to the micelles is likely. Also, the presence of hydrated silanols on "non-end-capped" columns reduces the effectiveness of added surfactants to the mobile phase, primarily due to interactions of these groups with the polar solvent. Other advantages of using micellar mobile phases in the analysis of triglycerides on reverse phase columns over traditional mobile phases include improved peak shape, which permits better integration and the use of less solvent. The use of a pseudo mobile phase in reverse phase chromatography provides an alternative in the analysis of hydrophobic solutes.

REFERENCES

1. Horvath, C., and W. Melander, American Laboratory, p. 17 (1978).
2. Plattner, R.D., JAOCS 58:638 (1981).
3. Plattner, R.D., and K. Payne-Wahl, Lipids 14:53 (1979).
4. McKay, V., and T. Ascah, Chromatographer 23:1 (1982).
5. Armstrong, D.W., American Laboratory, p. 14 (1981).
6. Graham, J.A., and L.B. Rogers, Chrom. Sci. 18:614 (1980).
7. Armstrong, D.W., and S.J. Henry, J. Chromatogr. Sci. 5:657 (1980).
8. Horvath, C., W. Melander and I. Molnar, J. Chromatogr. 125: 129 (1976).
9. Lockmuller, C.H., and D.R. Wiler, J. Chromatogr. Sci. 17:574 (1979).
10. Scott, R.P.W., Ibid. 18:297 (1980).
11. Fendler, J., and E.J. Fendler in Catalysis in Micellar and Macromolecular Systems, p. 315, Academic Press, New York, NY, 1975.
12. Singleton, J.A., and H.E. Pattee, JAOCS 61:761 (1984).

[Received August 8, 1984]

Chlorinated Long-Chain Fatty Acids. Their Properties and Reactions. XII. The Dechlorination Pathways of Sodium 9(10)-Chloro-10(9)-Oxooctadecanoates in Aqueous Sodium Hydroxide Solution

M. KETOLA and **U. LESKINEN**, Department of Chemistry and Biochemistry, University of Turku, SF-20500 Turku 50, Finland

ABSTRACT

The dechlorination pathways of the equal mixture of 9-chloro-10-oxo- and 10-chloro-9-oxooctadecanoic acids in aqueous sodium hydroxide solution were investigated. The reaction product mixture of these acids, isolated after dechlorination, was found to contain α-hydroxyoxo and long-chain alkanedioic acids at a weight ratio of 15 to 1. The most abundant compounds formed were 9-hydroxy-10-oxo and 10-hydroxy-9-oxooctadecanoic acids. The minor reaction products consisted of Favorskii rearrangement products, 2-heptyl-1,11-undecanedioic, 2-octyl-1,10-decanedioic and 2-nonyl-1,9-nonanedioic acids. On the other hand, the expected α,β-unsaturated oxoacids could not be detected in the reaction product mixture.

INTRODUCTION

We previously have described the alkaline dechlorination of an equal mixture of 9-chloro-10-oxo- (*1a*) and 10-chloro-9-oxooctadecanoic acids (*1b*), which was found to occur easily and at a rate comparable to those of the corresponding chlorohydrins, i.e. *threo-* and *erythro*-9(10)-chloro-10(9)-hydroxyoctadecanoic acids under similar conditions (1,2). In general, the dehalogenation of α-haloketones by alkoxide bases may yield various reaction products, such as Favorskii esters (carboxylic acid derivatives), α-hydroxy ketals, α-hydroxy ketones, α-alkoxy ketones and α,β-unsaturated ketones (3). In our continuing studies on the reactions of chlorinated long-chain fatty acids, the present paper deals with dechlorination pathways of an equal mixture of *1a* and *1b* in aqueous sodium hydroxide solution as checked by product analysis using chromatography and spectroscopy.

EXPERIMENTAL

Model compounds.

