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CHEMICAL INVESTIGATION OF THE INTERNAL SECRETION OF THE SPERM BLUE WHALE

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ABSTRACT

Ambergris, which is an internal secretion of the sperm blue whale, was extracted with 96% ethanol. The saponified and unsaponified portions have been studied in detail. The number of fractions and components of the unsaponified matter as separated by column and thin layer Chromatography confirm the presence of ambrein (triterpenoid) as well as some other sterols. The saponified portion is reported to contain stearic. oleic, linoleic, archidic and betenic acids. More than one compound have been found in each fraction.

Introduction

Ambergris is an internal secretion of the sperm blue whale (*pyseter carodon*) (Dannenfeldt, 1982). It is either obtained directly from the intestine of the whale or is found washed ashore in small fragments ranging in weight from several hundred grams to several kilograms as compact masses. As a result of the action of sunlight and oxygen for long periods of time, often for several years, ambergris undergoes an aging process during which the strong stercoraccous odor disappears. The finest and most valuable ambergris is pale gray to golden yellow or, in very rare cases, chalky white (Ohloff, 1982).

The first chemical investigation of the constituents of ambergris was carried out in Paris in 1820 by Pelletier and Caventon, who found that its major component is tricylic triterpene and that the odor of ambergris is due to the presence of oxidation products of ambrein. Since this finding many other constituents have been isolated from ambergris, including ambrein derivatives, epicorprostanol, coprostanone, chlolestanone, cholesterol, epicholestanol porphyrine, copper and fatty acids (Governo, Rocco & Manfred, 1977). In this study we have carried out chemical investigation in detail for various saponified and unsaponified matter of Ambergis powder and various new components are reported.

Materials

The ambergris used in this study was purchased from the local market in Riyadh, Saudi Arabia. It was gray to yellowish gray in color.

Method

Extraction and Saponifiction:

A mass of 120 grams of fine powder of ambergris was used. This was packed in a thimble in a soxhlet extractor (500 ml. capacity) and extracted with 96% alcohol, for 16 hours. The alcohol was distilled off under vacuum and the dark reddish oily residue (107g.) was saponied by refluxing for two hours with 5% alcoholic KOH. The product was then chilled and diluted with 300 ml. of distilled water.

The unsaponfied matter was extracted with 200 ml. chloroform from the alkaline mixture above. The chloroform fractions were combined, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure in a rotary evaporator to give the residue (A.100 g.).

The saponfiable portion in the aqueous medium was rendered acidic using dilute HCl and extracted with three volumes of 200 ml. of chloroform. The three extracts were combined and dried over anhydrous $Na_2SO_4$and the solvent evaporated under vacuum to give residue (B. 7g.).

A) *Column Chromatography of the unsaponifiable matter (Residue A):*

Residue A was dissolved in the minimum amount of Chloroform, was then triturated with silica gel G(70-240 mesh), and blended homogenouslyt all the chloroform was evaporated under room temperature. The resulting mixture was applied to the top of a prepared column of silica gel (70 x 240 mesh) and column chromatographic separation was commenced using gradient eluoisn technique. Ali quotes of 1.5 liter of each of petroleum ether (40-60°C): Chloroform mixtures in the ratios of 100:00; 80:20; 60:40; 50:50; 20:80; 00:100, diethylether (100%) and ethyl acetate (100%) were collected in 50 ml. fractions. These fractions were examined by thin layer chromatography (TLC) and combined accordingly. The solvent system was benzene -- methanol (98:2) using silica gel plates and cone. H_2SO_4 or iodine vapors as visualizing agents.

The residues of the combined fractions were designated FI; FII; FIII; FIV; FV; and FVI and were used for pharmacological evaluations. Each residue was prepared as an emulsion to give a final concentration of 5 mg/ml.

