Wax Esters of Barracudina Lipid: A Potential Replacement for Sperm Whale Oil

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ABSTRACT

A sample of barracudina, a small fish potentially available in large quantities off Nova Scotia, contained 17.7% body lipid of which 10% was triglyceride and 85% wax ester. The triglyceride, of calculated iodine value 48, was unusually rich in 14:0 (25.8%), 18:0 (4.3%), 20:0 (1.19%), 22:0 (0.45%) and 24:0 (0.75%). The wax ester fatty acids had a calculated iodine value of 126 and a "normal" marine oil fatty acid composition. The wax ester fatty alcohols contained 42.2% hexadecanol, 29.5% octadecenols, 8.2% eicosenols and 3.8% docosenols. Certain interrelationships between fatty alcohols and fatty acids are indicated by details of composition. The potential exploitation of barracudina lipid as a substitute for sperm oil in some uses appears possible.

INTRODUCTION

Technology in the fishing industry has always been capable of developing new fishing areas for traditional species, but more and more is modifying traditional fishing techniques to increase catches in areas otherwise already thought to be fully exploited. Midwater trawling is one such technique which is now being exploited in conjunction with modern echolocation apparatus and operation. Exploratory fishing off Nova Scotia in the spring of 1970 showed large echolocation returns which were investigated by midwater trawl. The final catch consisted of cod (*Gadus morhua* Linnaeus), but substantial tonnages of the white barracudina (*Paralepis rissoi krøyeri* Bonaparte 1840) were observed in the nets. Because of the size disparity between the barracudina and the 4.5 in. mesh of the net, which was designed for cod, only a few barracudina specimens were boated and brought ashore for examination. Our interest centered on the lipids.

Unexpectedly the lipids of the barracudina were found to be dominated by wax esters. Although the extent of the resource is at present unknown, it is evidently large, and it is possible that a fishery could be developed to process these fish with recovery of large amounts of oil potentially suitable as a substitute for oil from sperm whale *(Physeter catodon)*. We present the available data for evaluation on this basis and to stimulate interest in this presently untapped resource.

EXPERIMENTAL PROCEDURES

The area from which samples were received was Sydney Bight (off Sydney, Cape Breton Island, Nova Scotia, Canada). Fish were taken from 20-70 fathoms in water 115-160 fathoms deep, from 1680-1820 hr on or about April 1, 1970. They had been stored on ice for 6 days prior to examination.

Eight fish averaged 25.4 cm in length and 27 g in weight. Six fish were beheaded and eviscerated. The bodies were pooled (weight, 97.2 g) and extracted by the Bligh and Dyer procedure (1) with a lipid recovery of 17.7%. A Wijs iodine value of the total lipid was 126.4. The viscera were kept but later lost in a freezer failure.

Lipid (0.5 g) was applied to a chromatographic column of beads of styrene divinylbenzene gel and eluted with benzene (2). The pattern recorded by differential refractometry (see below) was unusual. Monitoring of fractions by thin layer chromatography (TLC) on silica gel (Eastman Chromagram) developed in petroleum ether-diethyl ether-acetic acid 90:10:1 showed that in addition to phospholipid fraction I (~0.8%), and a sterol ester fraction IV (~1%) there was a large wax ester component III overlapping the usual triglyceride component II. The lipid in benzene fractions II-III could be separated on Prekote Adsorbosil-5 (Applied Science Laboratories) silica gel plates (solvents as above) to give approximate recoveries (from original lipid) of 85% wax ester, 10% triglyceride, 3% free fatty acid and 1% combined mono- and diglycerides.

Individual TLC plate runs of all lipids, except wax esters, were esterified or transesterified by refluxing for 10 min with 7% BF_3 in MeOH (3). The wax ester bands were recovered and saponified by AOCS Method Ca-6b-53 with an increase in saponification time to 3 hr. The saponification mixture was acidified and an equal volume of water was added. Lipids were extracted with diethyl ether which was washed and dried over anhydrous sodium sulfate. The wax ester fatty acids and alcohols were separated by TLC on Prekote plates as described above. The recovered fatty acids were esterified as described above and the recovered fatty alcohols converted to acetates by refluxing with acetic anhydride for 30 min.

