract, day 49). The relative standard deviation (RSD) was found to range from 1.8% for the m/z 682 ion (the most intense of the sucrose ester peaks measured) to 11% for the m/z 640 ion. The m/z 724 ion, which is generally very weak, shows correspondingly poor precision, with a RSD of 72%.

RESULTS AND DISCUSSION

The DCI method was chosen because of its sensitivity and selectivity toward the sucrose esters (Einolf et al., 1983). The mass spectra are characterized by an adduct ion corresponding to $(M + NH_4)^+$, with major fragment ions resulting from loss of the unsubstituted ring $(M + NH_4 - 162)^+$ and loss of the substituted ring. Evidence for substitution of the ester on the glucose (and not the fructose) ring appears in a report (Schumacher, 1970) in which a glucose ester from an Oriental tobacco isolate was characterized. It is possible that in Schumacher's isolation procedure the sucrose ester was inadvertently hydrolyzed to produce the glucose ester. Further evidence for substitution on the glucose moiety of the sucrose esters was presented by Severson et al. (1983), showing that the C-6 position is substituted with an acetate group, while the C-2, C-3, and C-4 positions can be substituted with esters of varying chain lengths, i.e., from three to eight carbons in length. The TI-165 Tobacco Introduction variety was shown by these workers to contain a major isomer in the

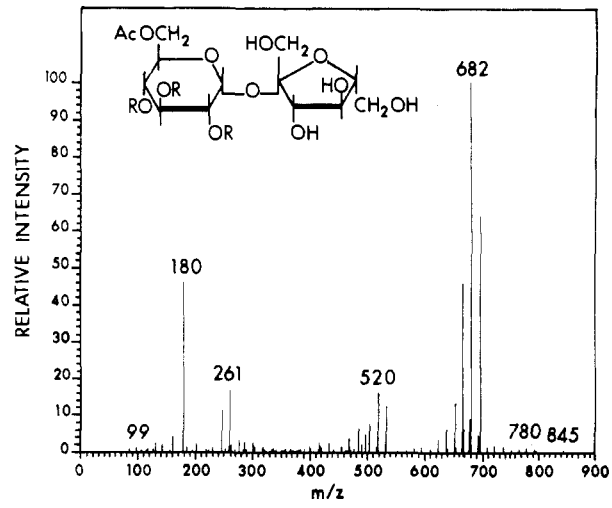

Figure 1. DCI mass spectrum of a purified sucrose ester fraction isolated from Oriental-Smyrna tobacco. Reagent gas used was ammonia; isotope peaks were not plotted.

sucrose ester fraction corresponding to the 6-*O*-acetyl-2,3,4-tri-*O*-(β-methylvaleryl) derivative of sucrose. The DCI analysis described here cannot differentiate the rings (glucose vs. fructose) but does indicate that one is substituted and one is not. The loss of the substituted ring leaves an ion corresponding in mass to the fructose ring at m/z 180. A spectrum of a purified sucrose ester fraction isolated from Oriental tobacco and the general structure of the esters are shown in Figure 1. The cluster of ions centered about m/z 682 represents $(M + NH_4)^+$ ions of the sucrose esters. These ions are subject to loss of 162 μ, and the resulting fragments appear as a similar cluster centered about m/z 520. The ion at m/z 180 (the fructose fragment) is also prominent in this mass spectrum.

The ions at m/z 640, 654, 668, 682, 696, 710, and 724 were monitored in the tobacco extract analysis. These ions correspond to the $(M + NH_4)^+$ species for the sucrose esters with different substituents. For example, the m/z 696 ion is most likely due to the sucrose ester having one acetate at the C-6 carbon of glucose and three β-methylvalerate substituents at C-2, C-3, and C-4 carbons of glucose (Severson et al., 1983). Since an internal standard (sucrose tetra-β-methylvalerate) was used in the analysis, m/z 752 $[(M + NH_4)^+]$ was also monitored.

