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Note

Gas chromatographic determination of low-molecular-weight carbonyl compounds in aqueous solution as their pentafluorophenylhydrazones

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Previously, we reported that the photolysis of aliphatic α-amino acids in the presence of mercury(II) chloride resulted in the formation of methylmercury, arising from apparent fragmentation of the alkyl residue of the α-amino acids[1].

Recently, it has been found that photochemical alkylation of inorganic mercury proceeds in a more complicated manner in neutral media[2]. In considering the mechanism of the alkylation, we were confronted with the problem that it was necessary to measure quantitatively the low-molecular-weight carbonyl compounds produced as intermediates in the photolysis of α-amino acids. Low-molecular-weight carbonyl compounds are known to be extremely soluble in water. The reaction between carbonyl compounds and 2,4-dinitrophenylhydrazine is known to be an effective derivatization process which yields compounds that are extractable with organic solvents.

On the other hand, gas chromatography (GC) requires the previous preparation of suitable volatile derivatives. Attal *et al.*[3] and Mead *et al.*[4] reported on the GC of carbonyl compounds using pentafluorophenylhydrazine (PFPH). This method has been applied to lower aliphatic carbonyl compounds by Hoshika and Muto[5]. However, they showed only typical chromatograms for standard solution of pure pentafluorophenylhydrazones which were prepared in methanol solution, and we are not aware of any report dealing with an aqueous solution containing lower aliphatic carbonyl compounds. This paper describes a procedure for the GC determination of low-molecular-weight carbonyl compounds in aqueous solution as their pentafluorophenylhydrazones and its application to such compounds produced by photolysis of α-amino acids.

EXPERIMENTAL

Reagents

Pentafluorophenylhydrazine (PFPH) was obtained from Aldrich (Milwaukee, Wisc., U.S.A.). The buffer solution was 0.6 *M* sodium phosphate solution (pH 7.0). The internal standard (IS) solution was a 0.025% solution of *p*-xylylene dichloride in ethyl acetate.

Apparatus and conditions

A Shimadzu GC-4APF gas chromatograph equipped with a flame-ionization detector (FID) was used. The GC conditions were as follows: a 2-m glass column packed with 3% XE-60 on 80–100-mesh Celite 545 (AW DMCS), column temperature 120°, detector temperature 150°, injection temperature 150° and chart speed 0.25 cm/min.

Standard procedure

To 0.5 ml of sample solution, add 0.5 ml of PFPH solution (1.5 mg/ml; *ca.* $7.6 \cdot 10^{-3}$ *M*) and 1 drop of 0.6 *M* phosphate buffer (pH 7.0) in a 10-ml centrifuge tube. Mix well and allow to stand for 20 min at room temperature. Saturate the reaction mixture with sodium chloride, add 1 drop of 18 *N* sulphuric acid and extract with 0.2 ml of ethyl acetate containing 50 μg of *p*-xylylene dichloride as internal standard. Carry out the extraction in the cold. Remove excess of sodium chloride and the aqueous layer with the aid of a syringe with a long needle, dry by adding a small amount of sodium sulphate and apply an aliquot of the ethyl acetate extract on to the GC column.

RESULTS AND DISCUSSION

The relative retention times of twelve pentafluorophenylhydrazone derivatives and some compounds that are possible internal standards are given in Table I.

TABLE I

RELATIVE RETENTION TIMES OF THE PENTAFLUOROPHENYLHYDRAZONES OF TWELVE CARBONYL COMPOUNDS AND SOME COMPOUNDS THAT ARE POSSIBLE INTERNAL STANDARDS