Gas liquid chromatography (GLC) was carried out on several open-tubular columns coated with butanediol succinate (BDS) polyester, each 150 ft (ca. 50 m) in length and 0.01 in. (ca. 0.25 mm) ID. All operating temperatures were 170 C or 175 C, and pressure of carrier gas (helium) was 40 or 60 psig as dictated by column parameters. The apparatus was by Perkin-Elmer, either Model 226 or Model 900. Other details of GLC operations have been described elsewhere (4). Additional studies were carried out with a similar column-coated with Apiezon-L, operated at 180 C and 60 psig helium.

Parts of samples of methyl esters of fatty acids and acetates of fatty alcohols were hydrogenated for qualitative and quantitative examination of minor saturated fatty acids. This information also permitted comparison of chain lengths with those from detailed GLC studies. Composition

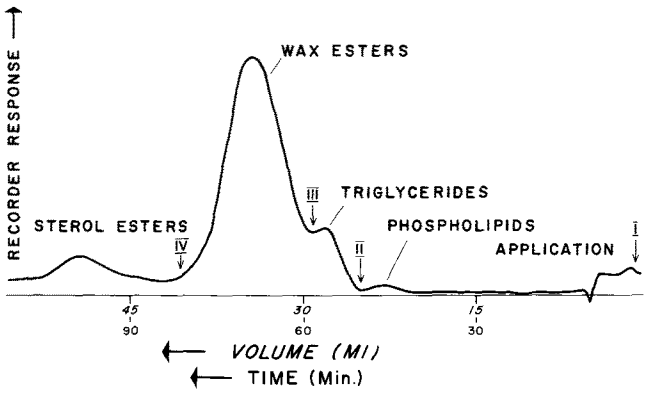

FIG. 1. Chart recording from differential refractometer monitoring benzene eluant from polystyrene bead column. Phospholipids (I) and sterol esters (IV) were ca.1% each of column load of 0.5 g of barracudina lipids, triglycerides (II) 10% and wax esters (III) 85%.

TABLE I

Comparisons of the Fatty Acid and Fatty Alcohol Compositions of Total and Specific Lipids From Barracudina Bodies[a]