Equal amounts of *1a* and *1b* were prepared by chromic acid oxidation of an equal mixture of *threo*-9-chloro-10-hydroxy- and *threo*-10-chloro-9-hydroxyoctadecanoic acids (15 g) in glacial acetic acid according to Corin et al. (4). The crude product (11 g) was recrystallized three times from petroleum ether (bp 40-60 C) at −17 C to give 5.8 g of *1* (39%): mp (uncorrected) 32.5-36.5 C; ^{1}H NMR(CCl_4) δ 0.90 (t, 3H, terminal $-CH_3$), 1.32 (m, chain $-CH_2$, 2.31 (t, 2H, $-CH_2CO_2H$), 2.63 (t, 2H, $-CH_2CO-$), and 4.10 ppm (broad s, 1H, $-CHCl-$); IR(KBr) 1725 (C=0), 1710 (COOH), and 680 and 600 cm^{-1} (C-Cl). MS of methyl ester mixture (*1a, 1b*), m/z (% rel. intensity): 349(0.2), 347(0.5), 317(1.3), 315(3.8), 310(1), 279(1.3), 250(1), 248(2.7), 185(100), 157(3.4), 157(7), 141(81), 125(31), 57(45), 55(62).

Spectroscopy

IR spectra were obtained with a Perkin Elmer 180 spectrophotometer in KBr. The viscous samples were run as thin films on KBr disks. ^{1}H NMR spectra were recorded on a Jeol PMK 60 spectrometer in CCl_4 with tetramethylsilane as internal reference. Mass spectra were taken on an LKB 9000 GC/MS instrument under electron impact at 70 eV.

Gas-liquid chromatography

A Hewlett Packard 5700A gas chromatograph was equipped with a flame ionization detector (FID) and a 2 m x 3 mm ID. stainless steel column packed with 3% Silar 10C on Chromosorb Q (80/100 mesh). The temperature was programmed from 220 to 260 C, 4 C/min. The GC/MS analyses were performed with a 2.4 m x 3 mm ID. glass column packed with 1% XE-60 on Gas-Chrom Q (100/120 mesh) by programming from 150 to 220 C, 5 C/min. The acids were methylated with diazomethane in diethyl ether containing methanol (9:1, v/v). The TMSi ethers of hydroxy ketonic acid methyl esters were prepared with BSTFA-IMCS (bis(trimethylsilyl)trifluoroacetamide-trimethylchlorosilane, 3:1, v/v) and heated at 75 C for 15 min.

Thin-layer chromatography

Analytical TLC was done on glass plates (20 x 20 cm) with

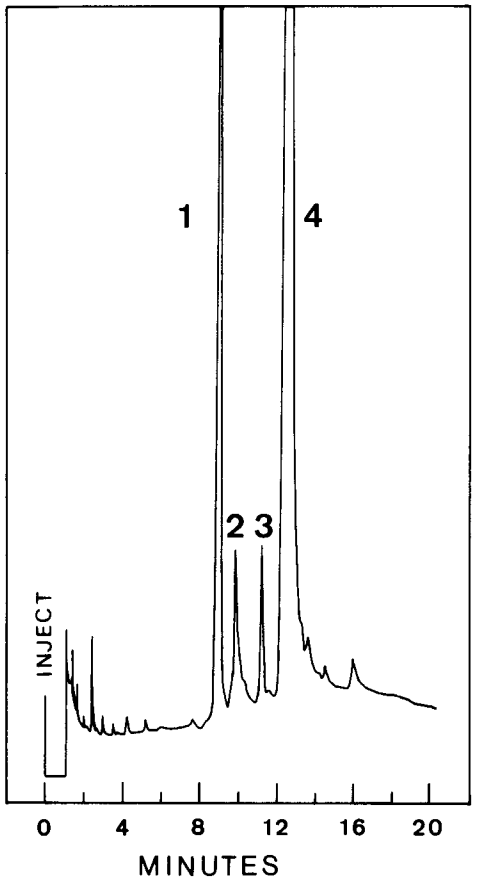


FIG. 1. Total ion current chromatogram of the methylated reaction products formed from *1 (1a,1b)* by aqueous alkali.

a layer of Silica Gel G (0.25 mm wet thickness). The reaction products were fractionated as acids by developing the plates twice with n-hexane-diethyl ether-acetic acid (70:30:1.5, v/v/v). The spots were visualized by charring with sulphuric acid (30%) or by spraying with an ethanolic solution (0.01%) of Rhodamine 6 G and viewing under UV light (254 nm). Preparative TLC was done in the same manner, except that a thicker layer of silica (0.5 mm wet thickness) was used. Separated fractions were scraped off and the compounds were eluted with diethyl ether and acetone.

