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Occurrence of Nitrosatable Amines in Some Nigerian Medicinal Plants

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The prevalence and incidence rate of certain cancers have been found to be highest in the tropical countries (Osunkoya, 1980). Liver cell carcinoma is another malignant tumor with high incidence in the tropics, and highest prevalence rate among males in tropical Africa (Edington 1978). The incidence of breast cancer which are common in Nigeria in particular and Africa at large have been associated with dietary factors such as drinks and food toxins (Quisenberry, 1960).

The presence of secondary amines; di-methylamine and diethylamine, methylamine, nitroso-methyl-n-propylamine have been reported in plants (Bush 1970). Levels found in tobacco samples range between 2 - 100 ppm. The occurrence, metabolism and functions of plant amines have been reviewed by Smith (1971). Nitrate is a normal constituent of human saliva, its concentration in that body compartment is directly related to the nitrate intake via food. In the mouth it is partially reduced by microflora present to nitrate, which is swallowed and can serve as nitrosating agent for secondary amines in the stomach.

The secondary amines have the imino-group which is very reactive and easily undergoes "nitration" to form the nitrosamines or nitroso compounds which have been shown to be toxic and carcinogenic (IARC 1976). Neurath (1968) reported that the high incidence of oesophageal cancer could be associated with the consumption of crude beers and other liquors in Zambia due to secondary amines, in addition to nitrosamines.

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Secondary amines, nitrates and nitrites are precursors in the formation of nitrosamines, and potentials exist for the nitrosation to occur in nature. Nitrates are normal constituents of plants and forage, and since secondary amines and nitrates occur frequently in plant foods and plant products, the nitrate content of these could therefore become a critical factor in the elaboration of nitrosamines in situ (Sander et al 1969).

The consumption of water or alcohol (illicitgin) extracts of medicinal plants roots (herbs) by most of the Ibadan populace as local remedies is suspected of causing illness and deaths related to cancer. This possibility was investigated analysing for nitrosatable amines in three medicinal plant roots believed to have antimalaria activity and frequently used for such purposes by the populace. The medicinal plants were Pleioceras barteri, Alostonia boonei and Morinda lucida.

MATERIALS AND METHODS

The medicinal plant roots were bought in the market, and were identified by the department of Forestry of the University of Ibadan, Nigeria. The plant roots were cleaned to remove soil particles, and then stored in plastic bags to avoid contamination.

The method of Singer and Lijinsky (1976) - the classical Hinsberg procedure for isolation of amines was employed in the isolation of the secondary amines, Approximately 50 gm of the plant root materials cut into small pieces were stuffed into a round bottomed flask, 200 ml of distilled water and 100 ml of 6N HCl were added. The mixture was left to stand for about 8 hr with agitation to extract plant amines. The mixture was then adjusted to pH 10 with 20% NaOH. To control foaming, 50 ml saturated $Ba(OH)_2$ was added. The mixture was steam distilled into a flask containing 5 ml 2N HCl. To remove steam volatile neutral compounds, 10 ml of ether solutions was added to the extract and kept over-night, while the resulting aqueous solution was concentrated on a rotary evaporator (bath temperature 55°C). The amines in the concentrate were derivatised by boiling the concentrate with 0.60 gm p-toluene-sulphonyl chloride and 20 ml of 20% NaOH under reflux and crushed ice (3 parts) which provided a temperature of -40°C to -50°C. After refluxing for 20 min the mixture was acidified to pH 1 with 6N HCl, and extraction carried out continuously for 1 hr by shaking with ether. The mixture was then separated using a separating funnel, and the ether solution containing the primary and secondary p-toluene-sulphonamides was evaporated under nitrogen steam (gas). The resulting crystals were then boiled under reflux for 20 min with 20% KOH (10 ml) and continuous extraction for

1 hr with ether, removed the secondary amine p-toluene-sulphonamides. The ether solution was then evaporated under nitrogen steam (gas) until no more solvent odour was perceived. The p-toluene-sulphonamide crystals were then taken up in 5 ml ethyl alcohol.

The pooled samples of the secondary amines p-toluenesul-phonamides of the different medicinal plant roots were analysed colorimetrically using the methods of Dowden (1938) and Pribly and Nedbalkova (1967) and by thin layer chromatography.

