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Benzo[a]pyrene Formation in the Pyrolysis of Selected Amino Acids, Amines, and Maleic Hydrazide

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The benzo[a]pyrene content in the pyrolyzates of methionine, proline, tryptophan, valine, phenethylamine, N,N-dimethyldodecylamine, and maleic hydrazide was determined by using an isotopic dilution method developed for the estimation of benzo[a]pyrene in tobacco smoke. Pyrolyses at 650 °C gave lower yields of benzo[a]pyrene than those at 850 °C, and at 850 °C the benzo[a]pyrene yields are dependent on the structure of the substance pyrolyzed rather than on elemental composition alone.

Polynuclear aromatic hydrocarbons are produced in varying amounts in the high-temperature thermolysis of simple aliphatic hydrocarbons (Badger, et al., 1965), alkylbenzenes (Badger et al., 1960), amino acids which are found in tobacco and other agricultural and food products (Higman et al., 1970; Patterson et al., 1969, 1971, 1973, 1975), maleic hydrazide and N,N-dimethyldodecylamine which are used as sucker control inhibitors (Patterson et al., 1972). Benzo[a]pyrene has been reported in some of these pyrolyses and in tobacco smoke (Patterson et al., 1972; Davis, 1968; Schmeltz et al., 1964), and the methods used for its separation and identification have been diverse and sometimes unsatisfactory. In a continuation of our studies of the pyrolytic products obtained from compounds found in, or related to, tobacco we have adapted an isotope dilution–spectrofluorometric method for the determination of benzo[a]pyrene in tobacco smoke to determine the benzo[a]pyrene content in the pyrolyzates of four structurally different amino acids (methionine, proline, tryptophan, and valine), 2-phenethylamine, N,N-dimethyldodecylamine, and maleic hydrazide.

EXPERIMENTAL SECTION

Materials. DL-Leucine, DL-methionine, DL-proline, DL-tryptophan, DL-valine, (Nutritional Biochemicals, Cleveland, Ohio), 2-phenethylamine, maleic hydrazide (Eastman Organic Chemicals, Rochester, N.Y.), and N,N-dimethyldodecylamine (Armour Industrial Chemical Company, McCook, Ill.) were used as received.

Cyclohexane, benzene, hexane, chloroform, ether, and pentane (Burdick and Jackson, Inc.), distilled-in-glass solvents, and methanol (J. T. Baker) low-in-acetone grade (BK-9070) were used as received after being checked to assure that they were free of fluorescent impurities in the region used for benzo[a]pyrene analysis.

Aluminum oxide G (Type E) and aluminum oxide H (Merck, Darmstadt) were used in the thin-layer chromatographic separations.

Acetylated cellulose (20% acetylated) was obtained from Macherey, Nagel and Co.

Florisil (60/100 mesh) was obtained from Floridin Co. 2,5-Diphenyloxazole was obtained from Beckman Instruments, Inc.

[8,9-¹⁴C]Benzo[a]pyrene (Volk Radiochemical Co., V 3905; sp act. of 20 mCi/mg) was purified by preparative thin-layer chromatography (1.5 mm alumina [Merck,

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PF-254] and developed with hexane–benzene [8:2]).

METHODS

Spectrofluorometric measurements were taken using an Aminco-Bowman, 4-8202 (American Instrument Co.) spectrofluorometer equipped with a 416-992 xenon lamp and a 1 p21 photomultiplier tube.

Scintillation counts were measured using a Beckman LS-100 Liquid Scintillation System.

Pyrolysis and Separation of Neutral Fractions. The pyrolyses were carried out in the apparatus previously described (Patterson et al., 1968) using 10 mL of Berl Saddles, a nitrogen flow of 60 mL/min, and a rotating screw device for the introduction of the samples into the pyrolysis tube.

The pyrolyzate was collected in two cooled (dry ice-chloroform–carbon tetrachloride) traps and extracted with ether, and the ether-soluble material was separated into acidic, basic, and neutral fractions by extraction with successive portions of 5% HCl and 5% NaOH saturated with NaCl.