Dechlorination

A mixture of *1* (700 mg, 2.1 mmol) and an aqueous solution of NaOH (100 ml, 4.2 mmol) was stirred for 4.5 hr at 50 C. After acidification with acetic acid, the aqueous reaction mixture was extracted with diethyl ether (5 x 40 ml). The combined extracts were washed with water and dried over anhydrous sodium sulphate for 24 hr. Evaporation of the solvent in vacuo gave a viscous residue (630 mg), which was further fractionated by TLC and then analyzed by GLC, spectroscopy and GC/MS.

RESULTS

Fractionating of Reaction Product Mixture

The products isolated after dechlorination of *1a, 1b* showed two distinct spots on TLC. The reaction product mixture (200 mg) was divided into two fractions by preparative TLC with four plates. The combined organic material of the upper fraction (Spot 1, Rf 0.60) was 12 mg, and that of the lower one (Spot 2, Rf 0.42) was 174 mg. The total recovery was 93%. In the gas chromatogram of the methylated reaction product mixture presented in Figure 1, two predominating peaks (Peaks 1 and 4) could be observed. The separate GLC of both TLC fractions showed that Spot 1 included exclusively the compounds giving Peak 1, and that Spot 2 contained predominantly compounds giving Peak 4 together with the very small constituents giving Peaks 2 and 3 (Fig. 1).

MS of the methyl esters of Peak 1 (*2a, 2b, 2c*), m/z (% rel. intensity): 342(0.1), 311(1), 283(5), 278(1), 251(3), 250(2), 244(3), 230(5), 216(4), 200(8), 198(12), 186(24), 172(33), 157(12), 155(19), 154(15), 143(21), 139(16), 138(21), 125(11), 112(11), 111(14), 98(59), 97(31), 87(82), 84(29), 83(35), 74(59), 69(56), 55(100).

MS of the methyl esters of Peak 4 (*3a, 3b*), m/z (% rel. intensity): 328(0.2), 310(0.3), 297(2), 279(3), 187(40), 185(11), 173(14), 172(10), 169(23), 157(49), 155(100), 144(20), 141(36), 87(69), 83(30), 74(43), 71(51), 69(43), 57(51), 55(74), 43(57). MS of the silyl ethers of the methyl esters of Peak 4 (*3a, 3b*): 400(0.4), 385(6), 369(3), 353(0.5), 273(6), 259(48), 245(32), 229(16), 215(56), 201(7), 155(25), 141(12), 129(12), 109(14), 103(21), 95(17), 83(22), 75(21), 73(100).

DISCUSSION

The dechlorination reactions of chloroketonic acids (*1a, 1b*) with aqueous sodium hydroxide are outlined in Scheme 1. The reaction product mixture was isolated after dechlorination and examined for its major constituents by GC/MS, and IR and NMR. Figure 2 shows the MS fragmentation patterns for the methylated and silylated derivatives of the principal reaction products.

