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SHORT COMMUNICATION

Determination of the homologue composition of some alkyltrimethyl quaternary ammonium antibacterial agents by gas chromatography

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RECENT work has shown that the micellar properties of quaternary ammonium surface-active agents may be linked with their antibacterial action (Weiner, Hart & Zografi, 1965). Micelle formation and antibacterial activity both depend markedly on the chain length of the hydrocarbon groups attached to the quaternary nitrogen, and other biological properties may also depend on this aspect of their structure (Hart & Nissim, 1966). Commercially produced materials are mixtures of homologues and a method is described below for measuring the homologue composition.

Previous work (Metcalfe, 1963) has described the gas chromatography of long chain quaternary ammonium compounds on an alkaline column. Under the published conditions a mixture of the corresponding tertiary amines was produced, the analysis of which provided a measure of the homologue composition of the original material. We were unable to reproduce these experiments but the following method proved successful. Two types of column were used and results seem to be essentially the same for both. Column A consisted of 10% silicone elastomer (S.E. 30) on kieselguhr, whilst column B consisted of a 5 ft column packed for its initial 2 ft with 20% potassium hydroxide on kieselguhr followed by 10% potassium hydroxide + 10% Apiezon L on the same support for the remainder of its length. The long chain quaternary ammonium compound in the form of its hydroxide was used in the analysis. Under the conditions described in the experimental section the normal β-elimination took place to yield an olefin, accompanied by the alternative mode of decomposition, namely loss of methanol, to give the corresponding tertiary amine. When a pure sample of tetradecyltrimethylammonium bromide was analysed in this way two peaks resulted, one

TABLE 1. HOMOLOGUE COMPOSITION OF SOME ALKYLTRIMETHYL QUATERNARY AMMONIUM COMPOUNDS

Compound	Approximate composition %				
	$C_{(10)}$	$C_{(12)}$	$C_{(14)}$	$C_{(16)}$	$C_{(18)}$
Sample A (cetrimide)	—	24	65	9	2
" B "	—	27	62	11	—
" C "	—	21	69	10	—
Morpan T	1	9	86	2	2
" CHA	1	6	10	69	14
" O	—	7	4	3	86

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from 1-dimethylaminotetradecane and the other from tetradec-1-ene. The peaks were identified by using standards of the authentic substances; decomposition was shown to be quantitative. The result of analysing cetrimide B.P. and some other samples of quaternary ammonium compounds in this way is shown in Table 1. A typical chromatogram (cetrimide sample) is shown in Fig. 1. The approximate percentage of each component present was expressed as a percentage of the total peak area (internal normalisation). Errors involved in applying this method are probably not serious since the detector response variation within a homologous series for a flame ionisation detector is small (Warrington, 1961) and the difference in response between olefin and amine was shown to be about 5% for equal weights of dimethylaminotetradecane and tetradec-1-ene.

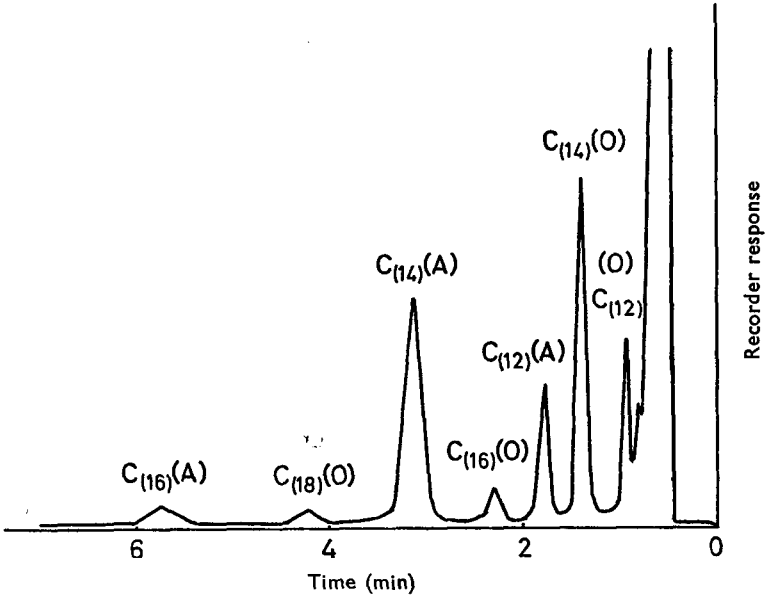

FIG. 1. Chromatogram of cetrimide, Sample A. (O) = olefin; (A) = tertiary amine.

The results for the cetrimide samples show that these products contain about 25% of $C_{(12)}$ compounds. The effect of this is to improve the solubility of the products although recent results (Weiner & others, 1965) suggest that it may also affect their antibacterial activity. Results for two of the Morpan samples in the Table agree reasonably well with the homologue composition of the alcohols used in the production of the compounds (see also the experimental section). The unsaturated components present in Morpan CHA were not detected under the conditions used.

MATERIALS

Tetradecyltrimethylammonium bromide was kindly supplied by Mr. J. E. Adderson (Adderson & Taylor, 1964). *Cetrimide B.P. samples.* Sample A was obtained from Glovers (Chemicals) Ltd., samples B and C from Imperial Chemical Industries Ltd. *Morpans* were donated by Glovers (Chemicals) Ltd. The percentage composition of the alcohols from which they were prepared was as follows: Morpan T, $C_{(14)}$ 95; Morpan CHA, $C_{(14)}$ 10, $C_{(16)}$ 70, $C_{(18)}$ 17, unsaturated material 3. *Tertiary amines and* 1-*alkenes* ($C_{(10)}$–$C_{(18)}$) were obtained from Kodak Ltd. The $C_{(14)}$ compound of each series was purified by preparative gas chromatography. Analysis of the other samples showed that several homologues were present and the retention times of the main peaks were determined. Their identities were checked by plotting the logarithm of the retention times against the number of carbon atoms in the chain when a linear plot was obtained.

APPARATUS AND METHODS

(i) A Pye "Series 104", model 4 flame ionisation isothermal chromatograph was used for the analysis of the pure sample of tetradecyltrimethylammonium bromide. A 4 mm diameter circular, stainless steel column, 5 ft long, was packed with acid-washed Celite (80/100 mesh) coated with 10% w/w S.E. 30. Column temperature, 240°; injection heater, 290°; carrier gas nitrogen, 20 ml/min.

0·003 ml of a test solution containing approximately 2% w/w tetradecyltrimethylammonium bromide and a reference solution containing 0·62% w/w tetradec-1-ene and 1-dimethylaminotetradecane respectively were used. In each case the solvent—methanol—contained 2% w/w potassium hydroxide.

(ii) A Pye Panchromatograph, with a flame ionisation detector was used for the cetrimide and Morpan samples. A 4 mm diameter glass column, 5 ft long, was packed for 3 ft with acid-washed Chromosorb W (60–80 mesh) coated with 10% w/w Apiezon L and 10% w/w potassium hydroxide, and for the initial 2 ft with Chromosorb W coated with 20% w/w potassium hydroxide. Column temperature, 240°; carrier gas argon at approximately 60 ml/min 0·005 ml of solutions each containing 4% w/w of the quaternary ammonium compound and approximately 1·2% w/w potassium hydroxide in methanol were injected.

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