Separation and Determination of Benzo[a]pyrene Content in the Neutral Fractions. A known amount (approximately 4×10^4 CPM) of [¹⁴C]benzo[a]pyrene was added to the cyclohexane mixture of the neutral fraction, the mixture clarified with anhydrous sodium sulfate for several minutes, filtered, and concentrated to approximately 1 mL with a rotary evaporator, keeping the temperature of the bath below 45 °C. The residue was taken up in hexane and applied to a 60/100 mesh Florisil column (2 cm diameter) deactivated with 3% water and packed to a depth of 8 cm in hexane. After the aliphatic hydrocarbons were eluted with hexane, the aromatic hydrocarbons were eluted with hexane–benzene (3:1) and collected as a single fraction. The eluent containing the aromatic hydrocarbons was concentrated to approximately 0.5 mL (rotary evaporator) and then applied as a streak to a 0.3-mm alumina thin-layer chromatography plate. A reference spot of authentic benzo[a]pyrene was applied adjacent to the streak and the plate was developed in a Chromaflex sandwich chamber (Kontes Glass Co.) with pentane–ether (85:15) or isooctane–toluene (9:1) until the band coincident to the benzo[a]pyrene reference spot was well separated. The portion of alumina containing the benzo[a]pyrene band was transferred to a 15-mL centrifuge tube and the impure benzo[a]pyrene desorbed with four 4-mL portions of methanol. The combined methanol extracts were evaporated to dryness, and the residue was dissolved by two treatments of 0.2 mL of benzene and applied as a streak to a 0.3-mm 20% acetylated cellulose thin-layer chromatography plate. A reference spot of authentic benzo[a]pyrene was placed adjacent to the streak

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Table I. Weight of Neutral Fraction and Benzo[a]pyrene Yield per Mole of Compound Pyrolyzed

Compound pyrolyzed	Temp, °C	Weight[a] of neutrals	Weight[b] of benzo-[a]-pyrene	Weight[a] of styrene
N,N-Dimethyl-dodecyl-amine	850	16.00	57	1.3
N,N-Dimethyl-dodecyl-amine	850[c]	14.74	28	1.8
Maleic hydrazide	850	6.76	11	0.3
Maleic hydrazide	850[c]	6.33	17	0.1
Methionine	850	1.36	2.1	0.01
2-Phenethyl-amine	850	8.77	8.0	0.1
2-Phenethyl-amine	650	32.79	1.1	20
Proline	850	5.32	25	0.3
Tryptophan	850	48.98	2.1	d
Valine	850	1.48	11	0.04
Valine	650	3.34	0.05	d

[a] Reported in g/mol of substance pyrolyzed (Patterson et al., 1972, 1973, 1975, 1976). [b] Reported in mg/mol of substance pyrolyzed. [c] Air is used as a carrier gas instead of nitrogen. [d] Not detected.

and the plate developed with methanol–chloroform (3:1) until the benzo[a]pyrene band was well separated. The portion of cellulose containing the benzo[a]pyrene band was transferred to a 15-mL centrifuge tube and extracted with four 4-mL portions of methanol. After evaporating the methanol solution to dryness, the residue was dissolved in 1.6 mL of toluene (vessel was washed with four 0.4-mL portions) and transferred to a 2.5-mL centrifuge tube. The volume was adjusted to 2.0 mL, and any remaining cellulose particles were removed by centrifugation. The fluorometric relative intensity of the benzo[a]pyrene in the solution was measured using excitation and emission wavelengths of 378 and 405 nm, respectively. The sensitivity of the photomultiplier microphotometer was adjusted to give a known reading with a standard solution of benzo[a]pyrene before the measurement of the analytical samples. The concentration of the isolated benzo[a]pyrene was obtained from the linear portion of a calibration curve of a plot of the logarithm of corrected relative intensity vs. the logarithm of the benzo[a]pyrene concentration. The entire solution was transferred to a scintillation vial, 12 mL of a toluene solution of 2,5-diphenyloxazole (4 g/L) was added, and the residual activity was counted. A secondary fluor was not needed in the counting system employed. The weight of benzo[a]pyrene in the pyrolyzate sample was calculated from the isolated benzo[a]pyrene weight and the final and initial specific activities in the usual way (Linnenbom, 1951).

