Modified Hofmann Degradation for the Analysis of *n*-Alkylbenzyldimethylammonium Chlorides by Gas Chromatography II

Benzalkonium Chloride

By H. MITCHNER* and E. C. JENNINGS, JR.

The modified Hofmann degradation with gas chromatography of degradation products has been applied to a study of benzalkonium chloride. Quantitation is possible to 0.05 percent and the determination of alkyl group distribution is simultaneously possible. A good correlation was found between the Hofmann degradation and the USP catalytic hydrogenation for determination of alkyl ratios.

BENZALKONIUM CHLORIDE is a germicide and preservative which has found increasingly greater use. Its germicidal effectiveness is based on its content of a mixture of *n*-alkyl dimethylbenzylammonium chlorides, the most important of which are the C_{12} and C_{14}. The United States Pharmacopeia requires compendia labeled material to contain not less than 40% of the C_{12}-alkyl, and that C_{14} be not less than 20% of the total alkyldimethylammonium chloride content with the sum of the C_{12} and C_{14}-alkyls not less than 70% of the total (1).

The analysis of the alkyl substituent in benzalkonium chloride poses a difficult analytical problem. For the official analysis the USP applies a gas chromatographic assay following an Emde degradation. This allows a determination of alkyl group ratios but is not quantitative and requires specialized hydrogenation equipment. A new gas chromatographic approach to the analysis of *n*-alkyl dimethylbenzalkonium chlorides employing a modified Hofmann degradation (2) was found applicable to the analysis of benzalkonium chloride.

The modified Hofmann degradation was applied directly to several commercial benzalkonium chloride preparations. The catalytic hydrogenation procedure utilized in the benzalkonium chloride monograph of the USP (1), which is essentially the procedure of Warrington (3) and the special apparatus of Southworth (4), was compared with the Hofmann degradation procedure with similar results.

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* Present address: Quality Control Division, Syntex Laboratories, Inc., Palo Alto, CA 94304

APPARATUS AND REAGENTS

In addition to the apparatus employed previously in this study (2), special adaptation of the Parr hydrogenation apparatus for microsamples was used.

For further calibration of the F&M 810R-12 gas chromatograph, pure alkyldimethylamines with even numbered alkyl groups C_8 to C_{18} were obtained from Matheson, Coleman, and Bell. These compounds were used as received and corrections for minor alkyldimethylamines were made, based on gas chromatograms of these materials.

Samples of benzalkonium chloride[1] from several manufacturers and a standard benzalkonium chloride from the USP reference standards,[2] as distributed by the United States Pharmacopeial Convention, Inc., were obtained.

PROCEDURE

Based on the label assay of the commercial benzalkonium chloride preparations, aqueous concentrations of 100 mg./ml. were prepared. For the modified Hofmann degradation, 1.8 ml. of the benzalkonium chloride solution was transferred with a Pasteur disposable pipet actuated with a 5-ml. Hamilton syringe. An equal volume of 8 *N* KOH was added to give final concentrations of 50 mg./ml. of benzalkonium chloride in 4 *N* KOH. Ampuls were sealed and heated 1.0 hr. at 110°. After cooling the ampul under tap water and shaking to thoroughly remix any separated phases, the ampul was opened, and 5 μl. was injected into the chromatograph. All conditions of operation were the same as used in the previous study (2).

For the catalytic hydrogenation, 2.0 ml. of the benzalkonium chloride solution was transferred to the micro glass reaction flask and 35 mg. of palladium chloride (10%) on charcoal was added. After initial evacuation of the reaction flask, hydrogen was introduced to a final pressure of 35 psig and the reaction was allowed to proceed until no further pressure drop was obtained. The procedure of the USP (1) was continued for the recovery of the alkyldimethylamines formed.

The calibration runs on the gas chromatograph

[1] These materials were taken from file samples and may not reflect current production material.
[2] USP Reference Standards, New York, N. Y.

