[Chem. Pharm. Bull. 23(6)1374—1376(1975)]

UDC 547.574.3.08 : 543.544 : 543.51

Identification of 2,4-Dinitrophenylhydrazones of Volatile Carbonyl Compounds by Combined Gas Chromatography-Mass Spectrometry[1]

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(Received November 2, 1973)

Some of 2,4-DNPH of volatile carbonyl compounds were analyzed by mass spectrometry combined with gas chromatography using a column packed with OV-101 (3%). The mixture of 2,4-DNPH of volatile carbonyl compounds could be resolved on the column under the condition with linear program at a rate 5°/min from 140°—300°. The 2,4-DNPH of volatile carbonyl compounds could be easily read from the peak with highest mass in the spectrum. The M^+-60 and M^+-42 were observed in the mass spectra of 2,4-DNPH of aliphatic aldehydes over carbon number five. The 2,4-DNPH of furan compounds also do exhibit the parent peak and fragment ions corresponding to the original compounds. M^+-NO and M^+-NO_2 were not significant in the mass spectrum of 2,4-DNPH of the volatile carbonyl compounds. From the result it was found that 2,4-DNPH of volatile carbonyl compounds were not decomposed during gas chromatography.

It has become apparent that volatile carbonyl compounds have a close relation with flavor which is one of the most important characteristics of roasted foods. In foodstuffs, however, volatile carbonyl compounds are present only in a small amount. Thus, volatile carbonyl compounds have been usually analyzed as their 2,4-dinitrophenylhydrazones (2,4-DNPH). The 2,4-DNPH of volatile carbonyl compounds could be directly analyzed by gas chromatography.[3] Although this gas chromatographic method affords good separation of 2,4-DNPH of volatile carbonyl compounds, insufficient result is sometimes obtained for identification. This problem could be resolved by mass spectrometry combined with gas chromatography. In the previous report, the volatile carbonyl compounds in peated malt were identified as their 2,4-DNPH by mass spectrometry combined with gas chromatography.[4]

This paper deals with the mass fragmentation of 2,4-DNPH of volatile carbonyl compounds separated by gas chromatography.

Experimental

Materials——The 2,4-DNPH of aldehydes were prepared by shaking 0.2 ml of each aldehyde with 200 ml of a saturated solution of 2,4-dinitrophenylhydrazine in 2N HCl, and allowing the mixture to stand at room temperature over night. The formed precipitate was collected by filtration, washed with 2N HCl and with water, and dried over silica gel in a vacuum desiccator. For the mass spectrometry combined with gas chromatography, the 2,4-DNPH was dissolved in chloroform to give 0.5% solution.

Conditions of Mass Spectrometry Combined with Gas Chromatography——The mass spectrometric analysis was carried out using an LKB 9000 instrument connected with a gas chromatograph under the following conditions: 3 m × 3 mm coiled glass column was packed with 3% OV-101 coated on Chromosorb GAW (80/100 mesh); column oven temperature was programmed from 140° to 300° at 5°/min; carrier gas, helium (30 ml/

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1) A part of this work was present at the 126th Annual Meeting of Nishi Nippon Branch of the Agricultural Chemical Society of Japan, Nagasaki, October, 1971.

2) Location: *a) Iwase, Matsudo-shi, Chiba*; *b) Bunkyo-cho, Nagasaki.*

3) *a*) J. Soukup, R.J. Scarpellino and E. Danielczik, *Anal. Chem.*, 36, 2255 (1964); *b*) Y. Shimizu, S. Matsuto, Y. Ito and I. Okada, *J. Agr. Chem.* (Japan), 43, 211 (1969).

4) M. Deki and M. Yoshimura, *Chem. Pharm. Bull.* (Tokyo), 22, 1754 (1974).

min); separator temperature, 320°; ion source, 300°; electron energy, 70 eV; accelarating voltage, 3.5 kV. Individual 2,4-DNPH of carbonyl compounds were also introduced *via* a direct probe into the ionization source on a Hitachi Model RMU-7 double-focussing mass spectrometer.

Results and Discussion

In the mass spectrum of 2,4-DNPH of formaldehyde, the molecular ion (*m/e* 210) and the fragment ions at *m/e* 183, 91, and 63 were clearly observed, and the fragment ion at *m/e* 63 revealed the strongest intensity in total fragment ions of the 2,4-DNPH (Fig. 1, A). The prominent ion at *m/e* 183 seemed to be due to elimination of $(HCN)^+$ from molecular ion. This fragmentation mode was suggested by the presence of metastable ion at *m/e* 159.4. The mass spectrum of 2,4-DNPH of acetaldehyde showed a molecular ion at *m/e* 224. The relative abundance of the molecular ion is 95% comparing with the base peak at *m/e* 79 (Fig. 1, B).

