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IDENTIFICATION OF 24-ETHYLCHOLESTANES AS MOLECULAR INDICATORS OF VASCULAR PLANTS SOURCE INPUTS IN COASTAL SEDIMENTS FROM NIGER DELTA, NIGERIA

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ABSTRACT

The identification of ααα-24-ethylcholestane, 2α-methyl-24-ethylcholestane and 3β-Methyl-5α- stigmastane in extracts of sediments from the coastal areas of the Niger Delta of Nigeria was achieved by gas chromatography-mass spectrometry (GC-MS) technique. The concentrations of the 24-ethylcholestanes varied between 0.05 and 1.40 mgkg-1 in the entire study area. The distributions of the 24-ethylcholestanes, which are known to be diagenetic products of plant sterols (phytosterols) in our samples, suggest possible source inputs from vascular plants around the coastal vegetation of the Niger Delta environment.

Keywords: 24-ethylcholestanes, steranes, phytosterols, source inputs.

INTRODUCTION

The main goal of many geochemical studies is to determine the sources and relative proportions of organic species in the samples being analyzed [1-7]. Sediments provide a dynamic and long-term reservoir for organic species. These organic species include lipids (solvent-soluble organic matter), such as hydrocarbons, fatty acids, alcohols and macromolecular organic matter. All these are derived from natural biogenic, geologic and industrial sources [8-13]. Lipid classes have been used as molecular tracers to understand the origin and reactivity of organic matter in marine ecosystem.

Steranes are diagenetic products of steroids. The steroids form a group of structurally related compounds which occur ubiquitously in eukaryotic organisms (ranging from microorganisms to macroalgae) and vascular plants. Sterols (from which "steroids" is derived) occur in animal and plant oils and fats. The sterols that are obtained from animal sources are often collectively referred to as zoosterols, and those obtained from plant sources as phytosterols. The sterols in plants (phytosterols) often carry extra alkyl groups at C-24 which are derived from methionine [14]. Thus, steroids and their environmental and geological derivatives are an important group of marker compounds providing valuable information about sources of organic matter in soils, recent sediments and ancient sedimentary rocks, as well as crude oils [15 -22]. Diagenesis of organic matter occurring in water and sediments modifies the structure of precursor steroids in complex way. Examples of these processes include oxidation of sterol to steranes [20], dehydration of hydroxyl group (of sterols) leading to the formation of steradienes and so on.

In tropical African regions such as Nigeria, molecular indicator compounds in sediments and other environmental samples are rarely studied. For this purpose, this paper reports the occurrence of steroid derivatives which are important marker compounds providing valuable information about sources of organic matter in recent sediments from the Niger Delta region of Nigeria. This is the first systematic study carried out to identify the sources and relative proportions of sterols as molecular marker compounds in recent sediments from our study area.

MATERIALS AND METHODS

Study area

The fan-shaped Niger Delta, which is the third largest in the world after the Mississippi (USA) and Pantanal (South-West Brazil), lies between latitudes (4 and 6)^0 north of the equator and longitudes (5 and 9)^0 east of the Greenwich Meridian. The North-South extension, north of the equator, is expressly defined by the Great Atlantic Ocean in the South to Aboh (Delta State) in the North where River Niger forks in Rivers Nun and Forcados at a village called Obotor [23]. The East-West extension is from the boundary of the Bonny River to River Sapele, Delta State. The geographical Niger Delta is just about 25, 640km^2 in size; made up of 7, 400km^2 low land area, 11, 700km^2 fresh water swamp, and 1, 140km^2 salt barrier islands as ecological zones [23].

