Spectrophotometric Determination of Camphor-aldehydes. I.

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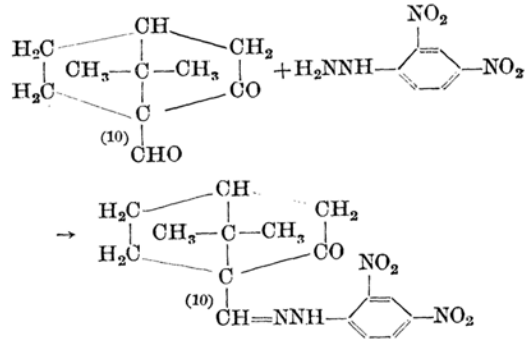
Introduction

In 1934, the senior author and his co-workers[1] reported the results of their studies on the chemical synthesis of hydroxycamphors and their carbonyl derivatives. Afterwards, much progress in physiological and clinical aspects was made, thanks to the efforts of numerous investigators. In connection with the chemical and biological determination of their unstable camphor-aldehydes in O_2 atomosphere, many procedures have also been carried out by several authors, but twenty years have passed since that time, while no reliable reports have appeared about the physico-chemical estimation of these camphor-aldehydes except those by ordinary iodometry, bisulfite-CrO_3 titration method etc. On the other hand, studies on elctrophotometric assay of various compounds have been markedly improved, and it is especially well known that Roe[2],[3] and coworkers succeeded during 1942-4 in colorimetrically determining dehydro-ascorbic acid with 2,4-dinitrophenylhydrazine (DNP). Therefore, the authors have recently attempted to prepare pure 2,4-dinitrophenylhydrazones of β-oxocamphor and π-oxocamphor, in order to undertake spectrophotometric estimation of camphor-aldehydes with the standard crystals of their 2,4-dinitrophenylhydrazones. Experiments are outlined as follows:

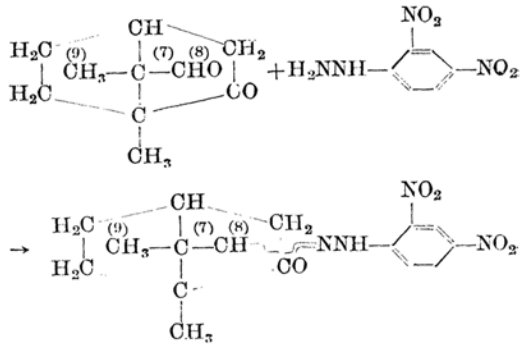
1) The absorption spectra of β-oxocamphor-2,4-dinitrophenylhydrazone and π-oxocamphor-2,4-dinitrophenylhydrazone were exactly determined in isopropylalcohol solution by a Beckman spectrophotometer to give absorption maxima, $E^{1\%}_{1\,cm}$ 356 mμ=658 and $E^{1\%}_{1\,cm}$ 354 mμ=678, respectively.

2) The E value of camphor-aldehyde-hydrazone in the solution to be tested was measured electrophotometrically, and the real amount of the camphor-aldehydes contained was calculated from the above standard E value.

β(10) Apocamphor-1-aldehyde



π(8 or 9) Apocamphor-7-aldehyde



Experimental

(1) Preparation of Camphor-aldehyde-2,4-dinitrophenylhydrazones.—β (10) Apocamphor-1-aldehyde-2,4-dinitrophenylhydrazone: Freshly prepared crystals of β(10) apocamphor-1-aldehyde (β-oxocamphor) from β-(10) hydroxycamphor by means of chromic acid oxidation and sodium bisulfite purification were dissolved in alcohol and treated by hot saturated 5N HCl solution of 2,4-dinitrophenylhydrazine. After a few minutes heating, the hydrazone separated out as an insoluble amorphous precipitate which when recrystallized from hot alcohol formed yellow needle crystals, yield almost quantitative; m. p. 212°C. (uncorr.)

Found: C, 54.61, 54.67; H, 5.75, 4.90; N, 16.12, 16.34, Calcd. for $C_{16}H_{18}O_5N_4$: C, 55.46; H, 5.24; N, 16.18%

π(8 or 9) Apocamphor-7-aldehyde-2,4-dinitrophenylhydrazone: Crystals of π(8 or 9) apocamphor-7-aldehyde (π-oxocamphor), freshly prepared

(1) Y. Sahashi and co-workers, *Scient. Pap. Inst. Phys. Chem. Res.* **25**, 47 (1934).
(2) Roe, Kuether, *J. Biol. Chem.*, **147**, 399 (1943).
(3) A. Fujita, *Vitamins*, **4**, 53 (1951).

