CHROMATOGRAPHIC ANALYSIS OF SOME CONSTITUENTS OF MARINE-ANIMAL OILS[1/]

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Most existing methods for the determination of lipid mixtures of natural origin are laborious, time-consuming, and uncertain. This is particularly true in the case of marine-animal oils, which are the most complex of these lipids. This study was undertaken to determine whether the separation and identification of lipid constituents of marine oils could be accomplished with the aid of the silicic acid-impregnated glass-fiber filter paper technique of Dieckert and Reiser. This method allows for the separation of microgram quantities of lipids.

EXPERIMENTAL

Several well characterized lipids were used as reference standards with 10 different solvent systems. R_f values, a measurement of position on the paper which may be used as an identifying index, of the various combinations were determined, each value being the average of at least five tests. Because of the large proportion of triglycerides occurring in marine oils, the mixtures were first separated on a column into 5 groups with 5 solvent systems according to the method of Fillerup and Mead. The eluates were concentrated and then chromatographed on the silicic acid paper.

A certain amount of variation, more pronounced with some of the reference compounds than with others, made absolute R_f values unreliable. For this reason, reference compounds were chromatographed simultaneously with the unknowns. Ascending chromatography was the method used.

The similarity of R_f values for vitamin D_3 and cholesterol in all solvents tried presented a problem which was solved by the addition of iodine to the isooctane ether solvent system. Vitamin D_3 remained at the point of origin while the R_f value for cholesterol was 0.30.

Densitometers were used in an attempt to make the chromatograms quantitative as well as qualitative. Substances such as plastic sprays, collodion, light cedarwood oil, plastic spray over light cedarwood oil, silicone water-repellent spray, and glycerine were used to minimize the irregularity in density in the paper.

RESULTS AND DISCUSSION

Of the five fractions obtained by the column separation, the first three contained all the lipids used in the reference compounds. The fourth contained the fatty acids, and the fifth the phospholipides. This study was not concerned with the fatty acid constituents so the fourth fraction was disregarded. The first fraction contained vitamin A palmitate, cholesteryl palmitate, squalene, hexadecyl palmitate, and the tocopherols. All the triglycerides came through in the second fraction with a part of the aliphatic alcohol. The remainder of the latter appeared in fraction three along with vitamin A alcohol, cholesterol, and vitamin D_3. The 7-dehydrocholesterol, according to preliminary tests, should have appeared in the third fraction. However, it was not recovered from the mixture containing all the reference compounds. The

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fifth fraction contained the phospholipides. In the oil samples studied, phosphatidylethanolamine was the only phospholipide demonstrable. Ninhydrin tests for phosphatidylethanolamine were much less sensitive than the chromatograms. Tests with Dragendorff's reagent for lecithin were negative.

Vitamins A alcohol and D_3 seemed to undergo some change during the process of separation and concentration which made them difficult to demonstrate consistently in the natural oils. "Super D" cod-liver oil showed spots corresponding to triglycerides, vitamin D_3, vitamin A alcohol, phosphatidylethanolamine, and an unidentified component. Silmo cod-liver oil and menhaden oil chromatograms indicated the presence of squalene, triglycerides, vitamins A alcohol and D_3, and phosphatidylethanolamine.

Most of the substances used to rectify the paper for density measurements darkened the paper and were unsatisfactory after drying. Glycerine gave better results than the others but the wet paper was difficult to handle. The lipid spots were too large to be measured with the reflection densitometer. With the transmission densitometer, differences between portions of the paper were often as great or greater than the differences between the paper alone and the charred spots. The spots from a chromatogram were irregular and their variation in size and shape made their densities impossible to measure accurately with this method.

SUMMARY AND CONCLUSION

A scheme for the separation and identification of some constituents of marine animal oils was developed. A preliminary separation on a silicic acid column with five solvent systems was followed by further separation and identification on silicic acid impregnated glass fiber filter paper. This method can be used successfully for qualitative determinations, but the irregularities in the density of the glass paper prevent an accurate quantitative assay.

COMMON MARINE WORMS USED FOR BAIT

The two common marine bait worms are the "sandworm" or "clamworm" and the "bloodworm."

The clamworm (Nereis virens) is usually taken by digging it from its burrow in the muddy or sandy bottom of estuaries during periods of low tide. These burrows vary in depth from a few inches to about eighteen inches or more.

The bloodworm (Glycera dibranchiata) is found in a similar habitat with most of the commercial digging taking place in Maine and extending into Canada.

The greatest use of these worms is in the sport fishery from Maryland to Connecticut with a large demand existing for them in the Long Island Sound area. The worms should be at least 6 or 7 inches long and may be used to catch bluefish, fluke, scup, gray sea trout, sea bass, striped bass, blackfish, kingfish and flounder.

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