

## Note

### Analysis of benzalkonium chlorides by gas chromatography

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Benzalkonium chlorides are widely used as an antimicrobial preservative in pharmaceutical preparations and sanitary products, and are a mixture of predominantly C<sub>12</sub>, C<sub>14</sub> and C<sub>16</sub> alkylbenzyltrimethylammonium chlorides.

Various analytical methods have been reported for the determination of benzalkonium chlorides by high-performance liquid chromatography (HPLC)<sup>1–5</sup> and gas chromatography (GC)<sup>6–16</sup>. HPLC analysis is available but difficult to use in combination with mass spectrometry in our laboratory. We wished to establish a GC method for the routine analysis of benzalkonium chlorides since their identification can be facilitated using GC–mass spectrometry (MS). These compounds are non-volatile and thus need to be converted into more volatile derivatives before GC<sup>11–13</sup>. The Hofmann degradation for ammonium compounds is one such conversion method<sup>14–16</sup>. Tanakano *et al.*<sup>16</sup> reported that a mixture of alkyldimethylamines, alkylbenzylmethylamines and  $\alpha$ -olefins is obtained from benzalkonium chlorides by Hofmann degradation with sodium methoxide, but this approach requires too severe reaction conditions to decompose them.

In previous papers<sup>17,18</sup>, we have reported that long-chain alkyltrimethyl- and dialkyldimethylammonium compounds can be analyzed by GC as the corresponding  $\alpha$ -olefins and alkyldimethylamines which are formed by the Hofmann degradation with potassium *tert.*-butoxide under mild conditions. In the present work, a GC method for benzalkonium chlorides employing this degradation technique has been developed.

## EXPERIMENTAL

Dodecylbenzyltrimethylammonium chloride (I) was obtained from Kao Soap (Tokyo, Japan), tetradecylbenzyltrimethylammonium chloride (II) and hexadecylbenzyltrimethylammonium chloride (III) from Tokyo Kasei Kogyo (Tokyo, Japan) and octadecylbenzyltrimethylammonium chloride (IV) from Aldrich (Milwaukee, WI, U.S.A.). Standard solutions were prepared so as to contain 1 mg/ml of each compound in benzene–dimethyl sulphoxide (DMSO) (8:2). All other chemicals were of analytical grade.

### Degradation procedure

An aliquot of the standard solution containing 2–10 mg of compounds I–IV was pipetted into a 25-ml Pyrex glass test-tube with a ground glass stopper. The total volume was made up to 25 ml with benzene–DMSO (8:2). After the addition of 100 mg of potassium *tert.*-butoxide, the solution was vigorously shaken and allowed to stand for 10 min at room temperature. The reaction mixture was transferred to a 100-ml separating funnel. The test-tube was rinsed with two 5-ml portions of benzene. The rinsing solutions were added to the reaction mixture in a separating funnel and then shaken with 20 ml of 10% sodium chloride solution. The benzene layer was separated and transferred to a 100-ml round-bottom flask. After the solvent had been evaporated to dryness under reduced pressure, the residue was dissolved in 5 ml of acetone and 5  $\mu$ l of this solution were injected into the gas chromatograph.

### Gas chromatography

GC analysis was carried out with a Shimadzu Model GC-15A gas chromatograph equipped with a hydrogen flame ionization detector. Chromatography was performed on a glass column (2 m  $\times$  3 mm I.D.) packed with 5% SE-30 on Chromosorb W AW DMCS (80–100 mesh) under the following conditions: nitrogen flow-rate 40 ml/min; injection port and detector temperature 250°C; column temperature program from 140 to 230°C at 5°C/min.

### RESULTS AND DISCUSSION

Benzalkonium chlorides shown in Fig. 1 were degraded with potassium *tert.*-butoxide by essentially the same manner as described in previous papers<sup>7,8</sup>. However, the formation of  $\alpha$ -olefins and alkyldimethylamines as degradation products was found to vary with the reaction temperature. In searching for an optimum condition for alkyldimethylamine formation the effect of the reaction temperature for compounds I–IV was examined. As shown in Fig. 2, alkyldimethylamine formation was found to be accompanied by a decrease in that of  $\alpha$ -olefin at temperatures lower than 40°C. These results indicate that the debenylation of benzalkonium chlorides with potassium *tert.*-butoxide proceeds selectively at lower temperature to give alkyldimethylamine, and on the other hand the  $\beta$ -elimination reaction at the nitrogen-bonded alkyl chain predominates at temperatures higher than 80°C to give

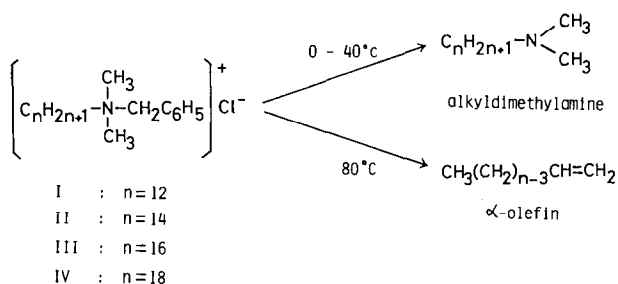


Fig. 1. Degradation scheme of benzalkonium chlorides with potassium *tert.*-butoxide in benzene–DMSO (8:2).

