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GAS-LIQUID CHROMATOGRAPHIC DETERMINATION OF BENZETHONIUM CHLORIDE

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SUMMARY

Benzethonium chloride (I), used as a detergent, disinfectant and pharmaceutical preparation, was determined by gas-liquid chromatography of the reduction product obtained by treatment with sodium borohydride and nickel(II) chloride. The reduction product of I was identified as 2-{2-[*p*-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy}ethyl-dimethylamine by mass spectrometry, nuclear magnetic resonance spectroscopy and elemental analysis. In the reduction, the benzyl group of I was eliminated as toluene. The procedure is suitable for the assay of 10-100 $\mu\text{g/ml}$ of aqueous preparations of I, and useful for the routine analysis of I, as the reduction takes place quantitatively, the reagents are relatively safe to handle and there is no need for complicated apparatus such as in catalytic hydrogenation.

INTRODUCTION

During studies on the determination of quaternary ammonium compounds, we have studied the determination of benzethonium chloride, used as a detergent, disinfectant and pharmaceutical preparation, by gas-liquid chromatography (GLC). The analysis of benzethonium chloride has previously been performed by spectrophotometry¹⁻¹¹, titrimetry¹²⁻¹⁸, gravimetry^{11,19-21} and thin-layer chromatography²². The disadvantage of these methods is the lack of specificity for the determination of benzethonium chloride. On the other hand, GLC of the benzethonium salt, which is non-volatile, has previously been carried out by a thermal decomposition method^{23,24}, and has not been used in quantitative analysis because of the appearance of multiple peaks and the poor response.

In previous papers, we reported that cationic surfactants based on N-alkylpyridinium salts²⁵ and N-alkyldimethylbenzylammonium salts²⁶ could be determined by GLC of their reduction products obtained by reduction with a mixture of sodium borohydride and a transition metal salt, *e.g.*, nickel(II) chloride. This paper deals with the GLC determination of benzethonium chloride by use of the reduction system of sodium borohydride (NaBH_4) and nickel(II) chloride (NiCl_2).

EXPERIMENTAL

Apparatus

GLC was carried out with a glass column (1 m × 0.3 cm I.D.) on a Hitachi Model 073 gas chromatograph equipped with a hydrogen flame-ionization detector (HFID). Mass spectrometry was performed on a JEOL JMS-D300 mass spectrometer. Nuclear magnetic resonance spectroscopy (NMR) was carried out with a Hitachi Model R-20 (60 MHz) instrument.

Materials

All chemicals were of analytical-reagent grade. Freshly distilled diethyl ether was used in the extraction of the reduction products.

Benzethonium chloride (I) was commercially available.

Synthesis of 2-{2-[p-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy}ethyl dimethylamine (II) from I by the NaBH₄-NiCl₂ reduction system

I (4.48 g, 0.01 mol) and NiCl₂ · 6H₂O (2.38 g, 0.01 mol) were dissolved in methanol (300 ml). To the solution was added NaBH₄ (18.9 g, 0.5 mol) in small portions at 25°C with stirring, which was continued for 1 h at room temperature. A black precipitate was formed and hydrogen was evolved. The precipitate was filtered off and washed with methanol, and the filtrate and the washings were combined and concentrated to one third of the initial volume under reduced pressure. To the residue was added water (50 ml) and the mixture was extracted with *n*-hexane (three 50-ml volumes). The *n*-hexane layer was dried over sodium sulphate and evaporated to dryness. The resulting residue was distilled under reduced pressure to give a colourless oil, b.p. 168–181°C/4 mmHg, in 82.7% yield. Elemental analysis: Calculated for C₂₇H₃₅O₂N, C 74.72, H 10.97, N 4.36%; found, C 74.42, H 11.11, N 4.39%.