Although the ammonia reagent gas is selective, additional tobacco component peaks are present in the mass spectrum but do not interfere with the sucrose ester analysis. As an example, a partial DCI mass spectrum obtained from a cured Oriental tobacco leaf extract is shown in Figure 2. The most intense peak in this spectrum is due to solanesol and occurs at m/z 648 $[(M + NH_4)^+]$. The peak at m/z 630 is most likely due to a dehydration ion from the solanesol adduct while the compound responsible for the m/z 664 ion has not yet been identified. The internal standard peak at m/z 752 is also present in this scan. The associated cluster of ions around m/z 520 is present, although not shown in Figure 2. At lower masses ($<m/z$ 500) the spectrum is cluttered with ions, but these do not interfere with the analysis. It is not possible to rule out the presence of interfering components at the monitored masses in the quantitative analyses (m/z 640, 654, etc.) with our instrumentation, but experiments with DCI mass spectrometry/mass spectrometry (McLafferty and Bockhoff, 1978) could verify that the monitored parent ions are derived solely from the sucrose esters. A high-resolution mass spectrum would also indicate whether there are interfering isobaric ions. The fact

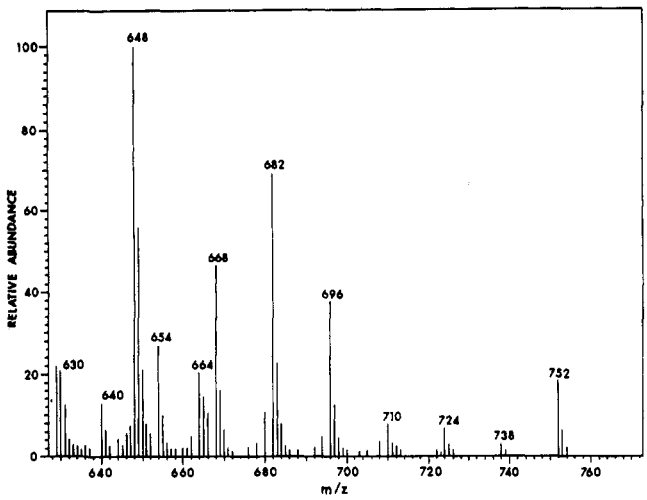

Figure 2. Partial DCI mass spectrum of the Oriental-Smyrna tobacco fraction used for quantitating sucrose esters in freeze-dried fresh green leaf. The peak at m/z 752 corresponds to $(M + NH_4)^+$ for the internal standard, sucrose tetra-β-methylvalerate.

that the DCI mass spectrum of the crude tobacco isolate (Figure 2) so closely approximates that of the purified sucrose ester fraction (Figure 1) suggests that interfering components, if present at all, are minimal. Differences in sucrose ester ion intensities between the two samples can be attributed to the use of an HPLC purification procedure for the sample in Figure 1, resulting in some discrimination among the esters.

Results of quantitation of the four components at m/z 654, 668, 682, and 696 are shown in Figure 3 for the three tobacco types studied. There are similarities in the behavior of these esters as a function of the growth time. In the Oriental plant the sucrose esters reach the first maximum at day 42, while this maximum occurs somewhat later (day 49) for bright and for the m/z 654 component in burley (the low data point at day 42 is assumed to be experimental deviation). This maximum is followed by a minimum at day 55 in both bright and Oriental, while the minimum in the burley m/z 654 is again later, at day 62. The last data point represents tobacco that was harvested at day 66 from transplant and was immediately subjected to a curing process. Thus, the last data point represents an additional 4 days of growth from the day 62 data point and the effects of the curing procedure used on that particular type of tobacco. The day 66 data show an increase over those of day 62 for all ions in Oriental and burley tobacco and for the m/z 654 and 696 ions in bright tobacco. The relative increase in sucrose ester content for burley and Oriental (from 200 to 300% for some compounds) vs. that for bright (about 20%) in these cured samples is an indication that the slower curing process, involving lower temperatures for the air- and sun-cured tobaccos, enhances the formation of sucrose esters, while the higher temperatures encountered in the oven-curing process do not favor ester formation to the extent that the other curing processes do. These differences may also be due to actual tobacco weight loss due to biochemical processes within the leaf in the air- and sun-cured varieties over the longer curing times (several days for the flue-curing vs. several weeks for air- and sun-curing). These conclusions are also apparent from the data presented in Figure 4, wherein the total sucrose ester content of each tobacco type is plotted as a function of days of growth from transplant. Figure 4 also shows the dramatic difference in sucrose ester level for the three tobacco types, particularly after the curing process. A comparison of the data in Figure 4 with data reported by Johnson and Severson

