Preparation and Stability of a New Series of Sterically Hindered Esters of Vitamin A. I

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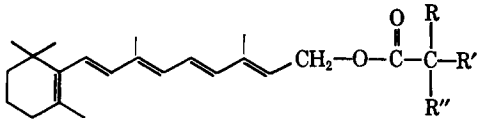
A series of α,α-dialkyl substituted aliphatic esters of vitamin A was prepared. The stability of these compounds was compared with vitamin A palmitate in hydroalcoholic acidic media, alkaline media, and under oxidative conditions. In all cases steric hindrance on the α-carbon of the fatty acid group increased the stability of the esters in solution. Under oxidative conditions it appeared that a long aliphatic chain was necessary for maximal stability. These results indicate that an ester such as vitamin A α,α-dimethylpalmitate could demonstrate optimal stability under all the conditions examined.

FORLANO AND HARRIS (1) demonstrated that the introduction of an electronegative group in the α-position of the acid portion of vitamin A esters increased stability toward acid-catalyzed elimination in anhydrous ethanol. The rate of anhydrovitamin A formation in anhydrous acidic media was slower for the α-chloropropionate than it was for either the palmitate or acetate. However, the resultant polarization of the ester linkage in the α-chloropropionate also rendered the ester more susceptible to solvent-catalyzed elimination in the hydroalcoholic media. These results suggested that vitamin A esters should be prepared from acids containing electropositive groups in the α-position to provide greater stability toward solvent-catalyzed degradation.

Vitamin A trimethylacetate (I) (R,R′, R″ = CH_3) was the first ester prepared using electropositive groups in the acid portion of the ester.



I, R, R′ and R″ = CH_3 or larger alkyl groups

This ester was more resistant toward solvolysis in an alcoholic medium but it also proved to be more stable than the acetate or palmitate toward acid-catalyzed elimination. Since this ester possessed greater stability in both media, it became obvious that steric and electromeric effects were both important considerations in the stability of vitamin A esters. The steric hindrance exerts an "umbrella-type" blocking effect which mechanically prevents reactive reagents from attacking the carbonyl group of the ester. The data obtained in this study showed that there was a direct relation between acidic and basic solution stability and the size of the groups in the α position of the ester. α,α-Disubstituted fatty acid esters were considerably more stable than the palmitate in anhydrous ethanolic 0.01 *N* HCl, 95% and 70% aqueous ethanolic 0.1 *N* HCl, 70% aqueous ethanol, and in alcoholic base. This suggested that reactions which involve either hydrolysis of the ester linkage or lysis of the alkyl-oxygen bond as the initiating step of the degradation, can be diminished appreciably by introducing steric hindrance on the carbon atom adjacent to the carbonyl group.

Oxidation studies of the sterically hindered esters were not very promising when compared with the palmitate in a vegetable oil (Wesson oil). These tests were run at a level of 1,000,000 units of vitamin A ester per gram of solution with and without antioxidants. Only the α,α-methylethylcaproate of the sterically hindered series of esters exhibited any appreciable resistance to oxidation, but it was not as resistant as the palmitate. When the pure compounds used in this study were subjected to oxidation tests, the crystalline pivalate (trimethylacetate) appeared to be the most stable ester. Since the pivalate is a solid and the others are liquid, it was not unreasonable to assume that its apparent stability was due to reduced surface area or to a coating of oxidized material on the surface which prevented the uniform penetration of oxygen through the crystal. This produced results that were misleading as far as the rate of oxidation under use conditions were concerned; consequently, all the oxidation tests were performed in Wesson oil at a level of 10^6 units of vitamin A per gram of solution. This procedure eliminated the variables of physical form and molecular concentration, since these solutions were all of the same molarity. The results of this test indicated that good oxidative stability in vitamin A esters required a high degree of liposolubility and a long aliphatic chain. Molecular models of the palmitate showed that it is possible for the aliphatic portion of the molecule to orient itself alongside the vitamin A