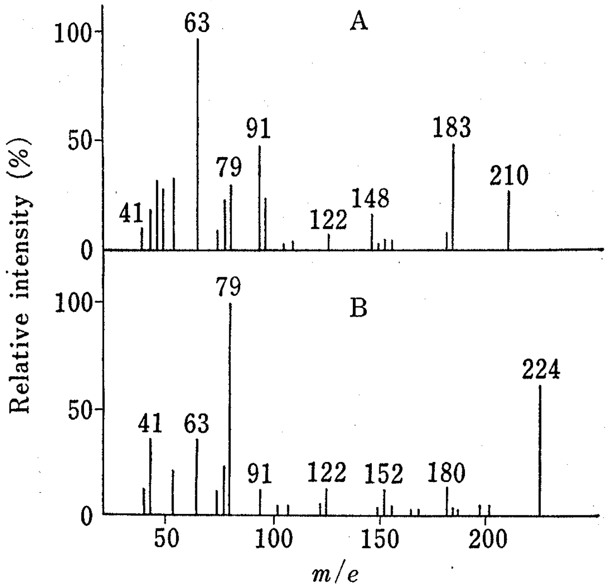

Fig. 1. Mass Spectra of 2,4-DNPH of Formaldehyde(A) and Acetaldehyde (B)

The *m/e* 180, 152, and 122 are common fragment ions to 2,4-DNPH of carbonyl compounds, and it is clear that these fragment ions correspond to dinitrobenzene ring from the results obtained by high resolution mass spectrometry. For example, an intense peak at *m/e* 180 in 2,4-DNPH of propylaldehyde corresponded to a fragment ion of $C_6H_4N_4O_3$ which occurred by removal of oxygen atom and subsequent loss of C_3H_6. This fragmentation mode is suggested by the presence of metastable ion at *m/e* 136.1. The 2,4-DNPH of branched aldehydes showed similar spectra to 2,4-DNPH of linear compounds with abundant molecular ion and common fragment ions at *m/e* 122, 152, 180, M^+-60, and M^+-42. The M^+-60 and M^+-42 were