B) Investigation of the Saponifiable Fraction (Residue B):

The saponifiable fraction in residue B was investigated by Gas Liquid Chromatography. The fatty acids in this residue were derived into their methyl esters. The residue obtained above was dissolved in 100 ml of absolute methanol and 2.5 ml of conc. HCl were added and the mixture was refluxed on a boiling water bath for about three hours the methanolic solution was reduced to 1/2 volume under vacuum on a rotary evaporator, diluted by an equal volume of water and extracted with ether (3x5ml). The ether layer was washed with 150ml of H_2O, separated and dried over anhydrous Na_2SO_4. The ether was evaporated under vacuum and the residue (7g) was subjected to GLC analysis as a chloroform solution.

C) Gas-Chromatographic Conditions

A varian Gas Chromatograph model 3700 with dual F.I.D. was used. The liquid phase was 3% OVL, adsorbed on Chromosorb Q. Support was packed in 70-240 glass column (3m of 0.33mm I.D.). The conditions were: oven temperature was programmed from 100°C, (start) to 300°C., injection port temperature 170°C, detector temperature 250°C, carrier gas was nitrogen at 40m1/min., hydrogen flow 300ml/min.: sample size 1 lit.

Individual components were identified by comparison of retention times of peaks separated with those of authentic methylated fatty acids and confirmed by peak enrichment (spiking) technique (Fig. 1 and Table-1).

The quantitation of individual components was calculated on the peak area relative to the total area of peaks in the sample (Table 2).

Results and Discussion

Table-1 illustrates the number of fractions and components of the unsaponified matter as separated by column and thin layer chromatography. Of these we were interested to detect the triterpenoid compound ambrein to confirm the authenticity of our purchased Ambergris (Takayaka, *et. al*., 1970; Opoyke, 1976.) On spectroscopic analysis of the eluted and recrystallized component it gave the same spectral data reported for ambrein (Ruzicka *et. al*, 1946; Lederer & Stoll, 1950; Takayaka, *et. al*., 1970). The infra red spectrum indicated the presence of a hydrocarbon with an alcoholic group and the NMR spectrum was typical to that reported by Takayaka *et. al*. (1970). These spectra confirmed the material to be genuine ambergris. The confirmation of the authenticity of the sample was thought of utmost importance since the material is valuable and subjectable to adulteration. However, other components of terpeniodal nature and positive to general tests for terpeniods or sterols were detected. Liberman, Burchard tests were perormed together with non-specific tests such as Vanillin/H_2SO_4 conc. H_2SO_4, iodine vapor and phosphoric acid components visualized under visible and U.V. light. The other components exhibited the R_f values as shown in Table-1.

The saponified matter as analysed by GLC technique indicates the presence of several fatty acids (Fig. 1). Stearic acid was about 24%, oleic acid Linoleic acids 16%, archidic acid 5%, while betenic acid constituted about 5% of the total saponified matter. The oleic and linoleic acid were not well dissolved on the GLC parameters described before and hence their relative concentrations were summed up together.

Several authors have discussed the constituents of ambergris (Gunther; Mookherjee; and Cambie, R.C.). They discussed it as volatile constituents, while other tried to synthesize these volatile constituents. However, little or nothing is known about the Pharmacological activity of the nonvolatile constituents of ambergris in their mixture of as individuals except the well-known natural sterols such as cholesterol.

Table 1: The fraction investigated chemically as separated by column and TLC from the unsaponified residue I

Fraction No	Elute on Column	No.spots on TLC	R_f Value on TLC	Chemical nature
F (I)	P.E.(100%)	2	0.740, 0.952	Sterol or terpenoid in nature
F (II)	P.E. + C(80:20)	3	0.740, 0.835 0.910	do
F (III)	P.E. + C(60:40)	3	0.345, 0.792 0.835	do
F (IV)	P.E. + C(50:50) P.E. + C(20:80)	4	0.132, 0.345 0.521, 0.835	do
F (V)	C:E (50:50)	6	0.132, 0.221 0.345, 0.444 0.521 and 0.835	do
F(VI)	E + A (50:50)	4	0.027, 0.132 0.345 and 0.835	do

P.E. = Petroleum ether P.E. + C = Petroleum ether plus chloroform

C:E = Chloroform plus ether; EtA = Ethylacetate
(0.835 is Common Component in all fractions).

