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Sterically Hindered Esters of Vitamin A III: Biological Availability of Vitamin A from Sterically Hindered Esters

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Abstract □ Previous studies demonstrated that sterically hindered esters of vitamin A, such as the α,α-dimethylpalmitate and α-methyl-α-ethylcaproate, had some superior chemical properties compared to the palmitate. The biological availability of vitamin A from these esters, compared with the palmitate and cod liver oil as controls, was determined in rats. Maximum availability of vitamin A was obtained with the palmitate followed by cod liver oil. Availability from the α,α-dimethylpalmitate was 71% that of the palmitate, while the α-methyl-α-ethylcaproate had a poor availability pattern.

Keyphrases □ Vitamin A—biological availability from sterically hindered esters □ Biological availability—vitamin A from sterically hindered esters □ Biological assay—comparison of vitamin A esters

A previous study by Forlano and Harris (1) suggested that maximal stability of vitamin A esters could be obtained by the introduction of electropositive groups in the α-position of the esters. Based on this concept, sterically hindered esters of vitamin A were prepared and their chemical stability pattern was determined. Some of the more promising compounds in this series were the vitamin A pivalate, α,α-dimethylvalerate, triethylacetate, α-methyl-α-ethylcaproate, and α,α-dimethylpalmitate. These were subjected to chemical evaluation tests such as oxidation, base-catalyzed hydrolysis, acid-catalyzed elimination, and acid-catalyzed isomerization (2, 3). It was then considered desirable to determine the biological availability of the esters that showed the best overall chemical stability when compared with the palmitate. Vitamin A α-methyl-α-ethylcaproate (2) and the α,α-dimethylpalmitate (3) were chosen, with cod liver oil and vitamin A palmitate functioning as standards. Two parameters were considered in the biological tests: (*a*) the amount of biological activity by a growth assay (4), and (*b*) the death rate in the various groups of rats, which was a further indication of the animals' ability to utilize the vitamin A ester (5).

EXPERIMENTAL

Chemical and spectrophotometric assays for vitamin A preparations may not give a true indication of the biological availability of vitamin A derivatives, especially new vitamin A derivatives that have unknown biological availability patterns. For this reason, a biological assay is very valuable in the final determination of potency.

The procedure used was a modification of the USP bioassay (5). It was based on the fact that the growth rate of a rat varies directly with the consumption of vitamin A (4). Vitamin A esters that are completely available will have higher growth rates than equimolar doses of esters that are not completely absorbed. Animals will die from avitaminosis A when fed derivatives that are poorly absorbed or not absorbed at all (5).

Table I—Average Weight (in Grams ±1 Standard Deviation) of Rats Fed Different Forms of Vitamin A

Days	Control Group	Cod Liver Oil Group	Vitamin A Palmitate Group	Vitamin A α-Methyl-α-ethylcaproate Group	Vitamin A α,α-Dimethylpalmitate Group
0	61	63	60	63	62
7	85	90	85	85	90
13	108	110	108	108	108
23	138	153	156	136	144
25	146 ± 13.5	165 ± 12.4	167 ± 9.2	141 ± 11.2	152 ± 9.2
30	144 ± 18.0	189 ± 14.3	187 ± 12.2	138 ± 18.1	167 ± 8.9
35	146 ± 14.2[a]	208 ± 15.4	210 ± 12.6	155 ± 16.9[b]	179 ± 12.8
38	156[c,d]	212 ± 8.9	216 ± 12.8	164 ± 14.0[e]	186 ± 13.8
41	157[d]	220 ± 10.2	232 ± 13.7	166 ± 17.0[e]	196 ± 11.6
45	144[d]	227 ± 10.8	242 ± 14.5	158 ± 24.3[e]	201 ± 12.9
52	128[d]	247 ± 11.5	264 ± 14.3	165 ± 26.4[a]	211 ± 15.8
55	—[f]	250 ± 11.1	271 ± 15.3	160 ± 27.8[g]	212 ± 18.8

[a] Represents 50% mortality. [b] Represents 30% mortality. [c] Represents 80% mortality. [d] No standard deviation was run in these groups because only one rat remained. [e] Represents 40% mortality. [f] Represents 100% mortality. [g] Represents 60% mortality.

