Journal of Chromatography, 187 (1980) 413–417

CHROM. 12,353

Note

Gas chromatographic determination of low-molecular-weight carbonyl compounds in aqueous solution as their O-(2,3,4,5,6-pentafluorobenzyl) oximes

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(Received August 31st, 1979)

Pentafluorophenylhydrazine (PFPH)[1,2] and O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (pentafluorobenzyloxyamine, PFBOA)[3,4] have been used as derivatizing agents in the gas chromatographic (GC) determination of keto-steroids. Hoshika and Muto[5] reported the use of PFPH in the GC of lower aliphatic carbonyl compounds. We have considered these reagents as derivatizing agents in the GC determination of low-molecular-weight carbonyl compounds in aqueous solution. In a previous paper[6], we described the use of PFPH in the GC determination of these carbonyl compounds.

Later, PFBOA was found to be more suitable for this purpose, and this paper compares the utility of PFBOA and PFPH.

EXPERIMENTAL

Reagents

PFPH was obtained from Aldrich (Milwaukee, Wisc., U.S.A.). PFBOA hydrochloride was synthesized from pentafluorobenzyl bromide (Aldrich) and N-hydroxyphthalimide (Tokyo Kasei, Tokyo, Japan) according to the procedure of Youngdale[7]. White plates (m.p. 115°) were obtained. Elemental analysis: calculated for $C_7H_5NOF_5Cl$, C 33.69, H 2.02, N 5.61%; found, C 33.69, H 1.97, N 5.52%.

The internal standard (IS) solution was a 0.025% solution of *p*-chlorobenzyl chloride in ethyl acetate.

Apparatus and conditions

A Shimadzu Model GC-4APF gas chromatograph equipped with a flame-ionization detector(FID) was used. The GC conditions were as follows: 2-m glass column packed with 3% XE-60 on 80–100-mesh Celite 545 (AW DMCS); column temperature, 90°; detector temperature, 120°; injection temperature, 120°; chart speed, 0.25 cm/min.

Standard procedure

To a 0.5-ml aliquot of sample solution in a 10-ml centrifuge tube was added 0.5 ml of PFBOA solution (0.2 mg/ml; *ca.* $8.0 \cdot 10^{-4}$ *M*). The mixture was shaken well and allowed to stand for 40 min at room temperature (overnight if the sample

solution contained ketones). To the reaction mixtures, saturated with sodium chloride, was added 1 drop of 18 *N* sulphuric acid and the mixture was extracted with 0.2 ml of ethyl acetate containing 50 μg of *p*-chlorobenzyl chloride as internal standard. Excess of sodium chloride and the aqueous layer were removed with the aid of a syringe with a long needle, a few grains of anhydrous sodium sulphate were added to dry the ethyl acetate extract and an aliquot was injected on to the GC column.

RESULTS AND DISCUSSION

The retention times of thirteen O-pentafluorobenzyloximes (O-PFBO) relative to the internal standard obtained on different columns are given in Table I.

TABLE I

RELATIVE RETENTION TIMES OF THE O-PENTAFLUOROBENZYLOXIMES OF CARBONYL COMPOUNDS

Parent compound	*Stationary phase and column temperature*			
	3% XE-60 (90°)	*3% XF-1105 (100°)*	*3% SE-30 (80°)*	*2% OV-17 (70°)*
PFBOA	0.62	0.69	0.88	0.65
HCHO	0.19	0.27	0.29	0.19
CH_3CHO	0.32, 0.34*	0.48, 0.51*	0.57, 0.59*	0.43
C_2H_5CHO	0.47, 0.51*	0.80	1.05	0.72
n-C_3H_7CHO	0.80	1.41	1.83, 1.92*	1.30, 1.38*
iso-C_3H_7CHO	0.57	1.01	1.32	0.88
n-C_4H_9CHO	1.35, 1.44*	2.68	3.33, 3.52*	2.52, 2.68*
iso-C_4H_9CHO	2.72	1.91, 1.99*	2.55, 2.72*	1.76, 1.94*
CH_3COCH_3	0.40	0.70	0.88	0.65
$CH_3COC_2H_5$	0.61	1.13	1.50	1.04
CH_3CO-*iso*-C_3H_7	0.73	1.48	2.08	1.24, 1.43*
CH_3CO-*iso*-C_4H_9	1.16	2.36	3.38	2.08
$C_2H_5COC_2H_5$	0.85	1.74	2.43	1.61
C_2H_5CO-*n*-C_3H_7	1.27	2.70	3.95	2.61
$ClC_6H_4CH_2Cl$**	1.00	1.00	1.00	1.00

* Double peaks.