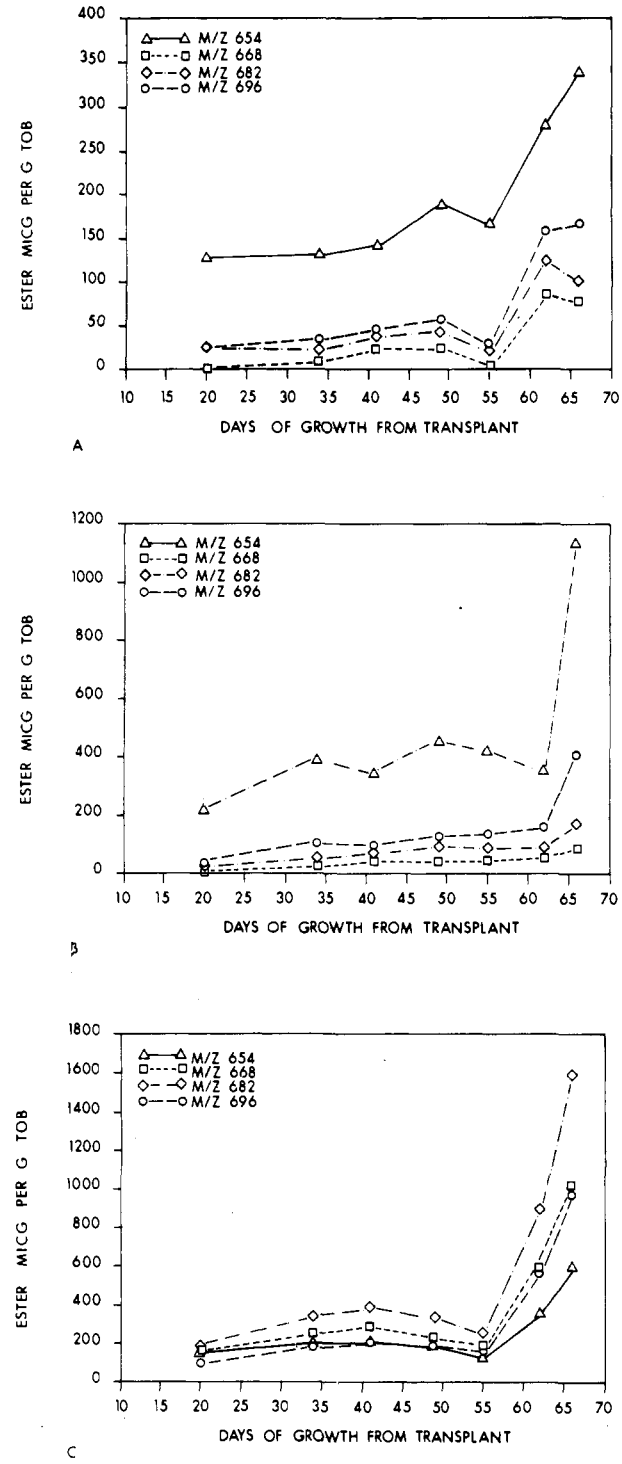

Figure 3. Individual sucrose ester levels of (A) flue-cured Coker 319 bright, (B) burley Kentucky 14, and (C) Oriental-Smyrna tobacco leaves as a function of growth time after transplant. Note that the last data point (day 66) includes curing. Values are peak areas of monitored ions compared to the peak area of the internal standard ion and assume equal response factors.

(1982) shows reasonable agreement. In their report of total sucrose esters as determined by a GC method, values ranging from not detected to 1335 ppm were found for various Tobacco Introduction varieties of *Nicotiana tabacum*. The corresponding value for the Coker 319 bright mature green leaf from our study is 752 ppm, a value in the middle of the range reported by Johnson and Severson.

