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GAS–LIQUID CHROMATOGRAPHIC DETERMINATION OF N-ALKYLPYRIDINIUM SALTS

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SUMMARY

Long-chain N-alkylpyridinium salts (I–V) used as detergents and disinfectants were readily determined by the gas–liquid chromatography of the reduction products (VI–X) obtained by treatment with sodium borohydride and nickel(II) chloride. The procedure is useful for the routine analysis of N-alkylpyridinium salts, as the reduction takes place quantitatively and the reagents are relatively safe to handle, instead of the need to use a complicated apparatus for catalytic hydrogenation. The reduction products of I–V were identified as N-alkylpiperidines (VI–X), *i.e.*, the perhydrogenated products, by mass spectrometry and elemental analysis. The reduction system described serves as a novel and convenient method for the synthesis of N-alkylpiperidines.

INTRODUCTION

In continuing studies of the analysis of cationic surfactants, we have now studied the determination of long-chain N-alkylpyridinium salts by gas–liquid chromatography (GLC). The long-chain N-alkylpyridinium compounds (alkyl = C_{10}–C_{18}) are used as disinfectants, and N-cetylpyridinium chloride is well known as an antiseptic detergent.

As these pyridinium salts are non-volatile, their GLC has previously been performed after conversion into their reduction products[1] or thermal decomposition products[2,3]. However, such conversion methods have not been used in quantitative analysis because of the appearance of multiple peaks and the poor response.

In previous papers, we reported that herbicides based on N-alkylbipyridylium derivatives, such as diquat[4], paraquat[4] and morphamquat[5], could be determined by GLC of their perhydrogenated products obtained by reduction with a mixture of sodium borohydride ($NaBH_4$) and a transition metal salt, *e.g.*, nickel(II) chloride

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TABLE I

N-ALKYLPIPERIDINES (VI–X)

No.	*R*	*Boiling point (°C/mmHg)*			*Appearance*	*Formula*	*Analysis (%)*	
		*Experimental**	*Literature*				*Calculated*	*Found*
			Value	*Reference*				
VI	n-$C_{10}H_{21}$	128/8	150–152/13	7	Colourless oil	$C_{15}H_{31}N$	C 79.92	79.85
							H 13.86	14.05
							N 6.21	6.20
VII	n-$C_{12}H_{25}$	161/5	114–116/0.4	8	Colourless oil	$C_{17}H_{35}N$	C 80.56	80.75
							H 13.92	14.20
							N 5.53	5.40
VIII	n-$C_{14}H_{29}$	171/5	186–187**	6	Colourless oil	$C_{19}H_{39}N$	C 81.06	81.04
							H 13.96	14.11
							N 4.98	4.79
IX	n-$C_{16}H_{33}$	176–177/1	148–151/0.1	7	Colourless oil	$C_{21}H_{43}N$	C 81.47	81.41
							H 14.00	14.08
							N 4.52	4.24
X	n-$C_{18}H_{37}$	199–200/5 (32.5-34)***	126–127/8	7	Colourless solid	$C_{23}H_{47}N$	C 81.82	81.37
							H 14.03	13.96
							N 4.15	4.04

* All uncorrected.
** Melting point (HCl salt).
*** Melting point.

($NiCl_2$). This paper deals with the GLC determination of long-chain N-alkylpyridinium salts by use of the reduction system of sodium borohydride and nickel(II) chloride.

EXPERIMENTAL

Apparatus

GLC was carried out with a glass column (1 or 2 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 Shimadzu Model LKB-9000 mass spectrometer.

Materials

N-Decylpyridinium bromide(I), N-tetradecylpyridinium bromide(III) and N-stearylpyridinium bromide(V) were prepared by reaction of pyridine and the corresponding alkyl bromide in the usual way[6]. N-Laurylpyridinium chloride(II) and N-cetylpyridinium chloride(IV) were commercially available.

Reagents

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

Synthesis of N-alkylpiperidine (VI–X) from N-alkylpyridinium salts (I–V) by the $NaBH_4$–$NiCl_2$ reduction system

N-Alkylpyridinium salts (I–V: 2.76–4.13 g, 0.01 mol) and $NiCl_2 \cdot 6H_2O$ (2.38 g, 0.013 mol) were dissolved in methanol (300 ml). To the solution was added $NaBH_4$ (11.35 g, 0.03 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 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 benzene (three 50-ml volumes). The benzene layer was dried over sodium sulphate and evaporated to dryness. The resulting residue was distilled under reduced pressure to give a colourless oil in 70–75% yield, as shown in Table I.

