SHORT COMMUNICATION

The Docosahexaenoic Acid of Marine Organisms

ABSTRACT

Preparative gas chromatography has been used for the purification of the highly unsaturated C_{22} acid present in cod liver. Confirmation that this acid is $\Delta^{4,7,10,13,16,19}$ docosahexaenoic acid has been obtained by purely physicochemical means using NMR and mass spectrometry.

The fatty acids of the lipids of aquatic plants and animals are characterized by the presence of a wide range of highly unsaturated acids. The structures of several of these acids have been established by Klenk and Brockerhoff (1). However, there has been some controversy about the identity of the highly unsaturated C_{22} acid present in considerable quantities in the lipids of many species of phytoplankton and fish oils. On the basis of their earlier work, Klenk and Brockerhoff (1) considered this compound to be $\Delta^{4,7,10,13,16,19}$ docosahexaenoic acid [22:6 ($\Delta^{4,7,10,13,16,19}$)] and this identification was accepted by Ackman and Burger (2). Later, Klenk et al. (3) examined the fatty acids from various species of phytoplankton which were thought to contain this acid, but the most unsaturated C_{22} acid which they were able to detect was docosapentaenoic acid. This acid was subsequently shown by ozonolysis to be the $\Delta^{7,10,13,16,19}$ isomer. In view of this disparity, the structure of this acid was reinvestigated. While this work was in its later stages, Harrignton and Holz (4) published a preliminary account of the fatty acids of *Gyrodinium cohnii*, of which this acid was the major component. They were able to confirm, by classical chemical methods, that the acid was in fact 22:6 ($\Delta^{4,7,10,13,16,19}$), as originally proposed by Klenk and Brockerhoff (1). It was thought worthwhile to publish the results of our study which fully agreed with this structure, as it was carried out by a completely independent, purely physicochemical procedure.

The methyl ester of the highly unsaturated acid was isolated from the mixed methyl esters of the fatty acids of cod liver oil in 30% yield by preparative gas liquid chromatography in a modified Pye Panchromatograph. The separation was carried out isothermally at 180 C with a 2.7 m x 1.25 cm bore glass column packed with 15% of diethylene glycol succinate on 80-100 mesh acid-washed silanized Chromosorb W. The ester, which had a retention time of 5 hr was condensed in a zigzag capillary cooled to -80 C.

Mass spectrometry of the methyl ester showed the major peak to be at 342 amu, as would be expected for methyl docosahexaenoate. NMR spectroscopy was used to confirm the number of double bonds in the compound and to ascertain their position. This technique has been used by Hashimoto et al. (5) with unresolved mixtures of highly unsaturated methyl esters of fish oils to demonstrate that the unsaturation is of the divinyl methane type (-CH=CH-CH_2-CH=CH-). The NMR spectrum of our compound, which closely resembled those published by Hashimoto et al. (5) showed the following important diagnostic features: (a) a triplet at 4.7 τ arising from olefinic groups adjacent to methylene groups; (b) a singlet at 6.3 τ arising from the carbomethoxy group; (c) a triplet at 7.1 τ due to methylene groups adjacent to two olefinic groups; (d) a group of peaks in the region 7.5-8.0 τ due to the α-methylene group and the methylene group adjacent to a single olefinic group; and (e) a triplet at 9.0 τ arising from the terminal methyl group.

In the quantitative interpretation of the NMR data the integrator reading corresponding with one proton was first assessed. This value was assumed to be one third of the average integrator reading for the peaks at 6.3 τ and 9.0 τ which arise respectively from the carbomethoxy and terminal methyl groups. Consideration of the integrator reading for the triplet at 4.7 τ led to the conclusion that the compound contained 5.7 ± 0.3 double bonds, in satisfactory accord with 22:6. Examination of the integrator reading for the triplet at 7.1 τ showed the presence of 4.75 ± 0.4 methylene groups adjacent to two double bonds. This is consistent with a docosahexaenoic acid having its double bonds in the skipped methylene configuration. Three isomers of this type can exist; these may have the terminal double bond in the $\omega 2$, $\omega 3$ or $\omega 4$ position. If the acid under consideration was of the first type, the triplet existing at 9.0 τ would not have been present, but would be replaced by a doublet at 8.0-8.5 τ arising from the terminal vinyl group. On the other hand, if the skipped methylene chain commenced at the $\omega 4$ position, the α-methylene group would be sandwiched between a double bond and the carboxylic acid group. The NMR spectrum of the compound under consideration showed no evidence of the couplet at 6.5-7.0 τ which would arise from a methylene group having this configuration. This led to the conclusion that the compound had the $\omega 3$ structure, and was 22:6 ($\Delta^{4,7,10,13,16,19}$) as first postulated by Klenk and Brockerhoff (1).

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