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chain and thus afford a mechanical barrier against attack by oxygen. The relationship between acid chain length and resistance to oxidation clearly showed that chain length was a critical factor. On the basis of resistance to acid degradation, alkaline hydrolysis, solvent-catalyzed degradation, and oxidation, the α,α-methylethylcaproate was found to be most stable of the sterically hindered esters. The major disadvantage of this compound was its inability to resist oxidation to the same extent as the palmitate. Based on the observed results it was theorized that a vitamin A ester of maximal stability should have (*a*) steric hindrance in the α-position of the acid group, and (*b*) a long aliphatic chain. An ester that could fulfill these requirements would be vitamin A α,α-dimethylpalmitate. The study of the preparation and properties of this ester will be discussed in a future publication.

The balance of this paper deals with the methods of preparation, determination of degradation rates, and comparisons of the stability of these esters. Vitamin A β-cyclopentylpropionate and tetrahydrophthalate were also included in this study. While they are not classical aliphatic-type fatty acid esters, it was desirable to observe the effect of other types of steric hindrance.

EXPERIMENTAL

Preparation of the Esters

The esters were prepared and purified using the method described by Forlano and Harris (1). Essentially, vitamin A alcohol dissolved in a methylene chloride–pyridine mixture was treated with the respective acid chloride or anhydride. The acids or anhydrides were obtained from K & K Laboratories, Jamaica, N. Y. The reaction mixtures were purified by alumina column chromatography, and the esters were isolated as viscous yellow oils after evaporating the solvent. The pivalate crystallized from ethanol upon refrigeration as a yellow solid (m.p. 52–55°), and the monotetrahydrophthalate was crystallized from warm petroleum ether (30–60°) and had an m.p. 94–97°. The esters were identified by their IR and UV spectra which are typical for vitamin A esters along with the absorptivity (*a*) 327 mμ, which is a specific identification factor for each ester. The *a* and the wavelength of maximum absorption for each of the esters are presented in Table I. Vitamin A alcohol and acetate are also included in this table for comparison purposes. They were not prepared in this study.

TABLE I—OBSERVED *a* AND $\lambda_{max.}$ IN ISOPROPANOL FOR THE VITAMIN A COMPOUNDS USED IN THIS STUDY

Compd.	m$\mu_{max.}$	*a*
Trimethylacetate	327	130.8
Triethylacetate	327	115.0
α,α-Dimethylvalerate	328	120.9
α,α-Methylethylcaproate	327	118.1
β-Cyclopentylpropionate	326	122.0
Monotetrahydrophthalate	327	118.9
Palmitate	326	93.6
Alcohol	325	183.5[a]
Acetate	326	155.0[a]

[a] These values were obtained from "Merck Index," 7th ed., (1960).

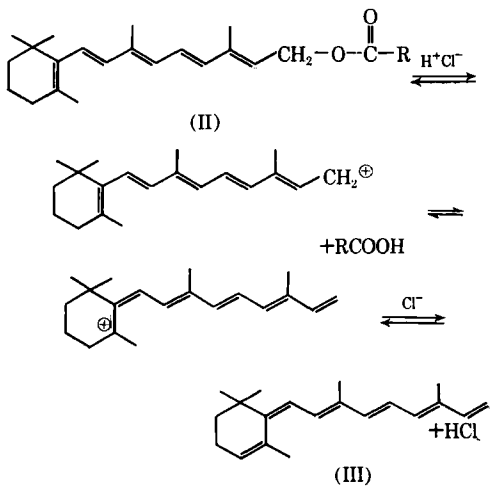
Stability Testing

In order to compare the stability of the hindered esters to that of the palmitate, they were subjected to the tests described below. Since vitamin A palmitate is the most stable ester commercially available, it was chosen as the standard with which to evaluate the new esters. The commercial material, however, was not suitable for these comparative tests, since it contained small quantities of antioxidants. Consequently, the standard used in these experiments was made by the same method used for the other esters.