The Niger Delta has been simply subdivided into fresh water zone, the mangrove and the coastal sand ridges [24]. The Northern part of the fresh water zone is often regarded as an extension of the lower Niger flood plain [25], and it is very susceptible to the annual floods during the rainy season. The southern part of the fresh water zone and most of the mangrove are swampy and hardly rise above 10m above mean sea level. This sub-zone covers a greater percentage of the Niger Delta. The strip of sand ridges and beaches lies close to the open sea and is bordered landwards by swamp areas with many creeks [23]. The Niger Delta region displays a typical dendritic

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drainage pattern. This is because most of the tributaries join their main rivers at oblique angles [23]. For example, Taylor Creek joins the main River Nun at Polaku, and the Epie Creek joins the River Nun/Ekole at Yenagoa. This also implies that there are many confluence towns in the Niger Delta. There is also a near parallel type of drainage, for example, New Calabar/Bonny River, Brass/Nun River, Forcados/Escravos River, Taylor Creek/Epie Creek and Orashi River.

The vegetation of the Niger Delta coastal areas is characterized by extensive freshwater and mangrove swamps. The fresh water swamps consist of stilt rooted trees and shrubs. The main vegetation of the mangrove swamps of the Niger Delta is dominated by the red mangrove which forms more than nineteen percent (19%) of the saline swamps [26]. The white mangroves occur scattered among the red mangroves and thrive in less water- logged places. Ferns, Nipa palms and herbs are found in areas where their salt content is not too high. The Niger Delta has provided the best conditions for the thriving of vegetation on the Nigerian coast.

By some estimates, over sixty percent (60%) of fishes caught between the Gulf of Guinea and Angola breed in the mangrove belt of the Niger Delta. The vegetation of the Niger Delta region is arranged in the form of storey/strata or layers [23]. There is the upper stratum occupied by very tall trees of heights 30m and above; followed by the middle tier trees with heights of 15-20m, while the lower layer has trees and shrubs with heights below 12m. The upper layer is normally occupied by tall trees like the Iroko (*Chlorophora excelsa*), Mahogany (*Khaya ivorensis*), and Abura (*Mitragyra macrophylla*) [27]. Palm trees (*Elaeis guineensis*) and others occupy the middle layer. The availability of sunlight is also partly responsible for the growth of the tall plants as it provides them with the necessary solar energy for the photosynthetic process.

Sampling

Samples of surface sediments were collected at different stations each along the coastal areas of Warri, Bonny,Qua Iboe and Imo rivers as depicted in the map of the study area (Figure-1) using a modified grab sampler ($0.1m^2$). Samples were removed from the middle of the grab to avoid contact with the inner metallic surface of the grab sampler, wrapped in aluminium foil and stored frozen at - 4 ^{0}C. The sampling was carried out based on the nature of potential anthropogenic inputs (Table-1). Prior to extraction, the samples were freeze-dried, crushed and sieved through a 230 mesh (< 63µm) sieve.

