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# Microdetermination of Vitamin A (Retinol) with N-Bromosuccinimide

**Fathy A. Khalifa**[§] **and Zeinab M. Turk***

*Department of Chemistry, Faculty of Science, Cairo University, Giza and*
**National Organization for Drug Control and Research, P.O. Box 29, Cairo, Egypt.*

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**Abstract**□A novel method for the microdetermination of vitamin A in its pure state and pharmaceutical preparation using NBS is reported. The new method is easy, simple and accurate.

**Keywords**□Microdetermination of vitamin A, N-bromosuccinimide.

Vitamin A is one of the most important vitamins required for the biological functions[1, 2]. The microdetermination of the vitamin in its pure form or pharmaceutical preparations seemed to be of great importance. As a continuation of the effort in this field[3, 4], we report here, a novel procedure for the microdetermination of the vitamin which is highly accurate and simple.

Thus it has been found that pure vitamin A (retinol) (**1**) reacted with NBS (**2**) in chloroform to give a product of molecular formula $C_{20}H_{26}Br_4O$ (**3**) corresponding to the reaction of four molecules of N-bromosuccinimide (**2**) with one molecule of vitamin A (**1**). The reaction production could be formulated as the 1', 2', 5', 6'-tetrabromo derivative of vitamin A (**3**). This is proved by elemental analysis, IR and NMR spectra.

The IR spectrum of the reaction product showed the absorption bands related to the presence of the OH, saturated CH, $CH_2$, $CH_3$ and -C=C- group. The $^1$H NMR spectrum of the reaction product revealed signals corresponding to the presence of five $CH_3$, four $CH_2$ and two vinylic protons in addition to the OH proton. The $^1$H-NMR spectrum revealed signals at 1.5 (d, 6H, two $CH_3$); 1.8 (s, 9H, three $CH_3$); 2.3 (t, 2H, C-5 $H_2$); 2.7 (m, 2H, C-4 $H_2$); 2.9 (t, 2H, C-3 $H_2$); 3.1 (s, 2H, $CH_2OH$); 5.2 (s, 2H, C-8' H and C-4' H) and 6.4 (s, br, 1H, $CH_2OH$). (cf. Experimental part). The four bromine atoms could be established however to substitute the four hydrogen atoms at positions 1', 2', 5' and 6' of the side chain of the molecule. It could be stated here that the other two protons at positions 4' and 8' could not be substituted even by the use of more than four equivalents of NBS. This could be explained by the fact that these positions are highly affected by the neighbouring methyl group (+ I effect) at positions 3' and 7' respectively. Structure (**3**) could however, be assigned for the tetrabromo derivatives of the vitamin. Succinimide (**5**) could be also isolated. The mechanism can be shown as follows: (in Scheme 1).

However, vitamin A (retinol) reacted with bromine in chloroform to afford a product corresponding to the addition of five molecules of bromine to one molecule of the vitamin. The reaction product can be formulated as the decabromo derivative (**6**).

The IR spectrum of the reaction product (**6**) did not show among its peaks those corresponding to the -C=C- bands indicating the full saturation of the vitamin during the bromination process.

On the other hand, no vinylic protons could be detected among the signals revealed from the $^1$H-NMR spectrum of the reaction product. This established further the complete saturation of the vitamin. The $^1$H-NMR spectrum revealed signals at 1.6 (d, 6H, two $CH_3$); 1.8 (s, 9H, three $CH_3$); 2.3 (t, 2H, C-6 $H_2$); 2.7 (m, 2H, C-4 $H_2$); 3.0 (t, 2H, C-3 $H_2$); 3.2 (s, 2H, $CH_2OH$); 4.6 (d, 4H, hydrogens at $C_{6'}$, $C_{5'}$, $C_{2'}$ and $C_{1'}$ and 5.4 (s, 2H, hydrogens at $C_{8'}$ and $C_{4'}$).

