• Letter to the Editor

A Simple Procedure for Obtaining Docosahexaenoic Acid

Highly unsaturated fatty acids have aroused conspicuous interest recently, especially for metabolic and nutritional studies. The availability of large quantities of these acids is of paramount importance for such investigations. Nevertheless relatively pure, commercially available fatty acids are prohibitively expensive for large-scale use.

This letter reports an improved fractionation technique for obtaining almost 90% docosahexaenoic acid in large quantities. The method provides a notable simplification over previous procedures (1,2,3). In this work the starting material was a commercially available fish oil with a high proportion of docosahexaenoic acid.

Menhaden oil fatty acids, purchased from Archer Daniels Midland Co. (I.V. 182, docosahexaenoic acid content, 10%) were fractionated in a centrifugal molecular still. Fractions of increasing mol wt and unsaturation were cut successively; they represented 5, 30, 21, 20, and 11%, respectively, of the original acids. A 749 g sample of the last fraction (I.V. 315, NE 320) was added dropwise in 1–2 hr to a stirred solution of 530 g of urea in 3.15 l of methanol at 35C under N_2. The mixture was heated to 45C for 1 hr and cooled slowly with stirring. After 18 hr at 16C the precipitate was filtered, washed with urea-saturated methanol, and discarded. The filtrate and washings were heated to 45C with 325 g of urea until solution occurred. After slow cooling the mixture was held at 13C overnight and filtered. The filtrate was evaporated to 1.5 l on a rotary evaporator under a partial N_2 atmosphere below 25C, cooled 8 hr at 13C, and filtered. One-sixth of the filtrate was rotary-evaporated successively with 400 ml and 250 ml of methanol saturated with urea at 25C. The final filtrate was poured into water; the lipid fraction was dissolved in petroleum ether, washed with water, and dried over sodium sulfate. The solvent was removed on a rotary evaporator to leave 31.5 g of docosahexenoic acid [or 189 g from all of the starting acids; I.V. 401; docosahexaneoic acid, 88% by gas-liquid chromatography (GLC)]. A sample of the acid was methylated by refluxing for 12 min with methanol (3.3 ml/g) and concentrated hydrochloric acid (1 ml/100 ml methanol). Colored impurities were removed by passing a petroleum ether solution of ester through a short column of Fisher silica gel (28–200 mesh). Residual impurities were removed by molecular distillation (I.V., corrected, 420.5; calculated from GLC, 420.7). All I.V. are by the Hanus method, which gives lower values for highly unsaturated lipids than the Wijs method. The correction factor is Wijs/Hanus = 436/416. Methyl docosahexaenoate and its saturated derivative, methyl behenate, were identified by GLC comparison with authentic compounds.

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REFERENCES

1. Stoffel, W., and E. H. Ahrens, Jr., J. Lipid Research, *1*, 139–146 (1960).
2. Hammond, E. G., and W. O. Lundberg, JAOCS, *30*, 438–441 (1953).
3. Privett, O. S., R. P. Weber, and E. C. Nickell, *Ibid.*, *36*, 443–449 (1959).

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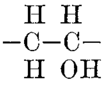
• Errata

JAOCS, *39*, page 454, October, 1962, Rheineck et al.: Chemistry and Technology of Some Drying Oil Fatty Acid Esters of Polyvinyl Alcohol. Under the subheading "Use of Other Solvents," the first paragraph should read: Polyvinyl alcohol is highly hydrogen bonded and has a solubility parameter calculated by Small's (29) method to be 9.1 for the unit.



$$\begin{array}{cc} H & H \\ -C- & C- \\ H & OH \end{array}$$

The parameter can be calculated from the sum of the values of groups or elements, thus

$$S = \frac{d \Sigma G}{M}$$

For polyvinyl alcohol this is:

M = molecular weight per unit, 44
d = density, 1.21
CH_2 = 1.33
CH = .28
OH = 1.70
Σ G = 3.31

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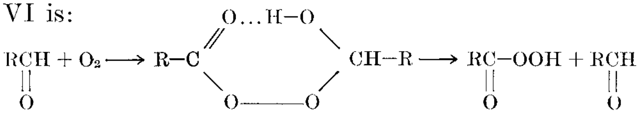
JAOCS, *39*, 480–487, November, 1962, Silbert: Fatty Pfroxides: Synthesis, Analysis, and Reactions. Page 483, first column, under the subheading "Aldehyde Oxidation," first paragraph, Structure VI is:

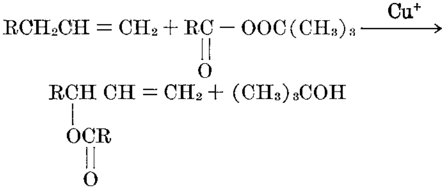


Page 484, first column, under heading "Polarography," reference in the first sentence should read: (1o).

Page 484, second column, under heading "Infrared," third paragraph, first sentence should read: The structure of hydrogen peroxide has been found to be. . . . The third sentence should read: Various peroxide types differ somewhat in the dihedral angles (Table II), being lower for peracids (72°), due to hydrogen-bonding, and slightly higher for di-t-butyl peroxide (123°) due to steric hindrance.[3]

Page 484, footnote #3, change 95C–120C to: 95°–120°.

Page 486, equation (28) should read:



$$RCH_2CH=CH_2 + \underset{\underset{O}{\|}}{RC} - OOC(CH_3)_3 \xrightarrow{Cu^+} \underset{\underset{\underset{\underset{O}{\|}}{OCR}}{|}}{RCH}\,CH=CH_2 + (CH_3)_3COH \qquad (28)$$

Page 487, reference 24 should read: Davies, A. G., R. V. Foster, and A. M. White, J. Chem. Soc., *1953*, 1541.