Nuclear Magnetic Resonance Determination of Bacteriostats

MARTIN W. DIETRICH and ROBERT E. KELLER, Research Department,
Organic Chemicals Division, Monsanto Company, St. Louis, Missouri

Abstract

A rapid high-resolution NMR method has been developed for the determination of soap and detergent bacteriostats. The method can be used to identify six common commercial bacteriostats (3,4,4′-trichlorocarbanilide; 3-trifluoromethyl-4,4′-dichlorocarbanilide; 4′,5-dibromosalicylanilide; 3,5-dibromosalicylanilide; 3,4′,5-tribromosalicylanilide; and hexachlorophene) and all two-component mixtures prepared from them. This method can also be used to determine the relative concentrations of these bacteriostats in all two-component mixtures. The precision and accuracy of the method are estimated to be within 3% absolute.

Introduction

BACTERIOSTATS are used extensively in soaps, detergents, and cosmetic preparations. This paper describes new methodology for common bacteriostats which are used either alone or as synergistic mixtures. Analytical work reported in the literature includes colorimetric (1–7), ultraviolet absorption (8–13), and gas chromatography (14) methods for the determination of 2,2′-methylenebis (3,4,6-trichlorophenol) (VI), better known as hexachlorophene. An ultraviolet method (15) is also reported for the determination of 3,4,4′-trichlorocarbanilide (I) and 3,4′,5-tribromosalicylanilide (V). No literature references were found for 3-trifluoromethyl-4,4′-dichlorocarbanilide (II), 4′,5-dibromosalicylanilide (III), or 3,5-dibromosalicylanilide (IV). The reported colorimetric and ultraviolet absorption techniques lack the specificity needed to identify all of the six bacteriostats or quantitatively measure each in two component mixtures. This is especially true for the determination of 3,4,4′-trichlorocarbanilide in admixture with 3-trifluoromethyl-4,4′-dichlorocarbanilide.

This paper describes a rapid proton nuclear magnetic resonance (NMR) procedure for identification of these six commercial bacteriostats. Relative concentrations can be determined in all two-component mixtures thereof.

Separation techniques needed to isolate the bacteriostats from different types of products prior to NMR analysis are not included. Reference should be made to the literature already cited for separation schemes used by others.

Experimental Section

The bacteriostats in this study were obtained from commercial sources and were used without additional purification. All NMR measurements were made with a Varian Associates A-60 NMR spectrometer, operating at the ambient probe temperature. Spectra were obtained at a sweep rate of 0.4 cps/sec. Peak areas were measured electronically by using the integrator, which is part of the A-60 spectrometer system. Chemical shift values are reported in ppm with respect to TMS = 0 and are considered accurate to within ±.03 ppm.

Sample Preparation

Individual components or mixtures of bacteriostats, free of interfering materials, may be examined directly by dissolving sufficient bacteriostat in dimethyl sulfoxide-d_6 to give ca. 10% solutions. Materials which absorb in the chemical shift intervals used for analysis, such as some other aromatic components, will interfere. To identify and measure the bacteriostats in commercial products, such as soap formulations, the bacteriostats must be isolated from the soap. Extraction procedures, such as the one reported by Jungermann and Beck (15), have proven satisfactory for this separation. Sufficient soap should be used so that at least 50 mg of bacteriostat will be obtained.

Results and Discussion

The major features of the NMR spectra of the six bacteriostats are summarized in Table I. Where possible, assignments of the individual peaks in these spectra have been made. Each of the bacteriostats gives a characteristic NMR spectrum. Enough differences exist in these spectra to allow ready identification of a given bacteriostat.

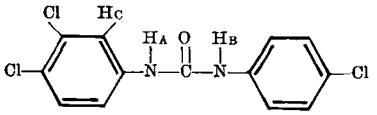
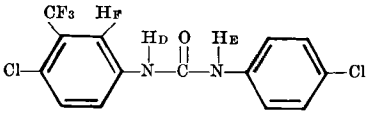
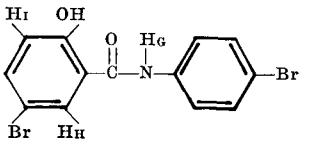
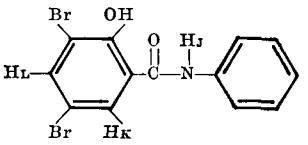
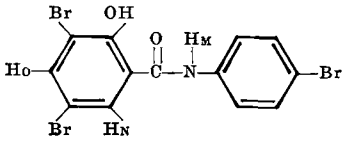
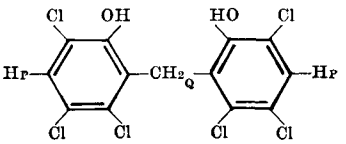
Comparison of these spectra indicated that NMR could also be used for the identification and measurement of each component in two-component bacteriostat mixtures. To confirm this procedure, 15 approximately equimolar mixtures were prepared to cover all two-component combinations of these materials. The NMR spectrum obtained from each mixture was sufficiently distinctive to permit identification of the components present.

