trans-6-Hexadecenoic Acid in the Atlantic Leatherback *Dermochelys coriacea coriacea* L. and Other Marine Turtles

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ABSTRACT

Depot fat from the Atlantic leatherback turtle (*Dermochelys coriacea coriacea* L.) was shown to contain ~3% of *trans*-6-hexadecenoic acid. Structural details were elucidated through comparative aspects of isolation techniques, NMR, IR, hydrogenation, oxidative fission, etc., and confirmed by similarity of properties with those of a sample of fatty acid of this structure isolated from the seed oil of *Picramnia sellowii*. One additional leatherback turtle oil sample, and depot fat from two other marine turtles, the loggerhead (*Caretta caretta caretta*) and ridley (*Lepidochelys olivacea kempi*) contained this acid, that from the ridley in a lower proportion. No corresponding C_{18} acid was detected in the leatherback oil.

INTRODUCTION

The lipids of marine reptiles have received scant attention in recent years, although several turtle oils were at one time studied by classical distillation techniques (1). The oil of the Atlantic leatherback turtle *Dermochelys coriacea coriacea* L. was examined in this laboratory by gas liquid chromatography (GLC) on packed columns and found to conform broadly in fatty acid composition to a general marine oil pattern (2). Oil from this species was recently reinvestigated on open tubular (capillary) GLC columns as part of a continuing study on monoethylenic fatty acid isomer compositions of marine lipids (3-8; also, Ackman et al., submitted for publication).

It is known that the elution sequence of the methyl esters of monoethylenic fatty acids, when all are of the same configuration (either *cis* or *trans*), is nominally similar on both polar and nonpolar GLC liquid phases when only the common range of fatty acid positional isomers is concerned. Basically this range, in the C_{18} acids for example, would include the 6-octadecenoic to the 15-octadecenoic acids. It is also apparent from published data that the elution order of the components in a mixture of *cis* and *trans* monoethylenic acids from this range, as well as for the other positional isomers, may be somewhat different on a nonpolar liquid phase as compared to a polar liquid phase (9-11). Comparison of the C_{16} monoethylenic fatty acid complex in a whole-oil GLC analysis of leatherback turtle oil on a polar column (Fig. 1) and a nonpolar column (Fig. 2) showed transposition of the two major 16:1 components. This observation of a nonconforming isomer stimulated a detailed investigation and resulted in the identification of *trans*-6-hexadecenoic acid in the leatherback and two additional marine turtle oils.

EXPERIMENTAL PROCEDURES

Detailed examination of the leatherback turtle oil was primarily based on the sample examined previously (2). The oil (a depot fat) was recovered from its fibrous matrix by extraction with chloroform. An additional sample of leatherback turtle fat, although somewhat oxidized, was treated similarly. These fats came from turtles recovered in Nova Scotian waters (12). Body fat samples from the loggerhead (*Caretta caretta caretta*) and ridley (*Lepidochelys olivacea kempi*) came from specimens captured respectively at Veracruz, Mexico, and in the Gulf of Mexico off Tamaulipas. Seed oil was recovered from *Picramnia sellowii*.

All oil samples were saponified and nonsaponifiable materials were extracted from soap solutions by AOCS methods. The fatty acids were recovered and converted to methyl esters by brief treatment with 5% BF_3-MeOH solution.

Basic analytical GLC was carried out with open tubular columns (150 ft x 0.01 in i.d.) coated with butanediol succinate (BDS) polyester or Apiezon-L (Ap-L) grease. The columns were purchased from the Perkin-Elmer Corp. and operated with a high split ratio (No. 1 or 2) in either Model 226 or Model 900 GLC units of this firm. Injection port temperatures were 250 C, and operating conditions for the respective columns were: BDS, 170 C and 50 psig helium; Ap-L, 190 C and 80 psig helium. Preparative GLC was carried out with an Aerograph A-90 (thermal conductivity) unit fitted with a 10 ft x 1/4 in. column packed with Chromosorb G (DMSC), 80/100 mesh, coated with 10% SE-30 silicone gum.