Stability of Vitamin A Esters in Anhydrous Ethanolic 0.01 *N* HCl—*Part I*—A sufficient quantity of vitamin A ester was added to anhydrous ethanolic 0.01 *N* HCl, such that the initial UV absorbance at the maximum for vitamin A esters (327 mμ) was 0.700. The solutions were maintained at a constant temperature of 22 ± 1°, and changes in UV absorbance at 327 and 391 mμ were measured every 30 min. Anhydrovitamin A, one of the major degradation products under the conditions of this experiment, has a maximum absorbance at 391 mμ and an absorbance at 327 mμ, the maximum for vitamin A, equal to 0.2 the absorbance at 391 mμ. Since vitamin A has a negligible absorption at 391 mμ, the following correction factor for the absorbance for vitamin A at 327 mμ was determined:

$$A_{327\ m\mu\ (corr.)} = \left[A_{327\ m\mu\ (obs.)} - (0.2\ A_{391\ m\mu\ (obs.)})\right]$$

A is the UV absorbance at the specific wavelength. A plot of log $A_{327\ m\mu(corr.)}$, a measure of the concentration of vitamin A in solution, *versus* time was linear; consequently, the first-order rate constants were determined from the slope of this line. Under these conditions, the principal degradation pathway proceeds from the ester (II) to anhydrovitamin A (III), as shown below.



In the following tables, the "stability factor" represents the ratio of the rate constant of the specified ester compared to the rate constant of vitamin A palmitate. The first-order degradation rates are given in Table II.

Stability of Vitamin A Esters in 95% Ethanolic 0.1*N* HCl—*Part II*—The testing procedure used in this case was similar to that used in Part I, except that the solvent here was 0.1 *N* HCl in 95% ethanol. The results are presented in Table III.

Stability of Vitamin A Esters in 70% Ethanolic 0.1 *N* HCl—*Part III*—The testing procedure used in this section is outlined under Part I, except that the solvent here was 0.1 *N* HCl in 70% aqueous ethanol, and the results are presented in Table IV.

Stability of Vitamin A Esters in 70% Ethanol—*Part IV*—Since the degradation rate at 22° was too slow to be measured satisfactorily, the procedure used in this section was similar to that used under Part I, except that the reaction was run at 42 ± 1°, and the solvent here is 70% aqueous ethanol. The results are given in Table V.

Alkaline Hydrolysis of Vitamin A Esters—*Part V*—The method used to determine the resistance to alkaline hydrolysis was a modification of the procedure of Isler *et al.* (2) where 2.0 mmoles of ester was dissolved in 50 ml. of *n*-butanol and 50 ml. of 90% aqueous ethanol containing 2.0 mmoles of NaOH. The solutions were maintained in a constant temperature bath at 42 ± 1° and 10-ml. samples were removed for assay at 45, 80, 170, 235, 285, 330, and 375 min. The quantity of base consumed, a measure of the rate of hydrolysis, was determined by titrating the samples with 0.008 *N* HCl using thymol blue indicator. Since a plot of log concentration *versus* time was linear, it appeared that the reaction followed first-order kinetics. The rates of hydrolysis were determined from the slope of the line and the results are presented in Table VI.

Oxidation Studies—*Part VI*—The first test involved direct exposure of 0.2 ± 0.05 Gm. of accurately weighed pure compound to air at 37°, evenly spread over the surface of a 20-ml. opened Wheaton vial. The rate of oxidation, however, was too rapid to measure accurately; consequently, the experiment was performed at 22°. The amount of vitamin A remaining at any time was determined by dissolving the entire sample in isopropyl alcohol and measuring UV absorbance at 310, 325, and 334 mμ. Since the degradation products of these esters interfered with the direct measurement of vitamin A from the absorbance at 325 mμ, a correction factor (3) was applied to determine the portion of the absorbance at 325 mμ due to vitamin A.