Detection of toluene in the reduction of I with the NaBH₄-NiCl₂ system

To a vial (5 ml) was added an aqueous solution (1 ml) of I (100 μg) and 0.02 M NiCl₂ (0.5 ml). The vial was sealed tightly with a double rubber stopper. The air phase was removed with a syringe, and then to the vial was added 2.6 M NaBH₄ (0.5 ml) with the syringe through the double rubber stopper. After 5 min, the gas (2 ml) generated together with hydrogen in the vial was withdrawn with a syringe, and injected into the gas chromatograph. GLC was carried out under the following conditions: (A) a glass column (2 m × 0.3 cm I.D.) packed with 25% silicone DC550 on C-22 (40–60 mesh) at 120°C with a nitrogen flow-rate of 30 ml/min, an injection port temperature of 170°C and attenuation 10² × 8; (B) the same sized column packed with 25% PEG 6000 on Chromosorb W AW DMCS (60–80 mesh) at 90°C with a nitrogen flow-rate of 30 ml/min, an injection port temperature of 150°C and attenuation 10² × 8. The volatile compound was identified as toluene by comparing its retention time in GLC with that of an authentic sample. The retention time was 4 min (A) and 4.6 min (B).

Reduction of I with the NaBH₄-NiCl₂ system on the gas-liquid chromatographic scale

To an aqueous solution (1 ml) of I (10–100 μg) was added 0.02 M NiCl₂ (0.5 ml) and 2.6 M NaBH₄ (0.6 ml) with stirring. The mixture turned black and hydrogen

was evolved. The mixture was allowed to stand for 1 h at room temperature, and was then extracted with diethyl ether (four 2-ml volumes). The combined organic layer was dried over sodium sulphate, acidified with a few drops of acetic acid and evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate (200 μ l) and 1 μ l of the solution was injected into the gas chromatograph.

GLC was performed on a glass column (1 m \times 0.3 cm I.D.) packed with 5% potassium hydroxide plus 5% PEG 20M on Chromosorb W AW DMCS (60–80 mesh) at 200°C with a nitrogen flow-rate of 50 ml/min, an injection port temperature of 250°C and attenuation 1 \times 8. The internal standard was 0.01% anthracene.

Influence of the amount of NaBH₄ on the reduction of I with the NaBH₄-NiCl₂ system

An aqueous solution (1 ml) of I (1 mg; 2.2 μ mol) was treated with 0.33 ml (6.6 μ mol) of 0.02 M NiCl₂ and different amounts of 2.6 M NaBH₄ (aqueous solutions of 110, 220, 440, 660, 880 and 1100 μ mol) at room temperature for 1 h. The resulting reduction product was subjected to GLC as described above.

Influence of the amount of NiCl₂ on the reduction of I with the NaBH₄-NiCl₂ system

An aqueous solution (1 ml) of I (1 mg; 2.2 μ mol) was treated with different amounts of 0.02 M NiCl₂ (aqueous solutions of 1.1, 2.2, 4.4, 6.6, 8.8 and 11.0 μ mol) and 2.6 M NaBH₄ (0.42 ml) at room temperature for 1 h. The resulting reduction product was examined by GLC as described above.

Rate of reduction of I with the NaBH₄-NiCl₂ system

An aqueous solution (1 ml) of I (1 mg; 2.2 μ mol) was treated with 0.02 M NiCl₂ (6.6 μ mol; 0.33 ml) and 2.6 M NaBH₄ (1.1 mmol; 0.42 ml) at room temperature for various times (15 min, 30 min, 1 h, 2 h and 3 h). The resulting reduction product were examined under GLC conditions as described above.

RESULTS AND DISCUSSION

Previously we have reported that the reduction of N-alkylpyridinium salts (detergent and disinfectant) and N-alkylbipyridylum salts (herbicide) with NaBH₄ alone gave the corresponding cyclic tertiary amines^{25,27} containing a few double bonds, but the reduction of these quaternary ammonium salts with NaBH₄ and NiCl₂ afforded the corresponding expected reduction products, *viz.*, N-alkylpiperidines²⁵ and N-alkylbipiperidines^{28,29}. Further, we have observed²⁶ that N-alkyldimethylbenzylammonium chloride (benzalkonium chloride: detergent and disinfectant) could not react with NaBH₄ alone, but treatment of the ammonium chloride with NaBH₄ and NiCl₂ gave N-alkyldimethylamine with reductive elimination of its benzyl group. Using a similar procedure to that reported previously, the NaBH₄-NiCl₂ reduction system was applied to the GLC determination of benzethonium chloride (I). The reduction product (II) thus prepared by treatment of I with NaBH₄-NiCl₂ was chromatographed in a glass column packed with 5% potassium hydroxide plus 5% PEG 20M on Chromosorb W AW DMCS at 200°C. As shown in Fig. 1, II is readily detected as a symmetrical peak.

