

CHROM. 9300

REGENERATION OF MONOCARBONYLS FROM THEIR 2,4-DINITRO-PHENYLHYDRAZONE DERIVATIVES

P. A. E. CANT and N. J. WALKER

New Zealand Dairy Research Institute, Palmerston North (New Zealand)

(Received May 7th, 1976)

SUMMARY

A method has been developed for regenerating monocarbonyl compounds from their 2,4-dinitrophenylhydrazone derivatives by passing a pentane solution of the hydrazones through a column of Celite impregnated with sulphuric acid. The use of heat or long contact with concentrated sulphuric acid was thus avoided. After regeneration the monocarbonyls were adsorbed from pentane on to a column of silicic acid and subsequently eluted in a small volume of diethyl ether prior to quantitative analysis by gas-liquid chromatography. The response of the flame ionization detector to a series of alkan-2-ones and *n*-alkanals was determined and the results compared to previously reported data.

INTRODUCTION

In the course of a study of monocarbonyl compounds present in whole milk powder, a method was required for their regeneration from the 2,4-dinitrophenylhydrazones (DNPs) in a form suitable for analysis by gas-liquid chromatography (GLC). The methods for carbonyl regeneration reported previously¹ all contained features which seemed undesirable for their application to milligram quantities of these reactive and volatile compounds. Some methods, for example, make use of heat or concentrated acid to liberate the carbonyls, while others require steam distillation to concentrate the products.

An alternative to the usual techniques of batch regeneration was suggested by the work of Parsons² and later of Craske and Edwards³, who demonstrated the reversibility of a 2,4-dinitrophenylhydrazine (DNPH) reaction column⁴ by passing a solution of DNP in cyclohexane over a column of Celite impregnated with 66% phosphoric acid. The yellow band adsorbed near the top of the Celite column was shown to be DNPH, while the eluate contained the regenerated parent carbonyl³. Yields of the lower ketones were high but there were large losses of the higher homologues and of the aldehydes studied. An investigation of this method of regeneration and its application to the four commonly encountered monocarbonyl classes (alkan-2-ones, *n*-alkanals, alk-2-enals and alka-2,4-dienals) is reported in this paper, together with a technique for concentrating the carbonyls on a column of silicic acid prior to

GLC analysis. A study of the response of the flame ionization detector (FID) to alkan-2-ones and *n*-alkanals is also discussed.

EXPERIMENTAL

Materials

Silicic acid (100 mesh; Mallinckrodt, St. Louis, Mo., U.S.A.) was washed with distilled water to remove fines and activated by heating for 48 h at 150°. Aluminium oxide (Brockman, activity II; BDH, Poole, Great Britain) was heated for 48 h at 150° before being deactivated by the addition of 6% (v/w) water. Celite (analytical grade; Johns Manville, Lompoc, Calif., U.S.A.) was used as received. *n*-Pentane (laboratory reagent; BDH) was made carbonyl-free by refluxing over sulphuric acid for 4 h, followed by distillation over potassium hydroxide pellets and passage through a column of activated silicic acid. Other solvents used were made carbonyl-free by the methods of Hornstein and Crowe⁵ or Schwartz and Parks⁴, except for ether (Analar; BDH) which was used as received. The monocarbonyls were obtained from the following sources: alkan-2-ones from Koch-Light (Colnbrook, Great Britain) and BDH; *n*-alkanals from Aldrich (Milwaukee, Wisc., U.S.A.) and K & K (Plainview, N.Y., U.S.A.); alk-2-enals and alka-2,4-dienals from Maumee Flavours and Fragrances (Danbury, Conn., U.S.A.). If not chromatographically pure, the compounds were distilled under reduced pressure. The DNPs were prepared from the monocarbonyls⁶ and their purity determined by thin-layer chromatography^{7,8} and mass spectrometry.