Parent compounds	*Stationary phase and column temperature*			
	2% OV-17, 100°	*3% SE-30, 100°*	*3% XE-60, 120°*	*3% XF-1105, 115°*
PFPH	0.67	0.19	0.44	0.25
HCHO	0.33	0.22	0.51	0.34
CH_3CHO	0.56	0.43*	0.61*	0.49
C_2H_5CHO	0.94	0.77	0.93	0.80
n-C_3H_7CHO	1.48	1.32	1.44	1.31
iso-C_3H_7CHO	1.00	1.00	1.00	1.00
n-C_4H_9CHO	2.78	2.81	2.34	2.50
CH_3COCH_3	0.69	0.68	0.69	0.65
$CH_3COC_2H_5$	1.30	1.15	0.97	1.03
CH_3CO-*iso*-C_3H_7	1.37	1.55	1.10	1.32
CH_3CO-*iso*-C_4H_9	2.32	2.48	1.73	2.05
$C_2H_5COC_2H_5$	1.70	1.81	1.22	1.50
C_2H_5CO-*n*-C_3H_7	2.58	2.90*	1.64*	2.20
I-C_6H_4-I	2.98	1.34	1.11	1.14
Br-C_6H_4-Br	0.52	0.54	0.30	0.34
Cl-C_6H_4-Cl	0.61	0.39	0.39	0.34
Cl-C_6H_4-CH_2Cl	0.64	0.48	0.43	0.38
$ClCH_2$-C_6H_4-CH_2Cl	2.39	1.50	1.56	1.11

* Double peaks.

Hoshika and Muto[5] reported that the separation of the peaks of PFPH and the formaldehyde pentafluorophenylhydrazone derivative was incomplete when either 5% SE-30 or a glass capillary coated with DEG 20M was used. As shown in Table I, however, the separation of the both of these peaks was adequate for the determination of formaldehyde on any of the columns we used. Fig. 1 illustrates a typical separation of some carbonyl compounds as their pentafluorophenylhydrazones using a 3% XE-60 column. *p*-Xylylene dichloride was used as the internal standard. A series of preliminary investigations was carried out in order to find suitable conditions for reaction and extraction, and the procedure described under Experimental was established. The condensation reaction proceeded more readily in neutral than in acidic media.

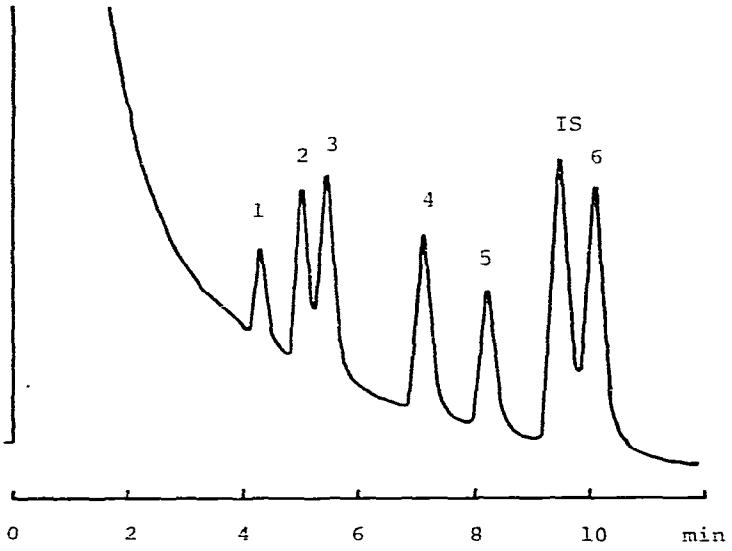

Fig. 1. Gas chromatogram of some carbonyl compounds as their pentafluorophenylhydrazones. Conditions: 3% XE-60, 2.0-m glass column, temperature programmed from 105° to 130° at 2°/min, FID. Peaks: 1 = formaldehyde; 2 = acetaldehyde; 3 = acetone; 4 = isobutylaldehyde; 5 = diethyl ketone; IS = *p*-xylylene dichloride; 6 = methyl isobutyl ketone.

Table II shows the effect of the reaction period at room temperature on the extent of the condensation reaction with 0.5 ml of a $7 \cdot 10^{-4}$ *M* solution of each carbonyl compound according to the procedure described. In general, aldehydes readily underwent quantitative reactions in 20 min, while the reactions with ketones proceeded slowly and showed a tendency to become even slower as the alkyl residue became larger. It seems likely that the lowering of the extent of reaction is due to steric hindrance of bulky, alkyl groups.

The reagent concentration was made about 10 times greater than those of the carbonyl compounds and the reaction period for aldehydes was fixed at 20 min at room temperature in order to obtain constant extents of reaction.

Of the carbonyl compounds tested, some gave double peaks on the chromatograms, possibly due to *syn*- and *anti*-isomers resulting from condensation reactions with PFPH.