Results are reported in Table I.

RESULTS AND DISCUSSION

The reproducibility of the isotope dilution method for the determination of benzo[a]pyrene in tobacco smoke was established. The standard deviation was seldom over 8% of the mean.

The Florisil column deactivated with 3% water provides an efficient method for the separation of aliphatic hydrocarbons (hexane eluent) from aromatic hydrocarbons (3:1 hexane–benzene eluent). A [¹⁴C]benzo[a]pyrene marker added to the original cyclohexane solution has been recovered with better than 99% yield from such a Florisil

column. Similar columns packed to a height of 20 cm and eluted with 250 mL of hexane–benzene also give near quantitative recoveries of benzo[a]pyrene.

Both pyrodegradation and pyrosynthesis appear to occur more extensively at 850 than at 650 °C. Thus the lower yield of neutrals (pyrodegradation) observed in the pyrolysis of valine and phenethylamine is accompanied by higher yields of benzo[a]pyrene (pyrosynthesis). In agreement with earlier studies (Fortina et al., 1966; Oro and Han, 1966), indicating that the optimum temperature range for the production of polynuclear aromatics from aliphatic compounds is between 800 and 850 °C, the 850 °C pyrolysis of valine resulted in the production of much higher yields of benzo[a]pyrene than did the 650 °C pyrolysis. Similar results were observed with the aralkylamine, phenethylamine.

In pyrolyses at 850 °C, there does not appear to be a direct relationship between the yields of neutrals and the production of benzo[a]pyrene. Thus, proline and N,N-dimethyldodecylamine gave low yields of neutrals and high yields of benzo[a]pyrene while tryptophan gave high yields of neutrals and low yields of benzo[a]pyrene.

The formation of benzo[a]pyrene in the pyrolysis of styrene (Badger and Buttery, 1958) suggests that styrene might be the precursor of the benzo[a]pyrene produced in the pyrolyses reported here. Although the yields of benzo[a]pyrene generally increase with increasing yields of styrene (Patterson et al., 1972, 1973, 1975, 1976), it is evident that styrene is not a necessary precursor to the formation of benzo[a]pyrene. Maleic hydrazide and proline give comparable yields of styrene but their benzo[a]pyrene yields vary by a factor of 2. Large quantities of styrene result from the pyrolysis of phenethylamine at 650 °C and little of the styrene undergoes pyrodegradation or pyrosynthesis at this temperature (Badger et al., 1965). At 850 °C, the yield of styrene is small and the yield of benzo[a]pyrene is less than that anticipated based on the amount of styrene produced at 650 °C. This lower styrene yield at 850 °C can be attributed to a change in the simple deamination pathway observed at 650 °C to paths favoring the generation of radical intermediates (such as benzyl, styryl, and phenyl) and their conversion to other stable products (Patterson, et al., 1973). Based upon the compounds pyrolyzed herein, the formation of benzo[a]pyrene appears to depend to a considerable extent upon the structure of the substance pyrolyzed and not upon the number of carbon atoms (or total atoms) present in the pyrolyzand. Species containing the same number of carbon atoms (valine, proline, methionine) produce wide variations in benzo[a]pyrene yields. Likewise, substances having essentially the same elemental composition (proline and valine) on pyrolysis generate differing amounts of benzo[a]pyrene.

A comparison of the gases obtained from the compounds pyrolyzed (Patterson et al., 1972, 1975, 1976) indicates that whereas all of the compounds in question produce ethylene and acetylene, only proline and N,N-dimethyldodecylamine produce butadiene. Proline and N,N-dimethyldodecylamine also yield larger amounts of benzo[a]pyrene than the rest of the compounds pyrolyzed. This points out the possible importance of C_4 units in the formation of benzo[a]pyrene. Although benzo[a]pyrene formation may come about or may be augmented via the participation of C_4 units, it is clear that the other compounds which do not yield C_4 units give rise to benzo[a]pyrene via different moieties such as C_2 fragments.