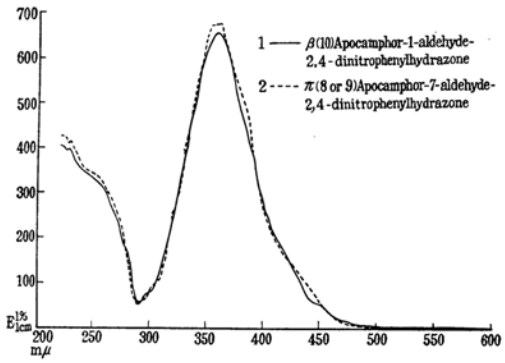


Fig. 1.—Absorption spectra of camphor-aldehyde-2, 4-dinitrophenylhydrazone in isopropylalcohol.

from π-bromocamphor according to Sahashi's method, were dissolved in alcohol and treated with hot saturated 5N HCl solution of 2, 4-dinitrophenylhydrazine by the same process mentioned above. The amorphous precipitate thus obtained was recrystallized from hot alcohol, yielding yellow needle crystals in a quantitative amount; m. p. 202°C. (uncorr.)

Found: C, 55.29, 55.49; H, 4.82, 5.09; N, 16.04, 16.01, Calcd. for $C_{16}H_{18}O_5N_4$: C, 55.46; H, 5.24; N, 16.18%

(2) **Spectrography of Camphor-aldehyde-2, 4-dinitrophenylhydrazones.** — 100 γ of camphor-aldehyde-2, 4-dinitrophenylhydrazone to be tested were dissolved in 10 cc. of isopropyl alcohol. The standard absorption of the above compounds were measured by a Beckman spectrophotometer with the following results.

	β(10) Apocamphor-1-aldehyde-2, 4-dinitrophenyl-hydrazone	π(8 or 9) Apocamphor-7-aldehyde-2, 4-dinitro-phenylhydrazone
Absorption-maxima	$E^{1\%}_{1\,cm}$	$E^{1\%}_{1\,cm}$
	356 mμ=658	354 mμ=678

(3) **Analytical procedure.—DNP Reagent:** 5g. 2, 4-dinitrophenylhydrazine (DNP) were dissolved in 200 cc. hot 5N HCl solution and filtered after cooling.

Procedure: A) 1 cc. of aq. β(10) oxocamphor solution was refluxed for about a minute with 10 cc. of the DNP reagent and allowed to cool. After 30 minutes the yellow precipitate was filtered, washed with water and dissolved in 100 cc. isopropyl alcohol. The solution thus obtained was diluted up to 10 γ/cc. (2000 cc.) The extinction was then measured by a Beckman spectrophotometer.

B) 1 cc. of aq. π (8 or 9) oxocamphor solution was heated for about a minute with 10 cc. of DNP reagent and treated as mentioned above. The separating hydrazone was dissolved in 100 cc. of isopropyl alcohol and diluted up to 10 γ/cc. (1000 cc.) The Beckman photometric observation was then carried out in the ordinary way.

Calculation: β(10) oxocamphor: Found, $E^{1\%}_{1\,cm}$356 mμ=528

$$\text{Hence,}\quad 10\,\gamma\times\frac{528}{658}\times 2000 = 16.04\ \text{mg.}$$

$$16.04\times\frac{166}{346} = 7.69\ \text{mg./cc.}$$

π(8 or 9) oxocamphor: Found, $E^{1\%}_{1\,cm}$354 mμ= 488

$$\text{Hence,}\quad 10\,\gamma\times\frac{488}{678}\times 1000 = 7.19\ \text{mg.}$$

$$7.19\times\frac{166}{346} = 3.44\ \text{mg./cc.}$$

C) Cases where the solution to be tested contains other emulsifying organic agents such as Tween, Span etc., it is best to carry out the separation of camphor-aldehydes by the process of steam distillation, and DNP reagent must be added to the distillate.

Summary

1) 2, 4-dinitrophenylhydrazones of β(10) apocamphor-1-aldehyde and π(8 or 9) apocamphor-7-aldehyde were prepared and their absorption spectra were exactly determined.

2) From the standard *E* values, the amount of the above compounds in an unknown solution can be calculated from the estimation by a Beckman spectrophotometer.

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