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Carcinogenic Polycyclic Aromatic Hydrocarbons in some Nigerian Foods

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A study of malignant diseases in the city of Ibadan (Nigeria) between 1960 and 1963 showed a high incidence of carcinoma of the liver, stomach, cervix and breast (EDINGTON and MCLEAN 1965). Subsequent surveys of the incidence of cancer of the alimentary tract also showed a high frequency of cancer of the stomach as compared with cancers of the tongue and oesophagus (EDINGTON and EASMON 1966, ODEBIYI 1972). It is believed that within this community malignancies of the stomach rank next to that of the liver. Little or no attention has so far been paid to environmental agents probably contributing to these malignancies within the population. Work done in other countries has shown that polycyclic aromatic hydrocarbons present in foods, especially smoked foods, could play a leading role in the initiation of carcinoma of the alimentary canal (BAILY and DUNGAL 1958, WOSTELOWICZ et al. 1957 and KAUFMAN et al. 1959).

The search for environmental contaminants responsible for the high incidence of cancer in Nigeria has, however, inadvertently focussed attention mainly on mycotoxins (BABABUNMI et al. 1977). There is no information on the presence of carcinogenic aromatic hydrocarbons in foods processed locally and their role in the initiation of cancer in this tropical region with very high cancer frequency (IARC 1971). We have, therefore, been motivated by this fact to investigate the extent of polycyclic aromatic hydrocarbon contamination of smoked fish and smoked meat in Nigeria, with a view to assessing their contribution to the high cancer frequency.

MATERIALS AND METHOD

Fish and meat samples used in this study were bought as displayed in the local (Ibadan) market and preserved in sealed polythene bags to avoid any extraneous contamination until the samples were analysed. Solvents used were of analytical grade and were further purified by redistillation (COMMINS et al. 1954).

Weighed samples of the fish were extracted with acetone for 8 hr with a soxhlet extractor. The acetone extracts from each species were pooled together and

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taken to dryness with a rotary film evaporator. The concentrate was taken up with either hexane or cyclohexane and re-extracted with dimethylsulphoxide (DMSO). The DMSO was then repartitioned between benzene and water (method of LIJINSKY and SHUBIK 1965). The acetone extracts from the meat samples were saponified with 5% alcoholic sodium hydroxide and treated according to the method of HOWARD et al. (1966). The resultant oily residue was first separated on a column of alumina, developed with cyclohexane and benzene and the fractions monitored by ultraviolet illumination. Each fraction was then run on thin layer chromatoplate of silica gel G using cyclohexane-pyridine (30:1 v/v) as solvent system (HOOD and WINEFORDNER 1968). 1.2 benzanthracene, 3.4 benzo(a)pyrene, 20 methylcholanthrene and 7.12 dimethylbenz(a)anthracene (Sigma. Chem. Co., St. Louis, Mo., U.S.A.) standards were run along with the unknown as markers. Fluorescent spots were eluted with a benzene-ethanol (3:1 v/v) mixture (LIJINSKY et al. 1963). Each extract was taken to dryness and redissolved in ethanol-cyclohexane mixture and re-run again on tlc. The ultraviolet spectra of the extracts were obtained with a Perkin-Elmer 137 UV-VIS spectrophotometer in ethanol. The corresponding fluorescence spectra were also obtained with a Perkin-Elmer MPF-3L fluorescence spectrophotometer. For quantitative estimations the spectrophotometric method of COMMINS (1958) was utilized in all cases. For recovery experiments 50 µg of the reference compounds in hexane were injected into 1 kg of unsmoked samples of the fish and meat, extracted and estimated as above.

RESULTS AND DISCUSSION

Results obtained in this study indicate the presence of three main polycyclic aromatic hydrocarbons (PAH) in the fish species studied (Table 1) while four components were present in the meat samples as identified by their Rf values on thin layer chromatography and their fluorescence. Comparison of the Rf values, fluorescence and ultraviolet spectra (Table 1) of the fractions with those of the standards showed that 1:2 benzanthracene and 3.4 benzo(a) pyrene were the two main carcinogenic compounds present and representing fractions I and II, respectively. 7.12 dimethylbenzanthracene and 20 methylcholanthrene were absent in all cases.

Figure 1 is an illustration of the fluorescence spectral characteristics of both the reference 3.4 benzo(a)pyrene and a corresponding isolate from the specimens under study.