The correction factor used in this experiment to determine the *a* 325 mμ for a 1:25,000 dilution of the sample, is shown below:

$$a_{(\text{corr.})\ (325\ \text{m}\mu)} = \frac{7A_{325(\text{obs.})} - 2.625A_{310(\text{obs.})} - 4.375A_{334(\text{obs.})}}{\text{weight of sample in Gm.}} \times 25$$

A plot of log *a* (corr.) *versus* time was a straight line; consequently, the first-order rate constants were calculated from the slope of the line. The results are shown in Table VII. There did not

Table II—Vitamin A Ester Degradation Rates in Anhydrous Ethanolic 0.01 *N* HCl

Vitamin A Ester	First-Order Rate Constant $(k^1/2.303) \times 10^4$ hr.$^{-1}$	Stability Factor
Palmitate	21.2	1.00
β-Cyclopentylpropionate	21.2	1.00
Monotetrahydrophthalate	11.4	1.85
Pivalate	3.4	6.25
α-Methyl-α-ethylcaproate (MEC)	3.4	6.25
α,α-Dimethylvalerate (DMV)	3.2	6.70
Triethylacetate (TEA)	2.7	7.87

Table III—Vitamin A Ester Degradation Rates in 95% Ethanolic 0.1 *N* HCl

Vitamin A Ester	First-Order Rate Constant $(k^1/2.303) \times 10^4$ hr.$^{-1}$	Stability Factor
β-Cyclopentylpropionate	19.6	0.99
Palmitate	19.4	1.00
Monotetrahydrophthalate	10.0	1.94
Pivalate	6.1	3.15
α-Methyl-α-ethylcaproate	5.0	3.88
α,α-Dimethylvalerate	5.0	3.88
Triethylacetate	4.5	4.30

Table IV—Vitamin A Ester Degradation Rates in 70% Ethanolic 0.1 *N* HCl

Vitamin A Ester	First-Order Rate Constant $(k^1/2.303) \times 10^4$ hr.$^{-1}$	Stability Factor
β-Cyclopentylpropionate	18.4	0.96
Palmitate	17.7	1.00
Monotetrahydrophthalate	11.8	1.50
Pivalate	6.0	2.96
α-Methyl-α-ethylcaproate	5.9	3.00
α,α-Dimethylvalerate	5.4	3.28
Triethylacetate	3.2	5.52

Table V—Vitamin A Degradation Rates in 70% Ethanol at 42°

Vitamin A Ester	First-Order Rate Constant $(k^1/2.303) \times 10^4$ hr.$^{-1}$	Stability Factor
Monotetrahydrophthalate	20.0	0.63
Palmitate	12.5	1.00
β-Cyclopentylpropionate	12.2	1.02
Pivalate	6.5	1.93
α,α-Dimethylvalerate	4.8	2.60
α-Methyl-α-ethylcaproate	2.7	4.63
Triethylacetate	2.2	5.70

Table VI—Rates of Vitamin A Ester Hydrolysis by NaOH in *n*-Butanol–90% Ethanol Mixture

Vitamin A Ester	First-Order Rate Constant $(k^1/2.303) \times 10^5$ min.$^{-1}$	Stability Factor
Palmitate	161.0	1.00
β-Cyclopentylpropionate	58.8	2.73
Pivalate	30.1	4.72
Monotetrahydrophthalate	34.9	5.24
α,α-Dimethylvalerate	13.4	12.05
Triethylacetate	8.1	20.00
α-Methyl-α-ethylcaproate	6.2	26.31

TABLE VII—OXIDATION RATES OF VITAMIN A ESTERS (PURE COMPOUNDS) EXPOSED TO AIR AT 22°[a]

Vitamin A Ester	First-Order Rate Constant ($k^1/2.303$) × 10^4 hr.$^{-1}$	Stability Factor
Palmitate	433.0	1.00
α-Methyl-α-ethylcaproate	228.0	1.90
β-Cyclopentylpropionate	216.0	2.00
Monotetrahydrophthalate[b]	175.0	2.47
Triethylacetate	142.2	3.05
α,α-Dimethylvalerate	71.4	6.06
Pivalate[b]	9.5	43.30

[a] It is noteworthy that the oxidation pattern of the pure compounds is quite different from oxidation in solution. This may be related to differences in physical form which is eliminated by solution studies. [b] Crystalline esters.

appear to be any induction period in this study, however, this is not surprising since no antioxidant was present.

