CCXLI. THE SEPARATION OF CARBONYL COMPOUNDS FROM WAXES

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FOLLOWING the discovery by Channon & Chibnall [1929] of *n*-nonacosan-15-one in cabbage leaf wax it has been customary in this laboratory to submit both the unsaponifiable and acid fractions of all waxes undergoing investigation to an analysis for the presence of ketonic groups, the method used being that of these authors, in which advantage is taken of the greatly increased solubility of the ketoximes in certain organic solvents. The procedure includes a fairly extensive fractional crystallization, and is thus not a particularly suitable one for a rapid examination of a large number of waxes: what is required in its place is a more direct mode of separation, preferably one in which the substance possessing a carbonyl group can, after appropriate treatment, be precipitated from solution and removed by filtration. For this reason the recent work of Anchel & Schoenheimer [1936], on the separation from unsaponifiable material of animal origin of ketones belonging to the sterol group by coupling them with reagents possessing acidic properties, is of great interest. The reagents that they use are *p*-carboxyphenylhydrazine (*p*-hydrazinobenzoic acid), carboxymethoxylamine and carboxymethylhydrazine, which form with the ketones acidic hydrazones or oximes, the alkali salts of which are water-soluble and may therefore be quantitatively separated from the ethereal solution of the other unsaponifiable material. The regenerated hydrazones or oximes are then split with either formaldehyde or pyruvic acid in alcohol solution, and the ketones recovered in excellent yield. If a similar condensation could be effected with long chain aliphatic carbonyl compounds then the method, modified if need be to allow for differences in solubilities, might clearly be of great use with wax products, and experiments have accordingly been carried out on these lines.

The only reagent which has so far been investigated is *p*-carboxyphenylhydrazine. Under the conditions described by Anchel and Schoenheimer (refluxing the ketone in 95 % alcohol for 2 hr. with the reagent and a few drops of acetic acid) very little coupling with a ketone such as myristone took place, or even in 24 hr., but in the presence of a few drops of pyridine the reaction was complete in 4 hr. As was expected, the Na and K salts are insoluble in cold water, and a study was therefore made of the solubilities of the hydrazone and its various salts. The relevant information gleaned can be summarized as follows:

(1) The hydrazone is practically insoluble in hot and cold water, but is soluble in cold ethyl alcohol and ether.

(2) The Na and K salts are practically insoluble in hot and cold water and in cold ethyl alcohol, but are readily soluble in cold ether.

(3) The Ba and Pb salts are very sparingly soluble in boiling methyl alcohol and are completely insoluble in boiling acetone. The Ca salt is also insoluble in boiling acetone, but is very freely soluble in ice-cold methyl alcohol.

Trial experiments with mixtures typical of the unsaponifiable fraction of waxes, e.g. ketone, primary alcohol and paraffin, showed that after treatment with the reagent and solution of the mixture in boiling methyl alcohol the hydrazone is precipitated in good yield as the Ba salt on the addition of boiling methyl alcoholic $\text{Ba}(\text{OH})_2$ and can be removed by filtration. Furthermore the regenerated hydrazone is readily split by alcoholic formaldehyde and the ketone is in all cases recovered in 75–80 % yield. The other components of the mixture remain in the clear methyl alcoholic filtrate and separate in almost quantitative yield and free from ketone on cooling to 0° . A further 10–15 % of ketone can be recovered by working over the final methyl alcoholic mother-liquor, but the product thus obtained is slightly contaminated with the other components of the mixture and in practice the extra operation has not been found worth the labour involved.

Both ketonic primary and secondary alcohols can be separated with the same facility as simple ketones, but in these cases the hydrazone is not readily split by alcoholic formaldehyde, and it is necessary to use alcoholic pyruvic acid. The application of the method to the separation of wax ketonic acids is under investigation.

The new method is rapid in operation and has already been applied to the separation of *n*-nonacosan-15-one from cabbage leaf wax and of palmitone and 10-hydroxypalmitone from sandal leaf wax [cf. Chibnall *et al.* 1937]. The following waxes have been shown to contain no ketone: apple cuticle wax, tea leaf wax, coffee bean wax and sugar-cane wax.

EXPERIMENTAL

p-Carboxyphenylhydrazine. “*p*-Hydrazinobenzoic acid” obtained from the Eastman Kodak Company was purified by the method of Anchel & Schoenheimer [1936].

