Regeneration of Carbonyl Compounds from 2,4-Dinitrophenylhydrazones with Sulfuric Acid[1]

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A simple, rapid method employing sulfuric acid and water to regenerate carbonyl compounds from 2,4-dinitrophenylhydrazones is presented. The method was evaluated by using a number of DNP-hydrazones of alkanals, alkan-2-ones, and 2-alkenals and was found to give recoveries ranging from 23 to 61%. The procedure makes possible rapid organoleptic observations and is well adapted for recovery of the free carbonyl compounds for other studies.

A DERIVATE REAGENT widely accepted for studies of carbonyl compounds is 2,4-dinitrophenylhydrazine.[3] The once serious problem of regenerating the free carbonyl compound from the hydrazone has been overcome in many procedures through the efforts of various investigators (1,5,6,7,8,9,11,12,13,14).

The published methods employ reagents that impart odors which impair sensory evaluation of the regenerated carbonyl compounds. Except for the levulinic acid method of Keeney (7) it is difficult to isolate the regenerated carbonyl compound free of impurities that interfere with subsequent analyses. A technique for regenerating carbonyl compounds directly into a gas chromatographic column was recently described by Ralls (11). The possibility of using this method for an organoleptic analysis was not discussed by the author but may have possibilities.

The procedure described herein employs sulfuric acid and distilled water as the regeneration reagents. The carbonyl compound is regenerated instantly and can be evaluated organoleptically without interfering odors. Also the free carbonyl compounds can be easily isolated for subsequent studies.

Experimental

2,4-Dinitrophenyl(DNP)hydrazones. All carbonyl compounds except the 2-alkenals were obtained from commercial sources. The 2-alkenals were synthesized by modifying the Radlove procedure (10) as described by Day and Lillard (4).

Authentic DNP-hydrazones were prepared by reaction of the carbonyl compounds with 2N HCl solutions saturated with DNP-hydrazine, or by the method of Shriner, Fuson, and Curtin (13). The DNP-hydrazones were purified by column chromatography (3) before regeneration.

Sulfuric Acid. Concentrated A.C.S. grade (sp. gr. 1.83–1.84).

Regeneration of Carbonyl Compounds for Odor Evaluation. The DNP-hydrazone (0.1 to 10 mg.) was placed in a dry 50-ml. Erlenmeyer flask and dissolved in 3 ml. of concentrated H_2SO_4. When the hydrazone was difficult to dissolve, more sulfuric acid was used, but heating to the concentrated H_2SO_4 was avoided. Ten ml. of distilled water were cautiously added while the contents of the flask were carefully rotated. The carbonyl compound was regenerated instantly.

Evaluation of the Regeneration Method. Efficiency of the regeneration procedure was determined by using known quantities of authentic DNP-hydrazones for regeneration. The freed carbonyl compounds, following regeneration, were steam-distilled into DNP-hydrazine-HCl solutions, extracted with hexane, chromatographed, and the recovered DNP-hydrazone was measured spectrophotometrically. Details of the procedure follow. Concentration of authentic DNP-hydrazone in chloroform solution was determined by measuring absorbance in a Beckman D.U. spectrophotometer at 358 mμ, 363 mμ, and 373 mμ, for alkanal, alkan-2-one, and 2-alkenal DNP-hydrazones, respectively, and the quantity of carbonyl compound calculated by the formula:

$$\frac{As \cdot MW \cdot 1000}{Am \quad (1000/X)} = \text{mg. carbonyl compound}$$

As = absorbance of the DNP-hydrazone solution
MW = molecular weight of carbonyl compound
Am = molar absorptivity
x = volume of chloroform solution for As measurement

An aliquot of the chloroform solution representing a known weight of carbonyl compound was transferred to a 50-ml. Claissen distilling flask, and the chloroform was removed at reduced pressure. One neck of the distilling flask contained a 25-ml. separatory funnel for adding acid and water, and the other neck was fitted with a steam injector tube. The side arm of the flask was connected through a small water condenser to a graduated cylinder containing 25 ml. of 5N HCl saturated with DNP-hydrazine. After chloroform was removed from the flask, the DNP-hydrazone was dissolved in 3 ml. of concentrated H_2SO_4. Ten ml. of water were then added through the separatory funnel, and the liberated carbonyl compound was steam-distilled into the DNP-hydrazine reagent. Fifty ml. of distillate were collected during the steam distillation, and the reaction mixture was allowed to stand 12 hrs. before it was extracted three times with 75-ml. quantities of hexane. The hexane extracts containing the DNP-hydrazone were combined, the hexane was removed, and the DNP-hydrazone(s) were chromatographed on a nitromethane-hexane-celite column (3). The DNP-hydrazones recovered in this manner were characterized by chromatographic behavior and U.V. spectra. Recovery of carbonyl compounds from the regeneration mixtures was ascertained by measuring absorbance of the chromatographed DNP-hydrazones and calculating the concentration by the same procedure outlined above.