Reduction of N-alkylpyridinium salts(I–V) with the $NaBH_4$–$NiCl_2$ system on the gas–liquid chromatographic scale

To the aqueous solution (1 ml) of N-alkylpyridinium salt (I–V: 5–140 μg) was added 0.02 *M* $NiCl_2$ (0.5 ml) and 2.6 *M* $NaBH_4$ (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 (100 μl), and 1 μl of the solution was injected into the gas chromatograph.

GLC was performed on a glass column (2 m × 0.3 cm I.D.) packed with 5% potassium hydroxide plus 5% PEG 20M on Chromosorb W AW DMCS (60–80

mesh) at 190°C with a nitrogen flow-rate of 30 ml/min (injection port temperature, 250°C; attenuation, 1 × 8). In the determination of IV and V, a shorter column (1 m × 0.3 cm I.D.) was used at 180°C (retention times relative to internal standard: IX = 0.85; X = 1.2). The internal standards were 0.01% *p*-anisidine for I, II and III, 0.02% γ,γ'-dipyridyl for IV and 0.02% diphenylamine for V.

Temperature-programmed GLC was carried out on dual glass columns packed with 1% SE-30 on Gas-Chrom Z, the column temperature being increased at 7.5°C/min from 100 to 250°C.

Rate of reduction of IV–IX with the $NaBH_4$–$NiCl_2$ system

An aqueous solution (1 ml) of IV (1 mg; 3 μmol/ml) was treated with 0.02 *M* $NiCl_2$ (9 μmol; 0.45 ml) and 2.6 *M* $NaBH_4$ (1.5 mmol; 0.58 ml) at room temperature for various times (15 min, 30 min, 1 h and 2 h). The resulting reduction products were examined under isothermal GLC conditions as described above.

Influence of the amount of $NaBH_4$ on the reduction of IV–IX with the $NaBH_4$–$NiCl_2$ system

An aqueous solution (1 ml) of IV (1 mg; 3 μmol) was treated with 0.45 ml (9 μmol) of 0.02 *M* $NiCl_2$ and different amounts of 2.6 *M* $NaBH_4$ (aqueous solutions of 15, 30, 60, 90, 150, 210, 300, 900 and 1500 μmol) at room temperature for 1 h. The resulting reduction products were subjected to GLC as described above.

Influence of the amount of $NiCl_2$ on the reduction of IV–IX with the $NaBH_4$–$NiCl_2$ system

An aqueous solution (1 ml) of IV (1 mg; 3 μmol) was treated with different amounts of 0.02 *M* $NiCl_2$ (aqueous solutions of 0.09, 0.15, 0.3, 0.6, 0.9, 1.5, 2.0, 3.0, 6.0, 9.0 and 12 μmol) and 2.6 *M* $NaBH_4$ (0.58 ml) at room temperature for 1 h. The resulting reduction products were examined by GLC as described above.

RESULTS AND DISCUSSION

Reduction of N-alkylpyridinium salts with $NaBH_4$ has been reported by several investigators[1,9]. On GLC, the reduction products emerged as two peaks composed of dihydro- and tetrahydro-N-alkylpiperidines[1]. On the other hand, the catalytic hydrogenation of these N-alkylpyridinium compounds gave N-alkylpiperidines (perhydrogenated products)[6], but the application of this method in GLC was undesirable because of the tedious procedure involved in handling the apparatus.