Lipid		Weight %						
		Acids[b] from total lipid	Acids from triglycerides	Acids from phospholipids	Free fatty acids	Acids from mono- and diglycerides	Acids[b] from wax esters	Alcohols[b] from wax esters
	12:0	0.23	0.20	0.22	0.47	ND	0.44	ND
Iso	13:0	0.16	0.25	ND	0.12	ND	0.16	ND
Aiso	13:0	Trace	Trace	ND	Trace	ND	Trace	ND
	13:0	0.12	0.20	0.15	0.13	ND	0.08	ND
Iso	14:0	0.16	0.45	0.35	0.22	ND	0.15	ND
	14:0[c]	8.01	25.85	2.53	13.88	1.66	6.23	3.84
Iso	15:0	0.63	1.25	0.36	0.79	0.50	0.42	0.13
Aiso	15:0	0.25	0.50	0.06	0.23	0.32	0.24	0.03
	15:0	0.65	1.52	1.00	1.13	0.55	0.53	0.48
Iso	16:0	0.08	0.23	0.16	0.11	0.13	0.06	0.22
	16:0[d]	7.52	17.94	26.98	18.38	15.93	6.27	42.16
Iso	17:0	0.20	0.27	ND	0.08	0.73	0.16	0.36
Aiso	17:0	0.03	0.07	ND	0.03	0.21	0.05	0.27
	17:0[e]	0.49	0.74	1.12	0.65	0.43	0.66	0.36
Iso	18:0	0.08	0.12	0.16	0.20	0.06	0.09	0.54
	18:0	0.87	4.28	22.62	6.80	32.18	0.41	7.06
	19:0	0.05	0.05	0.05	0.05	0.18	0.09	0.11
	20:0	0.63	1.19	7.48	2.19	19.20	0.23	0.23
	22:0	0.08	0.45	7.45	1.62	19.00	ND	0.04
	24:0	0.10	0.75	ND	ND	0.37	0.36?	ND
	14:1ω9	0.03	ND	ND	0.07	ND	ND	ND
	14:1ω7	0.09	0.05	ND	0.05	ND	ND	ND
	14:1ω5	0.36	0.71	0.02	0.33	0.06	0.26	ND
	15:1ω9	0.01	0.03	ND	ND	ND	ND	ND
	15:1ω8	0.02	0.05	Trace	0.27	ND	ND	ND
	15:1ω6	0.01	0.02	ND	ND	ND	ND	ND
	16:1ω9	0.82	1.23	0.16	0.65	0.06	0.74	ND
	16:1ω7	12.05	8.62	1.54	13.49	0.55	14.47	0.47
	16:1ω5	0.54	0.98	0.11	0.65	0.06	0.54	ND
	17:1ω9	0.24	ND	0.05	0.05	ND	0.06	ND
	17:1ω8	0.64	0.24	0.16	0.54	0.08	0.62	ND
	17:1ω6	0.16	ND	0.06	0.02	ND	ND	ND
	18:1ω9[f]	24.93	17.73	5.24	21.77	0.66	31.86	21.31
	18:1ω7	3.01	3.61	3.43	4.77	1.21?	2.17	7.31
	18:1ω5	0.22	0.24	0.11	0.22	ND	0.13	1.03
	19:1ω9	0.04	ND	ND	0.06	ND	0.06	ND
	19:1ω8	0.05	0.05	ND	0.10	0.02	0.09	ND
	19:1ω7?	0.03	ND	ND	ND	ND	ND	ND
	19:1ω6	0.03	ND	ND	0.01	ND	0.03	ND
	20:1ω11[g]	0.16	0.21	ND	0.26	0.18	0.29	0.12
	20:1ω9	3.92	3.35	0.39	3.92	0.72	3.73	7.22
	20:1ω7	0.35	0.13	1.36?	0.16	0.22?	0.31	0.90
	20:1ω5	0.03	0.05	0.48?	0.05	0.91?	0.01	0.11
	22:1ω13+11	3.65	1.88	0.65	3.19	1.19	4.26	4.47
	22:1ω9	0.86	0.51	ND	0.52	ND	1.05	0.75
	22:1ω7	0.03	0.06	ND	0.13	ND	0.06	0.30
	22:1ω5	ND	ND	ND	ND	1.66?	ND	ND
	24:1ω	0.16	ND	ND	0.21	ND	0.35	ND
	16:2ω6	0.05	ND	ND	0.11	ND	0.24	ND
	16:2ω4?	0.16	ND	ND	ND	ND	ND	ND
	18:2ω6	1.89	0.52	0.48	0.63	0.03	1.91	0.11
	20:2ω6	0.13	0.11	ND	0.13	ND	ND	ND
	22:2ω6	0.05	ND	ND	ND	ND	ND	ND
	16:3ω3	ND	ND	ND	0.16	ND	0.12	ND
	18:3ω6	0.05	0.10	ND	0.06	ND	0.12	ND
	18:3ω3	1.48	0.20	1.76	0.20	0.20	1.16	ND
	20:3ω9?	0.03	0.01	ND	ND	ND	ND	ND
	20:3ω6	0.05	0.04	ND	ND	0.18	0.04	ND
	20:3ω3	0.18	0.12	ND	ND	0.06	0.13	ND
	22:3ω6	0.10	ND	ND	ND	ND	ND	ND
	16:4ω3	0.37	0.12	1.62	0.12	0.24	0.43	ND
	18:4ω3	2.15	0.31	0.45	0.06	ND	1.89	ND
	18:4ω1?	0.05	0.01	ND	ND	ND	ND	ND
	20:4ω6	0.32	0.09	0.65	ND	0.04	0.40	ND
	20:4ω3	0.81	0.19	0.37	ND	0.05	0.77	ND
	20:4ω6	0.03	ND	ND	ND	ND	0.06	ND
	22:4ω3	0.04	ND	ND	ND	ND	ND	ND
	20:5ω3	9.12	0.54	8.65	ND	ND	7.53	ND
	21:5ω2	0.26	ND	ND	ND	ND	0.11	ND
	22:5ω6	0.15	ND	ND	ND	ND	0.10	ND
	22:5ω3	0.73	0.08	ND	ND	0.71?	0.41	ND
	22:6ω3	9.79	1.52	6.52	ND	ND	6.94	ND

[a]ND = not determined.

[b]Corresponding mole % composition available from authors on request.

[c]Includes 4,8,12-trimethyltridecanoic acid

[d]Includes 2,6,10,14-tetramethylpentadecanoic acid.