TABLE 1: Rf Values[1] Fluorescence[2] and Ultraviolet[2] Spectral Characteristics of Isolated Samples and some reference Carcinogens

Compounds	Rf	λ_{ex} (nm)	λ_{em} (nm)	U.V Peaks
1.2 benzanthracene	0.63	340	390	258, 284, 296 363, 382
3.4 benzo(a)pyrene	0.53	380	410	267, 286, 298 348, 367, 386
20-methylcholanthrene	0.59	360	400	223, 261, 285 295, 341, 358,378
7.12 dimethylbenz-anthracene	0.64	300	410	265, 287, 298 365, 383
Fractions				
I	0.63	340	390	257, 284, 296 363, 382
II	0.53	380	410	267, 286, 298 348, 367, 386
III	0.31	300	390	218, 249, 256 261, 360, 371
IV[3]	0.11	340	440	215, 256, 269

I. Rf values were obtained on silica gel G coated t.l.c. plates with cyclohexane-pyridine 30:1 v/v as solvent.

2. Both fluorescence and U.V spectral analysis were obtained in ethanol (SCHOENTAL and SCOTT 1949).

3. This fraction was only present in the meat samples but absent in all the fish samples.

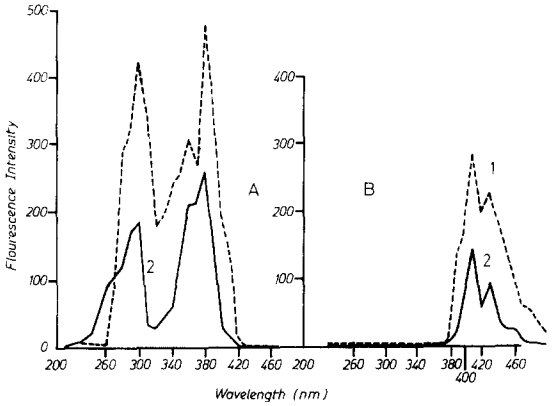

Fig. 1. Fluorescence spectra of benzo(a)pyrene: 1. Compound isolated from smoked meat (Suya) (----------). 2. Reference compound (——————). A. Excitation spectra at emission 410 nm and B. Emission spectra at excitation 390 nm.

The fluorescence characteristics of the fraction with Rf 0.11 were similar to that of Coronene reported by HOOD and WINEFORDNER (1968) though their measurements were at low temperature.

1.2 benzanthracene, a weak carcinogen, is present in all the species of fish and the smoked meat studied (Table 2).

TABLE 2: Concentration of 1.2 benzanthracene and 3.4 benzo(a)pyrene in fish and meat samples

Samples	1.2 benzanthracene (µg/kg)	3.4 benzo(a) pyrene (µg/kg)
Silver catfish (Chrysichthys nigrogitatus)	2.5 ± 0.5	Traces
Catfish (Clarias dahomeyensis)	3.5 ± 0.2	3.5 ± 0.5
African mudfish (Clarias lazera)	4.0 ± 0.5	4.5 ± 1.0
Moonfish (Citharinus spp.)	7.5 ± 0.5	2.1 ± 1.0
Snakehead (Ophiocephalus obscurus)	1.6 ± 0.5	Traces
Tilapia (Tilapia nilotica)	3.5 ± 0.5	3.8 ± 1.5
Recovery	68%	61%
Smoked meat (Suya)	15.5 ± 2.5	8.5 ± 2.0
Recovery	60%	58%

Lowest values were obtained in the silver catfish (Chrysichthys nigrogitatus) and the snakehead (Ophiocephalus obscurus) with 2.5 µg/kg and 1.6 µg/kg, respectively, while the moonfish (Citharinus spp.) had the highest concentration (7.5 µg/kg). The snakehead fish and the catfish had only traces of 3.4 benzo(a)pyrene. The remaining species contained between 2.1 to 4.5 µg/kg of 3.4 benzo(a)pyrene. The smoked meat (suya), however, contained a relatively high concentration of both 1.2 benzanthracene and 3.4 benzo(a) pyrene (15.5 µg/kg and 8.5 µg/kg), respectively. The high level of these carcinogens in the meat samples might be due to the fatty nature of the original meat from which 'suya' is usually prepared as this will result in greater pyrolysis and subsequent formation of the polyaromatic hydrocarbons under high temperature (LIJINSKY and SHUBIK 1964). Similarly, the fish samples are known to contain between 12% and 16% lipids (OSUJI 1974).

The presence of these carcinogens at the level shown (Table 2), therefore, might be a contributory factor to the reported high stomach cancer within this community (EDINGTON and EASMON 1966). In a similar study, BAILY and DUNGAL (1958), THORSTEINSSON and THORDANSON (1968) and THORSTEINSSON (1969), have shown the presence of polycyclic aromatic hydrocarbons at comparable levels in Icelandic smoked fish and singed meat and also associated this with the prevalence of stomach cancer in Iceland.

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