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[3] 2,4-Dinitrophenyl is hereinafter abbreviated DNP.

TABLE I

Recovery of Free Carbonyl Compounds after Regeneration from Their DNP-Hydrazones

Carbonyl compound carbon chain length	Mg. for regeneration	Mg. recovered	Percentage recovery
n alkanals			
n-C5	1.05	0.438	42
n-C10	0.681	0.333	49
n-C12	1.35	0.669	50
n-alkan-2-ones			
n-C5	0.997	0.328	33
n-C9	1.61	0.665	41
n-C11	0.82	0.502	61
n-2-alkenals			
n-C5	0.446	0.257	57
n-C7	0.475	0.257	54
n-C8	0.744	0.413	56
n-C9	1.27	0.538	42
n-C10	0.984	0.306	31
n-C11	1.11	0.252	23

Recoveries for a number of alkanals, alkan-2-ones, and 2-alkenals are presented in Table I. Aside from these compounds, a number of miscellaneous carbonyl compounds have been regenerated and their odors found to be typical of the authentic compounds. These included 3-methylthiopropanal, furfural, phenylacetaldehyde, cinnamaldehyde, and the bis-DNP-hydrazone of 2,3-butanedione.

Discussion

Low recoveries shown in Table I may be attributed in part, to the manipulation required to obtain the quantitative data. Part of the loss was caused by steam distillation because isolation by this procedure varies among compounds. The extent of reaction of the distilled carbonyl compounds with DNP-hydrazine reagent may be limited at the concentration used here (2). Consequently it is not known whether the procedure will regenerate each carbonyl compound equally well.

With the alkanals and alkan-2-ones, there was no evidence that the regeneration methods caused the production of additional carbonyl compounds. On regeneration of 2-alkenals however traces of additional carbonyl compounds were produced. The trace components appeared as faint bands on the chromatography column that were not identified. One compound moved before the original "enal," and two very small bands followed it. In every recovery the bulk of the carbonyl compounds regenerated from the DNP-hydrazone of the 2-alkenal was the original "enal."

The advantage of the sulfuric-acid regeneration method over other methods is that it is fast and simple, and the odor of the regenerated carbonyl is easily observed. This procedure has been particularly useful in flavor chemistry research and in studying oxidation products of lipids.

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[Received March 30, 1960]

The Preparation and Purification of Monoglycerides. I. Glycerolysis of Oils[1]

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Mono- and diglycerides were produced by reacting the following oils with glycerol: coconut, peanut, sesame, linseed, and sardine. It was shown that the yield of monoglyceride was not dependent upon the fatty acid composition of the oil but on the solubility of glycerol in oil, which is dependent in part on the temperature. An excess of glycerol above that which is soluble does not change the composition of the reaction product. At 180°C. no more than 45% monoglycerides can be formed by glycerolysis.

MONOGLYCERIDES are important commercial products used for various purposes. The two important processes used in industry for preparing monoglycerides are the direct esterification of fatty acids and glycerol and the glycerolysis of oils, *i.e.*, the reaction of an oil with glycerol.

Of the two methods, the glycerolysis method has become more important because the fatty acid does not have to be liberated from the fat. The method consists in heating the glycerol with fats at 180–250°C. in the presence of catalysts such as caustic soda or sodium alcoholates in amounts of 0.05 to 0.20% of the weight of fat used. Along with monoglycerides, di- and triglycerides are also produced. The reaction is conducted at a high temperature to hasten the rate of reaction and to increase the miscibility of the reaction mixture with glycerol. The extent to which the reaction can be carried out is limited by the comparative immiscibility of glycerides and glycerol. Any glycerol forming a second liquid phase cannot participate in the reaction.

The maximum amount of glycerol miscible and reactable with highly hydrogenated cottonseed oil plus 0.1% NaOH was determined by Feuge and Bailey (1). The solubilities of other oils such as coconut, linseed, and sesame in glycerol were determined by Choudhury (2). It was concluded from this work that the solubility of oil in glycerol was mainly dependent on temperature, and slightly dependent on molecular weight and the unsaturation of the oil.

[1] Presented at the 51st annual spring meeting, Dallas, Texas, April 4–6, 1960.