Anomalous High Iodine Value of Squalene and the Impact on Iodine Values of Shark Liver Oils

R.G. Ackman*, E. Macpherson, and A. Timmins

Canadian Institute of Fisheries Technology, DalTech, Dalhousie University, Halifax, Nova Scotia B3J 2X4, Canada

ABSTRACT: Squalene has six ethylenic bonds, but the experimental iodine values in two different solvent systems—chloroform and cyclohexane/acetic acid—were 25% higher than the theoretical values. We propose that this results from an additional halogen adding at each of the two terminal ethylenic bonds carrying two methyl groups. In the solvent system of cyclohexane alone, the excess is only 3–4% greater than the theoretical. Mixtures of squalene in seal oil confirmed the additivity of the experimental squalene high iodine value and the seal oil fatty acid iodine value with reasonable accuracy but depended on the skill of the operator in obtaining the titration end point for cyclohexane/acetic acid. This observation has particular relevance for shark liver oils and olive oils.

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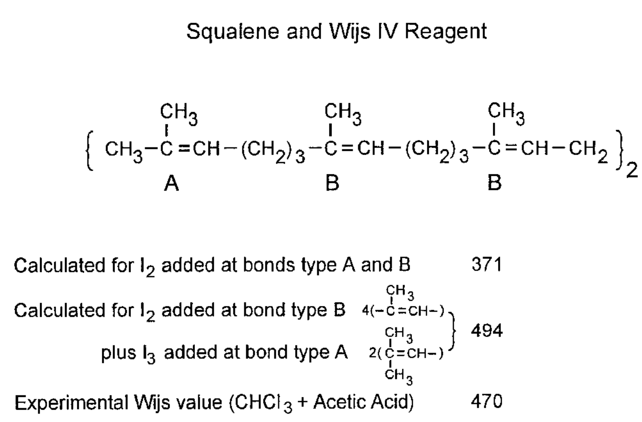
KEY WORDS: Iodine value, olive oil, shark liver oil, squalene.

The hydrocarbon squalene is most commonly associated with shark liver oils (1,2), although it is also important in the unsaponifiables of olive oil (3–5) and is even found in the deodorizer condensate from soy and canola oil refining (6). Not all shark liver oils are rich in squalene, but liver oils from some deep-water species have extremely low levels of polyunsaturated fatty acids and substantial contents of squalene (7). The iodine value (IV) commonly used in the characterization of marine oils can still be relatively high in such cases if squalene is present. Generally, the major part of the IV of marine oils is made up of the contributions from eicosapentaenoic acid (EPA or 20:5n-3, IV 421) and docosahexaenoic acid (DHA or 22:6n-3, IV 465). The total of these two fatty acids may be in the range of 10 to 30% in the fatty acids of common marine oils (8–10), and minor polyunsaturated fatty acids can add to the total with another 3–5% of fatty acids with quite high IV. Nevertheless, in our laboratory, agreement between the contribution of the fatty acid composition to the IV and of the portion of the IV calculated from the theoretical IV of squalene (371) was generally poor for shark liver oils.

At some time in the past 40 yr of research on marine oils in Halifax, chloroform replaced the carbon tetrachloride of the original Wijs reaction. No difference in IV was obvious as a result of this change, and we have published many papers with the term "Wijs iodine value" in the methods. However, to be completely correct, in this report we will describe our version of AOCS method Cd 1-25 (corrected 1991) as a "modified" Wijs method.

We have now determined the experimental IV of squalene of 99.5% purity (Sigma Chemical Co., St. Louis, MO) by the modified Wijs IV method to be 470 (technician A) or 468 (technician B). An explanation of the differences between the theoretical and experimental values may lie in the different structures of the ethylenic bonds in squalene. Four of the six ethylenic bonds of the squalene molecule have one methyl group attached (type B), but the terminal ethylenic bonds (type A) have two methyl groups attached. Scheme 1 shows that, if the iodine (actually iodine monochloride) adds halogen molecules equally to all six bonds, the calculated IV would be 371. However, if the terminal type A ethylenic bonds were to add three halogens instead of two, the sum of A + B contributions would lead to an IV of 494 for squalene.