Gas chromatography of free monocarbonyls

A Varian Assoc. (Walnut Creek, Calif., U.S.A.) Model 2800 gas chromatograph equipped with FIDs was used in conjunction with a Hewlett-Packard (Avondale, Pa., U.S.A.) Model 3380A digital integrator. Analyses were performed using a 3-m glass column (2 mm I.D.) packed with Varaport 30 (80–100 mesh; Varian Assoc.) supporting 10% (w/w) stabilized DEGA (Analabs, Hamden, Conn., U.S.A.), or a 2-m glass column (2 mm I.D.) packed with 10% (w/w) OV-210 (Applied Science Labs., State College, Pa., U.S.A.) on Varaport 30 (100–120 mesh). Conditions under which both columns were operated are as follows: carrier gas (nitrogen) flow-rate, 25 ml/min; detector temperature 250°; injector temperature 210°; injection, 2.5 μ l; attenuation 8×10^{-11} ; the column temperature was programmed from 50° (held for 6 min) to 220° at 10°/min. Standard solutions (800 ppm of each homologue) of alkan-2-ones and *n*-alkanals in pentane were prepared and analysed by GLC using the conditions described above. Both solutions contained tetradecane as an internal standard so that the responses of the FID could be compared with data reported previously^{8,9}.

Regeneration

Concentrated sulphuric acid (1.5 ml) and distilled water (1.5 ml) were mixed in a mortar and ground with 5 g analytical-grade Celite. When homogeneous, the mixture was slurried with pentane and packed firmly into a chromatographic tube (2 cm I.D.) fitted with a sintered disc. Excess solvent was drained from the column before the DNPs were applied in a minimum quantity of pentane. Liberated carbonyls were eluted with further pentane (30–100 ml). The presence of a yellow band

in the top third of the column packing was taken as evidence that regeneration had occurred.

To evaluate the efficiency of the regeneration column for each class of DNP, a solution containing approximately 7.5 μ moles of a selection of compounds (C_3 – C_{15}) within a class was passed through a column. The eluate was led directly onto a DNPH reaction column in order to reform the DNP derivatives, which were then adsorbed onto a column of deactivated (6%) alumina⁴. Traces of DNPH remained on the alumina when the DNPs were eluted with *n*-hexane–benzene (1:1, v/v). After being taken to dryness, the DNPs were redissolved in chloroform for optical density measurements at 365, 355, 373 and 390 nm for the alkan-2-ones, *n*-alkanals, alk-2-enals and alka-2,4-dienals, respectively.

Concentration

The pentane solution of free carbonyls eluted from the regeneration column was too dilute for direct GLC analysis. Although the relative volatility of the lower homologues precluded the use of conventional distillation techniques for quantitative concentration, the carbonyls could be adsorbed from pentane on activated silicic acid and subsequently eluted in diethyl ether. The dry silicic acid (0.5 g) was placed in a chromatographic tube (0.6 cm I.D.) and washed with 10–15 ml diethyl ether, followed by pentane, prior to the introduction of the carbonyl solution.

To evaluate recoveries from the silicic acid concentration column, 1-ml aliquots of standard solutions of alkan-2-ones, *n*-alkanals, and alk-2-enals (200 ppm) were adsorbed onto separate columns. A quantity of pentane (approximately the volume required to elute the appropriate class from the regeneration column) was passed through each column and the carbonyls were eluted subsequently with five 1-ml fractions of diethyl ether for GLC analysis.

The capacity of the silicic acid concentration column was established by successive additions of 1-ml aliquots of the 200-ppm alkan-2-one solution and 2 ml pentane until carbonyls were observed in the eluate.

Quantitative analysis following regeneration and concentration

To establish the overall efficiency of the regeneration–concentration system, a known weight of alkan-2-one DNPs (0.8 mg each, C_3 – C_{15}) was dissolved in pentane and passed in turn through the regeneration and concentration columns. The diethyl ether fractions containing free alkan-2-ones were analysed by GLC on the 10% DEGA column. Using response factors obtained from experimental data, the yields of individual ketones were calculated.

RESULTS AND DISCUSSION

Gas chromatography of free monocarbonyls

The FID responses to the alkan-2-ones and *n*-alkanals relative to tetradecane were used to calculate for each compound an effective carbon number, N_c , which are given in Table I, together with literature values where available. By definition⁸,

$$N_c = N_{\text{ref.}} \cdot \frac{\text{Peak area ratio, sample to reference}}{\text{Mole ratio, sample to reference}}$$

where $N_{\text{ref.}} = 14$ for tetradecane.

TABLE I
EFFECTIVE CARBON NUMBERS, N_c , FOR ALKAN-2-ONES AND *n*-ALKANALS
A, derived from relative response factors quoted by Dietz⁹; *B*, from ref. 8.