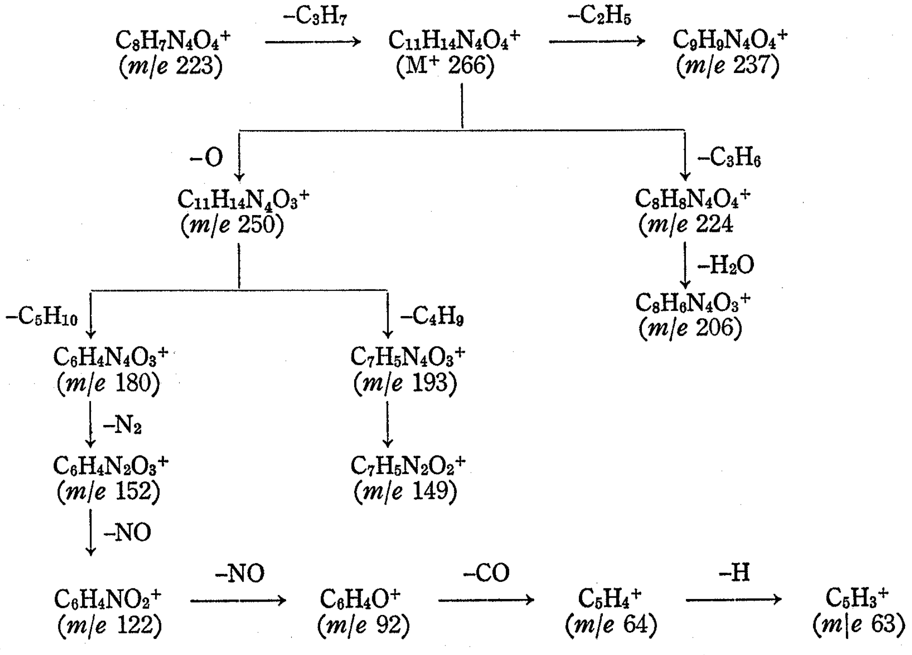

Chart 1

observed in the mass spectra of 2,4-DNPH of aldehydes over carbon number five, but not in those of under carbon number four. As noted earlier,[5] the 2,4-DNPH of *n*-valeraldehyde shows prominent peaks at *m*/*e* 224 and 206 resulting from molecular ion by McLafferty rearrangement and by subsequent loss of water. The similar fragmentation pattern was observed in the mass spectrum of 2,4-DNPH of isovaleraldehyde. It was confirmed by high resolution mass spectrometry that the ion at *m*/*e* 250 in the mass spectrum of 2,4-DNPH of isovaleraldehyde occurred with loss of oxygen atom from molecular ion, and the ion at *m*/*e* 180 was formed by elimination of $C_5H_{10}^+$ from *m*/*e* 250. The ions at *m*/*e* 152, 122, and 92 could also be produced by successive loss of N_2 and 2NO from the $C_6H_4N_4O_3^+$ (*m*/*e* 180). The fragment ion at *m*/*e* 206, which was considered to be formed by loss of the $C_3H_6^+$ and following loss of water from molecular ion, was confirmed to have a formula $C_8H_6N_4O_3$. The mode of this fragmentation was supported by occurrence of the metastable ion at *m*/*e* 189.4. M^+-NO and M^+-NO_2 which are characteristic for nitro-compounds are not significant in the mass spectra of 2,4-DNPH of carbonyl compounds (Chart 1).

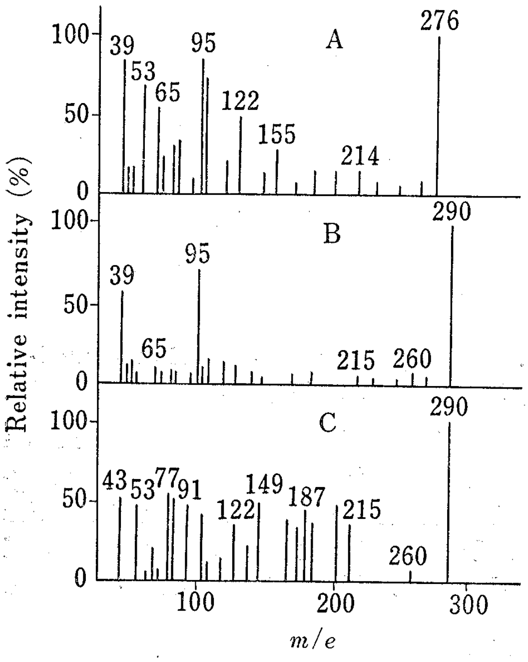

Fig. 2. Mass Spectra of 2,4-DNPH of Furfural (A), 2-Acetylfuran (B), and 5-Methylfurfural (C)

$$\begin{tikzcd}
\substack{C_{12}H_{10}N_4O_6^+ \\ (M^+\ 306)} \arrow[r, "\substack{m^*\ 271 \\ -H_2O}"] & \substack{C_{12}H_8N_4O_5^+ \\ (m/e\ 288)} \arrow[r, "m^*\ 50.8"] \arrow[dr] & \substack{C_6H_5N_2O^+ \\ (m/e\ 121)} \\
\substack{C_5H_3O^+ \\ (m/e\ 79)} & \substack{C_6H_3O_2^+ \\ (m/e\ 107)} \arrow[l, "-CO"'] & \substack{C_6H_3N_2O_4^+ \\ (m/e\ 167)} \arrow[l, "-2NO"']
\end{tikzcd}$$

Chart 2

In the spectrum of 2,4-DNPH of furfural, the molecular ion (*m*/*e* 276) was the base peak. The fragment ions at *m*/*e* 97 and 95, due to rearrangement ion produced by the cleavage of N-N bond, were prominent. This fragmentation mode was also observed in the spectrum of 2,4-DNPH of 2-aceytlfuran. The fragment ion at *m*/*e* 39, corresponding to cyclopropenyl cation which was formed by cleavage of furan ring, could not be found in the 2,4-DNPH of 5-methylfurfural (Fig. 2).

In the mass spectrum of 2,4-DNPH of 5-hydroxymethylfurfural, the molecular ion (*m*/*e* 306) showed a relative abundance of 30% comparing to the base peak at *m*/*e* 121, and oxonium ion (*m*/*e* 288) produced by loss of water from the molecular ion was observed. The fragment ion at *m*/*e* 121 was given by loss of the dinitrobenzene from the ion at *m*/*e* 288. This fragmentation mode could be explained by the presence of metastable ions at *m*/*e* 50.8 (Chart 2).

Acknowledgement We are grateful to Prof. H. Yoshimura, Kyushu University, for helpful advice. We also express our gratitude to Mr. Y. Ono, Central Customs Laboratory, for mass measurement.

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[5] *a*) D. Goldsmith and C. Djerassi, *J. Org. Chem.*, **31**, 3661 (1968); *b*) R.J.C. Kleipool and Th. Heins, *Nature*, **203**, 1280 (1964); *c*) C. Djerassi and S.D. Sample, *Nature*, **208**, 1314 (1965).