[e]Includes 3,7,11,15-tetramethylhexadecanoic acid.

[f]Includes small amounts of 18:1ω11.

[g]Includes small amounts of 20:1ω13.

TABLE II

Weight Percentages of Important Components of Wax Esters of Barracudina and Sperm Whale Body and Head Oils

Ester	Fatty acids			Fatty alcohols		
	Barracudina	Sperm body[a]	Sperm head[a]	Barracudina	Sperm body[a]	Sperm head[a]
14:0	6.2	3.3	14.4	3.8	3.2	11.0
14:1	0.3	2.4	33.3	---	---	---
16:0	6.3	8.1	2.8	40.2	24.9	49.7
16:1	15.7	26.9	9.5	0.5	9.6	4.4
18:0	0.4	1.1	0.2	7.0	4.3	3.5
18:1	34.2	33.3	4.9	29.6	44.9	27.3
20:1	4.3	10.9	0.9	8.3	5.0	0.5
20:5ω3	8.5	1.7	---	---	0.3	Trace
22:1	5.3	2.2	---	5.5	0.2	---
22:5ω3	0.4	0.8	0.2	---	0.6	Trace
22:6ω3	8.9	2.1	0.3	---	---	---

[a]Data from Reference 28.

data is given to two decimal places solely to show the relatively small proportions of certain components. Quantitative errors may be of the order of 5-10% in major (>10%) components, 10-25% for middle range components (2-10%) and correspondingly greater for lesser components. Identifications for many minor components are tentative; where important, these are referred to in the discussion. Not determined (ND) usually refers to a component not recognizable at the 0.5% level, if present, under the specific sample load and analytical conditions employed. The use of (?) in Table I indicates that the observed peak had the correct retention time for the component indicated in at least one analysis but could not be confirmed by other GLC data.

RESULTS AND DISCUSSION

Details of Lipids and Fatty Acid

The results of the gel chromatography (Fig. 1) were unexpected, but wax esters were recognized from the unpublished results of attempts to separate wax esters and triglycerides by this technique. The large wax ester peak also coincided with the solvent volume corresponding to free fatty acids and mono- and diglycerides. The phospholipids examined were recovered directly from the gel chromatography, but the neutral lipids were further separated by TLC prior to recovery of fatty acids and conversion to methyl esters.

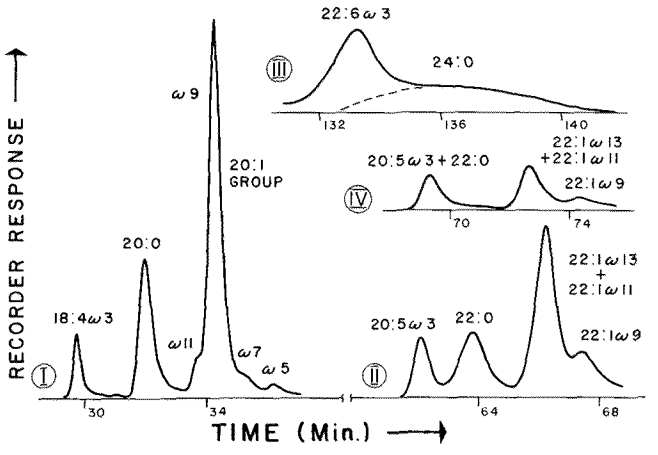

FIG. 2. Sections of recorder charts from open-tubular gas liquid chromatographic analyses of methyl esters of fatty acids from barracudina triglycerides. I-III, BDS column no. 18 of unusually low polarity operated at 175 C and 40 psig helium. IV, BDS column no. 20 of normal polarity operated at 175 C and 60 psig helium. Both columns of stainless steel tubing 150 ft x 0.01 in. and coated with butanediolsuccinate polyester. Note that 20:0, 22:0 and 24:0 peaks are twice the normal width for esters of unsaturated acids on column 18, and that 22:0 has a retention time identical with 20:5 ω3 on column 20.