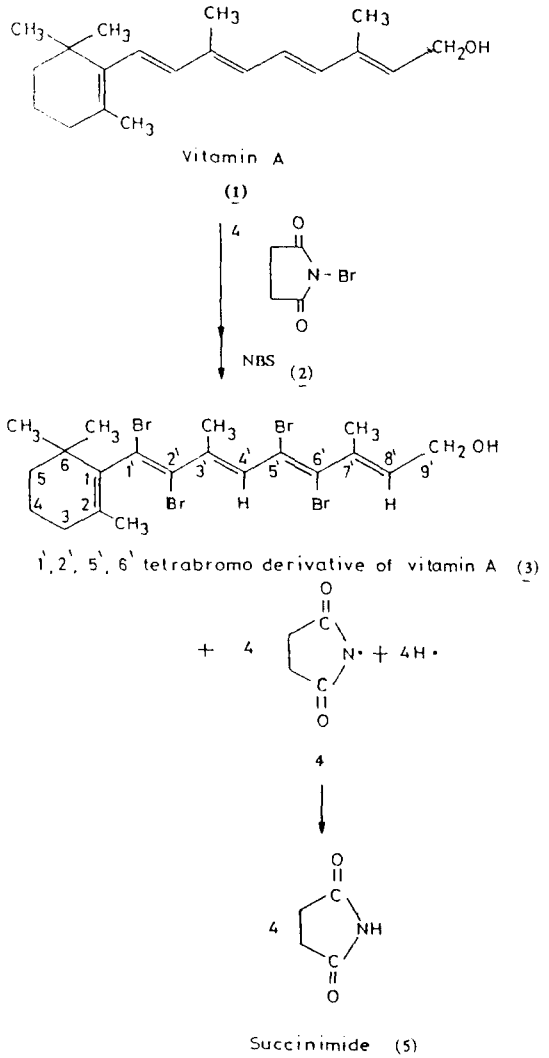
The method for the microdetermination of the pure vitamin was extended to cover the vitamin in its pharmaceutical preparations either tablets or capsules (Tables I and II).

## EXPERIMENTAL

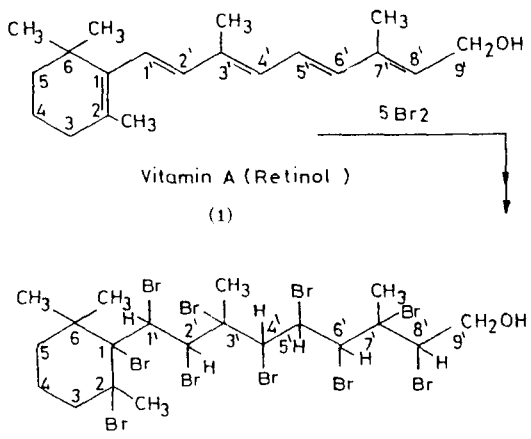
### *Reagents*

N-Bromosuccinomide (Prolabo), vitamin A alcohol (Retinol) (all trans retinol) (Sigma Chemical Co.),

[§] To whom correspondence should be adressed




Scheme 1






Decabromo derivative of vitamin A (6)

**Table I. Microdetermination of vitamin A* (retinol) by N-bromosuccinimide**

| | Taken (mg) | Found (mg) | Recovery (%) |
|---|---|---|---|
| 1. | 1.600 | 1.596 | 99.75 |
| 2. | 2.200 | 2.201 | 100.04 |
| 3. | 2.700 | 2.697 | 99.88 |
| 4. | 1.700 | 1.702 | 100.10 |

*Sigma Chemical Co.

Chloroform (Analar).

### *Reaction of vitamin A alcohol (Retinol) with N-bromosuccinimide*

A solution of vitamin A alcohol (all trans retinol) (0.2864g), 0.001 mol in chloroform (10 m*l*) was treated with N-bromosuccinimide (0.712g, 0.004 mol) in the same solvent (10 m*l*) at room temperature. The solution become hot whereby succinimide flauted after some time. After cooling succinimide was filtered off and crystallized in absolute ethanol to give succinimide with mp. and admixed mp. 125°C. The chloroform mother-liquor was removed in a stream of oxygen-free nitrogen at room temperature whereby the 1',2',5',6'-tetrabromo derivative of vitamin A **(8)** separated and filtered off and crystallized from absolute ethanol to give green colour crystals of **(3)** with mp. 95° (yield 99%). Analysis: $C_{20}H_{36}Br_4O$, Found C 39.8, H 5.9, Br 53.1; requires C 39.8, H 5.9, Br 53.11%. IR: 3500 $cm^{-1}$ (OH), 2980 (saturated $CH_2$ and $CH_3$) and 1630 $cm^{-1}$ (-C=C-). $^1$H-NMR: $\delta$ 1.5 (d, 6H, two $CH_3$), 1.8 (s, 9H, three $CH_3$), 2.3 (t, 2H, C-5 $H_2$), 2.7 (m, 2H, C-4 $H_2$), 2.9 (t, 2H, C-3 $H_2$), 3.1 (s, 2H, $CH_2OH$), 5.2 (s, 2H, C-8' H and C-4' H) and 6.4 (brs, 1H, $CH_2OH$).