In a separate study conducted after the summer growth period, plants were seeded and subsequently harvested at two early stages to determine when the sucrose esters first appear in the leaves. These stages were labelled "silver

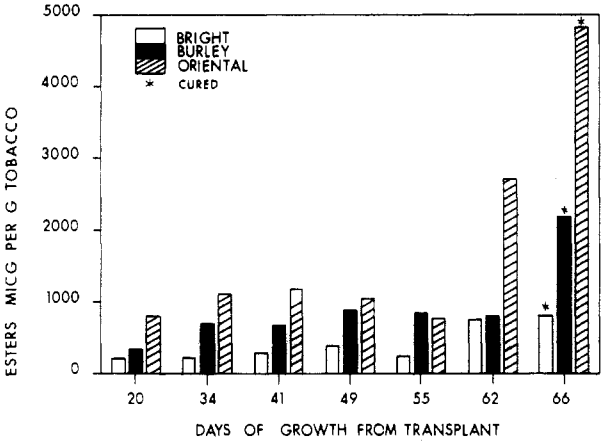

Figure 4. Total sucrose ester content of Coker 319 bright, burley Kenticky 14, and Oriental-Smyrna tobacco leaves as a function of growth time after transplant. Values are summed peak areas of monitored ions compared to the peak area of the internal standard ion and assume equal response factors.

Table I. Total Sucrose Ester Content[a] (μg/g of Tobacco) of Silver Dollar Size and Transplant-Size Tobaccos

tobacco type	silver dollar	transplant
Bright	274	475
Burley	218	420
Oriental	548	776

[a] Values are the summed peak areas of monitored ions compared to the peak area of the internal standard ion and assume equal response factors.

dollar" size (leaves about 35 mm in width, days 27–31 from seeding) and transplant size (days 46–52 from seeding). These growth periods are longer for these plants since the study was conducted later in the year. The results for total sucrose ester content in the whole leaves from these plants are given in the Table I. The data show a trend similar to those given in Figure 4, i.e., an increase in ester content as a function of plant maturity. When the individual ester levels are plotted as in Figure 5, however, the Oriental data show an interesting trend. In the silver dollar size plants, the m/z 654 component is the most abundant, with the m/z 668, 682, and 696 components showing decreasing levels. In the transplant-size tobacco, the m/z 668, 682, and 696 components increase at a faster rate than the m/z 654 component, with the m/z 668 component approaching a level comparable to that of the m/z 654. After transplant, the trend continues (Figure 3), with the m/z 654 component remaining at about 200 μg/g of tobacco through day 55 after transplant. The m/z 668 component was attributed to a sucrose tetraester composed of one C_2, two C_5, and one C_6 esters (Einolf et al., 1983; Severson et al., 1983). Similarly, a probable composition for the m/z 654 component is one C_2 and three C_5 esters. The reason for the shift to the higher carbon esters in the Oriental plant as a function of growth time is unclear, but it is significant that only the Oriental tobacco shows this trend.

The sucrose esters have been described in terms of the $(M + NH_4)^+$ value but have not been fully characterized by the DCI technique. The DCI procedure does assist in assigning structure by showing that only one of the two saccharide rings contains the substituents. These ester substituents are composed primarily of acetate, isovalerate, and methylvalerate groups, with the most likely substitution of the acetate at the C-6 carbon of glucose (Severson et al., 1983).

The results of this study lead to a question about the effect of stalk positon on the sucrose ester content. In the small scale study reported here it was not possible to