The results of the fatty acid analyses (Table I) were also unexpected in certain details. The total lipid fatty acids (calculated iodine value 118) were dominated by the wax ester fatty acids (calculated iodine value 136), modified mostly by the fatty acids of the triglycerides. The latter showed unexpected features distinguishing them from the triglycerides in most marine fish. Prominent among these were the high proportion (25.85%) of 14:0. Normal values for fish of similar size and habitat, but with triglyceride-based lipids, would be in the 5-10% range. The triglycerides of other fish with both triglycerides and wax esters in muscle lipid, i.e., *Ruvettus pretiosus* (5-7), *Lepidocybium flavobrunneum* (7), *Allocyttus verrucosus* (8) and several lantern fish (Myctophidae) (9), do not show excessive levels of 14:0. Associated with this acid were higher than usual proportions of *iso* and *anteiso* 15:0, and of 15:0 itself. The level of 16:0 was high, but not abnormal; but the trend to higher values for saturated fatty acids extended very markedly into 18:0 at 4.3% (normally 1-2%) and the higher acids 20:0 (1.2%), 22:0 (0.5%) and 24:0 (0.8%). Because of the presence of the 20:0 at just over 1% of the barracudina triglycerides, particular attention was also paid to the detection and measurement of 22:0 and 24:0. In addition to carrying out these analyses on the AP-L open-tubular columns, where they do not overlap with even chain components from other chain lengths (10), we were able to verify and determine these two acids on a BDS open-tubular column with an unusual low polarity for this polyester. Because of this property, the C_{20} acids did not overlap into 22:0, and most C_{22} appeared before 24:0 (Fig. 2). Triglycerides in some of the lantern fish with wax ester-rich lipids show elevated (4-8%) levels of 18:0 (9). Usually 20:0 is measured at ca. 0.1% in fish and marine mammal triglycerides, and 22:0 is barely detectable.

The special composition of the triglycerides is reflected in the calculated iodine value of 48. There is some indication that several of the triglyceride fatty acids are related more closely to the wax ester fatty alcohols than to the wax ester fatty acids. This view is based on the proportions of 16:0, 16:1 and 18:0, and on the monoethylenic isomer details for 18:1 fatty acids. On the other hand, 14:0, and especially the polyunsaturated fatty acids which are lacking in the wax ester fatty alcohols, cannot be related to the alcohols. The type of equilibrium relationship among the three lipids has been discussed elsewhere (11-15).

The phospholipids, free fatty acids, and mono- and diglycerides are possibly also interrelated as lipid hydrolysis in fish tissue tends to concentrate on phospholipids (16,17). The weight per cent recovery suggests only limited hydrolysis of phospholipids, and the high proportion of 14:0 and acids of the C_{15} group in the free fatty acids

suggest that triglyceride hydrolysis also took place (17). The monoethylenic isomer details are especially interesting since in the mono- and diglycerides 18:1 ω7 exceeded 18:1 ω9, plausibly reflecting an origin in the phospholipids. Previous work has shown that the 1 position is richer in 18:1 ω7 than the 2 position in phosphatidyl ethanolamine, or both positions in the phosphatidyl cholines (18). The proportion 20:1 ω7 > 20:1 ω9 of the phospholipids (compare also 18:1 ω7 and 18:1 ω9) requires further study, but this fraction could include monoacyl phosphatides from specific or partial hydrolysis. The components tentatively identified as 20:1 ω5 and 22:1 ω5 in the mono- and diglycerides are also unusual for teleost marine lipids.

The wax esters are from body lipid, possibly an important consideration when making comparisons with wax esters specifically associated with fish roe as in the family Mugilidae (mullets) (19,20) or gouramis (14,15). Comparisons are more realistically made with species where the wax esters are in the muscle. The fatty alcohols, however, conform to the usual pattern (11) in that the main component is *a*16:0 (The prefix *a* indicates a fatty alcohol). The presence of substantial proportions of *a*20:1 and *a*22:1 has been reported for *L. flavobrunneum* (7) and *A. verrucosus* (8), but most other species cited herein apparently contain only minor amounts (≤3%) of *a*20:1 and less *a*22:1. The monoethylenic isomer distribution in the C_{20} and C_{22} chain length of the alcohols has parallels in the fatty acids, and therefore supports the accepted view of reasonably free interconversion of acids and alcohols which is also applicable to the 18:1 alcohols in elasmobranch fishes (21). The low proportion of *a*16:1 is, however, evidently due to a conversion to *a*18:1 ω7 if one accepts that de novo synthesis of monoethylenic acids in fish implies desaturation in the $\Delta^{9,10}$ position (22). If, as suggested, the triglyceride acids are related to the wax ester alcohols, this could be part of a systematic chain length alternation effect for the shorter chain acids whereby 14:0 → *a*16:0 and 16:1 → *a*18:1. The high proportions of 16:1 ω9 to 16:1 ω7 in the triglyceride fatty acids is unusual and suggests that this alteration is reversible. The fatty alcohols lack polyunsaturated materials except for a trace of 18:2 ω6. Dihydrophytol was tentatively identified but is not included in the data.