Satisfactory separations were achieved on an alkaline column such as 5%

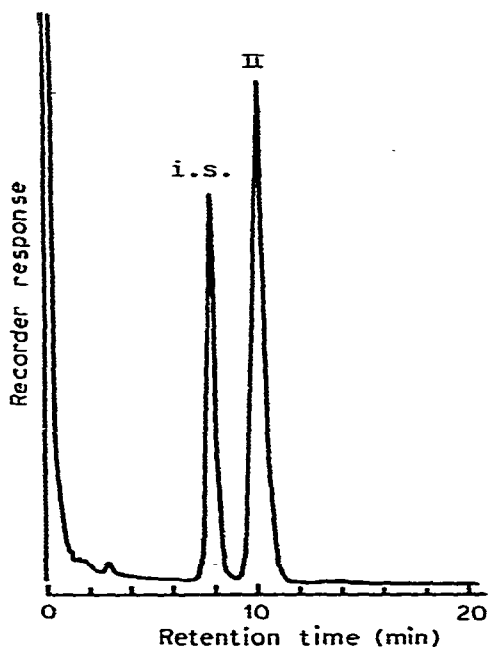


Fig. 1. Gas chromatogram of the hydrogenated product (II) derived from benzethonium chloride (I). Internal standard (i.s.): anthracene.

potassium hydroxide plus 5% PEG 20M, but neutral columns such as PEGS, PEG 20M, SE-30 and OV-17 exhibited slight tailing under isothermal conditions.

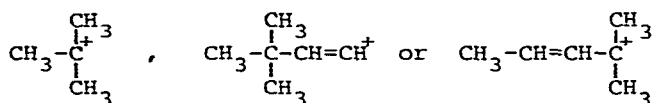
The structure of the reduction product (II) was clarified by an independent synthesis in the following manner. When a large excess of NaBH_4 was added to a methanolic solution of I and NiCl_2 , a black precipitate of nickel boride was immediately formed, with evolution of hydrogen. The reduction of I proceeded smoothly with the continuous evolution of hydrogen, and was complete in about 1 h to afford the hydrogenated product (II), which was purified by distillation under reduced pressure to give a colourless oil.

The mass spectrum of II exhibited a base fragment peak at m/e 58 due to the N-dimethylaminomethylene and/or methylene oxyethylene ions, and also showed a parent ion peak at m/e 321 and several fragment ion peaks as indicated in Scheme 1.

The proton nuclear magnetic resonance of II in CDCl_3 exhibited $-\text{C}(\text{CH}_3)_3$ protons at δ 0.70 ppm (9H, singlet), $>\text{C}(\text{CH}_3)_2$ protons at δ 1.33 ppm (6H, singlet), $-\text{CCH}_2\text{C}-$ protons at δ 1.70 ppm (2H, singlet), $-\text{N}(\text{CH}_3)_2$ protons at δ 2.26 ppm (6H, singlet), $-\text{OCH}_2\text{CH}_2\text{O}-$ protons at δ 3.96 ppm (4H, multiplets), $>\text{NCH}_2\text{CH}_2\text{O}-$ protons at δ 2.25 ppm (2H, triplets) and δ 3.65 ppm (2H, triplets), aromatic protons at δ 6.82 ppm (2H, doublets) and δ 7.26 ppm (2H, doublets), but not exhibited protons due to the benzyl group.

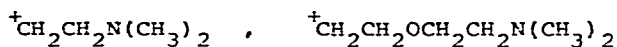
Based on these results and elemental analysis, the reduction product was identified as II.