### *Reaction of vitamin A (Retinol) with bromine*

To a solution of vitamin A (Retinol) (0.2865g, 0.001 mol) in chloroform (10 m*l*), bromine (0.8g, 0.005 mol) in the same solvent (10 m*l*) was added dropwise with shaking at room temperature. The solvent was removed under reduced pressure at room temperature and solid obtained was crystallized from absolute alcohol to give the decabromo-Vitamin A **(6)** which separated as pale yellow crystals from absolute alcohol with mp. 72°C (yield 99%). Analysis: $C_{20}H_{30}Br_{10}O$, Found C 22.1, H 2.7, Br 73.7; requires C 22.19, H 2.76, Br 73.66%. IR: 3500 $cm^{-1}$ (OH)

**Table II. Microdetermination of vitamin A in pharmaceutical preparations by NBS in tablets and capsules**

| | The name of capsule and tablets | Calculated amount of vitamin A (mg) | Found amount of vitamin A (mg) | Recovery (%) |
|---|---|---|---|---|
| (i) | Vitamin A 25000 I.U. capsule | 7.5 | 7.50 | 100.06 |
| | (PHARCO) each capsule contains vitamin | | 7.508 | 100.10 |
| | A palmitate (25,000 I.U = 7.5 mg vit. A) | | 7.5 | 100.00 |
| (ii) | A viton capsule (KAHIRA) each capsule | 15.00 | 15.005 | 100.03 |
| | contain vitamin A plamitate (50,000 I.U | | 14.990 | 99.9 |
| | = 15 mg. vit. A) | | 14.975 | 99.8 |
| (iii) | Abivit A 50,000 I.U. capsule (ABI) each | 15.00 | 15.010 | 100.06 |
| | capsule contains vit. A palmitate (50,000 | | 15.015 | 100.10 |
| | I.U = 15 mg vit. A) | | 15.000 | 100.00 |
| (iv) | Davitamon A 50,000 I.U. capsule | 15.00 | 14.982 | 99.88 |
| | (ORGANON) each capsule contains vit. | | 14.961 | 99.74 |
| | A palmitate (50,000 I.U = 15 mg vit. A) | | 14.970 | 99.80 |
| (v) | Dohyfral A 50,000 I.U tablets | 15.00 | 15.016 | 101.06 |
| | (DUPHAR) each tablet contains vit. A | | 15.020 | 100.13 |
| | palmitate (50,000 I.U = 15 mg vit. A) | | 15.019 | 100.12 |
| (vi) | Dagravit fort 50,000 I.U tablets | 15.0 | 14.899 | 99.32 |
| | (DAGRO) each tablet contains vit. A | | 14.885 | 99.23 |
| | palmitate (50,000 I.U = 15 mg vit A). | | 14.955 | 99.7 |
| (vii) | Ro. A-Vit tablets (ROCHE) each tablet | 15.0 | 15.010 | 100.06 |
| | contains vit. A acetate (50,000 I.U | | 14.908 | 99.38 |
| | = 15.0 mgVitamin A). | | 14.874 | 99.16 |

and 2980 $cm^{-1}$ (saturated $CH_2$ and $CH_3$). $^1$H-NMR: $\delta$ 1.6 (d, 6H, two $CH_3$), 1.8 (s, 9H, three $CH_3$), 2.3 (t, 2H, C-5 $H_2$), 2.7 (m, 2H, C-4 $H_2$), 3.0 (t, 2H, C-3 $H_2$), 3.2 (s, 2H, $CH_2OH$), 4.6 (d, 4H, hydrogens at C-6', C-5', C-2' and C-1') and 5.4 (s, 2H, hydrogens at C-8' and C-4').

***Microdetermination of vitamin A pure by N-bromosuccinimide:***

***Analytical reagents and solution***

Vitamin A alcohol (Retinol, all-trans retinol), (Sigma Chemical Co.); Chloroform, Analar. Potassium iodide; an aqueous solution of potassium iodide (10%). Starch: one per cent starch solution. N-Bromosuccinimide: 1% in glacial acetic acid. Standardized sodium thiosulphate (0.02 N).

***Microdetermination of vitamin A alcohol (1 mg = 3333.333 I.U.)***

To an accurate amount of vitamin A (1-3 mg) dissolved in chloroform (2 m*l*) in 50 m*l* Erlenmeyer flask an accurate solution of standardized NBS in glacial acetic acid (2 m*l*) was added. The flask was stoppered and left in the dark for ten minutes. Sulfuric acid (2N, 10 m*l*) and potassium iodide (4 m*l*, 10% w/v) were added. The liberated iodine was titrated against standardized sodium thiosulphate (0.02 N) solution to the starch end point (cf. Table I).

