Sterically Hindered Esters of Vitamin A II: Vitamin A α,α-Dimethylpalmitate

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Abstract □ A previous study showed that short-chain sterically hindered esters of vitamin A demonstrated better stability in solution than the commercial plamitate. The oxidative stability of the short-chain sterically hindered esters, however, was poor. This work indicated that a long aliphatic chain with steric hindrance in the α-position was necessary for maximal stability. A compound of this type, vitamin A α,α-dimethylpalmitate, was prepared and evaluated.

Keyphrases □ Sterically hindered vitamin A esters □ Vitamin A α,α-dimethylpalmitate—synthesis □ Stability—vitamin A α,α-dimethylpalmitate □ UV spectrophotometry—analysis □ IR spectrophotometry—analysis

The possibility of developing a vitamin A ester of optimum stability was suggested by the observation that steric hindrance in the α-position of the acid portion was required for good solution stability and that a long aliphatic chain was required for maximal oxidative stability (1, 3). Previously, Forlano and Harris (2) prepared a series of vitamin A esters containing an electronegative group such as chlorine in the α-position of the acid portion. These esters demonstrated increased stability in anhydrous ethanolic HCl compared to commercial vitamin A palmitate. Biologically inactive anhydrovitamin A, formed by the elimination of a molecule of water, was the main degradative pathway in acidic solutions. Since it also was observed that the resultant polarization of the ester linkage by the electronegative groups rendered these esters more sensitive to solvent-catalyzed elimination, the increased stability in anhydrous acidic media was somewhat academic.

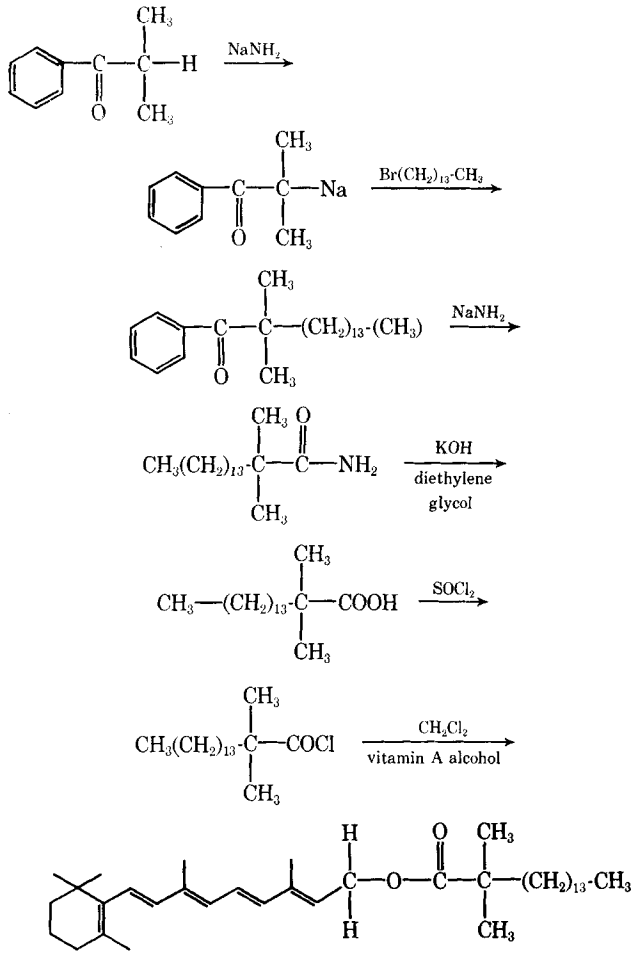
A careful re-examination of the Forlano and Harris data (2) indicated that the replacement of the electronegative groups with electropositive groups should yield esters having greater stability towards solvent- and base-catalyzed attack. A series of compounds incorporating electropositive groups in the α-position of the esters (1, 3) were demonstrated to resist such attack. The excellent stability pattern of these esters in alcoholic acid was quite unexpected, since it was assumed that the introduction of an electropositive group would increase the basicity of the carbonyl group and consequently favor proton-catalyzed attack. It became evident that steric factors as well as electronic effects were important parameters in the stability of vitamin A esters. These data indicated that reactions which involved either hydrolysis of the ester or lysis of the alkyl oxygen bond as the initiating step of the degradation were considerably decreased by the introduction of steric hindrance in the α-position of the acid. The increase in steric hindrance from a methyl to an ethyl group produced a slight but significant increase in solution stability. These short-chain (C_3–C_6) esters, however, lacked good oxidative stability, suggesting the need for a long aliphatic chain.