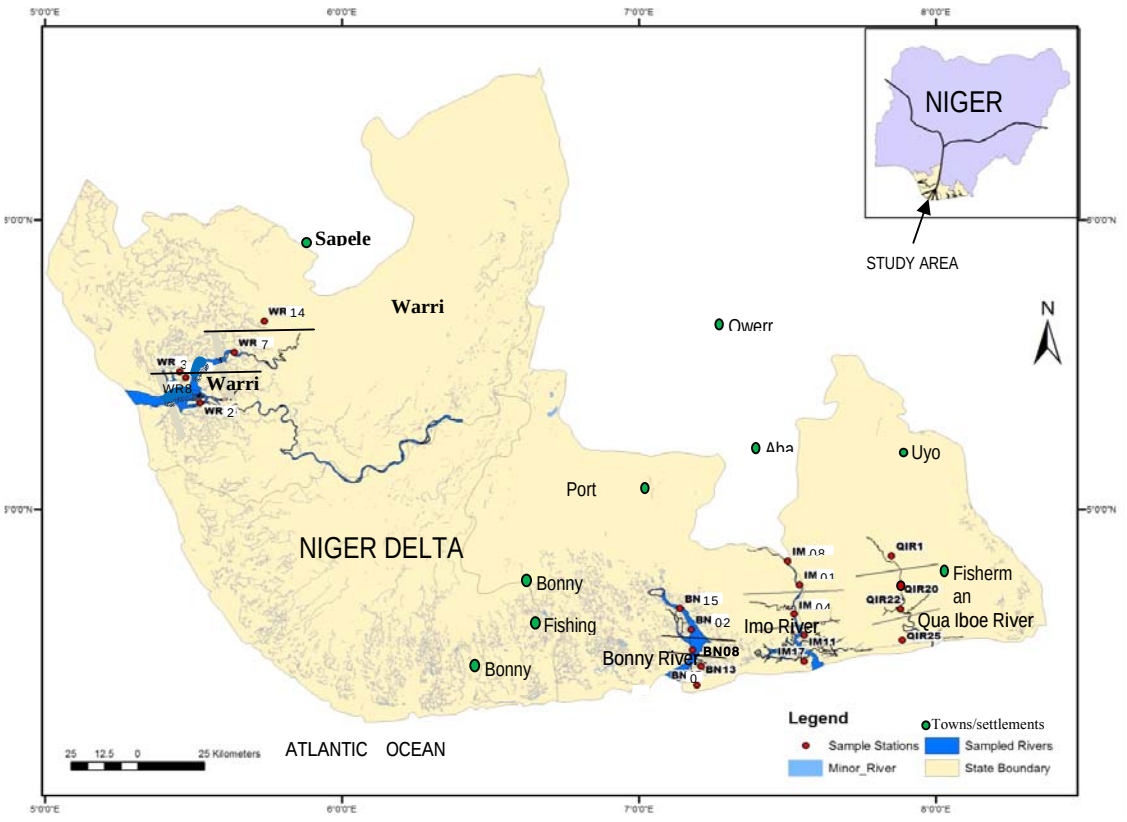

Figure-1. Map of study area showing sample stations.

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Extraction and fractionation

To minimize contamination, all glassware was cleaned with detergent solution, rinsed with distilled water, heated in an oven at 550^{0}C for eight hours to combust traces of surface organic matter, and finally rinsed with ANALAR grade dichloromethane. The total organic carbon (TOC) contents were determined using a LECO CNS analyzer. Extraction of the crushed and sieved (< 63μm) sediment samples for extractable organic matter (EOM) was carried out using a soxhlet apparatus [28]. The thimbles and the glass wool used in the extraction were soxhlet-extracted with dichloromethane for 20 minutes on a water bath. Powdered sediment sample (50g) was then placed in the extracted thimble. The thimble with glass wool was filled with dichloromethane and extracted for 18hrs. Extracts were desulphurized by addition of 30g activated copper (copper immersed in 20ml of 0.1M concentrated hydrochloric acid for ten minutes) into the round-bottom flask during extraction. Extracts obtained were evaporated to near dryness using a vacuum evaporator. The weight of extracts was determined as a measure of the amount of extractable organic matter (EOM), made up of asphaltenes and maltenes. Precipitation of asphaltenes from the extractable organic matter (EOM) was carried out using a mixture (1:30) of dichloromethane /petroleum ether (b.p. 40-60^{0}C), and centrifuged at 3, 000 rpm for about 20min [29]. The asphaltenes precipitated from EOM were discarded after weighing. The separation of maltenes obtained from the extracts into aliphatic, aromatic and hetero-fractions was carried out by column chromatography (column 30 x1.2cm) using activated silica gel (20g activated by heating in an oven for two hours at 400^{0}C) and alumina (neutral, 10g activated by heating in an oven for two hours at 500^{0}C) on top of the silica gel. The concentrated extract (2ml) was carefully added to the top of the column already clamped to position. The saturated (aliphatic) fraction was eluted with 50ml hexane, while 200ml of 1:1 dichloromethane/hexane mixture was used for the elution of the aromatic fraction [30]. Finally, a mixture (1: 2, 60ml) of methanol/ dichloromethane was used to remove the heterofractions.