TABLE II

EFFECT OF REACTION PERIOD ON EXTENT OF CONDENSATION REACTION WITH PFPH

Compound	*Reaction period (min)* *				
	0	*20*	*40*	*120*	*24 h*
HCHO	0.37**	0.58	0.57	0.52	0.54
CH_3CHO	0.33	0.67	0.67	0.62	0.64
C_2H_5CHO	0.60	0.62	0.64	0.65	0.68
n-C_3H_7CHO	0.49	0.46	0.51	0.54	0.53
iso-C_3H_7CHO	0.55	0.82	0.81	0.81	0.80
n-C_4H_9CHO	0.97	1.00	1.03	1.14	1.11
CH_3COCH_3	0.08	0.25	0.29	0.72	0.76
$CH_3COC_2H_5$	0.06	0.10	0.18	0.41	0.78
CH_3CO-*iso*-C_3H_7	0.02	0.07	0.08	0.19	0.83
CH_3CO-*iso*-C_4H_9	0.01	0.04	0.06	0.13	0.38
$C_2H_5COC_2H_5$	0.02	0.05	0.07	0.12	0.30
C_2H_5CO-*n*-C_3H_7	—	0.04	0.06	0.08	0.22

* At room temperature.

** The extent of the reaction was determined as the peak-area ratio of the compound peak to that of the internal standard (*p*-xylylene dichloride).

Ethyl acetate was a suitable solvent for the extraction of the hydrazone reaction products. Salting-out improved the extent of extraction. Extraction was carried out in acidic media to prevent an excess of PFPH from being extracted into the organic solvent together with the reaction hydrazone products. It would also be desirable to carry out extraction at reduced temperatures and dehydration by adding a small amount of anhydrous sodium sulphate. Standard solutions of pure hydrazones in ethyl acetate were stable.

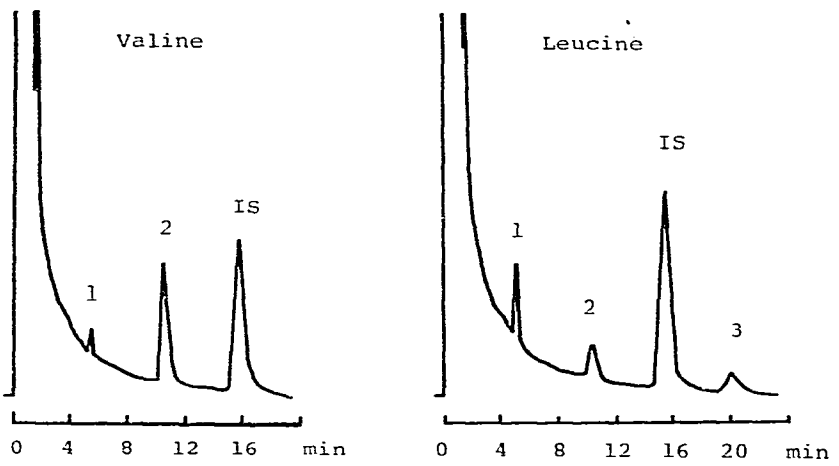

Fig. 2. Gas chromatograms of pentafluorophenylhydrazones of some carbonyl compounds produced from photochemical reactions of amino acids. Conditions: 3% XE-60, 2.0-m glass column, 120°, FID. Peaks: 1 = formaldehyde; 2 = acetaldehyde; IS = xylylene dichloride; 3 = isovaleraldehyde (?).

Sample solutions containing three carbonyl compounds (formaldehyde, acetaldehyde and isobutyraldehyde) were measured according to the procedure described, and the calibration graph for each was linear and passed through the origin for concentrations in the range 10–40 μg. Five repeated determinations on an identical sample solution containing 30 μg of isobutyraldehyde gave a standard deviation of 1.84%.

Solutions of DL-alanine, DL-valine, DL-leucine or DL-isoleucine (7.5 mmole) in 0.1 M phosphate buffer (pH 7.0) (300 ml) containing mercury(II) chloride (2.5 mmole) were irradiated in a quartz vessel with a 20-W blacklight lamp (300–400 nm) as a light source for 300 h and the mixtures were measured by the method described.

Fig. 2 shows the gas chromatograms obtained. For valine, two peaks corresponding to formaldehyde and isoburaldehyde were observed. For leucine, a peak thought to be isovaleraldehyde from its retention time, in addition to isobutyraldehyde and formaldehyde, was observed.

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