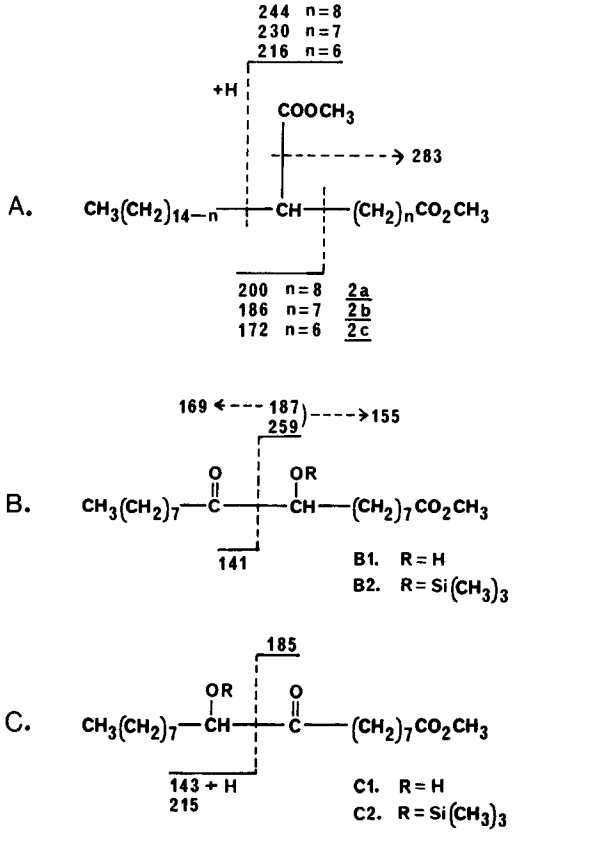


FIG. 2. MS fragmentation of derivatives of the dechlorination products of *1a,1b*. A: 2-heptyl-1,11-undecanedioic (*2a*), 2-octyl-1, 10-decanedioic (*2b*) and 2-nonyl-1,9-nonanedioic acid (*2c*) dimethyl esters; B1: methyl 9-hydroxy-10-oxooctadecanoate (*3a*); B2: its TMSi ether; C1: methyl 10-hydroxy-9-oxooctadecanoate (*3b*); C2: its TMSi ether.

Structures of the Reaction Product Constituents

The upper TLC fraction (Spot 1) contained compounds which gave the IR absorption bands at 2940-2860 (C-H), 1705 (COOH), and 720 cm^{-1} ($-(CH_2)_n-$, $n \geqslant 4$) typical of the long-chain alkanoic acids without any other functional groups. The NMR spectrum gave signals at δ 0.89 (t, 3H, terminal $-CH_3$), 1.31 (broad s, chain $-CH_2$), 2.31 (t, 2H, $-CH_2COOH$) and 10.4 (broad s, $-COOH$). These spectral data with the characteristic NMR signal at δ 2.73 (t, 1H, $-CH_2CH(R)CO_2H$) suggested that the compounds were alkyl-substituted dicarboxylic acids. The mass spectrum (Fig. 2A) of Peak 1 in Figure 1 revealed three isomeric dioic acids. The mass spectrum of methyl esters showed the low molecular ion peak at m/z 342 ($C_{20}H_{38}O_4$) with other salient peaks at m/z 311 ($M-CH_3O$), 276 ($M-2CH_3OH$) and 269 ($M-CH_2CO_2CH_3$). These ions with those in two series, 27 + 14n and 84 + 14n, are typical of the long-chain dibasic acid dimethyl esters (5).