The volatile compound generated together with hydrogen, except for II, in this reaction of I was identified as toluene by GLC using 25% silicone DC550 and 25% PEG 6000 columns on the basis of the experiment carried out in a vial. This demon-



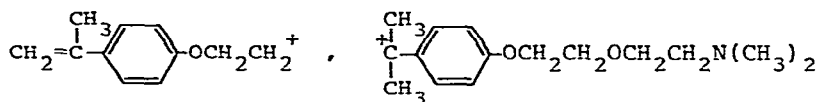
m/e 57

m/e 83



m/e 72

m/e 116

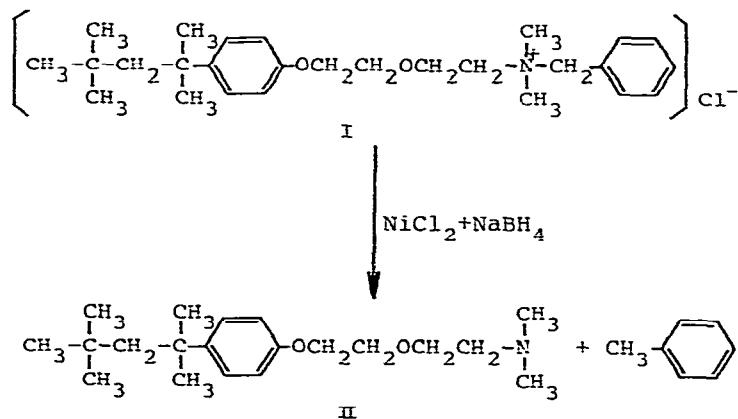


m/e 161

m/e 250

Scheme 1.

states that the reduction of I with NaBH_4 and NiCl_2 gave II with the release of a benzyl group from the molecule, as shown in Scheme 2. Consequently, it is clear that no methyl group is eliminated in this reduction. On the other hand, such a reduction did not proceed on treatment of I with NaBH_4 alone. These findings are similar to those of our earlier results²⁶ relating to N-alkyldimethylbenzylammonium chloride.



Scheme 2.

The conditions for the hydrogenation of I with NaBH_4 and NiCl_2 on the analytical (GLC) scale were examined in its aqueous solution. As shown in Fig. 2, the reduction of an appropriate amount of I (2.2 μmol in 1 ml of water), which corresponds to a high response in the determination, is dependent on the amount of NiCl_2 in the presence of a large excess of NaBH_4 (1.1 mmol in 0.43 ml of water). The hydrogenation of I to II proceeded quantitatively when more than 2.2 μmol of NiCl_2 (1.0 mol per mole of I) were used in the reduction system. In the presence of a definite

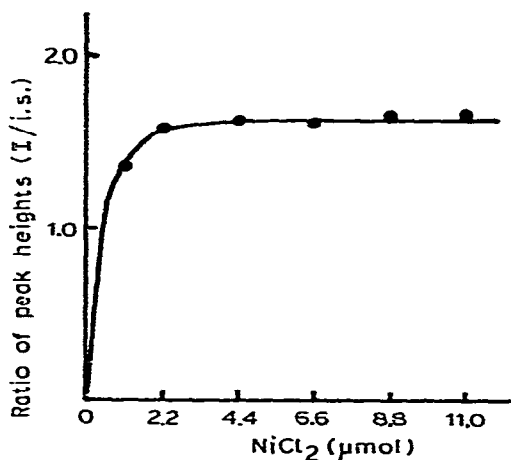


Fig. 2. Influence of amount of NiCl_2 on reduction of I to II with the NaBH_4 - NiCl_2 system. Internal standard (i.s.): anthracene.

amount of NiCl_2 ($6.6 \mu\text{mol}$), the reduction was complete with amounts of NaBH_4 in the range 111 – $1115 \mu\text{mol}$, as shown in Fig. 3. To avoid incomplete reduction, a large excess of reducing agent, such as the combination of 0.5 ml ($10 \mu\text{mol}$) of 0.02 M NiCl_2 and 0.6 ml (1.56 mmol) of 2.6 M NaBH_4 , is advisable for the GLC analysis of I. Fig. 4 demonstrates the hydrogenation under these conditions is complete within 1 h at room temperature.

