

## Methods for the Isolation and Characterization of Constituents of Natural Products

### XIII. Regeneration of 2,4-Dinitrophenylhydrazones on a Periodic Acid Column

D. P. SCHWARTZ AND C. R. BREWINGTON

*Dairy Products Laboratory, Eastern Marketing and Nutrition Division,  
Agricultural Research Service, U.S. Department of Agriculture,  
Washington, D.C. 20250*

Regeneration of 2,4-dinitrophenylhydrazones (DNPH) has been accomplished in a number of ways. An early method attributable to Conant and Bartlett (2) employed pyruvic acid as regenerator-acceptor; Demaecker and Martin (4) and Cullinane and Edwards (3) employed hydrochloric acid and acetone for cleavage and acceptor after reduction of the nitro groups with  $\text{SnCl}_2$ . Robinson (10) suggested the use of at least 80% formic acid solution and copper carbonate as a regenerating system for ketosteroids. Reduction of the nitro groups with chromous chloride and simultaneous cleavage of ketosteroid hydrazones with hydrochloric acid was described by Elks and Oughton (6). Keeney (8) outlined a method of cleavage which utilized levulinic and hydrochloric acids. DePuy and Pender (5) extended this method to other classes of DNPH and also to oximes. Ralls (9) was able to get limited regeneration of DNPH by heating with  $\alpha$ -ketoglutaric acid. Bassette and Day (1) used concentrated  $\text{H}_2\text{SO}_4$  and water to liberate the parent carbonyl from the derivative. The recent method of Wong and Schwartz (13) regenerates the parent carbonyl in the injection port of the gas chromatograph.

A statement by Feigl (7) that hydrazides, oximes, and hydrazones can be attacked by periodic acid prompted us to investigate the use of this reagent for cleaving 2,4-dinitrophenylhydrazones, especially since we had already developed a simple, two-phase periodic acid column technique for cleaving periodic acid-susceptible structures (12).

## EXPERIMENTAL METHODS

### *Reagents and Apparatus*<sup>1</sup>

Paraperiodic acid (G. Frederick Smith Co., Columbus, OH); magnesium sulfate, anhydrous (J. T. Baker Chemical Co., Phillipsburg, NJ, cat. no. 2506);  $\text{CCl}_4$  (Baker, cat no. 1512); Silicone fluid 200, viscosity grade 2.0 (Dow Corning Corp., Midland, MI); medicine droppers, approximately  $10 \times 0.5$  cm (i.d.).

### *Preparation of Periodic Acid-Sulfuric Acid Column*

One milliliter of a saturated solution of periodic acid and 1 ml of 2 N  $\text{H}_2\text{SO}_4$  were pipetted onto 8 g of  $\text{MgSO}_4$  in a mortar. The mortar was shaken gently to cover the liquid with  $\text{MgSO}_4$  and was then ground with a pestle until homogeneous. The impregnated  $\text{MgSO}_4$  was sieved through an 80 mesh screen and the material which passed stored at  $0^\circ\text{C}$ . To prepare a column, approximately 300 mg of the powder was transferred to a medicine dropper and packed by tapping on a bench top. The column was wetted with  $\text{CCl}_4$  and inspected for voids. These were removed by tapping or by stirring with a wire. Columns prepared in this manner will allow 0.5 ml of  $\text{CCl}_4$  to completely enter in 15–20 minutes.

### *Regeneration Procedure*

One micromole or less of the 2,4-dinitrophenylhydrazone was dissolved in  $\text{CCl}_4$  and 0.5 ml was pipetted on the column. The course of the reaction was easily followed by observing the accumulation of an orange-yellow component at the top of the column. The colorless effluent was collected together with a column volume of  $\text{CCl}_4$  used to wash out residual carbonyl.

