Studies on Kitol IV

Purification of Kitol by Chromatographie

By

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Previously the authors separated whale liver oil into Vitamin A fraction and Kitol fraction through their different solubility to the solvent.[1] This Kitol fraction showed Extinction value of 500 at 290 mμ, which is about of 70% pure, being $E\,\frac{1\%}{1\,cm}(290\,m\mu)=707$[2] in pure Kitol. The authors purified it through chromatographie and obtained light yellow glassy lump of $E\,\frac{1\%}{1\,cm}(290\,m\mu)=680$. Its melting point was 72°C[1] and it showed pure scarlet colour in the Carr-Price reaction. This colour showed its maximum some seconds later, being rather stable. The same colour is also obtained by conc. sulphulic acid, acid clay and glyceroldichlorhydorine. It had a slight odour like terpene and was easily soluble in petroleum ether acetone and chloroform; soluble in ethanol and methanol; but quite insoluble in water. It was unstable in the air and light. When it was left exposed in powdered form in the room for two days, $E\,\frac{1\%}{1\,cm}(290\,m\mu)$ decreased to 500. When kept in a dark room filled with CO_2 gas, it showed little change even two days later. When it was preserved in petroleum ether solution, it was so unstable that even when kept in the dark room, it oxidized rapidly and precipitated white insoluble matter. This precipitate was insoluble in olive oil too,[2] while easily soluble in chloroform and acetone, and soluble in ethanol and methanol. Alkaline treatment produced a red substance, which could be removed from Kitol as saponifiable matter. Absorption curve of Kitol obtained by the authors is as shown in the following figure.

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Experimental Part

300 g. of a mixture of liver oil of blue and fin whales caught in the Antarctic (containing 100,000 I.U. of Vitamin A) was saponified with 300 cc. of 20% alcoholic potash; and 32.5 g. of unsaponifiable matter was obtained therefrom. This unsaponifiable matter was dissolved in 1 L of petroleum ether; and from this solution 90% methanol was extracted 35 times, 500 cc. at each extraction. Most of

1) Melting point of crystalized Kitol is 88—90°C. (3)

2) Even when Kitol was preserved in olive solution it gave this white precipitate.

sterin and Vitamin A collected in the methanol layer. After the petroleum ether layer was washed several times with water and then dried, petroleum ether was distilled. When the residue was saponified again with a small amount of 20% alcoholic potash, unsaponifiable matter was obtained which was reddish yellow and viscous. Very thin chloroform solution of this unsaponifiable matter showed blue colour with the first drop of chloroform solution of antimony trichloride; but further addition of the same solution caused the blue colour to change instantly to cherry red. This is $E^{1\%}_{1\,cm}$ (290 mμ)=500. 500 cc. of petroleum ether solution of this substance was passed into an adsorption column of aluminium oxide. Aluminium oxide used in this experiment as adsorbent was a mixture of equal amounts of "BL6" manufactured by the Japan Aluminium Co. Ltd. and inactivated "BL6"[3]. When this adsorption column was developed by the use of petroleum ether, a yellow layer of Vitamin A was formed at the bottom a colourless layer of Kitol in the middle, and a light yellow layer of unknown matter at the top. When this chromatogramm column was further developed with petroleum ether, the yellow part of the Vitamin A layer finally separated from the alminium layer, moved into the vessel bellow as petroleum ether solution. The aluminium adsorbed was eluted with petoleum ether containing a few drops of methanol and washed with water to remove methanol. Then chromatogramm was again made and the slight amount of Vitamin A at the bottom was washed off. The top of this chromatogramm, light yellow in colour, adsorbed a matter which showed blue in the Carr-Price reaction. This matter was adsorbed a little easier by aluminium oxide than Kitol and was slower than Vitamin A in the appearance of blue in the Carr-Price reaction. Namely, when antimony trichloride was added to a mixture of Vitamin A and Kitol, blue colour appeared first; then the blue gradually changed to purple, then finally to red. But when antimony trichloride was added to a mixture of this unknown matter and Kitol, the first colour to appear was red, which gradually turned to purple. Moreover, when this mixture was dissolved in petroleum ether and extracted again and again with pure methanol, Kitol moved gradually to methanol, leaving this unknown matter in the petroleum ether layer. From the above facts, this unknown matter was thought to be of larger molecule than Kitol.

After removing the upper part of chromatogramm in which this unknown matter was adsorbed, the Kitol layer in the middle was eluted in the same way as in the above experiment. When this was passed into a very short adsorption column consisting of inactivated alminium oxide "BL6" to which 10% activated alminium had been added, most of the Kitol flowed down without being adsorbed and the

3) It was too adsorptive for "BL6" to separate Vitamin A from Kitol.

unknown matter which showed blue colour with antimony trichloride was adsorbed in the column. After shaking 300 cc of methanol with the above petroleum ether solution of Kitol (about 1.5 L)[4] and removing the methanol layer, the petroleum

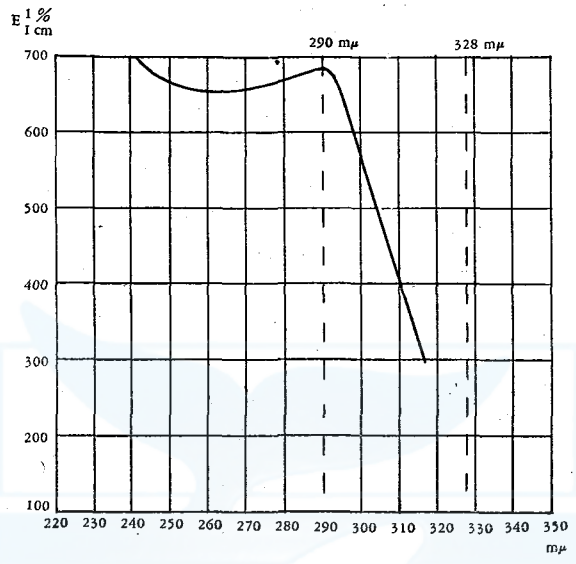

Fig. 1

layer was washed. After drying it, petroleum ether was distilled off to obtain about 5 g. of light yellow glassy lump which had a melting point of 72°C. This is E1% (290 mμ)=680.[5] It showed pure scarlet in the Carr-Price reaction.

References:

(1) The Scientific Reports of the Whales Research Institute No. 3 (1950), 85.
(2) F. B. Clough, H. M. Kascher, C. D. Robeson, J. G. Baxter: Science, 105 (1947) 436.
(3) Ditto.

4) to remove a slight amount of oxide produced in adsorption treatment.
5) 97% in Kitol purity.