From previous studies, therefore, it was determined that the features needed for optimum stability in a vitamin A ester are:

Table I—Time Required for Complete Oxidative Degradation of Vitamin A Esters at 64°

	Vitamin A Palmitate Commercial	Vitamin A α,α-Dimethyl-palmitate
Additives	BHA,[a] BHT[b]	BHA, BHT
Degradation time, hr.	64	184
Stability factor (ratio to vitamin A palmitate)	1.0	2.86

[a] Butylated hydroxyanisole. [b] Butylated hydroxytoluene.



Vitamin A α,α-dimethylpalmitate

Scheme I

Preparation of Vitamin A α,α-Dimethylpalmitate

Table II—Stability of Vitamin A Esters at 20° in the Presence of Diacetyl Tartaric Acid Mono- and Diglycerides With and Without Corn Oil

Time, days	DMP[a] units/g.	DMP[a] % Retained	DMP + Corn Oil units/g.	DMP + Corn Oil % Retained	VAP[b] units/g.	VAP[b] % Retained	VAP + Corn Oil units/g.	VAP + Corn Oil % Retained
0 (est.)	324,000	—	324,000	—	324,000	—	324,000	—
11	278,000	86	329,000	100	182,000	56	263,000	81
21	227,000	70	303,000	94	122,000	38	215,000	66
42	208,000	64	292,000	90	No UV[c]	0	182,000	56
90	158,000[d]	49	255,000[d]	79	No UV[c]	0	No UV[c]	0

[a] Vitamin A α,α-dimethylpalmitate. [b] Vitamin A palmitate. [c] No detectable vitamin A by UV method; samples had solidified indicating that considerable oxidation and polymerization had occurred. [d] Samples still fluid at this time.

1. Steric hindrance in the α-position of the acid portion, provided by electropositive alkyl groups, which protect the carbonyl and alkyl oxygen groups from reactive species through physical blockage and electronic effects.

2. A long aliphatic chain to protect the conjugated double bond system from oxygen.

Vitamin A α,α-dimethylpalmitate, Scheme I, which was not prepared previously theoretically appeared to satisfy all these requirements, and this report, describing its preparation and properties, shows that it does. The biological availability of vitamin A from the α,α-dimethylpalmitate and α,α-methylethylcaproate will be discussed in a future report.

EXPERIMENTAL

The α,α-dimethylpalmitic acid was prepared by the method of Bui-Hoi *et al.* (4). The acid chloride, prepared by reacting the acid with thionyl chloride, was subsequently condensed with vitamin A alcohol to form the ester.

Materials—Sodamide 90%,[1] isobutyrophenone,[2] 1-bromotetradecane,[3] and diethylene glycol.[1]

Procedure—Eighty-eight grams sodamide (2.03 moles) was added to 600 ml. boiling dry toluene in a three-neck flask equipped with a reflux condenser, dropping funnel, and thermometer; 300 g. isobutyrophenone (2.03 moles) was added dropwise to the stirring mixture and the system was refluxed for 1 hr. after the addition was completed. The reaction mixture was cooled to room temperature and 400 g. 1-bromotetradecane (1.45 moles) was added and stirring was initiated. After the initial evolution of heat subsided, the mixture was refluxed for 4 hr. with stirring. The cooled mixture was slowly poured into 5 l. 5% acetic acid solution cautiously, since some unreacted sodamide may be present.