There is no evident reason for the decrease of benzo[a]pyrene production from the pyrolysis of N,N-di-

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methyldodecylamine in air and the reversal of this trend in the case of maleic hydrazide. However, this observation is in agreement with the previously reported effect that air had on the production of the mixture of isomeric benzopyrenes during the pyrolysis of *N,N*-dimethyldodecylamine and maleic hydrazide (Patterson et al., 1972).

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Quantitation of Hexane-Extractable Lipids in Serial Samples of Flue-Cured Tobaccos

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Solanesol, fatty acids, sterols, neophytadiene, and hydrocarbon waxes were quantitated in two flue-cured varieties of tobacco obtained at six intervals: (1) green, before harvest; (2) ripe, at harvest; (3) cured; (4) redried; (5) aged 1 year; and (6) aged 2 years. Solanesol and neophytadiene concentrations increased during processing, while fatty acid concentrations generally decreased and sterol and hydrocarbon wax content remained essentially unchanged in both varieties. Lipid concentrations also varied with crop year. PAH content of smoke condensate was related to concentration of lipids in the cigarette tobacco.

The hexane-extractable fraction of tobacco contains lipids that are important to leaf quality and are precursors of polynuclear aromatic hydrocarbons (PAH) in smoke (Chortyk and Schlotzhauer, 1973; Schmeltz and Hoffmann, 1973). Most quantitative studies of tobacco lipids have been limited to individual compounds or classes of compounds. Thus, fatty acids and their esters (Chu et al., 1972; Chu and Tso, 1968; Tso and Chu, 1970; Hoffmann and Woziwodzki, 1968; Ellington et al., 1977), sterols and steryl esters (Ellington et al., 1977; Grunwald, 1975; Schmeltz et al., 1975; Davis et al., 1970; Cheng et al., 1971; Grunwald, 1970), neophytadiene (Ellington et al., 1977; Bilinsky and Stedman, 1962; Chortyk et al., 1975), hydrocarbon waxes (Ellington et al., 1977; Chortyk et al., 1975; Stedman and Rusaniwskyi, 1959), and solanesol (a C_{45} unsaturated alcohol) (Irvine et al., 1972; Severson et al., 1977), which constitute the major tobacco lipids, have been quantitated by various research groups. In most instances only one or two of these components have been analyzed in the same tobacco sample (Chu et al., 1972; Cheng et al., 1971).

Pyrolysis of specific components of the hexane extract of tobacco by Schlotzhauer and Schmeltz (1969) indicated

Table I. Total and Free Solanesol and Sterols

| | % dry leaf | | | |
| | Total[a] | | Free[b] | |
	NC95	PY	NC95	PY
	Solanesol			
G	0.986 ± 0.04	0.88	0.91	0.41
R	1.73 ± 0.06	0.93	1.57	0.81
C	2.60	1.45	1.99	1.02
RD	2.59	1.34	1.86	1.13
1 yr	2.07	1.52	2.00	1.34
2 yr	2.29	1.74	1.65	1.15
	Sterols[c]			
G	0.135 ± 0.006	0.17	0.09	0.08
R	0.161 ± 0.002	0.19	0.11	0.08
C	0.17	0.15	0.13	0.07
RD	0.17	0.17	0.10	0.10
1 yr	0.16	0.16	0.09	0.09
2 yr	0.16	0.24	0.08	0.09

[a] Standard deviations are given where three replicate samples were analyzed; other values represent averages of two analyses. [b] Analyses were performed on single samples only. [c] Cholesterol, campesterol, stigmasterol, and sitosterol.

that straight-chain aliphatic compounds, such as hexane, stearic acid, and dotriacontane, yielded significant quantities of aromatic products with alkyl side chains.

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