Previousiy we found[4] that the $NaBH_4$–$NiCl_2$ reduction system was the most effective for obtaining the perhydrogenated products in the reduction of N-alkylpyridinium salts with a combination of $NaBH_4$ and the salts of transition metal ions such as Ag^+, Co^{2+}, Ni^{2+}, Mn^{2+}, Fe^{3+}, Pt^{4+} and Cr^{6+}. Using a similar procedure to that reported previously[4], the $NaBH_4$–$NiCl_2$ reduction system was applied to the GLC determination of long-chain N-alkylpyridinium salts (alkyl = C_{10}–C_{18}). The reduction products (VI–X) thus prepared by the treatment of I–V with $NaBH_4$ and $NiCl_2$ were chromatographed in a glass column packed with 5% potassium hydroxide +5% PEG 20M on Chromosorb W AW DMCS at 190°C. As shown in Fig. 1, all hydrogenated products (VI–X) were readily resolved and each gave a single, symmetrical

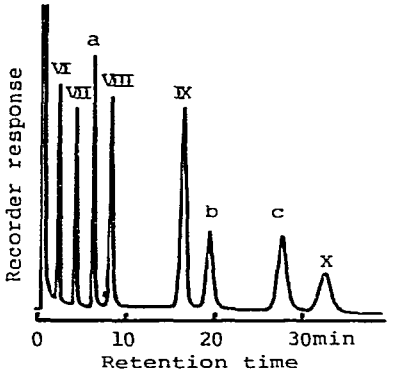


Fig. 1. Gas chromatogram of the perhydrogenated products (VI–X) derived from N-alkylpyridinium salts (I–V). Internal standards: a = *p*-anisidine; b = γ,γ′-dipyridyl; c = diphenylamine.

peak. The peaks emerged in order of the size of the alkyl groups, the retention time doubling for each additional carbon atom in the alkyl groups.

Satisfactory separations were achieved on alkaline columns such as 5% potassium hydroxide plus 5% PEG 20M or PEG 6000, but neutral columns such as PEGS, PEG 20M, PEG 6000, SE-30 and OV-17 exhibited slight tailing under isothermal conditions.

Temperature-programmed GLC of the reduction products (VI–X) was carried out with dual glass columns packed with 1% SE-30 on Gas-Chrom Z, the column temperature being increased at 7.5°C/min from 100 to 250°C. As alkaline columns cannot be used at high temperatures, an SE-30 column was employed. The peaks of the

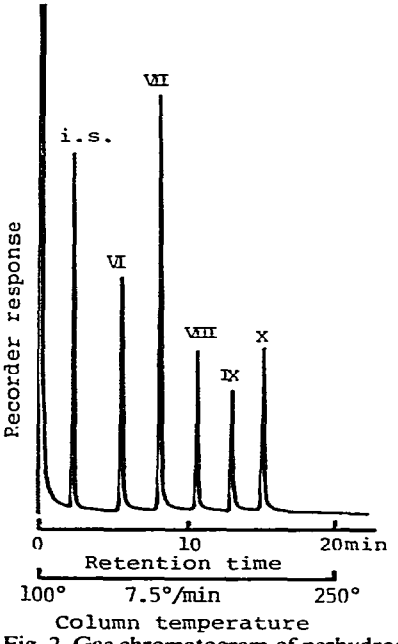
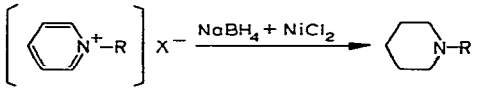


Fig. 2. Gas chromatogram of perhydrogenated products (VI–X) derived from N-alkylpyridinium salts (I–V) by temperature-programmed gas chromatography. Internal standard (i.s.): diphenyl.

products appeared in order of the number of carbon atoms in the alkyl group, the retention time increasing by about 5 min for each additional carbon atom, as shown in Fig. 2. The isothermal and temperature-programmed GLC behaviours of VI–X are similar to those of higher alkanes[10].

The structure of the reduction products (VI–X) was clarified by an independent synthesis in the following manner. When a large excess of $NaBH_4$ was added to a methanolic solution of pyridinium salts (I–V) and $NiCl_2$, a black precipitate of nickel boride[11,12] was immediately formed, with evolution of hydrogen. The reduction of these compounds (I–V) proceeded smoothly with the continuous evolution of hydrogen, and was complete in about 1 h to afford the hydrogenated products (VI–X). The resulting products were purified by distillation under reduced pressure and gave colourless oils, as indicated in Scheme 1.