Carbon number	N_c					
	Alkan-2-ones			<i>n</i> -Alkanals		
	Exptl. (DEGA)	<i>A</i>	<i>B</i>	Exptl. (DEGA)	Exptl. (OV-210)	<i>A</i>
3	2.01	1.99	2.06	—	—	—
4	3.16	3.07	3.16	2.69	1.21	3.12
5	3.92	—	—	3.87	2.37	—
6	4.99	—	—	4.58	3.53	—
7	6.07	5.66	—	5.58	5.18	6.14
8	6.96	7.17	—	6.67	6.56	6.99
9	7.91	—	—	7.83	7.58	8.74
10	8.99	—	—	8.51	8.66	—
11	9.96	—	—	9.65	9.79	—
12	—	—	—	10.55	10.92	—
13	11.92	—	—	—	—	—
15	13.74	—	—	—	—	—

A typical chromatogram of the alkan-2-ones is shown in Fig. 1.

The alkan-2-ones exhibit effective carbon numbers of one less than their chain lengths. This finding is consistent with the limited data reported⁹, and is attributed to the absence of response of the FID to the carbonyl carbon⁸. Perkins *et al.*¹⁰ have shown that the FID gives no response to the carbonyl carbon in fatty acids and aldehydes, and the data in Table I support this contention over a wider range of

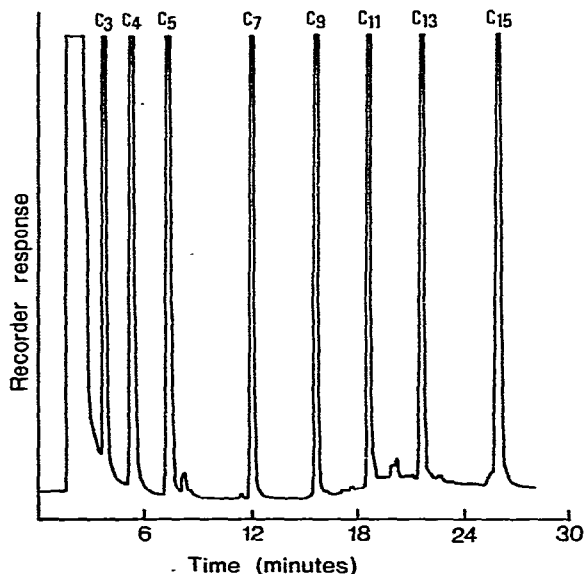


Fig. 1. Gas chromatogram of alkan-2-ones on DEGA column. For chromatographic conditions see text.

compounds than hitherto investigated, although the effective carbon numbers obtained on the DEGA column are slightly lower than expected. This anomaly may be due either to irreversible reactions occurring in the chromatographic system, or to the presence of impurities in the *n*-alkanals which deteriorate rapidly, even on storage at 4°. While each *n*-alkanal was analysed by GLC prior to being used for the standard solution, organic acids and polymeric material may not have been detectable. The difficulty in preparing pure alk-2-enals and alka-2,4-dienals, which are even less stable, prevented response factor data for these compounds from being obtained. The behaviour of the *n*-alkanals on the OV-210 column, however, suggests that the chromatographic system influences the observed FID responses, as the effective carbon numbers of the lower homologues are more than two less than the chain length.

Regeneration

The recoveries of the four DNP classes after passage through the regeneration column (Table II) are essentially quantitative for all but the alka-2,4-dienals. Low recoveries of the alka-2,4-dienals are probably a consequence of the comparatively high reactivity of these compounds, which are unstable on storage at 0°.

TABLE II

RECOVERIES OF DNP CLASSES FROM THE REGENERATION COLUMN

Recoveries are means of duplicate determinations.

<i>DNP class</i>	<i>Recovery (%)</i>
Alkan-2-ones	94
<i>n</i> -Alkanals	100
Alk-2-enals	94
Alka-2,4-dienals	61

No quantitative measure of the regeneration capacity of the column was obtained, but when used to regenerate 7.5 μ moles of DNPs, overloading occurred if either the Celite or the sulphuric acid had lost activity due to the absorption of atmospheric water. Both these materials needed to be stored in tightly closed containers. Inconsistent performance also resulted if the column was not packed tightly enough.