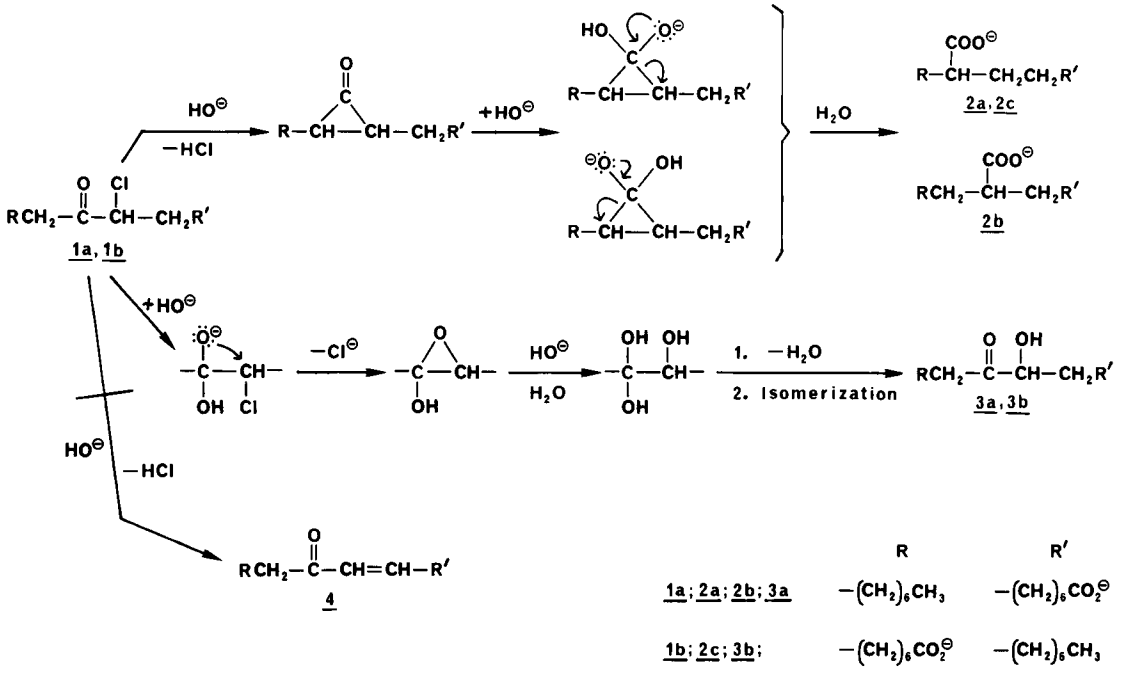
The ion at m/z 283 (M-59) was formed by the loss of the methyl ester group $-CO_2CH_3$ from the tertiary carbon atom C-2 (Fig. 2A). The rearrangement ion peaks at m/z 244, 230 and 216 ($M-(CH_2)_nCH_3 + H$, n = 6,7 or 8) were indicative of ions produced by the cleavage of the alkyl side chain from the C-2 carbon of each three isomeric C_{18} dioic acid esters *2a, 2b* and *2c,* respectively. The other characteristic fragment ions at m/z 200, 186 and 172 ($M-(CH_2)_nCO_2CH_3$, n = 6,7 or 8) showed fission of the 2,3-bonds of these three esters (Fig. 2A). From the electron impact fragmentation patterns and the chromatographic and spectoscopic data, three alkyl-substituted dicarboxylic acid esters giving Peak 1 in Fig. 1 were formulated as 2-heptyl-1,11-undecanedioic (*2a*), 2-octyl-1,10-decanedioic (*2b*) and 2-nonyl-1,9-nonanedioic acid (*2c*) dimethyl esters.

The lower TLC fraction (Spot 2) contained the predominant part of the reaction product mixture. Its compounds gave IR bands at 3460 (OH), 3120 (broad, intramolecular association OH...O=C), 1710 (COOH, C=O), and 720 cm^{-1} ($-(CH_2)_n-$, $n \geqslant 4$). The NMR spectrum gave signals at δ 0.89 (t, 3H, terminal $-CH_3$), 1.30 (broad s, chain $-CH_2$), 2.2-2.5 (m, 4H, $-CH_2CO-$ and $-CH_2CO_2H$), and 4.03 ppm (t, 1H, $-CH(OH)-$). The IR and NMR spectra were consistent with fatty acids containing hydroxy and oxo functions. The gas chromatogram (Peak 4 in Fig. 1) was found to contain two isomeric hydroxy ketonic acids (*3a, 3b*) whose structures were confirmed by MS fragmentations as methyl esters and their TMSi ethers (Figs. 2B and 2C). Methyl esters gave very weak molecular ion peaks at m/z 328 ($C_{19}H_{36}O_4$) followed by other high-mass ion peaks at m/z 310 ($M-H_2O$), 297 ($M-CH_3O$) and 279 ($M-H_2O-CH_3O$). The characteristic fragmentation of one ester *3a* (Fig. 2B) showed strong α-cleavage ions at m/z 141 and 187 (m/z 259 for the silylated ester) indicating that *3a* was 9-hydroxy-10-oxooctadecanoic methyl ester with α-cleavage between the C-9 and C-10 carbon atoms carrying hydroxyl and oxo groups, respectively. The other isomeric ester *3b* (Fig. 2C) gave the great fragment ions at m/z 185 and 144 (m/z 215 for the silylated ester) from which the compound was formulated as 10-hydroxy-9-oxooctadecanoic acid methyl ester. In both cases the ions containing TMSi ether groups predominated over those with oxo groups.