Harp seal oil contains about 20% marine-type polyunsaturated fatty acids but little squalene, and the IV for one oil sample is basically 151. To test the influence of the experimental IV for squalene, as it might influence the IV of marine oils such as shark liver oils, 10.0% squalene was added to this seal oil. The results of averaged duplicate determinations of



SCHEME 1

*To whom correspondence should be addressed at Canadian Institute of Fisheries Technology, DalTech, Dalhousie University, 1360 Barrington St., P.O. Box 1000, Halifax, Nova Scotia B3J 2X4, Canada.
E-mail: odorjr@dal.ca

TABLE 1
Tests of the Effect of Different Iodine Value Solvent Systems on Squalene, on a Seal Oil, and on a Mixture, Carried Out in Duplicate

Solvent	Squalene	Seal oil only	Seal oil with 9.2% squalene
Chloroform (our modified Wijs)	463, 464	145, 145	168, 169[a]
Cyclohexane only	384, 387[b,c]	140, 143	163, 162
Cyclohexane and glacial acetic acid	460, 463	138, 138	163, 158

[a]Calculated additive value 174.
[b]Determined on different days by technician A, averages of two analyses on each day.
[c]Independently, technician B obtained values of 380 and 378.

IV are as follows: experimental by modified Wijs IV, seal oil only = 151; experimental by modified Wijs IV, seal oil with 10% squalene = 182; calculated for seal oil with squalene from the modified Wijs IV = 136 + 47 = 183. This simple test served two purposes; first, it validated our experimental modified Wijs IV for squalene; second, it confirmed the additive values for marine oil plus squalene, and it suggested comparisons with newer IV reagent/solvent systems.

The AOCS replaced the halogenated solvents of method Cd 1-25 (corrected 1991) with cyclohexane in 1993 (method Cd 1b-87, revised 1990) and later with a mixture of cyclohexane and acetic acid as the solvent [Method Cd 1d-92 (95)]. Publication of Cd 1b-87 (cyclohexane) included the stipulation that it was for oils in the IV range of 15 to 70. The newer method Cd 1d 92 (95) had no such restriction but was originally tested with a fish oil with the low IV of 109. A detailed summary of this work as a collaborative study has been published (11). A fish oil of higher IV (*ca.* 193) has been used in another collaborative study with cyclohexane/acetic acid and was reported in detail (12). The recent publication of two papers on these two methods with fish oil (13,14) is therefore welcome. A different lot of seal oil with 9.17% squalene added was tested with the three solvent systems, with interesting results in view of these recent publications on marine oils and AOCS methods for IV determination. The cyclohexane solvent gave a result for squalene that was only slightly above the theoretical value of 371 (Table 1). In our hands cyclohexane/acetic acid showed a slight drop in the IV for a different seal oil when only cyclohexane was used to replace chloroform. The cyclohexane/acetic acid titration end point is difficult because the biphasic and agitated solvent mix is grey, making the starch-blue end point hard to determine. Any overshoot reduces the experimental IV, which is possibly the cause of a further reduction in the seal oil IV. A smaller amount of squalene was used, because of its high IV. The end point for squalene with cyclohexane plus acetic acid was accordingly easier to observe, giving better agreement with our modified Wijs method, although slightly less than observed previously.

These observations are a part of an ongoing examination of the best methods for the analysis of shark liver oils, or of any oil or oil product that contains squalene. Some olive oils contain as much as 12 g of squalene per kg (5), which could have a small but noticeable effect (4 IV units) on IV compared to that computed from the fatty acid composition. The proposed difference between interactions of iodine monochloride with the two types of ethylenic bonds of squalene calculates in a satisfactory manner and could be checked out with model monoethylenic compounds. No explanation can be offered for the lower IV for squalene obtained in cyclohexane. If shark liver oils that contain squalene are being characterized, cyclohexane and acetic acid is now the preferred system for IV, rather than cyclohexane alone.

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