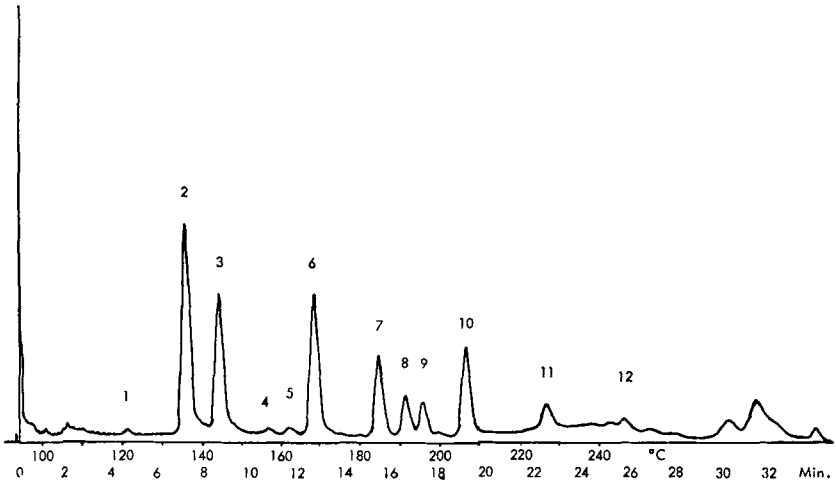

Fig. 1—Synthetic mixture of C_{14}, C_{16}, and C_{18} alkyl benzyldimethylammonium chlorides. Key: 1, *1-*$C_{12}H_{24}$; 2, *BDMA;* 3, *1-*$C_{14}H_{28}$; 4, *BMA;* 5, n-$C_{16}H_{34}$; 6, *1-*$C_{16}H_{32}$; 7, n-$C_{18}H_{38}$; 8, *1-*$C_{18}H_{36}$; 9, n-$C_{12}H_{25}OH$; 10, n-$C_{14}H_{29}OH$; 11, n-$C_{16}H_{33}OH$; 12, n-$C_{18}H_{37}OH$.

TABLE I—RELATIVE ELUTION TEMPERATURE AND DETECTOR RESPONSE FOR AMINE CALIBRATION COMPOUNDS

Carbon No.	*n*-Alkyldimethylamines RTr
C_8	0.38
C_{10}	0.66
C_{12}	1.00
C_{14}	1.34
C_{16}	1.65
C_{18}	1.95
Detector response (mcg./DU) $\times 10^{-6}$	37 ± 1

TABLE II—*n*-ALKYL DISTRIBUTION IN A SYNTHETIC MIXTURE OF C_{14}, C_{16}, AND C_{18}-ALKYLBENZYLDIMETHYLAMMONIUM CHLORIDES

1-Alkene	Synthetic Mixture, 2.3 mg./ml. Sample *1*	Sample *2*	Sample *3*	Av.	Calcd. as Mixed
C_{12}	0.0	0.0	0.0	0.0	0.0
C_{14}	45.6	42.8	42.5	43.7	48.0
C_{16}	43.4	43.2	42.4	43.0	41.6
C_{18}	11.0	14.0	15.0	13.3	10.4
	100.0	100.0	100.0	100.0	100.0

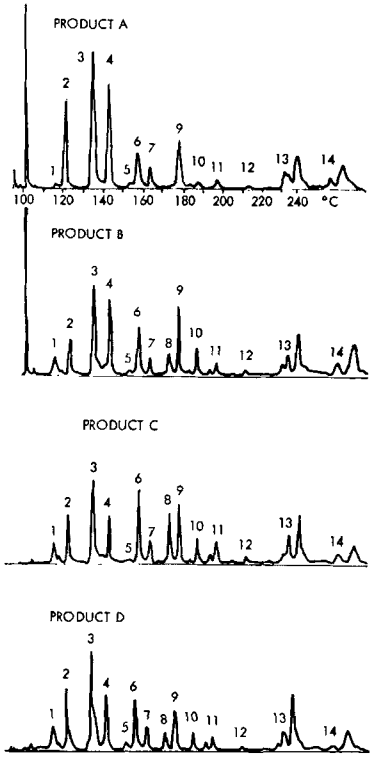

Fig. 2—Hofmann degradation of four commercial benzalkonium chlorides. Key: 1, n-$C_{12}H_{26}$; 2, *1-*$C_{12}H_{24}$; 3, *BDMA;* 4, *1-*$C_{14}H_{28}$; 5, *BMA;* 6, n-$C_{16}H_{34}$; 7, *1-*$C_{16}H_{32}$; 8, n-$C_{18}H_{38}$; 9, $C_{10}H_{21}OH$; 10, *1-*$C_{18}H_{36}$; 11, $C_{12}H_{25}OH$; 12, $C_{14}H_{29}OH$; 13, $C_{16}H_{33}OH$; 14, $C_{18}H_{37}OH$.

were temperature programmed runs (6°/min.) from 100–240° and linear plot of relative elution temperature to benzyldimethylamine for all alkanes, alkenes-1, and alkanols found in the modified Hofmann degradation against carbon number in the various homologous series gave straight line relationships. The same operating conditions were used also for the analysis of the catalytic hydrogenation products.

