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PRESENCE OF SQUALANE IN URBAN AQUATIC ENVIRONMENTS

GENKI MATSUMOTO* and TAKAHISA HANYA

Department of Chemistry, Faculty of Science, Tokyo Metropolitan University, Setagaya-ku, Tokyo 158 (Japan)

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SUMMARY

Ethyl acetate extracts of river waters and sediments, night-soil and sewage treatment plant effluents and sludges from the Tokyo area were analysed for squalane using combined gas chromatography-mass spectrometry after separation by silica gel column chromatography. Squalane was identified in all the samples studied and the concentration in river waters and sediments ranged from 0.46 to 1.7 $\mu\text{g/l}$ and from 0.86 to 15 μg per g dry sediment, respectively. Squalane is presumably derived from artificial materials rather than from natural sources, with the exception of fossil fuel products.

INTRODUCTION

Much attention has been paid to isoprenoid hydrocarbons as a biological marker in various sediments ranging in age from recent to precambrian rocks and petroleum. It is well known that pristane and phytane are widely distributed in sediments and petroleum. The entire series of acyclic regular (head-to-tail linked) isoprenoid hydrocarbons from C_5 to C_{40} have also been found in petroleum¹⁻⁶. Biological substances such as squalene (C_{30}), lycopene (C_{40}), solanesol (C_{45}) and phytol side chain of chlorophyll have been suggested as the precursors of these regular isoprenoid alkanes¹⁻⁶. The irregular isoprenoid hydrocarbon squalane has been found in the Soudan shale⁷, an African cretaceous shale⁸ and a Nigerian petroleum⁴. In the modern environment, regular isoprenoid hydrocarbons such as C_{18} (ref. 9), pristane and phytane have been found, but very little is known on the occurrence of irregular isoprenoid hydrocarbons, except for squalene, other than in living organisms. We report here the identification and estimation of squalane in river waters and sediments and some related samples collected from the Tokyo area.

EXPERIMENTAL

Apparatus

Care was taken to avoid contamination of samples, as described previously^{10,11}.

The gas chromatographic-mass spectrometric (GC-MS) measurements were

performed using a Shimadzu LKB 9000 instrument. A silanized glass column (2 m × 3 mm I.D.) was packed with 1% silicone OV-1 on Chromosorb W AW DMCS (80–100 mesh). The flow-rate of carrier gas (helium) was 30 ml/min. The column temperature was programmed from 100°C to 285°C at 6°C/min. The injection block, molecular separator and ion source were maintained at 290, 300 and 330°C, respectively. The mass fragmentogram was recorded for the mass number m/e 57 ($C_4H_9^+$) at 20 eV. Mass spectra were taken at 70 eV with an accelerator voltage of 3.5 kV.

Chemicals

Chemicals were used after treatment as described in previously^{10,11}. Authentic squalane and squalene were purchased from Wako (Osaka, Japan).

Samples

During 1975–1977, water samples were collected from the downstream (Chohfu) of Tama river and its tributaries (Ohkuri and Nogawa rivers) and Sumida river (Kototoi) in the Tokyo area, which are highly polluted by sewage¹⁰. Sediments were taken from the upper (Hamura) and downstreams (Chohfu and Gasu) of Tama river. F night-soil and T sewage treatment plant effluents and F night-soil and S and M sewage treatment plant sludges were collected from the Tama river basin, as possible sources of squalane in the rivers.

Water samples were collected using a 5-l stainless-steel bucket or with a 2-l glass bottle with glass stopper. The sample was immediately acidified (pH < 2) with concentrated hydrochloric acid and stored at *ca.* 5°C until analysis. Sediments were collected with an Eckman-Berge Dredge and stored in polyethylene bags at temperatures of *ca.* 5°C or –20°C, together with sludge.

Analysis of squalane

The water sample (1.0 l) was extracted three times with ethyl acetate (200 ml, 150 ml × 2). The ethyl acetate extract was evaporated to dryness, redissolved in 50 μ l benzene–ethyl acetate (1:1) and then chromatographed on a silica gel column (160 × 4 mm I.D., 100 mesh, 5% water). Two column volumes of hexane eluate (hydrocarbon fraction) were analysed using GC–MS. The method of extraction of organic matter from sediment (sludge) is described in detail elsewhere^{11,12} and will be noted here only briefly. Wet sediment or sludge (2–5 g) was extracted with ethyl acetate after saponification with 0.5 M potassium hydroxide in methanol. The ethyl acetate extract was analysed by the same method as the water sample.