Gas chromatography-mass spectrometry (GC-MS) analysis

The gas chromatography-mass spectrometry (GC-MS) analyses of the aliphatic and aromatic fractions from the sediment extracts were performed using an Agilent 6890 Series gas chromatograph (GC) interfaced to an Agilent 5973 Network Mass Selective Detector (MSD) and Agilent 7683 Series Injector. The GC Separation was achieved on a fused silica capillary column coated with DB 35 (60m x 0.25mm i.d., 0.25μm film thickness). The GC oven temperature was programmed from 50^{0}C (isothermal for 1 minute) to 120^{0}C at a rate of 20^{0}C/ min, then to 300^{0}C at a rate of 3^{0}C/ min. The final temperature was held for 45 minutes. Helium was used as the carrier gas. Samples were introduced into the cool on-column injector under electronic pressure control. The GC column outlet was connected directly to the ion source of the mass spectrometer. The GC-MS interface was kept at 280 ^{0}C, while the ion source and quadrupole analyzer were at 230 and 150^{0}C, respectively. The mass spectrometer was operated in the electron impact (EI) mode at 70eV ionization energy. Mass spectra were recorded from 45-550 Da (0 - 40min) and 50 - 700 Da (above 40 min). Data were acquired using the HP-MSD Chemstation Integrator. Individual compounds were identified by comparison of mass spectra with literature and library data.

Table-1. Concentrations of steranes in coastal sediments from Imo (IM), Qua Iboe (QIR), Warri (WR) and Bonny (BN) rivers.

Station	TOC (%)	Sterane concentrations (mg/kg)		
		24eSter	2aMSter	3bSter
IM01	3.18	nd	nd	nd
IM08	2.76	nd	0.36	0.46
IM04	4.26	nd	nd	nd
IM17	1.76	0.34	0.39	0.39
IM11	1.96	0.45	0.72	0.56
Total		0.79	1.47	1.41
QIR1	3.36	0.28	1.23	1.73
QIR22	3.12	nd	nd	nd
QIR20	3.48	nd	nd	nd
QIR25	0.44	nd	nd	nd
Total		0.28	1.23	1.73
WR8	2.71	0.05	0.05	0.04
WR2	2.37	0.59	0.44	0.35
WR7	3.27	1.40	0.67	0.68
WR3	2.98	0.86	0.31	0.27
WR14	1.26	nd	nd	nd
Total		2.9	1.48	1.34
BN02	1.45	nd	nd	nd
BN05	1.67	nd	nd	nd
BN08	1.43	nd	nd	nd
BN13	0.87	nd	nd	nd
BN15	0.97	nd	nd	nd

24eSter = ααα-24-ethylcholestane; 2aMSter = 2α-Methyl-24-ethylcholestane;
3bSter = 3β-Methyl-5α-stigmastane; nd = not detected