The alkan-2-ones required only 33% sulphuric acid (1 ml sulphuric acid and 2 ml water) for complete regeneration, and losses of the lower homologues were observed at the higher acid concentration (50%) necessary to regenerate the aldehyde classes.

The *n*-alkanals were regenerated readily using 50% sulphuric acid and were eluted from the column in 30 ml pentane, whereas the alkan-2-ones, alk-2-enals, and the alka-2,4-dienals required 100 ml pentane.

Regeneration of alkan-2-ones was examined on Celite impregnated with various concentrations of phosphoric acid, but efficiencies were low compared to the sulphuric acid-Celite columns. The use of concentrated hydrochloric acid (3 ml) on Celite was also investigated. While the yields of regenerated alkan-2-ones were as high as those obtained from the sulphuric acid-Celite column, the alk-2-enals

appeared to undergo a reaction, possibly with hydrochloric acid dissolved in the pentane eluent, to give a yellow discolouration on the top of the concentration column. The regeneration column did not appear to be overloaded in this instance, as the colour of the adsorbed DNPH extended for less than a third of the packed length.

Concentration

Recoveries of the free monocarbonyls from the silicic acid concentration column are presented in Table III. For all but C₃ and C₄ homologues, which may undergo a condensation reaction catalysed by the silicic acid, recoveries of the alkan-2-ones, *n*-alkanals, and alk-2-enals were quantitative. The lower recoveries of the C₁₀-C₁₂ *n*-alkanals when 30 ml pentane was passed over the silicic acid implies that this class was less strongly adsorbed than the others. As a consequence, the volume of pentane used to elute the *n*-alkanals from the regeneration column was kept to a minimum (30 ml).

Although usually required to adsorb less than 3 mg monocarbonyls, the silicic acid column was not overloaded on adsorption of at least 32 mg alkan-2-ones.

TABLE III
RECOVERIES FROM SILICIC ACID COLUMN

Carbon number	Recovery (%)				
	Alkan-2-ones		<i>n</i> -Alkanals		Alk-2-enals
	100 ml*	30 ml*	15 ml*	100 ml*	
3	71.5	—	—	—	
4	93.1	92.4	90.4	—	
5	98.3	102.1	104.9	—	
6	104.1	102.0	107.4	101.0	
7	102.4	101.4	106.5	101.5	
8	103.6	100.1	106.9	99.2	
9	102.2	97.1	104.2	101.1	
10	102.6	95.9	104.8	104.5	
11	102.2	92.0	101.2	100.0	
12	—	88.6	97.1	—	
13	99.9	—	—	—	
15	100.7	—	—	—	

* Volumes of pentane from which carbonyls were adsorbed on to the silicic acid.

Quantitative analysis following regeneration and concentration

The yields of alkan-2-ones obtained from a representative experiment by regeneration from the DNP derivatives and subsequent concentration on a silicic acid column are shown in Table IV.

Recoveries were greater than 90% for all alkan-2-ones except acetone, 50% of which was lost. While part of this loss could be attributed to the concentration step (see Table III) acetone was also lost during regeneration. However, when a solution of free alkan-2-ones was passed through the regeneration column no acetone

TABLE IV

YIELDS OF ALKAN-2-ONES FOLLOWING REGENERATION FROM DNPs AND CONCENTRATION ON SILICIC ACID

Carbon number	Carbonyl recovered (μg)		Yield (%)
	Experimental	Theoretical*	
3	99.1	198.9	49.8
5	265.0	265.8	99.7
7	313.0	309.4	101.2
9	354.0	362.5	97.7
11	407.2	388.6	104.8
13	409.5	423.2	96.8
15	408.9	452.0	90.5

* Calculated from weight of DNP in mixture.

was lost. It is likely, therefore, that acetone undergoes reaction at the time of cleavage of the hydrazone.

The yields of regenerated alkan-2-ones and *n*-alkanals were calculated using their response factors, but this method of analysis could not be applied to the unsaturated aldehyde classes because the inherent instability of these compounds prevented the determination of accurate response factors. However, an overall recovery factor relating the FID response of the regenerated compound to the known weight of DNP subjected to the regeneration procedure, allows an accurate determination of concentrations of that DNP in natural extracts.

Consequently, the method outlined above can be used to regenerate aliphatic monocarbonyl compounds conveniently, and in most cases quantitatively, under relatively mild reaction conditions.

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