### *Quantitative Aspects*

The quantitative aspects of the reaction were followed by reforming the DNPH of the carbonyl present in the colorless effluent. The exit tip of the periodic acid column was positioned on top of a small (1 g) column of Celite impregnated with a 60%  $\text{H}_3\text{PO}_4$  solution of 2,4-dinitrophenylhydrazine prepared as described by Schwartz and Parks (11). Following derivatization, the  $\text{CCl}_4$  effluent was passed directly over a 400 mg column of Dowex 50  $\times$  8 ( $\text{H}^+$ ) (used directly from the bottle and merely washed with 2 column volumes of  $\text{CCl}_4$ ) to remove 2,4-dinitrophenylhydrazine. The colored effluent was then adsorbed onto

<sup>1</sup> Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

alumina (11) and the DNPH eluted with benzene. Recovery was estimated spectrophotometrically in  $\text{CHCl}_3$  by comparing the absorbance to that of a standard.

#### *Experiments Using Silicone Oil as Solvent*

A major disadvantage of regenerating the DNPH by the above method was apparent when one wished to evaluate the odor of the regenerated carbonyl. To circumvent this situation we have used silicone oil with the specified viscosity as solvent for the DNPH. This oil has no odor and this attribute, together with its low viscosity, makes it quite suitable for odor evaluation of the regenerated carbonyl. The procedure was exactly the same except that silicone oil was substituted throughout. For some DNPH it was necessary to heat the silicone oil to effect solution.

### RESULTS AND DISCUSSION

Table 1 summarizes the regeneration information. The absence of data on the regeneration of unsaturated DNPH should be noted. This is due to the fact that oxidation of the double bond occurs to some extent. Although this is a serious limitation, it is felt that the procedure will still be of considerable utility due to its simplicity.

All of the compounds listed in Table 1 gave good yields of the parent carbonyl except formaldehyde which gave no yield. The aldehyde and keto esters originally gave yields in the vicinity of 60–80%, when run at a flow rate of 15–20 minutes/0.5 ml. This has been attributed to hydrolysis of the ester bond since decreasing the flow rate gave even lower yields whereas increasing the flow rate (through the use of a wider diameter column) gave much better yields.

The only DNPH investigated which did not regenerate were benzophenone and methyl- $\alpha$ -ketostearate. These came through the column unchanged. Formaldehyde DNPH was apparently regenerated but no free formaldehyde was detected in the effluent. It has been reported that formaldehyde is not detected in the effluent when terminal glycols are oxidized on a periodic acid column (12).

In order for a 2,4-dinitrophenylhydrazone to be cleaved, adsorption on the surface of the  $\text{MgSO}_4$  must first take place. Thus, no regeneration takes place when Celite is substituted for  $\text{MgSO}_4$  as the support. This is also probably a prerequisite for the oxidation of glycols and related compounds and explains the observation of Schwartz *et al.* (12) that Celite and glass beads impregnated with periodic acid fail to cleave periodate-susceptible structures.

Solvents with greater polarities than  $\text{CCl}_4$  do not work well or not at all because of the adsorption requirement. However, a 20% (v:v)

solution of benzene in  $\text{CCl}_4$  is satisfactory for most of the DNPH listed in Table 1. Short chain DNPH and DNPH with other functional groups in the parent compound can be regenerated using more polar solvents since they are more strongly adsorbed than are the longer chain DNPH.

Analysis of the regenerated carbonyl compounds as DNPH by thin-layer partition chromatography showed that no colored artifacts are produced in the procedure. With unsaturated DNPH, other spots, principally aldehydes produced from the oxidation of the double bond, were apparent.

Regeneration has also been accomplished on microgram quantities of DNPH by preparing microcolumns in melting point capillaries. In these instances, we have been able to inject the effluent from the column

TABLE I  
REGENERATION OF 2,4-DINITROPHENYLHYDRAZONES ON A  
PERIODIC ACID-SULFURIC ACID COLUMN

2,4-Dinitrophenylhydrazone	Flow rate (min/0.5 ml)	Amount over column ( $\mu$ moles)	Recovery of free carbonyl (%)
Ketones			
2-Nonadecanone	20	0.07	96
2-Undecanone	20	0.13	101
2-Hexanone	15	0.07	103
2-Butanone	15	0.17	96
2-Propanone	15	0.15	90
Acetophenone	18	0.08	102
3-Cholestanone	20	0.21	99
Aldehydes			
Octadecanal	18	0.08	102
Tridecanal	18	0.05	101
Butanal	15	0.35	98
Acetaldehyde	20	0.34	96
Formaldehyde	15	0.29	0
Benzaldehyde	18	0.06	99
Phenylacetaldehyde	17	0.27	82
Carbonyl esters <sup>a</sup>			
Ethyl levulinate	4.5	0.11	105
Methyl-12-ketostearate	6.5	0.15	90
Methyl-7-ketostearate	6.5	0.07	92
Glycolaldehyde stearate	6.5	0.07	92