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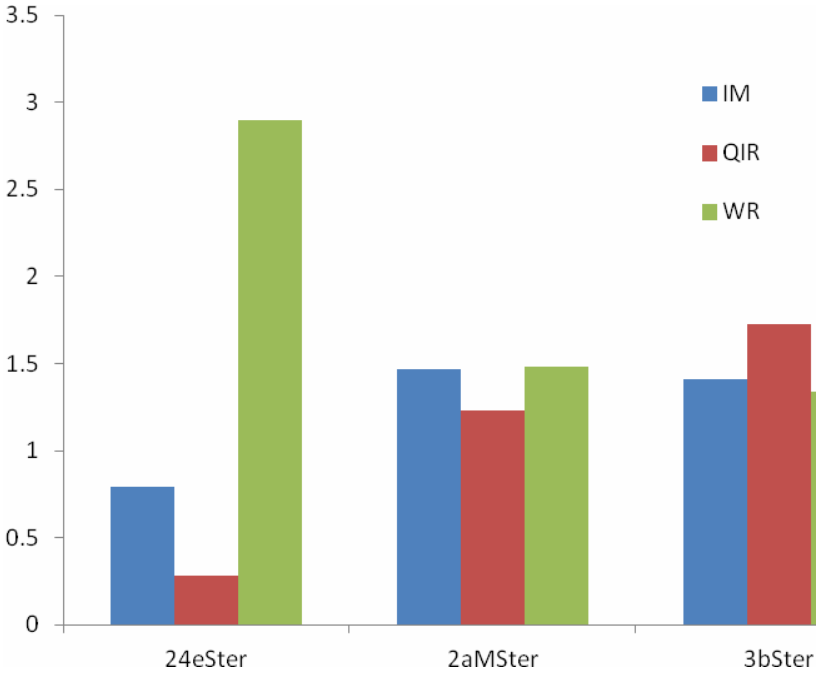
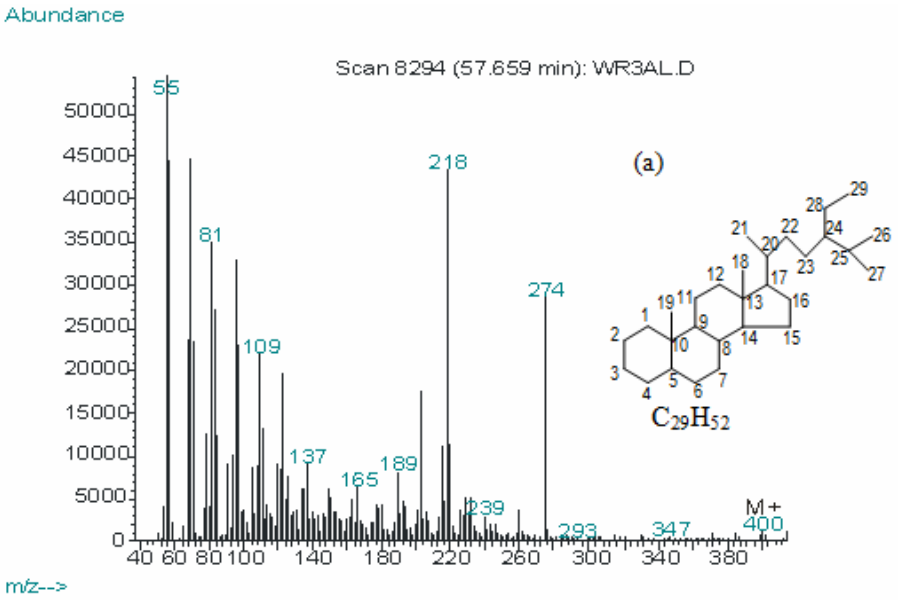


Figure-2. Variations in the total concentrations of steranes in coastal sediments from Imo (IM), Qua Iboe (QIR) and Warri (WR) rivers.



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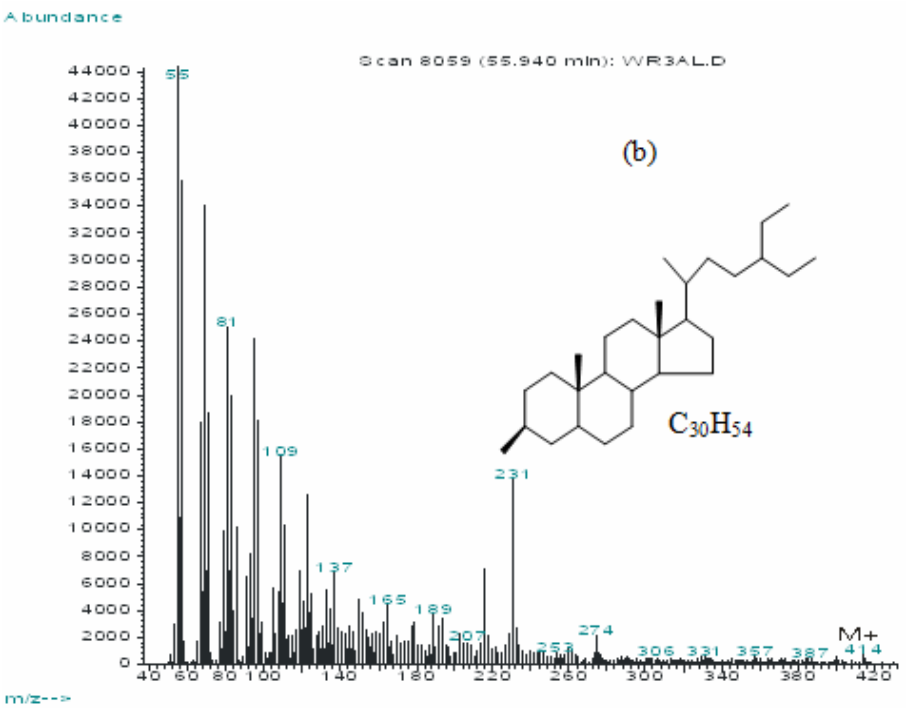