Subsequently, the oxidative stability of these esters was determined under use conditions in Wesson oil solutions with butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) as antioxidants. The esters were dissolved in commercial Wesson oil at a level of 1,000,000 units per gram of solution and the antioxidants at a level normally used in commercial preparations were added. Accurately weighed samples of 0.5 Gm. were placed into 20-ml. Wheaton vials and transferred to a 37° oven with free access to air. Since plots of log concentration of vitamin A *versus.* time in this case were not linear, the length of the "induction period" was considered the criterion of stability. This period represents the time lag before oxidation begins after the sample is exposed to air. Following the induction period, oxidation and destruction of the vitamin A molecule proceeds rapidly. The cyclopentylpropionate and monotetrahydrophthalate were not included in the solution experiments because of their insolubility in Wesson oil at the level of 10^6 units per gram. The lengths of these periods are given in Table VIII.

The oxidation tests in Wesson oil solutions were repeated without antioxidants and at a lower temperature (22°). The same conditions were used as in the previous section, except that the antioxidants were not included. Under these conditions it appeared that esters having the longer chain lengths in the acid portion have a measurable induction period which is not true of the short chain esters. The results are presented in Table IX.

TABLE VIII—INDUCTION PERIODS FOR VITAMIN A ESTER SOLUTIONS IN VEGETABLE OIL WITH ANTIOXIDANTS AT 37° (10^6 UNITS OF VITAMIN A PER GRAM)

Vitamin A Ester	Induction Period, hr.
Palmitate	161
α-Methyl-α-ethylcaproate	150
Triethylacetate	32
α,α-Dimethylvalerate	32
Pivalate	30

TABLE IX—INDUCTION PERIODS FOR VITAMIN A ESTER SOLUTIONS IN VEGETABLE OIL WITHOUT ANTIOXIDANTS AT 22° (10^6 UNITS OF VITAMIN A PER GRAM)

Vitamin A Ester	Induction Period, hr.
Palmitate	53
α-Methyl-α-ethylcaproate	41
α,α-Dimethylvalerate	0
Pivalate	0
Triethylacetate	0

SUMMARY AND CONCLUSIONS

The results of the stability testing of the new esters when compared to vitamin A palmitate indicate the following:

1. Steric hindrance in the α-position of the acid portion of vitamin A esters confers stability with respect to acidic or alkaline degradation.

2. Vitamin A α,α-dimethylvalerate (DMV), α-methyl-α-ethylcaproate (MEC), and triethylacetate (TEA), were four to eight times more stable than the palmitate in anhydrous and hydroalcoholic acidic solutions.

3. The DMV, MEC, and TEA esters were five times more stable than the palmitate in 70% aqueous ethanol.

4. The DMV, MEC, and TEA esters were approximately 20 times more stable than the palmitate in alkaline hydrolysis studies.

5. The α,α-methylethylcaproate was found to be the most stable sterically hindered ester of the series because of its superior stability in solution relative to the palmitate, and its ability to compare somewhat favorably with the latter ester when subjected to air oxidation studies.

6. Since the palmitate and α,α-methylethylcaproate both showed stability, it can be considered that a long aliphatic chain (C_6—C_{16}) in the fatty acid portion is necessary for resistance toward oxidation. A future publication will deal with a sterically hindered ester, having a long aliphatic chain such as vitamin A α,α-dimethylpalmitate.

7. The β-cyclopentylpropionate and monotetrahydrophthalate were not as stable as the sterically hindered aliphatic esters under any of the conditions examined.

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Keyphrases

Vitamin A esters, α,α-dialkyl substituted—synthesis

Degradation rates—sterically hindered vitamin A esters

Column chromatography—separation

IR spectrophotometry—identity

UV spectrophotometry—identity