The method of Vogel (1957) was used for the chemical synthesis of nitrosamines from plant roots as an invitro nitrosation analysis. 100 gm of the plant root material were treated with 50 ml of distilled water in a 2N solution of H_2SO_4 added dropwise. 80 gm sodium nitrite solution in a minimum of hot distilled water was gradually introduced into the flask. The resulting mixture was refluxed for about 4 hr to complete reaction and concentrate the mixture. The mixture was then distilled to dryness and the distillate was redistilled for four times and then finally redistilled with 6 ml of 2N H_2SO_4 to remove any amine base that might have passed over with nitrosamines. The distillate was then extracted in two 250 ml portions in separating funnels with 25 gm anhydrous potassium carbonate in 250 ml dichloromethane. The oily layer was collected and filtered. TLC and UV analysis of the oily liquid confirmed the presence of nitroso compounds.

The reliability of the procedure for extraction and quantification of the amines was tested by carrying out a recovery experiment on a standard amine, dimethy-lamine. 1 ml each, of the standard solution with concentration of 7 g-amine and 14 g-amine were added into 50 ml of distilled water respectively. Each content was then subjected to the same procedure of extraction and analysis of amine content as the test material (plant roots). The percentage recovery for both concentrations were calculated reference to the theoretical. 1 ml contains 8.155 gm DMA-HCL(14 g-N/ml).

RESULTS AND DISCUSSION

The dry weights of 50 gm wet weight of the medicinal root samples were determined and hence their moisture content, (Table 1). Recovery experiments were carried out with standard dimethylamine samples to test the reliability of the procedure employed. This was necessitated by the fact that most amines are volatile. The results show that the medicinal plant roots P barteri, A boonei, and M lucida have a concentration of 0.85, 0.91, and 0.90 mg alkylamine - N/kg dry root material respectively according to the method of Dowden (1938), while the method of Pribly and Nedbalkova

(1967) showed that the plant roots contain 0.80, 0.69 and 0.84 mg alkylamine - N/kg dry root material respectively under the experimental conditions, table 2a and 2b.

Table 1. Dry weight and moisture content

Root sample	Wet weight (gm)	Dry weight (mg)	Moisture content (%)
Pleioceras barteri	50	17.6	64.8
Alostonia boonei	50	13.2	73.6
Morinda lucida	50	20.2	59.6

*Average of three determinations.

Table 2(a) Secondary amine content of roots extract - Dowden (1938) method

Root sample	Vol. of extract (ml)	Vol. of derivative (ml)	Absorbance* of derivative	Amine concentration	
				g-N/kg root extract	mg-N/kg dry root
Pleiocetas barteri	356	5.0	0.40	3.0	0.85
Alostonia boonei	410	5.0	0.30	2.4	0.91
Morinda lucida	342	5.0	0.45	3.6	0.91

*Average of three determinations.

Table 2(b) Secondary amine content of plant roots extract - Pribly and Nedbalkova (1967) method

Root sample	Vol. of extract (ml)	Vol. of derivative (ml)	Optical* density of derivative	Amine concentration*	
				mg-N/kg root extract	Mg-N/kg dry root
Pleioceras barteri	356	5.0	0.16	2.8	0.80
Alostonia boonei	410	5.0	0.13	1.8	0.69
Morinda lucida	342	5.0	0.18	3.4	0.84

*Average of three determinations.

Table 3. Recovery study on dimethylamine standard solution treated as a test material

Initial DMA	Absorbance*	DMA reconvered (g/N)*	(%) recovery
7	0.9	5.4	77.1
14	1.81	10.6	75.1

Table 4. The Analysis of the plant extracts with 2,4-dinitro fluorobenzamide derivative of standard amines

Sample	Rf value*
Diethylamine	0.67
Dimethylamine	0.60
Morpholine	0.57
Piperidine	0.55
n-propylamine	0.34
Ethylanilamine	0.62
Pleiocceras barteri	0.58
Alostonia boonei	0.67
Morinda lucida	0.62

*Average of three determinations.

The Rf values on table 4, shows that the plant roots may contain dimethylamine, diethylamine, Morpholine, Piperidine and ethylaniline. The results appear to be in agreement with previous work, except for the fact that the presence of ethylaniline in plant materials has not been reported. Golovonya, (1975) reported the occurrence of dimethylamine, diethylamine, morpholine and piperidine in plant materials, while Smith (1971) also mentioned the occurrence of these amines among other amines.