The separated organic solution was washed with water until free of base, dried with anhydrous Na_2SO_4, and the toluene was removed by atmospheric distillation. The isobutyrophenone and 1-bromotetradecane were subsequently removed by vacuum distillation at 85–95° $_{15\ mm.}$ and 151–168° $_{8\ mm.}$, respectively. There were 486 g. α,α-dimethylpalmitophenone (1.41 moles) collected at 213–217° $_{6\ mm.}$ (87% yield) and this was added slowly to a suspension of 72 g. sodamide in 600 ml. boiling anhydrous toluene with stirring. The suspension was refluxed for 8 hr. and the cooled mixture was carefully washed with water to destroy any excess sodamide. Emulsification at this point can be reversed by distilling the mixture, since water forms an azeotrope with toluene which distils below the boiling point of both solvents. The residue was vacuum distilled using an air condenser, and 344 g. α,α-dimethylpalmitamide was collected by distilling between 229–235° $_{10\ mm.}$. Then 344 g. (1.21 moles) of the amide was hydrolyzed in 1250 ml. diethylene glycol containing 265 g. KOH by heating the mixture at 210–220° for 16 hr. with constant agitation. The cooled solution was diluted with three times its volume of water and made strongly acidic with concentrated HCl. The free fatty acid, which rose to the top of the solution, was removed with chloroform. The chloroform layer was washed with water until neutral and the solvent was removed. The residue was distilled under vacuum and 200 g. of a material corresponding to α,α-dimethylpalmitic acid was collected at 225–236° $_{20\ mm.}$. The boiling point was the same as that reported by Bui-Hoi *et al.* (4).

The acid was converted to 142 g. of the acid chloride, b.p. 148–150° $_{1\ mm.}$ by refluxing 197 g. of the former compound with 166 g. thionyl chloride in $CHCl_3$. Subsequently, 81 g. of the acid chloride was reacted with 80 g. vitamin A alcohol to yield 53 g. of vitamin A ester, purified by the alumina-column chromatographic procedure of Forlano and Harris (2). This material had an absorption maximum at 327 mμ, which is typical of vitamin A esters, and an a = 84.71 in isopropyl alcohol corresponding to 1.52×10^6 units of vitamin A/g. The IR spectrum was typical of a vitamin A ester showing no alcohol peaks. The heavy metal content was insignificant.

Vitamin A esters are not crystalline and because of their high molecular weight and lack of thermal stability they do not lend themselves to melting point or boiling point determinations. Consequently, the major determination of purity for vitamin A esters is the a determination (a measure of the concentration of vitamin A chromophore) at the vitamin A ester's UV maximum. The absence of shoulders on either side of the maximum indicated the absence of oxidative, isomerization and eliminative degradation products in the sample. The IR spectrum showed an absence of starting materials such as alcohols, carboxylic acids, or acid chlorides. The combination of column chromatography, proper UV maximum, and a value with an absence of shoulders and a good IR spectrum indicated that the product was chemically pure.

Testing Under Use Conditions—The new ester and commercial vitamin A palmitate were studied in a series of practical use tests. The first was a thin-film oxidation test at 64° by the previous method (1). The time required for the complete oxidation of the ester (Table I) indicated that the new ester was more resistant to oxidation than commercial vitamin A palmitate. All vitamin A assays were performed by the USP method (5).

Since vitamin A palmitate is the most stable commercial ester of vitamin A in resisting acid degradation, the relative stability of the α,α-dimethylpalmitate was determined in an acidic medium known to cause rapid degradation of the palmitate. The agent used was an acidic emulsifying agent, diacetyl tartaric acid mono- and diglycerides.[4] The tests were conducted by dissolving enough vitamin A ester in the emulsifying agent to produce an initial concentration of 325,000 units/g. In the second part of this experiment the esters were dissolved in a mixture of equal parts of the emulsifying agent and corn oil (Wesson Oil). The samples were stored at 20° and were periodically assayed by the USP method (5). Table II shows that commercial vitamin A palmitate was completely inactivated in 42 days while the α,α-dimethylpalmitate ester retained half its original potency for twice as long. Corn oil prolonged the stability of the samples possibly due to a reduction in concentration of the acidic emulsifying agent.