The relative elution temperature and average detector response in micrograms per disk unit of integration (mcg./DU) for the alkanes and alkanols have been previously reported (2). Table I shows the relative retention characteristics of alkyldimethylamines with respect to lauryldimethylamine under similar conditions.

TABLE III—ALKYL DISTRIBUTION OF FOUR BENZALKONIUM CHLORIDES BY THE MODIFIED HOFMANN DEGRADATION

Alkyl Group	Product A	Product B	Product C	Product D[a]
C_{12}	25.8	24.8	34.9	36.1
C_{14}	47.0	47.4	34.1	37.5
C_{16}	15.1	10.4	14.4	14.4
C_{18}	12.1	18.4	16.6	12.0
	100.0	100.0	100.0	100.0

[a] Prepared from the 93.5% concentrate.

RESULTS AND DISCUSSION

Alkyl Distribution Studies—To determine the applicability of the modified Hofmann degradation in conjunction with gas chromatography for the analysis of alkyl distribution of a mixture, such as benzalkonium chloride, a synthetic mixture was prepared from samples of C_{14}-alkyl-, C_{16}-alkyl-, and C_{18}-alkylbenzyldimethylammonium chlorides, previously analyzed by the same technique (2). As shown in Fig. 1, good resolution of all peaks was obtained, and reasonable replication of alkyl distribution between samples was achieved. (Table II.)

TABLE IV—ALKYL DISTRIBUTION OF PRODUCT E[a] BY THE MODIFIED HOFMANN DEGRADATION

	Lot No. →	02349	02269	02152	01920	01742	
Alkyl Group	Date Recd. →	3/29/66	11/30/65	8/2/65	12/21/64	7/9/64	Av.
C_{12}		57.2	50.2	52.2	51.2	50.0	52.2
C_{14}		34.9	36.1	35.6	35.4	34.4	35.3
C_{16}		6.7	12.4	10.7	13.1	12.7	11.1
C_{18}		1.2	1.3	1.5	0.3	2.9	1.4
		100.0	100.0	100.0	100.0	100.0	100.0

[a] Prepared from the 50% aqueous solution from samples received over a 2-year period.

TABLE V—COMPARISON OF THE CATALYTIC HYDROGENATION WITH THE MODIFIED HOFMANN DEGRADATION FOR THE ALKYL DISTRIBUTION IN BENZALKONIUM CHLORIDE

	Product A, Lot 1470 (50%)		Product A, Lot 0508 (50%)	
Alkyl Group	Catalytic Hydrogenation	Hofmann Degradation	Catalytic Hydrogenation	Hofmann Degradation
C_{12}	39.3	39.9	44.3	38.0
C_{14}	46.5	46.2	44.1	48.9
C_{16}	11.2	10.5	8.5	11.1
C_{18}	3.0	3.4	3.1	2.0
	100.0	100.0	100.0	100.0

TABLE VII—COMPARISON OF THE CATALYTIC HYDROGENATION WITH THE MODIFIED HOFMANN DEGRADATION FOR THE ALKYL DISTRIBUTION IN A USP REFERENCE STANDARD BENZALKONIUM CHLORIDE

Alkyl Group	Label Assay	Catalytic Hydrogenation	Hofmann Degradation
C_8	0.4	...	...
C_{10}	0.7	...	...
C_{12}	55.0	62.4	51.2
C_{14}	34.0	29.4	36.7
C_{16}	9.7	8.2	12.1
C_{18}	0.9	...	...
	100.7	100.0	100.0