The identification of squalane was based on the comparison of retention time and mass spectrum with that of the authentic compound. The estimation was performed by measurement of peak height on the mass fragmentogram. In addition, for *n*-alkanes, the peak height on the mass fragmentogram was calibrated with C_{16} , C_{20} , C_{24} , C_{28} and C_{36} alkanes. Based on four replicate addition experiments using authentic squalane, the recoveries of squalane for water and sediment were 85% (standard deviation, S.D. = 5.8%) and 92% (S.D. = 10%), respectively. A blank test showed that the contamination of squalane was less than 2 ng/l or 2 ng per g dry sample. Its presence does not affect the analytical results for our samples.

RESULTS

Identification

A typical mass fragmentogram of the hydrocarbon fraction obtained from the Tama river water sample is given in Fig. 1. Squalane occurs as the most prominent peak in the chromatogram, together with *n*-alkanes, pristane, phytane and an unresolved envelope of a complex mixture of hydrocarbons (UCMH) comprised of substituted naphthenic and aromatic compounds. The mass spectrum of squalane found in the river water sample is shown in Fig. 2, together with that of authentic

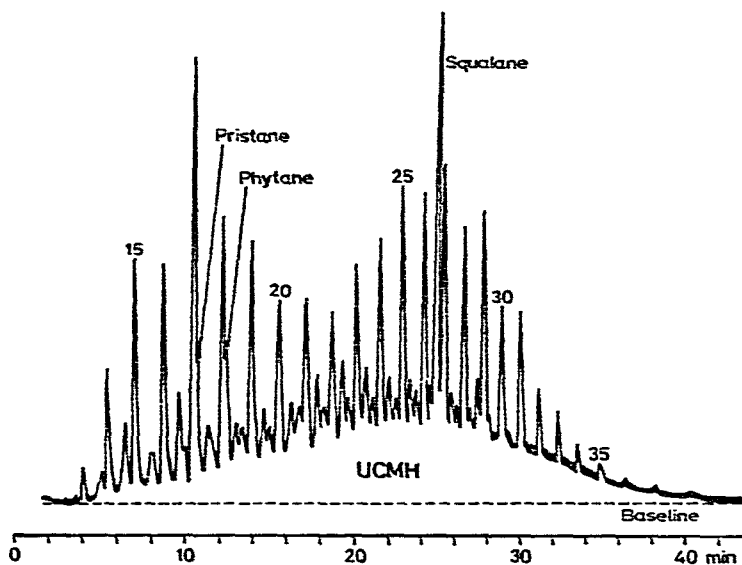


Fig. 1. Mass fragmentogram of the hydrocarbon fraction obtained from the Tama river water (Chohfu, December 21, 1977). Numbers at the peaks indicate the carbon chain length of *n*-alkanes. UCMH = Unresolved complex mixture of hydrocarbons.

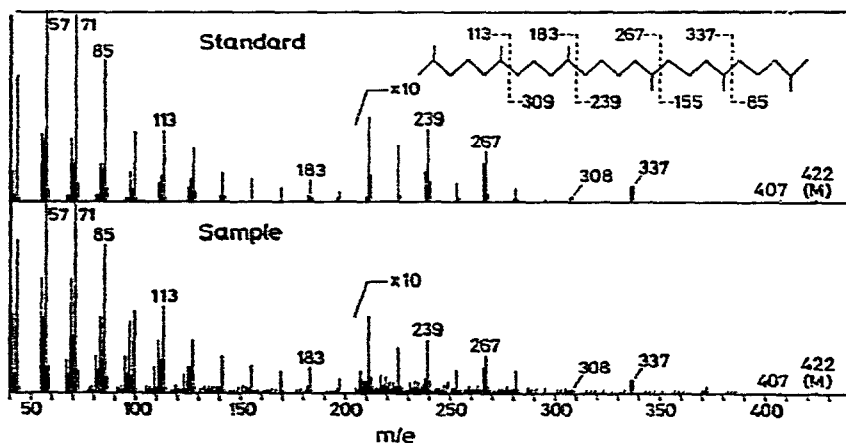


Fig. 2. Mass spectrum of squalane.

squalane. The major fragments of the sample coincide with those of authentic squalane, although there are several peaks due to UCMH.