I: R = n-$C_{10}H_{21}$, X = Br
II: R = n-$C_{12}H_{25}$, X = Cl
III: R = n-$C_{14}H_{29}$, X = Br
IV: R = n-$C_{16}H_{33}$, X = Cl
V: R = n-$C_{18}H_{37}$, X = Br

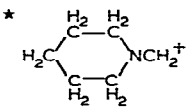
VI: R = n-$C_{10}H_{21}$
VII: R = n-$C_{12}H_{25}$
VIII: R = n-$C_{14}H_{29}$
IX: R = n-$C_{16}H_{33}$
X: R = n-$C_{18}H_{37}$

Scheme 1.

Elemental analyses of the products were consistent with the calculated values for N-alkylpiperidines. The mass spectra of VI–X exhibited a base fragment peak at m/e 98 due to the piperidino-N-methylene ion*, and also showed a parent ion peak corresponding to the N-alkylpiperidine. Based on these results, the reduction products (VI–X) were identified as N-alkylpiperidines, *i.e.* the perhydrogenated compounds of I–V. Thus this reduction serves as a novel and convenient method for the synthesis of N-alkylpiperidines.

The conditions for the perhydrogenation of the N-alkylpyridinium salts (I–V) with $NaBH_4$ and $NiCl_2$ on the analytical (GLC) scale were examined in aqueous solution by use of N-cetylpyridinium chloride (IV). As shown in Fig. 3, the reduction of an appropriate amount of IV (3 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 $NaBH_4$ (1.5 mmol in 0.58 ml of water). The hydrogenation of IV to VIII proceeded quantitatively when more than 3 μmol of $NiCl_2$ (1.0 mol per mole of IV) were used in the reducion system.

When less than 0.8 μmol of $NiCl_2$ was used, undesirable peaks due to by-products resulting from incomplete reduction of IV appeared. This indicates that the reduction system requires at least 1 mol of $NiCl_2$ per mole of IV for the complete hydrogenation of three double bonds in the pyridinium nucleus. In the presence of a

* 

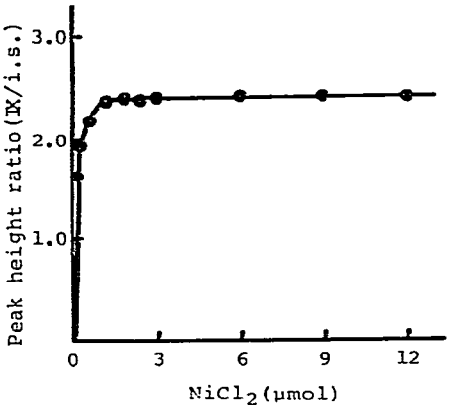
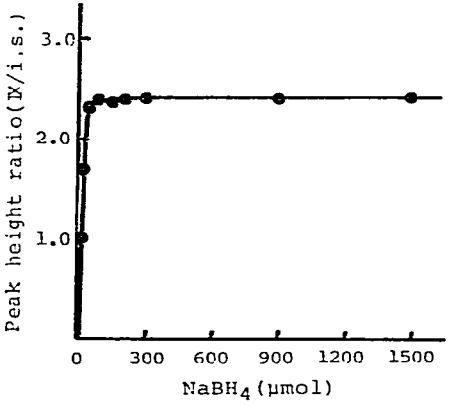


Fig. 3. Influence of amount of $NiCl_2$ on reduction of IV to IX with the $NaBH_4$–$NiCl_2$ system. Internal standard (i.s.): γ,γ′-dipyridyl.

definite amount of $NiCl_2$ (9 μmol), the reduction was complete with amounts of $NaBH_4$ in the range 90–150 μmol, as shown in Fig. 4. To avoid incomplete reduction, a large excess of the reducing agent, such as the combination of 0.5 ml (10 μmol) 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–V. Fig. 5 demonstrates that the hydrogenation under these conditions is completed within 1 h at room temperature.