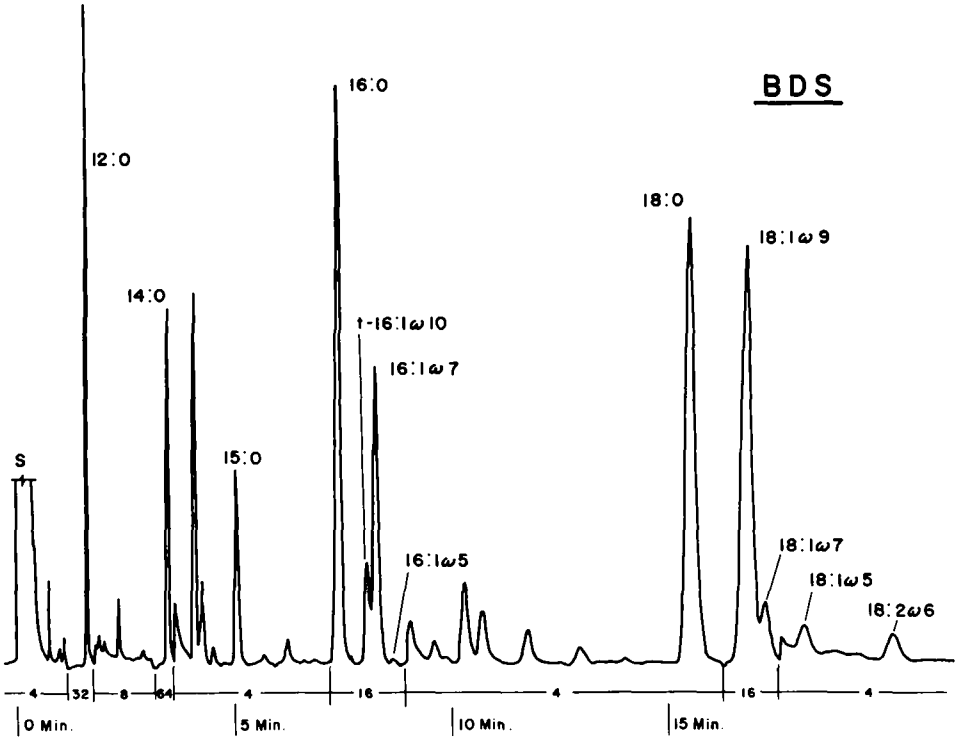

FIG. 1. Partial chromatograph recording from GLC analysis on an open-tubular column, with BDS coating, of methyl esters of fatty acids of leatherback turtle oil. S, solvent. Time and attenuations noted at bottom.

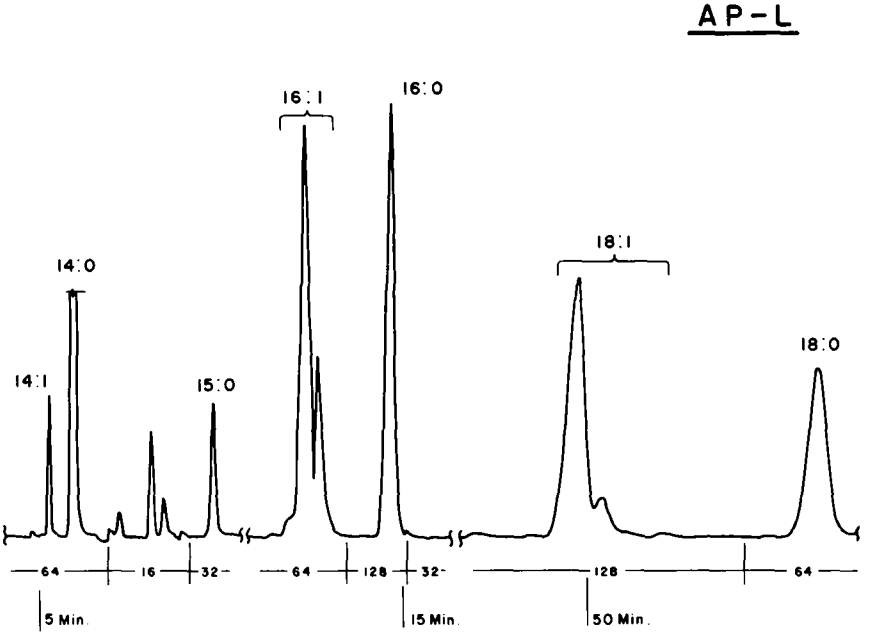

FIG. 2. Partial chromatograph recording from GLC analysis on open-tubular columns, with Ap-L coating, of sample shown in Figure 1.