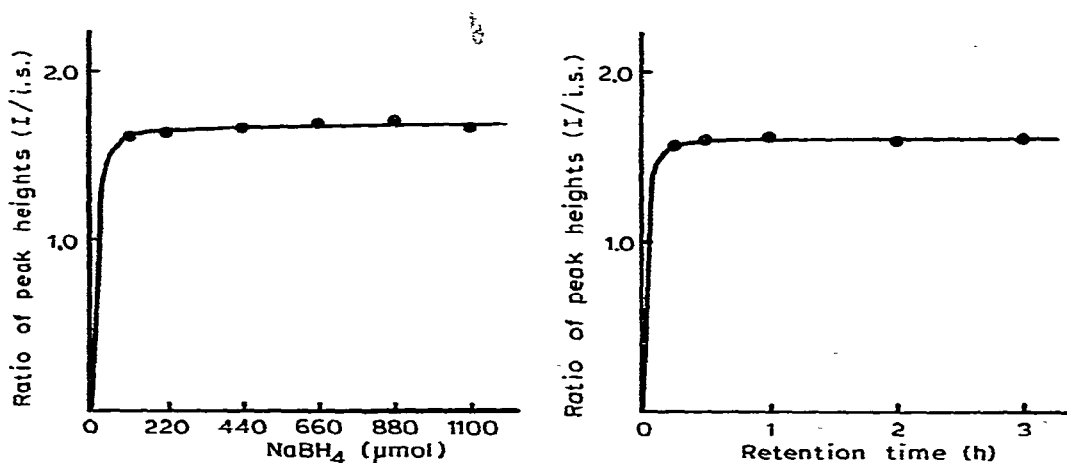


Fig. 3. Influence of amount of NaBH_4 on reduction of I to II with the NaBH_4 - NiCl_2 system. Internal standard (i.s.): anthracene.

Fig. 4. Reduction of I with the NaBH_4 - NiCl_2 system. Internal standard (i.s.): anthracene.

The determination of I under isothermal conditions was performed by the peak-height ratio method. The calibration graph for I (Fig. 5) showed good linearity using anthracene as the internal standard.

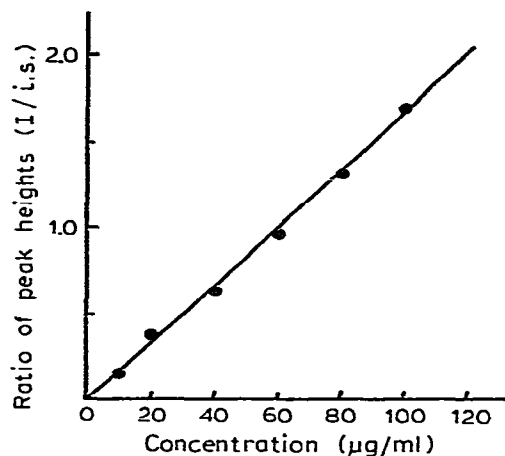


Fig. 5. Calibration graph for I. Internal standard (i.s.): anthracene.

The determination of I in several commercial preparations was carried out by this GLC method with satisfactory results, as shown in Table I.

TABLE I

DETERMINATION OF BENZETHONIUM CHLORIDE IN COMMERCIAL PREPARATIONS

Sample No.*	Aqueous solution of benzethonium chloride (% w/v)	Recovery (%)
1	10	91.3
2	10	97.4
3	4	91.4

* Samples: 1 = disinfectant; 2 = disinfectant containing 10% alkyl allyl polyether alcohol; 3 = gargle containing 0.47 ml/ml of ethanol and a flavouring.

CONCLUSION

The reduction of benzethonium chloride (I) with NaBH_4 and NiCl_2 proceeds complete to afford 2-{2-[p-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy}ethyl-dimethyl-amine (II) with the elimination of toluene, and I is readily determined by GLC of the reduction product (II). The procedure is suitable for the routine assay (10–100 µg/ml of aqueous preparation) of I, as the reduction takes place cleanly in aqueous medium at room temperature with easily handled reagents, without the need for complicated apparatus such as in catalytic hydrogenation. Further, the procedure would be applicable to the determination of other quaternary ammonium compounds in disinfectants and drugs.

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