The stability of the esters in the emulsifying agent was also determined at 28°. Table III shows that vitamin A palmitate was completely inactivated in half the time at 28° than at 20°. The slower rate of decomposition of the α,α-dimethylpalmitate was approximately the same at both temperatures.

[1] Matheson Coleman & Bell.
[2] Eastman No. 6272.
[3] Eastman No. 3558.

[4] Marketed as TEM-4C by Hachmeister Division, H. J. Heinz Co.

Table III—Stability of Vitamin A Palmitate and Vitamin A α,α-Dimethylpalmitate in Diacetyl Tartaric Acid Mono- and Diglycerides at 28°

Time, days	Vitamin A Palmitate, units/g.	Vitamin A α,α-Dimethylpalmitate, units/g.
1	301,000	283,000
10	202,000	—
15	—	243,000
21	No UV curve[a]	216,000
31	No UV curve[a]	177,000
41	No UV curve[a]	179,000
53	No UV curve[a]	158,000
73	No UV curve[a]	115,000

[a] At the time of assay, there was no UV absorption peak corresponding to vitamin A indicating complete loss of vitamin A potency.

The tests in Tables II and III were quite rigorous because the dispersing agent contained noticeable quantities of free acetic acid. The results clearly indicate the new ester's superior ability to withstand strong acidic conditions and acid-catalyzed degradation.

Further evidence of resistance to acid degradation may be found in the incidence of isomerization occurring in the diacetyl tartaric acid mono- and diglyceride mixtures. Isomerization, no doubt a pathway in acid-catalyzed degradation, is not readily detected because the molecule remains intact although there is a loss of biopotency. The degree of isomerization in terms of maleic values, as determined by reaction with maleic anhydride and colorimetric measurement (6), is given in Table IV. A value of approximately 33% represents the percentage of 13-*cis* isomers present and approaches isomeric equilibrium. The degree of isomerization of the α,α-dimethylpalmitate ester is smaller than that for the palmitate ester in the presence of the acidic emulsifier. Corn oil retarded isomerization and prolonged potency of the vitamin A esters probably by a reduction in concentration of the acidic emulsifying agent.

SUMMARY AND CONCLUSIONS

When the stability of a new ester of vitamin A was compared with commercial vitamin A palmitate, the following observations were made:

1. Vitamin A α,α-dimethylpalmitate was considerably more resistant to auto-oxidation than commercial vitamin A palmitate.

Table IV—Degree of Isomerization of Vitamin A/Esters (in Terms of Maleic Values[a]) in the Presence of the Acidic Emulsifier, Diacetyl Tartaric Acid Mono- and Diglycerides at 25°

Time, days	DMP[b]	DMP + Corn Oil	VAP[c]	VAP + Corn Oil
0	<5	<5	<5	<5
11	8.2	8.6	9.3	6.3
21	10.8	9.6	19.0	9.7
42	12.6	9.7	22.4	11.8
90	16.4	12.7	36.3	21.6

[a] Expressed as % of 13-*cis* isomers present; value of 33% indicates approximate isomeric equilibrium. [b] Vitamin A α,α-dimethylpalmitate. [c] Commercial vitamin A palmitate.

2. The new ester was more stable than vitamin A palmitate in the presence of the acidic emulsifier, diacetyl tartaric acid mono- and diglycerides.

3. Corn oil appears to retard the loss in vitamin A ester potency due to both acid-catalyzed degradation and isomerization probably by a reduction of the concentration of the acidic emulsifying agent.

4. Isomerization of the new ester is somewhat slower in the presence of the acidic emulsifier than the commercial palmitate.

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