<sup>a</sup> Hydrolysis of esters takes place on the periodic acid column when slow flow rates are used. Data on these esters were obtained using a 400 mg column in a chromatography tube 0.8 cm, i.d.

directly into a gas chromatograph for purposes of confirming the identity of the parent carbonyl.

#### SUMMARY

A method is described for regenerating carbonyl compounds at room temperature from 2,4-dinitrophenylhydrazones. A column of  $\text{MgSO}_4$  impregnated with periodic and sulfuric acids is used as the regenerant and  $\text{CCl}_4$  is used as the solvent for the 2,4-dinitrophenylhydrazone. Examples are given for saturated aliphatic and aromatic ketones, saturated aliphatic and aromatic aldehydes, keto- and aldehydo-esters, and a keto-steroid. Some oxidation of the double bond in unsaturated 2,4-dinitrophenylhydrazones occurs. The use of a low-viscosity silicone oil as solvent allows for odor evaluation of the regenerated carbonyl compound.

#### REFERENCES

1. Bassette, R., and Day, E. A., Regeneration of carbonyl compounds from 2,4-dinitrophenylhydrazones with sulfuric acid. *J. Amer. Oil Chem. Soc.* **37**, 482-483 (1960).
2. Conant, J. B., and Bartlett, P. B., Quantitative study of semicarbazone formation. *J. Amer. Chem. Soc.* **54**, 2881-2899 (1932).
3. Cullinane, N. M., and Edwards, B. F. R., Preparation of ketones by cleavage of 2,4-dinitrophenylhydrazones. *J. Chem. Soc. London* **1958**, 1311-1312.
4. Demaecker, J., and Martin, R. H., A method for the cleavage of 2,4-dinitrophenylhydrazones and its application to steroid ketones. *Nature (London)* **173**, 266 (1954).
5. DePuy, C. H., and Pender, B. W., Levulinic acid as a reagent for the hydrolysis of oximes and 2,4-dinitrophenylhydrazones. *J. Amer. Chem. Soc.* **81**, 4629-4631 (1959).
6. Elks, J., and Oughton, J. F., Compounds related to the steroid hormones. X. Recovery of 3-oxo-steroids from their 2,4-dinitrophenylhydrazones. *J. Chem. Soc., London* **1962**, 4729-4730.
7. Feigl, F., "Spot Tests in Organic Analyses," p. 130. Elsevier, New York, 1966.
8. Keeney, M., Regeneration of carbonyls from 2,4-dinitrophenylhydrazones with levulinic acid. *Anal. Chem.* **29**, 1489-1491 (1957).
9. Ralls, J. W., A rapid method for semi-quantitative determination of volatile aldehydes, ketones, and acids. Flash exchange gas chromatography. *Anal. Chem.* **32**, 332-336 (1960).
10. Robinson, R., Cleavage of the 2,4-dinitrophenylhydrazones of ketones. *Nature (London)* **173**, 541 (1954).
11. Schwartz, D. P., and Parks, O. W., Preparation of carbonyl-free solvents. *Anal. Chem.* **33**, 1396-1398 (1961).
12. Schwartz, D. P., Weihrauch, J. L., and Burgwald, L. H., Methods for the isolation and characterization of constituents of natural products. A periodic acid column procedure for the oxidation of vic-glycols, epoxides, and  $\alpha$ -hydroxy acids at the micromole level. *Anal. Chem.* **41**, 984-986 (1969).
13. Wong, N. P., and Schwartz, D. P., A versatile injector for the gas chromatograph. *J. Chromatogr. Sci.* **7**, 569-571 (1969).