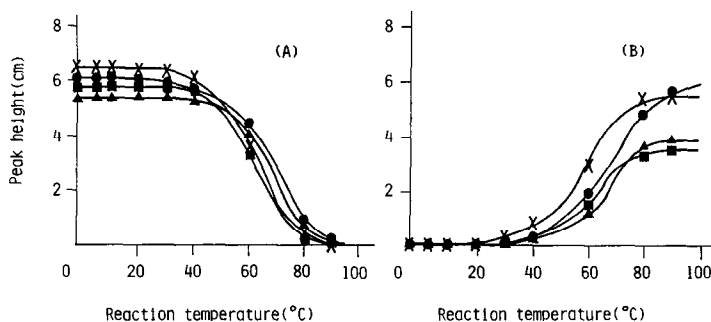


Fig. 2. Effects of reaction temperature on the formation of alkyldimethylamines (A) and  $\alpha$ -olefins (B) from the four benzalkonium chlorides, compounds I (●), II (×), III (▲) and IV (■).

$\alpha$ -olefin (Fig. 1). Thus, room temperature was selected for the GC analysis of benzalkonium chlorides as their alkyldimethylamines. The reaction was rapid under mild conditions in comparison with debenylation in the previously reported conversion methods<sup>11-16</sup> and was complete within 10 min at room temperature.

A typical gas chromatogram of compounds I-IV as their alkyldimethylamines on a 5% SE-30 column is shown in Fig. 3. Calibration graphs were constructed by plotting the peak heights of the alkyldimethylamines *versus* the concentrations of compounds I-IV. They showed good linearity over the concentration range 0.4-2 mg/ml. The minimum detectable amounts for compounds I-IV were found to be about 0.1 mg/ml.

For practical application *e.g.*, to a wet wiper containing benzalkonium chlorides, recovery tests were performed by adding 4 and 8 mg of compounds I-IV to an absorbent cotton (Table I). The cotton was extracted with ethanol under reflux for 60 min. The ethanol extract was filtered and evaporated to dryness. The analysis of

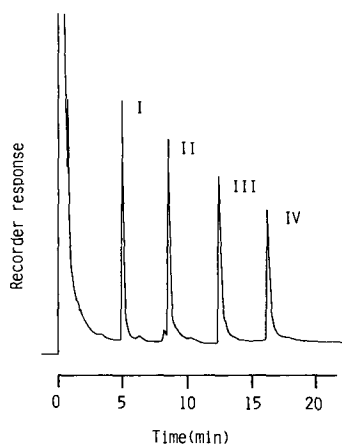


Fig. 3. Temperature-programmed gas chromatogram of the four benzalkonium chlorides as their alkyldimethylamines after degradation with potassium *tert.*-butoxide at room temperature, compounds I-IV: 6 mg.

TABLE I  
RECOVERIES OF BENZALKONIUM CHLORIDES FROM ABSORBENT COTTON

Compound*	Amount added (mg)	Average recovery (n=3) (%)	C.V. (%)
I	4	98.3	3.9
	8	103.4	1.9
II	4	104.2	1.4
	8	102.1	1.4
III	4	96.7	1.5
	8	92.4	3.3
IV	4	98.3	2.9
	8	96.3	4.5

\* Benzalkonium chlorides (I–IV) were dissolved in 10 ml of water, added to 1.7 g of absorbent cotton (The Pharmacopoeia of Japan, 11th ed.) and autoclaved at 120°C for 30 min.

compounds I–IV was performed by the method described. As shown in Table I, the average recoveries in three experiments were 92.4–104.2% and a good reproducibility was obtained with coefficients of variation (C.V.) of 1.4–4.5%.

We have applied this method to the determination of benzalkonium chlorides in 36 samples of commercial wet wipers. These were found to contain 0.06–0.28% in 10 samples as a mixture of C<sub>12</sub>, C<sub>14</sub> and C<sub>16</sub> alkylbenzyltrimethylammonium chlorides.

These experiments have demonstrated that the method proposed can be applied to routine analysis of benzalkonium chlorides.

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