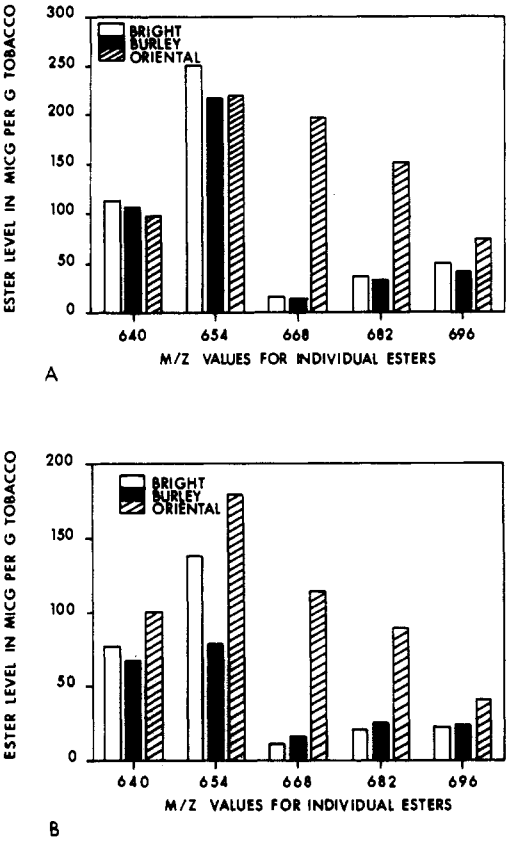

Figure 5. Individual sucrose ester levels in (A) transplant-size and (B) silver dollar size tobacco leaves. Values are the peak areas of monitored ions compared to the peak areas of the internal standard ion and assume equal response factors.

sample from only one stalk position during the growth period. However, a study is now under way in which plants will be raised to days 40–50 after transplant and leaves from the mid-stalk positions will be analyzed for their sucrose ester content. That is, the sucrose ester levels will be evaluated for each leaf in all the mid-stalk positions to determine whether there is any systematic variation in sucrose ester content. Some variation in total sugars has been reported for a bright tobacco variety, but this variation is minimal in leaf positions 7–14 (Walker, 1968). Variation in sugar content has also been reported for three different bright tobaccos as a function of leaf maturity, but no consistency was shown (Moseley et al., 1963).

In conclusion, the sucrose ester content of tobacco has been determined by using a novel quantitative technique that because of its sensitivity and selectivity requires a minimal sample cleanup procedure. A growth study was completed for three different tobacco varieties by sampling at various positions in the mid-stalk region of the plant. In most cases the esters were observed to maximize after 40 days from transplant, decrease somewhat, and again maximize to a higher value as the plant reached maturity.

ACKNOWLEDGMENT

We thank Grover Newell and Roger Bass for the plant materials and for helpful discussions concerning sampling procedures. Don Magin is also gratefully acknowledged for his suggestions about the data analysis and the Sep-PAK procedure. We are grateful to Howard Spielberg for his suggestions and encouragement. We thank Trish Sinkiewicz and Anne Donathan for typing the manuscript and Chuck Nilles for drawing the figures.

LITERATURE CITED

Cotter, R. J. *Anal. Chem.* **1980**, *52*, 1589A–1606A.

Einolf, W. N. In "Mass Spec Handbook Of Service"; Manura, J. J., Ed.; Scientific Instrument Services, Inc.: Pennington, NJ, 1983; p 75.
Einolf, W. N.; Magin, D. F.; Chan, W. G. *Int. J. Mass Spectrom. Ion Phys.* **1983**, *48*, 335–338.
Hancock, R. A.; Walder, R.; Weigel, H. *Org. Mass Spectrom.* **1979**, *14*, 507–511.
Johnson, A. W.; Severson, R. F. *Tob. Sci.* **1982**, *26*, 98–102.
McLafferty, F. W.; Bockhoff, F. M. *Anal. Chem.* **1978**, *50*, 69–76.
Moseley, J. M.; Woltz, W. G.; Carr, J. M.; Weybrew, J. A. *Tob. Sci.* **1963**, *7*, 67–75.
Schumacher, J. N. *Carbohydr. Res.* **1970**, *13*, 1–8.
Severson, R. F.; Arrendale, R. F.; Chortyk, O. T.; Green, C. R.; Johnson, A. W., 37th Tobacco Chemists' Research Conference, Washington, DC, Oct 1983, Abstract 11.
Severson, R. F.; Arrendale, R. F.; Chortyk, O. T.; Johnson, A. W., 33rd Southeastern Regional Meeting of the American Chemical Society, Lexington, KY, Nov 1981, Abstract 68.
Walker, E. K. *Tob. Sci.* **1968**, *12*, 58–65.