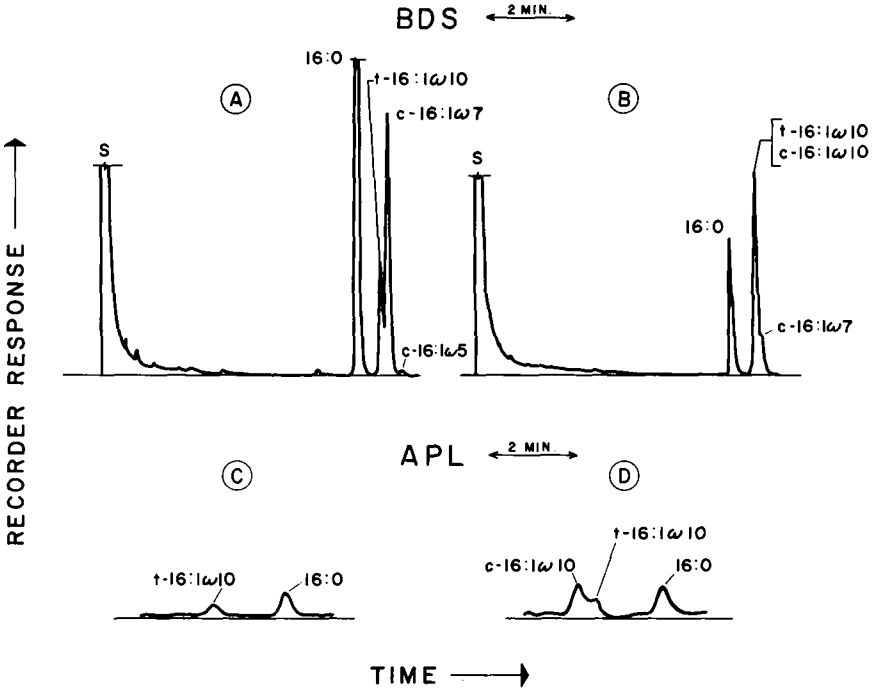

FIG. 3. (A) Chromatograph recording from GLC analysis on open-tubular column, with BDS coating, of methyl esters of C_{16} fatty acids from loggerhead turtle oil as isolated by preparative GLC (note: polyenes not obvious at this attenuation). (B) Chromatograph recording from GLC analysis on open-tubular column, with BDS coating, of methyl esters of C_{16} fatty acids from *P. sellowii* seed oil as isolated by preparative GLC. Note that both *cis*- and *trans*-6-hexadecenoic acid are present in the first monoene peak. (C) Partial chromatograph recording from GLC analysis on open-tubular column, with Ap-L coating, of *trans*-6-hexadecenoic acid after GLC and TLC-$AgNO_3$ isolation (16:0 added for reference). (D) Partial chromatograph recording from GLC analysis on open-tubular column with Ap-L coating of sample shown in B.

Silver nitrate chromatographic procedures were either large scale (~100 mg) by column, using Florisil-$AgNO_3$ as described elsewhere (3), or semipreparative thin layer chromatography (TLC), using plates prepared with Supelcosil 12D Lot 45E (Supelco Inc., Bellefonte, Pa.). Plates were activated for 1 hr at 100 C before use and developed at room temperature in benzene-hexane, 1:1. Visualization was by spraying with 0.2% 2',7'dichlorofluorescein and scanning under UV light.

NMR spectra were measured at 60 m H_z with a Varian A 60 spectrometer. Solutions in $CDCl_3$ (with TMS standard) were made up in 40 μl integral sphere microcells. IR spectra were obtained on samples of pure film (NaCl plate) with a Perkin Elmer 237.

Ozonolysis was carried out in methanol with oxidative work-up (13). Products were identified through direct GLC of C_5 - C_{12} monocarboxylic acids (14) and by in situ esterification with 2,2-dimethoxypropane (13) for study of methyl esters of mono- and dicarboxylic acids.

RESULTS

Preparative Florisil-$AgNO_3$ chromatography failed to give an adequately clean separation of the methyl ester of the unknown (*trans*-6-hexadecenoic acid) from total marine oil fatty acid esters owing to the rapid elution behavior of certain of the range of marine oil *cis* isomers in several chain lengths commonly found in marine oils (3-8, 15). More economy of effort was achieved by isolation of the C_{16} methyl esters by preparative GLC followed by TLC on the Supelcosil plates. The unknown C_{16} material gave a clearly defined spot between 16:0 and a major component presumed to be palmitoleate (*cis*-9-hexadecenoate) on the basis of an R_f similar to that of methyl oleate (*cis*-9-octadecenoate) (16). The R_f value of the unknown was essentially the same as that of methyl elaidate (*trans*-9-octadecenoate). The unknown recovered from the TLC plates was identical in GLC behavior to the unusual C_{16} component illustrated in Figures 1 and 2. This

isolative technique was reproducible and applicable without difficulty to all samples. Column chromatography was however applicable to the C_{16} fraction (order of elution: 16:0, unknown, palmitoleate), but was used only for a few large-scale preparations on pooled preparative GLC effluents.