The fatty acids in the barracudina wax esters are low in 16:0 and high in 18:1. This trend is also especially apparent in acids of the wax esters of the lantern fish (9), and to some extent in *A. varrucosus* (8), *R. pretiosus* (5-7), *L. flavobrunneum* (7) and *Latimeria chalumnae* (23). The ratio for 18:1 ω9 relative to 18:1 ω7 in the wax esters is much higher than is commonly observed in triglycerides for marine fish. This, taken with the high percentage for 18:1 ω9, implies de novo synthesis of the latter. Barracudina are meso- or bathypelagic, and wax ester containing 18:1 may be important in buoyancy control (24,25). The figures for primary food source C_{18} acids such as 18:2 ω6, 18:3 ω3 and 18:4 ω3 are higher in the wax ester acids than in the triglyceride acids but so are the longer chain successor acids 20:4 ω6, 20:5 ω3, 22:6 ω3, etc., with no detectable pattern or preference by type. These "normal" C_{20} and C_{22} polyunsaturated acids are also found in the wax ester fatty acids of some, but not all, of the other fish species in which body wax esters have been examined (5,7-9).

Potential as a Replacement for Sperm Oil

Oil from sperm whales *(Physeter catodon)* is not permitted entry to the U.S. since this animal is considered an endangered species (26). It would therefore be useful to be able to substitute barracudina oil. An oil from commercial reduction would be mostly the wax esters and triglycerides. This mixture would be akin to sperm body oil in some respects, but the degree of unsaturation would be higher as indicated by the Wijs iodine value of 126.4 for total lipid extract. The iodine value of sperm body oil is usually in the range 70-96 and the unsaponifiable matter content is 17-44%. The latter figure, when doubled, gives the rough percentage of wax ester in the oil. A defined part of the sperm oil is the spermaceti, a wax ester which may be recovered from either the head (case) oil, or body oil, by crystallization. The fatty alcohol portion of the barracudina wax esters is probably fully acceptable for use as a fatty alcohol product or as a wax ester component. A more detailed examination of combined acid and alcohol moieties (27) would indicated the potential of the oil for other uses typical of sperm oil. However the barracudina fatty acids contain somewhat higher levels of polyunsaturated fatty acids than are desirable (see Table II for comparison) although these acids are normal components of sperm body oil. Confusion exists in the literature, as authors often do not have defined sperm whale oil samples to work with, and it is difficult to tell if the sample is spermaceti, head oil, body oil or a commercial oil made from all the oil in the whale. One recent detailed report (28) is available for comparison (Table II). Polyunsaturated fatty acids have, however, also been independently identified in sperm whale oil (29), as have the corresponding alcohols (29,30). A selective hydrogenation of the barracudina oil would reduce the content of oxidation-susceptible *cis*-methylene-interrupted fatty acids (31). The effect of this treatment on other properties of the oil and wax esters remains to be investigated.

For such an evaluation and to develop a fishery, larger amounts of barracudina are needed. Since echosounding indicated schools measurable in dimensions of miles in the Sydney Bight catch of April, and in June barracudina were reported as "abundant" off Cape George, Newfoundland (MacPhail, private communication), there could be a very large resource, although at present it is not known if the schools could be located annually. The probable existence of such schools is otherwise known from examination of the food and feeding habits of the swordfish *Xiphias gladius* (32). In addition to location problems and development of suitable fishing methods for barracudina, it would also be necessary to evaluate reduction or solvent extraction methods and the nutritive value of the meal produced at the same time. While the protein properties of meals made from most fish are satisfactory, it would be desirable to ensure that residual wax ester lipid had no adverse effects in feeding. Such fears are probably groundless at the usual levels involved in food formulations for poultry, if other experience with wax esters is reproducible and applicable (6,33).

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