Received for review December 19, 1983. Accepted March 6, 1984.

Determination of Polycyclic Aromatic Hydrocarbons in Some Canadian Commercial Fish, Shellfish, and Meat Products by Liquid Chromatography with Confirmation by Capillary Gas Chromatography–Mass Spectrometry

James F. Lawrence* and Dorcas F. Weber

Eighteen polycyclic aromatic hydrocarbons, 11 of which are known carcinogens (oral, inhalation, dermal, or other) were determined in a variety of selected food commodities available in Canada including smoked and unsmoked seafood products, meat spreads, and fried and char-broiled meats. All samples were carried through a saponification step followed by a liquid–liquid partition, a Florisil column cleanup, and then a second liquid–liquid partition before analysis. Reversed-phase liquid chromatography with fluorescence detection compared well with capillary gas chromatography–mass spectrometry. Total carcinogenic PAH levels ranged from not detectable up to several hundred micrograms per kilogram in some cases (oil from a can of smoked oysters). Most samples contained levels in the low-to-sub micrograms per kilogram range.

Polycyclic aromatic hydrocarbons (PAH) have been the subject of numerous studies related to foods and to their possible effects on human health due to the carcinogenicity of a number of members of this class of compounds. A recent volume of the *Journal of Environmental Pathology and Toxicology* was completely devoted to PAH (Santondonato et al., 1981). Analytical methodology and the occurrence of PAH in foods have been the subject of a comprehensive review (Howard and Fazio, 1980). PAH contamination arises from several sources including processing of food (smoking, direct drying, cooking, natural sources (Suess, 1976), and environmental contamination of air, water, or soil (Tilgner, 1970), the latter being considered as the most important. Grimmer (1968) pointed out that vegetables, not smoked foods nor grilled meat, may be the greatest source of PAH to humans. It has also been estimated that food intake may in fact surpass tobacco smoking as a major contributor to PAH exposure (Santodonato et al., 1981). In Canada publications have appeared concerning the PAH content of smoked and char-boiled foods (Panalaks, 1976) and fish, mollusks, and lobsters (Dunn and Fee, 1979).

A number of approaches have been studied for the extraction of PAH from foodstuffs (Crosby et al., 1981; Lintas et al., 1979; Dunn and Fee, 1979; Kolarovic and Traitler, 1982). The methodology chosen for the present work was based on the extraction procedure developed by Grimmer and Böhnke (1975) with some modification that included the use of a deactivated Florisil column cleanup (Basu and Saxena, 1978) and a dimethylsulfoxide partition for additional purification (Obana et al., 1981; Haenni et al., 1962).

Table I. PAH Used in This Study

compd no.	IUPAC name	abbreviation
1	fluoranthene	FL
2	pyrene	PY
3	benzo[*b*]fluorene	BbFL
4	3,6-dimethylphenanthrene	DMP
5	benz[*a*]anthracene	BaA
6	perylene	Per
7	benzo[*a*]pyrene	BaP
8	dibenz[*ac*]anthracene	DacA
9	dibenz[*ah*]anthracene	DahA
10	picene	Pi
11	indeno[1,2,3-*cd*]pyrene	IP
12	anthanthrene	An
13	dibenzo[*ae*]pyrene	DaeP
14	9,10-diphenylanthracene	DPA
15	dibenzo[*ai*]pyrene	DaiP
16	dibenzo[*ah*]pyrene	DahP
17	chrysene	Ch
18	dibenzo[*al*]pyrene	DalP

High-pressure liquid chromatography (HPLC) with fluorescence detection has been found to be very suitable for the quantification of PAH in a variety of biological samples (Crosby et al., 1981; Panalaks, 1976; Obana et al., 1981; Joe et al., 1982) with detection limits often in the sub micrograms per kilogram range. The selectivity of the sample purification and detection makes the method very suitable for routine monitoring. However, often it is necessary to confirm high or suspicious results by some other technique to ensure that false positives are eliminated as much as possible. In this regard, gas chroma-

Food Research Division, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2.

0021-8561/84/1432-0789$01.50/0