The lower TLC fraction also contained two minor constituents giving Peaks 2 and 3 in Fig. 1. Peak 2 was not due to the expected methyl ester (*4*) with α,β-unsaturated carbonyl structure (MW. 310). The mass spectrum gave a weak molecular ion peak at m/z 326 ($C_{19}H_{34}O_4$), followed by strong α-cleavage ion peaks (% rel. intensity) at m/z 214(2), 185(100), 141(48) and 125(32). These ions indicated that the compound was 9,10-dioxooctadecanoic acid methyl ester. The ions at m/z 185 and 141 were generated by α-cleavage between the C-9 and C-10 carbon atoms with two oxo groups. This dioxo acid apparently is no real reaction product of the alkaline dechlorination of *1 (1a, 1b)* but an impurity formed during the synthesis of the model compound mixture. Peak 3 originated from the starting material *(1a, 1b).*

Dechlorination Pathways

In alkaline water solution, α-haloketones are subjected to several competing reactions, the extent of which depend on reaction conditions and the structures of reacting components (3,6,7). The possible dechlorination pathways of the α-chloro oxo acids *1a, 1b* outlined in Scheme I include (i) 1,3-elimination (Favorskii rearrangement) giving dicarb-



SCHEME I.

oxylic acid derivatives (*2a, 2b, 2c*), (ii) nucleophilic addition-elimination giving α-hydroxy oxo acids (*3a, 3b*), and (iii) formation of α,β-unsaturated oxo acids (*4*) through dehydrochlorination by the 1,2-elimination mechanism. Besides the first two reactions the occurrence of reaction (iii) also is described in the older chemical literature (3). The reaction product analysis showed, however, that α,β-unsaturated acids (*4*) were not formed under the reaction conditions applied here. Therefore, the reaction (iii) was of no importance and could be ignored. Thus, the overall dechlorination of *1a, 1b* actually involved only two reactions, (i) and (ii), yielding dioic and hydroxy oxo acids in a weight ratio of 1 to 15.

The removal of chlorine from *1a, 1b* may occur either before or after addition of hydroxide ion to carbonyl group, i.e. through reactions (i) and (ii), respectively. Reaction (i) is so-called normal Favorskii rearrangement (6,7) involving the formation of a cyclopropanone, which further undergoes ring opening to give two dicarboxylic acid derivatives from both *1a* and *1b* (Scheme I). The dioic acids *2a* and *2c* are formed from *1a* and *1b*, respectively, while the same acid *2b* may be created from both chloro oxo acids applied.

The reaction product analysis showed that dechlorination of *1a, 1b* occurred predominantly through reaction (ii), giving α-hydroxy ketonic acids *3a* and *3b* as principal products (Scheme I). The initial reaction involves nucleophilic attack of hydroxyl ion to carbonyl group, followed by displacement of chloride ion by negatively charged oxygen to give hydroxyepoxy derivatives. The further reactions beyond this intermediate are not fully understood. The epoxy ring obviously is opened with a base producing trihydroxy intermediates, which lead to the final products *3a* and *3b* through dehydration and isomerization reactions. The opening of epoxy ring by an acid reagent can also occur during separation of dechlorination products from the acidified reaction product mixture.

The dechlorination reaction in Scheme I may also play a role in pulp bleaching processes. The treatment of pulp containing resin with chlorine dioxide leads to the formation of α-chlorooxo derivatives of alkenoic fatty compounds as shown by oleic and elaidic acids and their partly dehydrochlorinated derivatives (unpublished results from this laboratory). After treatment with chlorine, pulp is subjected to alkaline extraction, where the alkali-labile compounds formed, e.g. α-chlorooxo compounds may react with alkali and transform into more water soluble derivatives. The high reactivity of α-chloroketones toward alkali is also demonstrated by the unpublished observation that the resin of fully bleached softwood sulphite pulp was found to contain very little if any fatty compounds with α-chloroketonic structure.