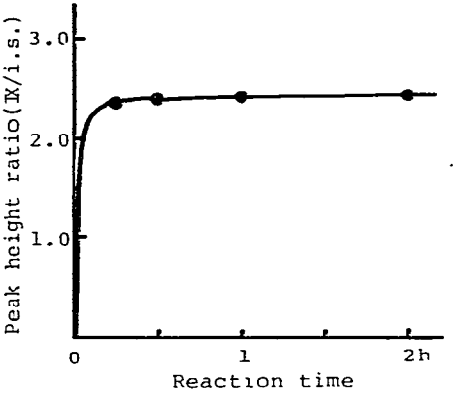
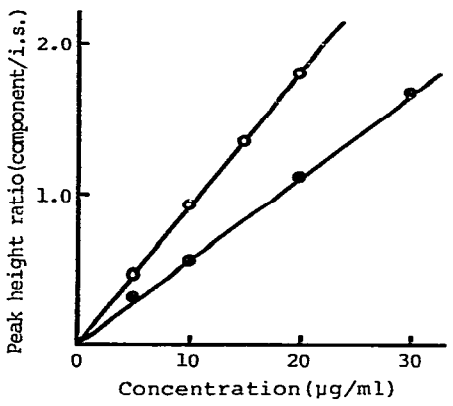


Fig. 4. Influence of amount of $NaBH_4$ on reduction of IV to IX with the $NaBH_4$–$NiCl_2$ system. Internal standard (i.s.): γ,γ′-dipyridyl.

Fig. 5. Reduction of IV with the $NaBH_4$–$NiCl_2$ system. Internal standard (i.s.): γ,γ′-dipyridyl.

The quantitative determination of N-alkylpyridinium salts (I–V) under isothermal conditions was performed by the peak-height ratio method. The calibration graphs obtained with perhydrogenated products for I–V (Figs. 6 and 7) showed good linearity using *p*-anisidine as internal standard for I–III, γ,γ′-dipyridyl for IV and diphenylamine for V.



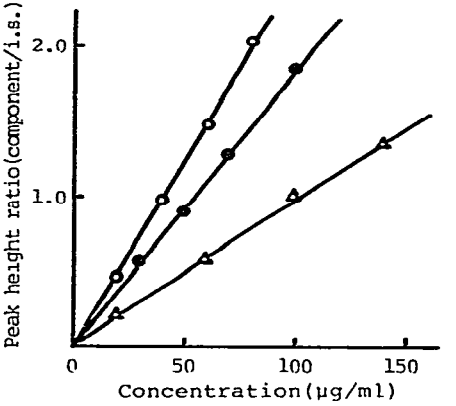


Fig. 6. Calibration graphs for I (○) and II (●). Internal standard (i.s.): *p*-anisidine.

Fig. 7. Calibration graphs for III (○), IV (●) and V (△). Internal standards (i.s.): III, *p*-anisidine; IV, γ,γ'-dipyridyl; V, diphenylamine.

The limits of determination for the N-alkylpyridinium compounds (I–V) using an HFID were 5 μg/ml for I and II, 10 μg/ml for III and 20 μg/ml for IV and V.

The reproducibilities and average recoveries for the analyses of I–V are illustrated in Table II.

TABLE II

PRECISION OF ANALYSIS

Parameter	*Analysis No.*	*Compound*				
		I	*II*	*III*	*IV*	*V*
Amount added (μg)	–	16.9	20.0	12.1	30.0	30.0
Amount found (μg)	1	17.7	19.4	10.6	27.5	27.7
	2	18.2	19.3	11.7	29.2	30.9
	3	16.8	19.9	12.7	29.4	30.6
	4	16.8	20.4	12.2	31.9	31.3
	5	17.5	19.9	12.4	31.2	31.4
	6	16.9	19.6	12.4	30.8	30.2
	7	15.4	19.6	12.1	30.1	30.2
	8	16.0	20.9	11.7	29.2	31.5
	9	17.6	20.0	12.2	30.0	29.3
	10	17.7	21.0	11.8	28.4	30.5
Mean (μg)	–	17.1	20.0	12.0	29.8	30.4
Standard deviation (μg)	–	0.8	0.6	0.6	1.3	1.1

CONCLUSION

Three double bonds in the N-alkylpyridinium nucleus are completely hydrogenated by the $NaBH_4$–$NiCl_2$ reduction system, and I–V are readily determined by

the GLC of their perhydrogenated compounds (VI–X) obtained by this reduction.

The procedure is suitable for the routine determination of N-alkylpyridinium compounds in detergents, disinfectants and drugs, as the reduction takes place cleanly in aqueous medium at room temperature with easily handled reagents, without the need for the complicated apparatus previously employed[6]. Further, our procedure would be applicable to the determination other quaternary ammonium compounds in disinfectants and drugs. Details of these analyses will be reported in the near future.

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