Concentration

Squalane is found generally as the major constituent of the hydrocarbon fraction and its concentration in river waters ranges from 0.46 to 1.7 $\mu\text{g/l}$, depending upon the sampling locations (Table I). Squalane has also been identified in night-soil and sewage treatment plant effluents. In the river sediments, the concentrations of squalane range from 0.86 to 15 μg per g dry sample. Large amounts of squalane are also found in sludges.

TABLE I
SQUALANE FOUND IN URBAN AQUATIC ENVIRONMENTS

Sample	Sampling data	Concn.	Squalane/ <i>n</i> -alkanes* ratio
<i>Water</i>			
Tama R. (Chohfu)	Dec. 21, 1977	0.92 $\mu\text{g/l}$	0.10
Ohkuri R.	Aug. 29, 1977	0.46 $\mu\text{g/l}$	0.21
Nogawa R.	Dec. 16, 1975	1.7 $\mu\text{g/l}$	0.13
Sumida R. (Kototoi)	July 9, 1975	1.3 $\mu\text{g/l}$	0.093
F night-soil treatment plant effluent	Dec. 16, 1975	5.3 $\mu\text{g/l}$	0.35
T sewage treatment plant effluent	June 26, 1975	0.23 $\mu\text{g/l}$	0.37
<i>Sediment and sludge</i>			
Tama R. (Hamura)	July 6, 1977	0.86 $\mu\text{g/g}$	0.054
(Chohfu)	July 21, 1977	15 $\mu\text{g/g}$	0.26
(Gasu)	Dec. 9, 1976	4.6 $\mu\text{g/g}$	0.065
F night-soil treatment plant sludge	Nov. 9, 1976	14 $\mu\text{g/g}$	0.13
S sewage treatment plant sludge	Nov. 6, 1976	84 $\mu\text{g/g}$	0.76
M sewage treatment plant sludge	Nov. 6, 1976	26 $\mu\text{g/g}$	0.24

* Sum of the C_{15} – C_{33} contents.

DISCUSSION

Squalane is known to be present in a Nigerian crude oil but its calculated concentration is very small (2 ppm)⁴. In addition, squalane is generally not a major constituent of petroleum and petroleum products. Thus the ratio squalane/*n*-alkanes (sum of the C_{15} – C_{33} contents) is assumed to be very small. However, the ratios for river waters and sediments are considerably higher, ranging from 0.093 to 0.21 and from 0.054 to 0.26, respectively (Table I). The ratios in night-soil and sewage treatment plant effluents and sludges are similar to those of the river waters and sediments. Therefore petroleum and petroleum products can hardly be the major source of squalane. Furthermore, squalane is rarely found in living organisms, thus the most probable source of squalane in our samples seems to be due to urban activities. Squalane is widely used as a lubricant, transformer oil, ingredient of watch and chronometer oils, perfume fixative, skin lubricant, ingredient of suppositories and carrier of lipid-soluble drugs¹³.

Squalene is also a possible source of squalane if subsequent biotic and/or abiotic hydrogenation occurs in the natural environment. Squalene is present in large amounts in shark liver oil and in smaller amounts (0.1–0.7%) in olive oil, wheat germ oil, rice bran oil and yeast. It is also known to be an intermediate in the biosynthesis of cholesterol. Furthermore, squalene is used as a bactericide, as an intermediate in the manufacture of pharmaceuticals, organic colouring materials, rubber chemicals, aromatics and surface active agents¹³. However, no squalene has yet been found in our water and sediment samples, although it has been isolated from surface sea-water in a coastal region¹⁴ and from a water sample collected at Chohfu on Tame river¹⁵. Besides, no squalane has been detected from areas unpolluted by man, even when the organic content of the sample was extremely high^{12,16}. Therefore, the contribution of squalene to the content of squalane in aquatic environments is thought to be small.

These results imply strongly that squalane is largely derived from artificial sources with the exception of petroleum products, rather than from natural sources. Squalane may be introduced to urban river waters and sediments directly and/or through night-soil and sewage treatment plants by our daily activity. Consequently, squalane in the aquatic environment is considered to be one of the indicators of human activities.

CONCLUSION

Squalane was identified in river waters and sediments, night-soil and sewage treatment plant effluents and sludges obtained from the Tokyo area. Its concentration in river waters and sediments ranged from 0.46 to 1.7 $\mu\text{g/l}$ and from 0.86 to 15 μg per g dry sediment, respectively. Squalane is most likely to be derived from artificial materials, except for fossil fuel products, rather than natural sources. This analytical method is also useful for determining very low concentrations (ng/l or ng per g dry sediment) of squalane in water and sediments.

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