REFERENCES

1. Ketola, M., K. Pihlaja and K. Grönlund, Finn. Chem. Lett. 29(1978).
2. Ketola, M., Acta Chem. Scand. 27:1328 (1973); Pihlaja, K., M-R. Lyytinen and M. Ketola, Acta Chem. Scand. B 30:953(1976).
3. Kende, A.S., Organic Reactions 11:262(1960).
4. Corin, P.A., J.F. Spencer and A.P. Tulloch, Can. J. Chem. 39:846(1961).
5. Ryhage, R., and E. Stenhagen, Arkiv Kemi 14:497(1959); Ibid. 23:167(1964).
6. Saunders, W.H., and A.F. Cockerill, Mechanisms of Elimination Reactions, John Wiley, New York, 1973, Chapt. 10.
7. Buncel, E., Carbanions: Mechanistic and Isotopic Aspects, Elsevier, Amsterdam, 1975, Chapt. 5.

[Received July 7, 1984]

♣Determination of Ascorbyl Palmitate by High Performance Liquid Chromatography

THELMA S. VICENTE, EDWARD H. WAYSEK* and WINIFRED M. CORT,[1]
Hoffmann-LaRoche Inc., Product Development & Applications, Nutley, NJ 07110

ABSTRACT

An HPLC method for the determination of ascorbyl palmitate in vegetable oil and lard has been developed. Chromatographic conditions consist of a diamine column, a mobile phase of 70:30 (v/v) methanol:0.02M monobasic potassium phosphate buffer, pH 3.5, and UV detection. Samples were extracted with methanol. An overall average recovery value of 96.7% was obtained for ascorbyl palmitate in five representative vegetable oils and lard.

INTRODUCTION

Ascorbyl palmitate (L-ascorbic acid, 6-hexadecanoate) has been shown to be very effective in the protection of vegetable oils (soybean, corn, peanut, safflower and sunflower) against oxidation (1). Although ascorbyl palmitate (AP) is not as efficient in the protection of animal fats per se, it effectively potentiates alpha- and gamma-tocopherol. AP also has been shown to synergize BHT, BHA, TBHQ and PG in safflower oil emulsions (2) and to synergize tocopherol in citrus oils and vitamin A (3). Recently (4) AP was shown to extend the stability and quality of frying fats.

Ascorbyl palmitate has been used widely in Europe for years. Klaui (5) demonstrated the activity of AP in butter fat, vegetable oils, vitamin A, beta-carotene, ethyl linoleate and ethyl arachidonate for both AP alone and in combination with alpha-tocopherol. Pongracz (6) has experimented extensively with AP in paste mixtures and demonstrated efficacy in butter and butter oil, salad dressings, biscuits, dried potatoes, ice cream mix and dried milk products.

In the United States ascorbyl palmitate is listed in the Code of Federal Regulations, Title 21, under section 182.3149 as a chemical preservative that is generally recognized as safe when used in accordance with good manufacturing practice. Although ascorbyl palmitate itself does not occur in nature, it is enzymatically broken down into ascorbic acid and palmitic acid, which are natural ingredients in food. Unlike many antioxidants, its use is not limited to 0.02% of the fat or oil, and AP may be used at higher levels if necessary.

A number of methods are reported in the literature for the analysis of ascorbyl palmitate. Budslawski and Pogorzelski (7) have reported a colorimetric procedure for the determination of AP. TLC procedures for AP have been reported by Alary et al. (8), Van Peteghem and Dekeyser (9), Pujol Forn (10) and De la Torre Boronat et al. (11). Woollard (12) and Melton et al. (13) have described HPLC

*To whom correspondence should be addressed.
[1]Present address: Cort Consultants, 4935 Brandywine Drive, Sarasota, FL 33583.