***Calculation***

$$\text{Amount of vitamin A in mg} = \frac{(a-b) \times c \times d \times M_1}{4a\ M_2}$$

where:

a = m*l* of (0.02 N) sodium thiosulphate for the blank.
b = m*l* of (0.02 N) sodium thiosulphate for the sample.

c = exact mls of 1% N-bromosuccinimide
d = concentration of NBS per 1 m*l*, in mgs.
$M_1$ = Molecular weight of vitamin A = 286.46
$M_2$ = Molecular weight of N-bromosuccinimide = 178

***Microdetermination of vitamin A (Retinol) in pharmaceutical preparations by N-bromosuccinimide***

In capsules and tablets, vitamin A can be estimated in tablets and capsules by the following two steps:
(1) Saponification and isolation of the unsaponifiable matter from the tablets and capsules.
(2) Microdetermination of the separated Vitamin A with NBS.

(i) vitamin A 25,000 I.U. capsule (PHARCO). Each capsule contains vitamin A palmitate 25,000 I.U. = 7.5 mg vitamin A.

(ii) Aviton capsule (KAHIRA): Each capsule contains vitamin A palmitate (50,000 I.U. = 1.5 mg vitamin A).

(iii) Abivit A 50,000 I.U. capsule (ABI): Each capsule contains Vitamin A palmitate (50,000 = 15 mg vitamin A).

(iv) Davitamon A 50,000 I.U. capsule (Organon): Each capsule contains vitamin A palmitate (50,000 I.U. = 15 mg vitamin A).

(v) Dohyfral A 50,000 I.U. tablets (Duphar): Each tablet contains vitamin A palmitate (50,000 I.U. = 15 mg vitamin A).

(vi) Dagravit fort 50,000 I.U. tablet (Dagro): Each tablet contains vitamin A palmitate (50,000 I.U. = 15 mg. vitamin A).

(vii) Ro.A-vit. tablets (ROCHE): Each tablets contains vitamin A acetate (50,000 I.U. = 15 mg vitamin A).

***Analytical reagents***

Diethyl ether, peroxide free (Merck). Chloroform (Analar); Ethyl alcohol absolute (E. Merck), Aqueous potassium hydroxide solution: potassium hydroxide (50g) was dissolved in 100 m*l* of freshly boiled water, mixed and cooled. This solution was prepared daily.

Potassium iodide: (10% w/v) N-Bromsuccinimide: 1% in glacial acetic acid previously prepared in part II and standardized. Stndardized sodium thiosulphate (0.02 N), starch: 1% solution.

***Sample preparation***

1 Capsule or tablet contains calculated (50,000 I.U., 15 mg vitamin A) was taken in conical flask 250 m*l* and distilled water 10 m*l* was added than it was put on water bath for 10 minutes. The remaining solid was crushed with a blunt glass rod, and warmed for 5 minutes longer. Absolute alcohol (23 m*l*), glycerol (7.0 m*l*) and potassium hydroxide (3 m*l*, 50 w/v) were added. The mixture was refluxed for 30 minutes then cooled and distilled water was added to conical flask. Then the mixture was extracted by ether three times each time 50 m*l*. The ethereal layer was washed several times with distilled water tell free from alkali then dried with sodium sulphate (anhydrous) then filtered and ether was evaporated in the presence of nitrogen gas. The residue was dissolved in 15 m*l* of chloroform.

Then an accurately standardized solution of NBS in glacial acetic acid (10 m*l*, 1% w/v) was added. The flask was stoppered and left in the dark for 10 minutes. Sulfuric acid (10 m*l*, 2 N) and KI (5 m*l*, 10% w/v) were added and the liberated iodine was titrated with standard sodium thiosulphate (0.02 N) to the starch end point (cf. Table II).

***Calculation***

$$\text{The amount of vitamin A} = \frac{(a-b) \times c \times d \times M_1}{4a\ M_2} \text{ in mg}$$

where:

a = m*l* of $Na_2S_2O_3$ (0.02 N) for the blank.
b = m*l* of $Na_2S_2O_3$ (0.02 N) for the sample.
c = exact m*l* of 1% NBS added.
d = concentration of NBS in mg per m*l*
$M_1$ = molecular weight of vitamin A = 486.46
$M_2$ = molecular weight of NBS = 178.

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