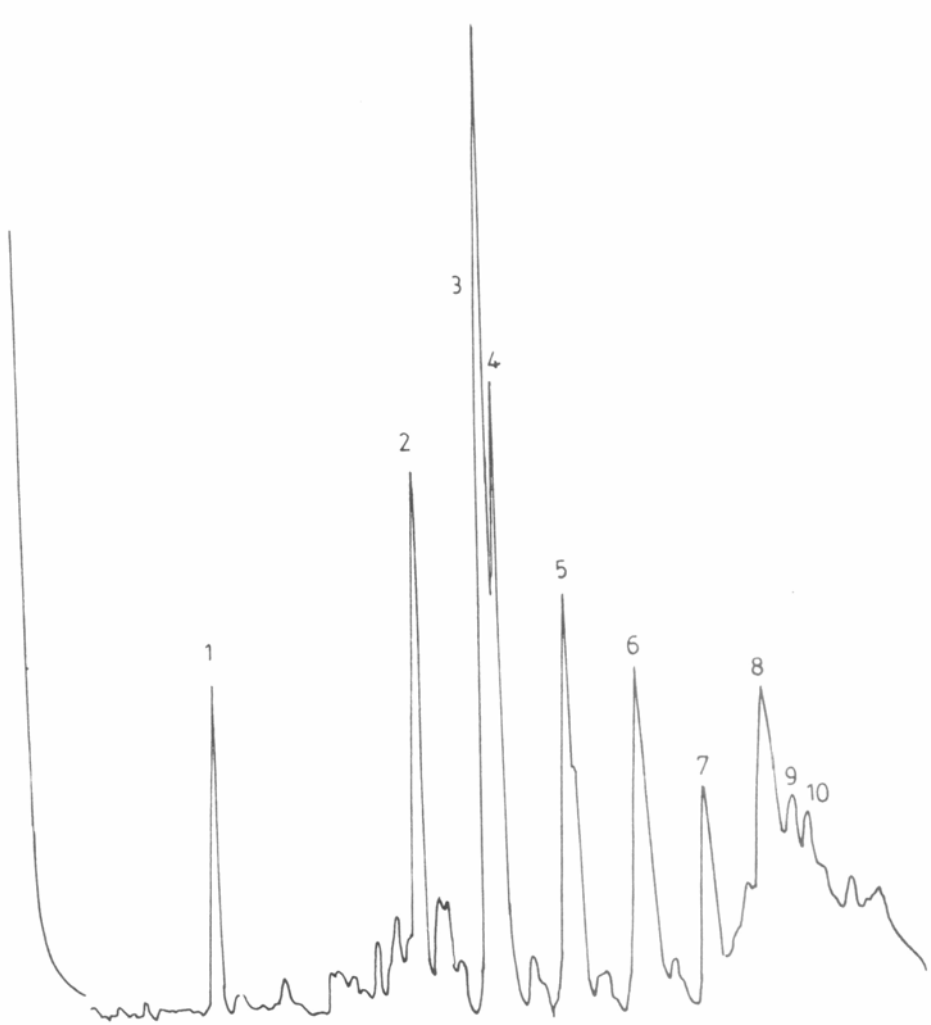

FIG. 1. GLC CHROMATOGRAM OF FATTY ACIDS METHYL ESTERS OF AMBERGRIS

Table-2: The identified fatty acids as methylesters and their relative concentrations in the saponifiable fraction (residue II) as separated by GLC on 3% OVL liquid phase.

Peak No.	Nature	Relative Concentration
3	Steric acid	24%
4	Oleic/Linolic acids	16%
5	Arachidic acid	5%
6	Betenic acid	45%
7	Peaks 1, 2, 7, 8, 9 & 10 are unknown.	

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