TABLE VI—MAJOR VOLATILE IMPURITIES PRESENT IN FOUR COMMERCIAL BENZALKONIUM CHLORIDES BASED ON PEAK AREAS

Carbon No.	Product A			Product B			Product C			Product D		
	Alkane	Alkanol	BMA	Alkane	Alkanol	BMA	Alkane	Alkanol	BMA	Alkane	Alkanol	BMA
C_{10}	...	825	...	...	875	...	...	765	...	...	515	...
C_{12}	55	170	...	65	200	...	75	275	...	100	195	...
C_{14}	...	60	...	...	100	...	...	90	...	...	65	...
C_{16}	635	255	...	690	310	...	995	1020	...	740	280	...
C_{18}	265	240	...	315	350	...	585	200	...	225	220	...
Total disk units	955	1550	160	1070	1835	115	1655	2350	95	1065	1275	160
mcg.[a]	26.9	54.6	7	30.1	64.6	5	46.6	82.8	4	30	44.9	7
Total impurities, mcg.		88.5			99.7			133.4			81.9	
Total material injected, mcg.		261			252			250			278	
% Impurity		33.9			39.6			53.3			29.5	
% Quat.[b]		57.8			54.4			54.4			49.9	
Total		91.7			94.0			107.7			79.4	

[a] Based on detector responses given in Table I. [b] Based on detector responses given in Table I for BDMA and distributed molecular wt. of quaternary ammonium chlorides.

Four different commercial benzalkonium chlorides were analyzed after Hofmann degradation. Qualitatively similar chromatograms were obtained (Fig. 2), but analysis of peak areas showed considerable variation in the alkyl ratio distribution (Table III). This study was expanded to evaluate possible lot to lot variations in a commercial product used for an extended time and samples of benzalkonium chloride (product *D*) as received over a period of 2 years were analyzed for their alkyl distribution. Constant alkyl ratios were obtained (Table IV). Two lots of product *A* were also analyzed with similar good agreement for alkyl distribution between lots (Table V).

Impurities in Benzalkonium Chlorides—In addition to the expected alkenes, samples of benzalkonium chlorides showed the presence of impurities of alkanols, alkanes, and benzylmethylamine. Many of these impurities have been previously characterized (2) and can be identified in chromatograms by their retention data. Considerable variation in the amounts of the impurities between different benzalkonium chlorides were noted. An analysis of the peak areas of the major impurities in the chromatograms from Fig. 2 is shown in Table VI. The volatile impurities were a significant contribution to the content of the products and were from 30–53%. No evaluation of nonvolatile impurities was made.

Quantitation—The use of the modified Hofmann degradation approach for quantitative results is based on the formation of benzyldimethylamine by any of the quaternaries present, irrespective of the alkyl substituents. The ratios of the alkenes found allows an assignment of the amount of the particular quaternary. Quantitation was possible to a lower limit of 0.05%.

Comparison of Catalytic Hydrogenation and Hofmann Degradation—The official USP method for the determination of alkyl distribution in benzalkonium chloride requires specialized hydrogenation equipment in addition to a temperature programmed gas chromatograph (1). In the chemical work-up after hydrogenation and before analysis, many of the impurities possibly present in the original compounds or created with hydrogenation are eliminated by the USP procedure. The ratio of the amines resulting from the hydrogenation of the benzalkonium chloride are obtained from the chromatograms. A comparison of the USP procedure with the modified Hofmann degradation was made for two lots of product *A* (Table V) and for a USP reference standard (Table VII). The results obtained by both methods showed that the differences between methods were within the limits of variation for either method on a single sample.

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N-Aralkyl-*N*-methylaminoethyl Carbanilates as Hypocholesteremic Agents

By WILLIAM J. ROST, BLAINE M. SUTTON, BENJAMIN BLANK, FRANCIS R. PFEIFFER, WILLIAM L. HOLMES, NICHOLAS W. DITULLIO, and EDWIN B. INGRAM

A group of aralkylaminoethyl esters and ureas of substituted carbanilic acids was prepared and studied for hypocholesteremic activity in eucholesteremic mice. Biological results indicated that modification of either terminal aromatic ring altered the activity of the resultant compound. The *meta* and *para* methyl substituted carbanilates of aralkylmethylaminoethanol consistently exhibited the most desirable effect. In the dicarbanilate series of aralkyl or aryl substituted iminodiethanols, no appreciable activity was seen.

A previous publication (1) reported on the preparation of a series of *N*-aralkyl-*N*-methylaminoethyl carbanilates and their local anesthetic activity. Since that report, additional biological testing revealed that certain members of the series possessed hypocholesteremic activity in experimental animals. The novelty of this activity as well as the potential value of a safe effective agent exhibiting these properties prompted the study of this type of compound in greater detail. This paper describes preparation of additional examples in this class of compounds, their effect on cholesterol metabolism in the mouse, and chemical structure-biological function relationships in the series.

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