Table II—Average Weight Gains of Rats in Grams (Column A) and the Percent Increase as Compared to Vitamin A Palmitate Standard (Column B)

Days	Control		Cod Liver Oil		Vitamin A Palmitate		Vitamin A α-Methyl-α-ethylcaproate		Vitamin A α,α-Dimethylpalmitate	
	A	B	A	B	A	B	A	B	A	B
0	—	—	—	—	—	—	—	—	—	—
7	23.4	—	27.5	—	24.9	100	22.5	—	28.0	—
13	47.0	97.5	47.5	98.5	48.2	100	45.0	93.4	45.7	94.8
25	84.0	79.0	101.3	95.3	106.3	100	78.2	73.6	90.0	84.7
35	84.8[a]	50.0	144.8	96.8	149.6	100	85.0[b]	56.8	116.5	77.9
45	83.0[c]	45.7	164.1	90.4	181.6	100	95.0[d]	52.3	137.9	75.0
55	—[e]	—	186.3	88.8	209.9	100	97.0[f]	46.2	149.6	71.3

[a] Represents 50% mortality. [b] Represents 30% mortality. [c] Represents 80% mortality. [d] Represents 40% mortality. [e] Represents 100% mortality. [f] Represents 60% mortality.

Fifty young male rats, weighing between 60 and 65 g. each, were randomly divided into five groups. All groups received a vitamin A-free diet[1] and water *ad libitum*. In addition, each group, except a control which received plain cottonseed oil, was given 0.1 ml. of a cottonseed oil solution of the vitamin A source containing the molar equivalent of 78 USP units once a week. The freshly prepared dose was administered with an oral syringe placed directly down the rat's throat.

The five groups were designated as follows:

1. Control group (cottonseed oil alone).
2. Cod liver oil group (cod liver oil USP diluted with cottonseed oil).
3. Vitamin A palmitate group [23 mg. of vitamin A palmitate (Pfizer) dissolved in 50 ml. of cottonseed oil].
4. Vitamin A α-methyl-α-ethylcaproate group [18 mg. of this ester (2) dissolved in 50 ml. of cottonseed oil].
5. Vitamin A α,α-dimethylpalmitate group [25 mg. of this ester (3) dissolved in 50 ml. of cottonseed oil].

The rats were weighed at regular intervals, using a conventional triple-beam balance. The average weights for the different groups are presented in Table I, and the average weight gains are presented in Table II.

RESULTS AND DISCUSSION

The control group required 55 days for complete mortality; however, at 35 and 38 days, there was 50 and 80% mortality, respectively. There were definite signs of vitamin A deficiency, such as loss of weight and xerophthalmia, at 30 days. These figures represent a typical vitamin A depletion pattern for rats in this weight group (4).

During this same 55-day period, the vitamin A palmitate group had the greatest rate of weight gain when compared to the other groups receiving equivalent amounts of vitamin A. For this reason, the palmitate group was considered the standard to which the other groups were compared. The average weight gain for the cod liver oil group was 89% that of the vitamin A palmitate group, while for the α-methyl-α-ethylcaproate group it was 46% in the rats that survived (60% death rate in this group), and for the α,α-dimethylpalmitate group it was 71% with no deaths.

The 40% surviving rats in the α-methyl-α-ethylcaproate group demonstrated an appreciable degree of variability in their weight-gain pattern as indicated by the large standard deviation. These data indicate that the rats that died could not effectively utilize this ester to an appreciable extent. The surviving rats also experienced considerable difficulty in their utilization of this compound, as suggested by their poor and erratic growth-rate pattern. None of the survivors had a weight-gain pattern comparable to the vitamin A palmitate group. One could speculate that the steric hindrance in the α-position of this ester may have caused substantial resistance to the action of esterases required to hydrolyze these compounds.

The availability pattern in the cod liver oil group was comparable to that of the vitamin A palmitate group, indicating that cod liver oil esters are reasonably well utilized.

There were no deaths in the vitamin A α,α-dimethylpalmitate group, even though the esters had steric hindrance in the α-position. The average weight gain of 71% after 55 days showed that while this ester was not as efficiently utilized as the palmitate, there was sufficient utilization to maintain life and growth.

Only the control and vitamin A α-methyl-α-ethylcaproate groups showed the physical signs of avitaminosis A. The rats in these two groups, in which death occurred, showed the following symptoms of avitaminosis A: (*a*) loss of weight; (*b*) conjunctivitus; (*c*) puffing of fur; (*d*) odd gait while walking, indicating improper bone formation; and (*e*) death.

If the biological availability patterns from the two sterically hindered esters are compared with those of nonsterically hindered esters, one can see that steric hindrance in the α-position of the ester exerts an inhibition of biological utilization. It is possible that steric hindrance in the α-position could affect the rate of enzymatic hydrolysis of these esters, with a subsequent decrease in utilization.

[1] Nutritional Biochemicals Corp., Cleveland, Ohio.

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