Table 5. In-vitro-n-nitrosation of root extractives of Pleioceras bartel, Alostonia boonei and Morinda lucida

Root sample	Vol. of Acid 2N H_2SO_4 (ml)	Nitrate concentration (gm/L)	TLC detection of nitrosamine in root extract concentrates		
			P barteri	A boonei	M lucida
20	10	10	-	-	-
40	20	20	-	-	-
60	30	30	-	-	-
80	40	40	-	-	-
100	50	80	+	+	+

- not detected
+ detected

The invitro n-nitrosation of the root extractives showed that increase in concentration of root materials, acidity and sodium nitrite enhanced nitrosamine formation. in the lower concentration, however, it is most likely that nitrosamines were also formed, but in trace quantities, hence they were not detectable as shown on table 5. In this study, 100 gm wet weight of root sample, 50 ml 2N H_2SO_4 and 80 mg/L weight of sodium nitrite generated clearly detectable amount of nitrosamine. 100 gm wet weight of root sample is approximately 35.2 gm dry weight of sample, and could contain 1.6 gm alkylamine - N/kg quantity of secondary amine. These values when compared with values from similar investigations appear insignificant. Telling et al (1975) generated detectable nitrosamine in the rat stomach using 2000 gm/kg of amine, which is by far greater than the value of amine involved in this study. Telling and his colleagues also detected nitrosamines in the rat stomach when they applied 100gm/L and 100gm/L sodium nitrite to 200 gm/kg and 1000 mg/kg secondary alkylamines respectively. These nitrate values are far more in excess of the quanity used for the invitro N-nitrosation experiment; hence the N-nitrosation of secondary amines depends largely on the nitrate concentration and also favourable in acid. The H_2SO_4 used in this study would generate sufficient acidity comparable to pH 3.4 at which reaction rates of nitrosation show maximum value (Mirvish, 1970). The pH of gastric juice is usually in the neighbourhood of 2, and therefore the implication of this on N-nitrosation in the stomach is obvious. Hence, the amount of nitroso compounds produced in the stomach-will depend partly on the nitrosation kinetics.

The continuous ingestion of these concoctions is likely to result in the possible accumulation of secondary alkylamines with time; levels which could generate carcinogenic nitrosamines in vivo. Consequently, the build up of secondary amines in the body must be responsible in part, for liver cancer one of the most common types of cancer in Africa.

REFERENCES

Bush LP (1970) Principal aliphatic secondary amines of barley tobacco. Beitr Tabak Forschung 5: 276 - 278.

Dowden HO (1938) The determination of small amounts of dimethylamine in biological fluids. Biochem J 32: 455 - 459.

Edinton GM (1978) The pattern of cancer in the Northern savanah of Nigeria with special reference to primary liver cell carcinoma and the Burkitt lumphoma Nig Med J 8: 281 - 289.
Golovonya RV (1975) Analysis of volatile amines contained in food stuffs as a possible precursors of N-nitroso compounds. In: IARC Sci Publ No 14: 247 - 254.
IARC (1976) In: Environmental N-nitroso compounds: Analysis and formation. IARC Sci Publ No 14: 136 - 141.
Mirvish S (1970) Kinetics of dimethylamine nitrosation in relation to nitrosamine carcinogenesis J Natl cancer Inst 44: 633 - 639.
Neurath G and Luttich W (1968) Gas chromatographic separations of 4-nitroazobenzen-4-carbonamides of primary and secondary amines. J Chromatog 37: 205 - 212.
Osunkoya BO (1980) Cancer in the Tropics. In: Smith RL and Bababunmi EA (ed) Toxicology in the tropics p4 - 10.
Pribly M and Nedbalkova I (1967) spectrophotometric determinations of dimethylamine and dimethyl-formamide. Z Anal Chem 233. 261 - 267.
Quisenberry WB (1960) Socio-cultural factors in cancer in Hawaii, Ann NY Acad Sci Article 17 p 1795.
Sander J and Burkle G (1969) Induction of Maligant tumors in rats by simultaneous feeding of nitrite and secondary amine, Z Kresbsforsch 73: 54 - 56.
Singer MS and Lijinsky W (1976) Naturally occurring nitrosatable compounds I Secondary amines in foodstuff J Agric Food Chem 24 (3): 550 - 55.
Smith TA (1971) The occurrence, metabolism and functions of amines in plants. Biol Rev 46: 201 - 241.
Telling GM, Hoar DO, Caswell D and Collins AJ (1975) In: Environmental N-nitroso compounds: Analysis and formation, IARC Sci Publ No 14: 247 - 254.
Vogel AI (1975) Textbook of practical organic chemistry 3rd Ed. ELBS.