** Internal standard.

A typical GC separation of some carbonyl compounds as their O-PFBO derivatives is illustrated in Fig. 1; *p*-chlorobenzyl chloride was used as the internal standard. The procedure was essentially the same as that described previously[6].

A calibration graph was constructed by plotting the ratio of the peak height of a carbonyl compound to that of the internal standard, and a straight line passing through the origin was obtained for formaldehyde, acetaldehyde, isobutyraldehyde and diethyl ketone in the range 1–50 μg in 0.5 ml of aqueous solution. The reproducibility of the method for five replicate determinations on an identical sample solution containing 30 μg of isobutyraldehyde was examined; the standard deviation was 1.48%, which was less than that obtained with PFPH (1.84%).

When some carbonyl compounds reacted with PFBOA (marked with asterisks in Table I), the two peaks in each instance can be considered to correspond to *syn*-

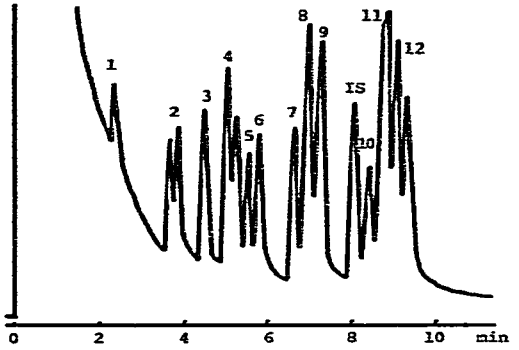

Fig. 1. Gas chromatogram of some carbonyl compounds as their pentafluorobenzyloximes. Conditions: 3% XE-60, 2.0-m glass column, temperature programmed from 80° to 120° at 4°/min, FID. Peaks: 1 = formaldehyde; 2 = acetaldehyde; 3 = acetone; 4 = propionaldehyde; 5 = isobutyraldehyde; 6 = methyl ethyl ketone; 7 = methyl isopropyl ketone; 8 = *n*-butyraldehyde; 9 = diethyl ketone; 10 = methyl isobutyl ketone; 11 = ethyl *n*-propyl ketone; 12 = *n*-valeraldehyde; IS = *p*-chlorobenzyl chloride.

and *anti*-isomers resulting from condensation reactions with PFBOA. A similar phenomenon was observed with PFPH.

The utility of PFBOA was compared with that of PFPH. As shown in Fig. 2, the PFPH concentration required was about ten times greater than those of the aldehydes being determined, in order to complete the reaction, whereas the formation of the O-PFBO derivatives was easily achieved with a much lower concentration of PFBOA. As can be seen in Table II, the condensation reaction was complete in 20 min at room temperature, after which the measured values were constant. Using isobutyraldehyde, the peak-area ratios of both O-PFBO and hydrazone derivatives to the same internal standard (*p*-chlorobenzyl chloride) were measured at column temperatures of 90° (Fig. 2) or 120°. The values obtained were O-PFBO 0.58 and hydrazone 0.28 at 90°, and O-PFBO 0.62 and hydrazone 0.28 at 120°. Hence the value for the O-PFBO derivative was approximately double that of the hydrazone at each temperature. The condensation reaction of PFBOA with ketones also proceeded

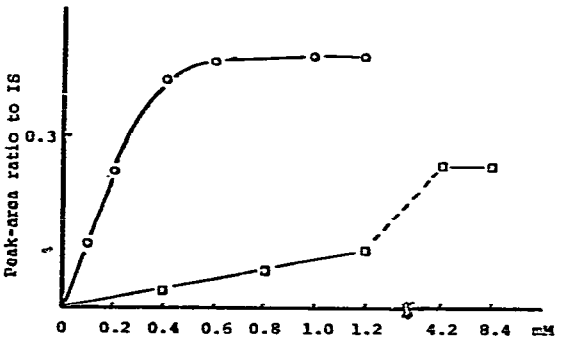

Fig. 2. Effect of PFBOA (○) and PFPH (□) concentration on reaction with *iso*-C_3H_7CHO (0.7 mM). The measurements were carried out according to the procedure described under Experimental and that in the previous paper[6]. IS: *p*-chlorobenzyl chloride for the determination of both derivatives produced. Column temperature: 90°.

TABLE II

EFFECT OF REACTION PERIOD ON EXTENT OF CONDENSATION REACTION WITH PFBOA

Reaction at room temperature. The values given for extent of reaction are the peak-area ratios of the compound peaks to that of the internal standard (*p*-chlorobenzyl chloride).