On hydrogenation the unknown gave a material indistinguishable from 16:0 by various GLC techniques. The IR spectrum showed a strong absorption band at 10.33 μ. On a semi quantitative basis this absorption, and that of the rest of the spectrum, were similar to that of methyl elaidate, and the general absence of specific NMR details suggested a double bond in the central portion (approx Δ^4 to Δ^{11}) positions (17). There was no spectral evidence for methyl branching or methyl substitution at the double bond. Oxidative fission of material recovered from chromatographic steps indicated >96% purity in terms of 10:0 monocarboxylic acid (identified as acid and as methyl ester) and 6:0 dicarboxylic acid (identified as methyl ester). An ozonolysis of total C_{16} fraction from the preparative GLC gave 16:0, 10:0, 7:0 mono- and 6:0 and 9:0 dicarboxylic acids in approximately the proportions indicated by open-tubular GLC analysis of the total C_{16} fraction.

The above results indicated that the unknown methyl ester from leatherback turtle oil should be the methyl ester of *trans*-6-hexadecenoic acid. The melting point of the acid recovered after saponification of ester (poorly defined crystals from petroleum ether) was 33-34 C, and is consistent with this proposed structure on the basis of similarity to melting points listed for various C_{18} monoethylenic acids (18). The GLC behavior of the ester on BDS and Ap-L vis a vis methyl palmitoleate was also compatible with this structure when comparisons were made with published retention data for methyl esters of C_{18} monoethylenic acids (9), and with data obtained in our laboratory for these materials on BDS and Ap-L open-tubular columns supporting the broad applicability of the literature data (10).

Subsequent to our identification of this acid in leatherback turtle oil a sample of *P. sellowii* seed oil became available. Recovery of the methyl ester of *trans*-6-hexadecenoic acid, previously indicated as a component of this oil (19,20), gave complete coincidence of components in several TLC and GLC systems tested (see below).

DISCUSSION

Trans-6-hexadecenoic acid amounted to 2-3% of the total fatty acids in the initial sample of oeatherback turtle oil, about the same in a different sample (somewhat oxidized), and in loggerhead depot fat, but was not as obvious (<1%) in the fat from the ridley. The presence of this acid in three different marine species of diverse origin would seem to indicate the deposition of this acid from a common food source. There is no apparent occurrence of either *trans*-6- or *trans*-8-octadecenoic acid which might be related. A preliminary screening of a number of other marine lipids for *trans*-6-hexadecenoic acid suggests that the occurrence of *trans*-6-hexadecenoic acid is limited to animals such as the marine turtles and the ocean sunfish (*Mola mola*) which are known to feed heavily on jellyfish (21). Further research on this basic source of this acid is planned.

Comparative features of the GLC behavior of some methyl esters of hexadecenoic acids are shown in Figure 3. A precise study of retention data remains to be carried out, but it may be noted that the *cis*- and *trans*-6-hexadecenoates (ratio 2:1) fail to separate from each other on this BDS column and effectively occupy the position usually assigned to *cis*-7-hexadecenoate in analyses of the methyl esters of fatty acids from marine lipids. In the alternative viewpoint of structure and GLC retention times, where significance is assigned to the ω value (carbon chain moiety terminating in the methyl group), this means that, in the 16:1 acids, *cis* ω10 and *cis* ω9 do not really separate from each other, but separate from *cis* ω7. Paralleling this, in the 18:1 acids, *cis* ω12 (petroselinate) and *cis* ω9 (oleate) show no separation on BDS columns with as many as 50,000 plates, although a small but useful separation of *cis* ω11 and *cis* ω9 is observed (3,22), but all of these 18:1 acids separate from *cis* ω7. In screening seed oils for petroselinic and related acids by open tubular GLC (polar columns) it might therefore be useful to examine the minor C_{16} acids as indicators of the probable presence of C_{18} analogues not detectable directly.

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