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Note

Gas-liquid chromatography of vitamin A_1 alcohol and vitamin A_1 acetate

Vitamin A and its derivatives are rapidly degraded in the presence of light or oxygen. This leads, in many cases, to difficulties in the determination of vitamin A. However, against reductive reagents vitamin A is very stable[1]. Catalytic hydrogenation of vitamin A converts it into the stable compound perhydro-vitamin A (ref. 2).

In the experiments described the conditions under which vitamin A_1 alcohol and vitamin A_1 acetate can be hydrogenated were determined. In addition, a gas–liquid chromatographic (GLC) method was developed for the qualitative and quantitative evaluation of the hydrogenation products of vitamin A_1 acetate and vitamin A_1 alcohol.

Material and methods

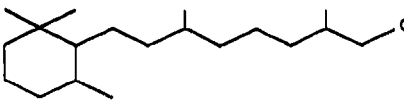
A solution of vitamin A* in 96% ethanol was prepared and an aliquot containing up to 25 mg of vitamin A was transferred to a 15-ml vacutainer tube. Five to ten milligrams of PtO_2 were added as a catalyst and the volume was made to a total of 2 ml with 96% ethanol. The vacutainer stopper was put in place. A 1½-in. 20G needle attached to a three-way stopcock was inserted through the vacutainer stopper. The air in the vacutainer was twice removed by vacuum and replaced with hydrogen gas. A final hydrogen pressure of 5 to 10 p.s.i. was used. The vacutainer tube was then shaken until the reaction mixture was colourless (approx. 5 min) and subsequently allowed to stand at room temperature overnight. The following day, 0.2 ml of 96% ethanol containing 4.2 mg of methylheptadecanoate was added as an internal standard to the tube containing hydrogenated vitamin A. After mixing and permitting the PtO_2 to settle, a portion of the supernatant was transferred to a storage tube. A measured amount (1.6 μl) of this solution containing the hydrogenation products of vitamin A_1 alcohol or vitamin A_1 acetate was analyzed by GLC in a Bendix GC-2500 equipped with a flame ionization detector. A 6-ft. glass column (¼ in. O.D.) was packed with 5% OV-1 on acid-washed and DMCS-treated 80–100 mesh Chromosorb W and operated isothermally at 230°. The inlet and transfer zone temperatures were 240°. Helium was used as the carrier gas at a flow-rate of 40 ml/min. Peak areas were determined by the use of the electronic digital integrator Vidar, Autolab-6300.

Results and discussion

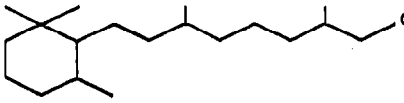
The absorption of vitamin A at 325 mμ was used to monitor the reaction. The presence of the catalyst without hydrogen did not cause any decrease in absorption over a period of 25 min. The hydrogenation was found to be slow and incomplete

* Vitamin A_1 alcohol was supplied by F. Hoffmann-La Roche & Co. Ltd., Basel, Switzerland. Vitamin A_1 acetate was purchased from Distillation Products Industries, Division of Eastman Kodak Co., Rochester, N.Y., U.S.A.

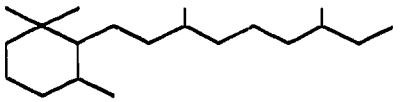
when less than 3 p.s.i. of hydrogen was used or when less than 5 mg of catalyst was present. With 5 to 10 mg of catalyst and 5 to 10 p.s.i. of hydrogen the absorption was zero within 20 min. Under these hydrogenation conditions two products are formed. One of the products, mol. wt. = 280(1), is the same whether the compound is vitamin A_1 alcohol or vitamin A_1 acetate. The second product is the saturated alcohol, mol. wt. = 296(2), or the saturated ester, mol. wt. = 338(3), depending upon whether the starting compound was in the alcohol or acetate form.



(1)



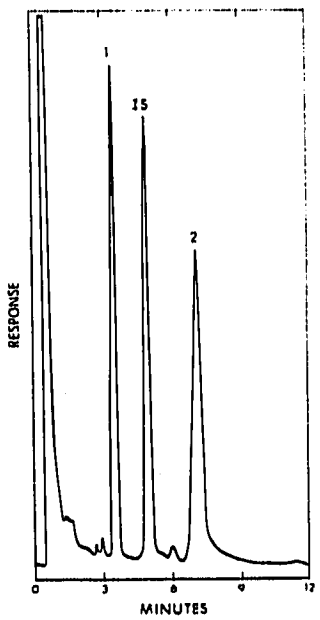
(2)



(3)

The identities of the products were confirmed by use of a mass spectrometer coupled to a gas chromatograph.

Gas chromatograms obtained under the conditions described above for the hydrogenation products of vitamin A_1 alcohol and vitamin A_1 acetate are shown in



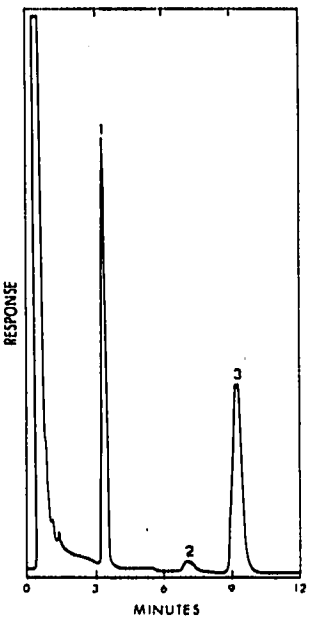


Fig. 1. Gas chromatogram of hydrogenation products of vitamin A_1 alcohol. See text for explanation of 1, 2 and internal standard (IS).

Fig. 2. Gas chromatogram of hydrogenation products of vitamin A_1 acetate. See text for explanation of 1, 2 and 3.

Figs. 1 and 2, respectively. The peaks for compounds 1, 2 and 3 are well separated. The fact that a small amount of compound 2 was detected in the chromatogram shown in Fig. 2 suggested that the vitamin A_1 acetate used contained a small amount of vitamin A_1 alcohol.

The amount of the hydrogenation products from vitamin A_1 alcohol, represented by their peak areas or by the sum of their peak areas, increased linearly with increasing amounts of vitamin A_1 alcohol present in the initial solution (Fig. 3). The

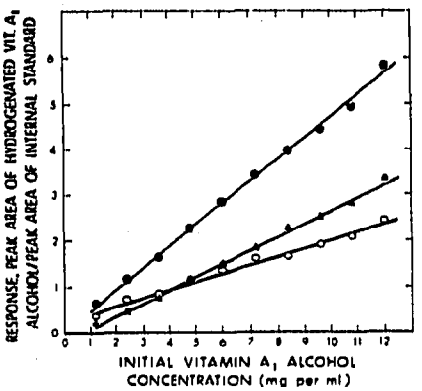


Fig. 3. Initial vitamin A_1 alcohol concentration in relation to the amount of the hydrogenation products 1 and 2 and the sum of both, measured in relation to a constant amount of internal standard. ●, 1+2/IS; ○, 1/IS; ▲, 2/IS.

curves in Fig. 3, especially the one calculated using the sum of the peak areas for both hydrogenation products, indicated that within the tested limits there was a linear correlation between the initial concentration of vitamin A_1 alcohol and the amounts of hydrogenation products determined by GLC.

The method described herein seems to be very suitable for the qualitative and quantitative determination of vitamin A_1 alcohol and vitamin A_1 acetate.

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