Compound	*Reaction period*				
	20 min	*40 min*	*1 h*	*2 h*	*24 h*
HCHO	1.78	1.88	1.82	1.82	1.63
CH_3CHO	0.85	0.88	0.82	0.85	0.71
C_2H_5CHO	0.92	0.82	0.88	0.85	0.75
n-C_3H_7CHO	0.81	0.79	0.82	0.77	0.71
iso-C_3H_7CHO	1.01	1.00	1.09	1.00	0.95
n-C_4H_9CHO	0.68	0.66	0.68	0.68	0.68
iso-C_4H_9CHO	0.56	0.52	0.56	0.50	0.78
CH_3COCH_3	0.44	0.60	0.86	1.06	1.23
$CH_3COC_2H_5$	0.18	0.31	0.38	0.58	1.18
CH_3CO-*iso*-C_3H_7	0.07	0.12	0.15	0.25	1.11
CH_3CO-*iso*-C_4H_9	0.05	0.07	0.09	0.14	0.60
$C_2H_5COC_2H_5$	0.07	0.12	0.17	0.21	0.98
C_2H_5CO-*n*-C_3H_7	0.03	0.04	0.06	0.11	0.97

slowly, being analogous to the reaction of PFPH but proceeding further to completion (Table II, Fig. 3), a measurable yield obtained within 24 h.

Nambara *et al.*[3] heated the reaction mixture in pyridine at 60° for 1 h in order to accelerate the condensation between PFBOA and keto-steroids. Koshy *et al.*[4] heated the mixture in benzene and pyridine at 65° for 30 min to prepare the O-PFBO derivatives of steroids with keto groups. In our experiment, the condensation reaction was carried out in aqueous solution. It can be seen in Fig. 3 that heating improved the yield, but the yield decreased gradually with an increase in the heating period, giving a value after 60 min that was considerably lower than that obtained after 24 h at room temperature.

With aldehydes, heating of the reaction mixture also did not give very good

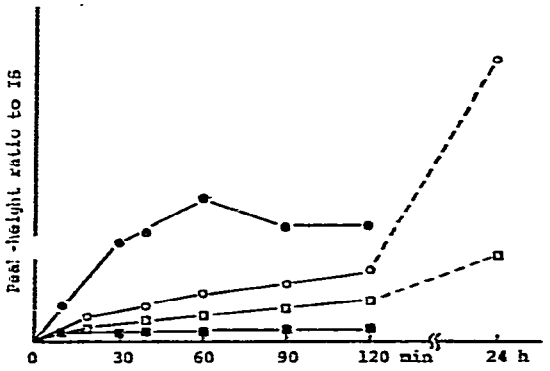

Fig. 3. Effect of reaction temperature and reaction period on the condensation reactions of diethyl ketone with PFBOA or PFPH. Reaction: ○, with PFBOA at room temperature; ●, with PFBOA at 70°; □, with PFPH at room temperature; ■, with PFPH at 70°.

results, and the procedure at room temperature was much more satisfactory with regard to the yield of the reaction and deviation of the values obtained from repeated determinations on an identical sample solution. A decrease in the reagent concentration in the reaction mixture led to an additional advantage with PFBOA that the removal of the unreacted reagent was almost complete. PFBOA in aqueous solution was slowly hydrolyzed on storage for more than a week and the pentafluorobenzyl alcohol liberated was extracted, giving an undesirable peak that overlapped that of the O-PFBO derivative of acetone on the gas chromatogram. The O-PFBO derivatives were much more volatile than the corresponding pentafluorophenylhydrazones, and therefore the GC separation could be carried out at lower temperatures. In this work, the column temperature was maintained at 90° for all separations, which was 30° lower than that used with the pentafluorophenylhydrazones.

The extent of the condensation reaction with PFPH was greater in neutral media, whereas the reaction with PFBOA proceeded readily in weakly acidic media (pH 4–6), so that the use of a buffer solution was unnecessary.

The stability of the O-PFBO derivatives was studied using isobutyraldehyde, and it appeared that the derivative was stable in ethyl acetate at room temperature for at least a few days. Because of the ease of preparation of the derivatives, the complete removal of unreacted reagent and the increase in volatility of O-PFBO, the reagent would be more suitable for the GC determination of carbonyl compounds and the derivatives would be extremely sensitive to electron-capture detection.

ACKNOWLEDGEMENT

The authors are indebted to Mr. T. Ueda for technical assistance.

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