Reaction with n-nonacosan-12-one. 0.5 g. of ketone (M.P. 74.5°) was added to 20 ml. of 95 % ethyl alcohol containing 0.5 g. of the reagent, 0.3 ml. of acetic acid and 0.3 ml. of pyridine and the whole refluxed for 4 hr. After cooling the mixture was run into 100 ml. of dilute HCl to remove the pyridine and the precipitated hydrazone filtered off and washed with water. It was then distributed between 4 % K_2CO_3 solution and ether to remove excess reagent as the water-soluble salt. The ethereal solution was then shaken with dilute HCl, the washed solvent removed and the residue taken up in 5 ml. of 95 % ethyl alcohol. On standing at room temperature for some hours no ketone crystallised out, showing that the coupling was probably complete. 10 ml. of alcohol were then added, together with 0.25 ml. of 40 % formaldehyde solution and the whole refluxed for 5 hr. to split the hydrazone. The alcohol was then removed, the residue partitioned between K_2CO_3 solution and water and the ethereal solution evaporated. Recrystallization from 10 ml. of warm alcohol gave 0.42 g. of ketone, M.P. 74.5° . The yield was 84 %.

Separation of a ketone and paraffin. 0.2 g. of *n*-nonacosan-12-one and 1.0 g. of mixed paraffins (M.P. 67.5°) were treated as before with 0.2 g. of the reagent, 0.3 ml. of acetic acid, 0.3 ml. of pyridine and 50 ml. of 95 % alcohol. After removal of the pyridine the washed precipitate was dissolved in 80 ml. of boiling methyl alcohol, 2 ml. of a saturated solution of CaCl_2 in methyl alcohol and 10 ml. of a saturated solution of baryta in methyl alcohol were then added and the boiling continued for another 10 min. The presence of CaCl_2 , which introduces CaO into the mixture helps to make the precipitation of the Ba salt of the

hydrazone more complete. The mixture was filtered on a hot water funnel and the residue washed three times with boiling methyl alcohol. The Ba salt was then treated with warm dilute HCl, the free hydrazone extracted with ether, the washed solvent removed and the residue taken up in 15 ml. of warm 95% alcohol. 0.25 ml. of formaldehyde was then added and the solution refluxed for 5 hr. The recovered ketone (M.P. 74.5°) weighed 0.15 g. (yield 75%). The methyl alcoholic filtrates mentioned above were cooled in the ice-chest. The material which separated was filtered off, taken up in warm acetone, filtered free from Ba salts and the solution cooled to 0°. 0.98 g. of paraffin, M.P. 67.5° crystallized out.

Separation of n-nonacosan-15-one from cabbage leaf wax. 9 g. of wax, from 1 kg. of dried leaves, gave 6 g. of clean white unsaponifiable material, M.P. 63–63.5°. This was treated with 0.9 g. reagent, 1 ml. of glacial acetic acid and 1 ml. of pyridine in 200 ml. of 95% alcohol. The Ba separation was applied and the ketone crystallized from alcohol (0.8 g.; M.P. 79.2°). Recrystallized once from acetone (charcoal) it melted at 80.5–80.7°, unchanged when mixed with synthetic *n*-nonacosan-15-one. The yield was more than twice as great as that obtained by Channon & Chibnall [1929].

Reaction with cocceryl alcohol (15-keto-n-tetratriacontanol). 0.3 g. of the ketonic alcohol (M.P. 100.3–100.6°) was coupled as before, using 0.25 g. of reagent. The Ba separation was applied and the hydrazone split by refluxing with 15 ml. of 95% alcohol containing 1 ml. of pyruvic acid for 5 hr. Recrystallized from ethyl alcohol the recovered ketonic alcohol (0.23 g.) melted at 100.2–100.5°. The hydrazone is not split readily by formaldehyde.

SUMMARY

p-Carboxyphenylhydrazine, which has been used recently by Anchel & Schoenheimer to separate ketones belonging to the sterol group from other unsaponifiable material of animal origin, condenses with the long-chain ketones, ketonic primary and ketonic secondary alcohols of waxes to give acidic hydrazones.

The barium salts of the hydrazones are insoluble in boiling methyl alcohol and can thus be readily separated from the other components of the unsaponifiable fraction of waxes, which are soluble.

The regenerated hydrazones are readily split with alcoholic formaldehyde or pyruvic acid, and the ketones or ketonic alcohols are recovered in an overall yield of 75–80%.

In conclusion I should like to thank Prof. A. C. Chibnall for suggesting this research and for advice.

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