Figure-3. Representative mass spectra of (a) ααα-24-ethylcholestane and (b) 3β-Methyl-α-stigmastane in aliphatic fraction of station WR3 from Warri River.

RESULTS AND DISCUSSIONS

The sampling stations, percentage total organic carbon (% TOC) contents, concentrations of biomarker steranes are presented in Table-1. Variations in the total concentrations of biomarker steranes detected in the Niger Delta study area are given in Figure-2. Significant differences ($p < 0.05$) were observed in the mean concentrations of steranes with respect to stations and compound types based on the results of the Two-way analysis of variance conducted on the data set. The total organic carbon (% TOC) contents ranged from 0.44-4.26, which is typical for estuarine sediments [12]. Tetra cyclic triterpane hydrocarbons detected in coastal sediments from the study area are ααα-24-ethylcholestane; 2α-Methyl-24-ethylcholestane and 3β-Methyl-5α-stigmastane. The identification of these compounds is based primarily on their mass spectra and GC retention time in the m/z 218, 231 key ion fragmentograms [31]. Representative mass spectra of two of these sterol biomarkers are shown in Figure-3. Stations from Warri and Qua Iboe rivers recorded the highest and lowest total concentrations of ααα-24-ethylcholestane, respectively (Figure-2). Almost equal proportions of 2α-Methyl-24-ethylcholestane were recorded for stations from Imo and Warri rivers as depicted in Figure-2. However, these sterol biomarkers were not detected in stations from Bonny River. This may not be unconnected with the predominantly mangrove vegetation around the coastal areas of the river. The total sterane concentrations for Imo, Qua Iboe and Warri stations were in the ranges of 0.79 -1.47mgkg^{-1}, 0.28 -1.73 mgkg^{-1} and 1.34-2.90 mgkg^{-1}, respectively.

Diagenesis of organic matter occurring in water and sediments modifies the structure of precursor steroids in complex way. Cholestane, a saturated tetracyclic hydrocarbon conforming to the general molecular formula C_nH_{2n-6}, is obtained from cholesterol. During diagenesis cholesterol is converted to cholestane by dehydration and reduction. Sterols in plants (phytosterols) often carry extra alkyl groups at C-24 [14]. Sterol biomarkers found in our samples carry extra ethyl groups at C-24 (Figure-3), suggesting possible source inputs from vascular plants. This submission further supports earlier report by [32] of dominant biogenic/terrestrial (CPI>1) source inputs to sedimentary organic matter in the study area. Thus, steranes, like the hopanes are abundant in sediments, rocks and petroleum because their precursors (sterols) are so common in living organisms. Steranes are additional molecular markers found generally at lower concentrations than the hopanes.

CONCLUSIONS

Specific organic compounds e.g. biomarkers can be used to infer the contribution of organic carbon from different sources. The occurrence of ααα-24-ethylcholestane, 2α-methyl-24-ethylcholestane and 3β-Methyl-5α-stigmastane in sediments from the study area is strongly suggestive of organic matter inputs from vascular plant sterols (phytosterols) which had been diagenetically transformed in recent benthic sediments. The sparse population of vascular plants around the coastal areas of the Niger Delta could be implicated for the erratic distributions of the sterol biomarkers in the sediments

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analysed. We recommend that further studies should be carried out to infer the contribution of organic carbon in sediments by other biological systems based on current approaches in biomarker geochemistry.

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