Quantitative Analysis of Two-Component Mixtures

To evaluate the NMR technique for quantitative measurements, the spectrum of each mixture was carefully integrated. All mixtures, except that of IV and V, gave integral curves at 60 mc/sec, indicating one or more fully resolved peaks for each component. By extrapolation of the chemical shift difference, the mixture of IV and V will give a spectrum with fully resolved peaks at 100 mc/sec. All fully resolved peaks for each mixture are listed in Table II. The peaks usable for analysis depend on the particular bacteriostat mixture encountered. In some cases a specific peak may be used in all mixtures; in other cases the same peak may not be used. Also, in some mixtures, more than one peak of a particular component may be used for analysis.

The relative concentration of each component can be determined directly from the relative areas of the analysis peaks. One or all of the possible analysis peaks for each component may be used. If an impurity which interferes with the measurement of the area of a specific peak is detected, this peak, of course, should not be used for analysis. If no interfering impurities are detected, all analysis peaks should be included. The comparison of results obtained in this manner with those obtained by basing analysis on a single peak of each component provides a means of detecting low levels of interfering materials. The mole percentage of each component is determined from the expressions

TABLE I
NMR Parameters of Soap Bacteriostats

Compound		Chemical shift: Assignment	Chemical shift: δ(PPM)[1]	Peak multiplicity[2]
	(I)	H_A	9.00	s
		H_B	8.92	s
		H_C	7.88	d (J = 2.5 cps)
		other ArH	7.2–7.6	m
	(II)	[4]H_D	9.20	s
		[4]H_E	8.98	s
		H_F	8.13	m
		other ArH	7.2–7.6	m
	(III)	H_G	10.5	s
		H_H	8.12	d (J = 2.5 cps)
		H_I	6.98	d (J = 8.7 cps)
		other ArH	7.5–7.9	m
		OH	Undetected[3]	
	(IV)	H_J	10.6	s
		[4]H_K	8.32	d (J = 2.5 cps)
		[4]H_L	7.95	d (J = 2.5 cps)
		other ArH	7.0–7.8	m
		OH	Undetected[3]	
	(V)	H_M	10.7	s
		[4]H_N	8.27	d (J = 2.6 cps)
		[4]H_O	8.00	d (J = 2.6 cps)
		other ArH	7.6–7.8	m
		OH	Undetected[3]	
	(VI)	H_P	7.68	s
		H_Q	4.38	s
		OH	Undetected[3]	

[1] Referred to internal TMS.
[2] s = singlet, d = doublet, m = multiplet.
[3] Presumed to be undetected because of exchange broadening.
[4] Tentative structural assignment.

TABLE II
Quantitative Peaks for Two-Component Mixtures

Components: X	Components: Y	Resolved peaks[1]: X	Resolved peaks[1]: Y	Analysis expression[2]: A_X	Analysis expression[2]: A_Y
I	II	H_C	H_F	A_C	A_F
I	III	H_A,H_B	H_H	½ ($A_A + A_B$)	A_H
I	IV	H_A,H_B	H_K	½ ($A_A + A_B$)	A_K
I	V	H_A,H_B	H_N,H_O	$A_A + A_B$	$A_N + A_O$
I	VI	H_A,H_B	H_Q	$A_A + A_B$	A_Q
II	III	H_D,H_E	H_I	½ ($A_D + A_E$)	A_I
II	IV	H_D,H_E	H_K	½ ($A_D + A_E$)	A_K
II	V	H_D,H_E	H_N	½ ($A_D + A_E$)	A_N
II	VI	H_D,H_E,H_F	H_Q	⅓ ($A_D + A_E + A_F$)	½ A_Q
III	IV	H_I	H_K	A_I	A_K
III	V	H_A	H_N	A_I	A_N
III	VI	H_H,H_I	H_Q	$A_H + A_I$	A_Q
IV	V	H_K[3]	H_N[3]	A_K	A_N
IV	VI	H_K,H_L	H_Q	$A_K + A_L$	A_Q
V	VI	H_N,H_O	H_Q	$A_N + A_O$	A_Q

[1] The chemical shifts of these peaks are found in Table I.
[2] A_X = area of analysis peak(s) for component X.
A_Y = area of analysis peak(s) for component Y.
[3] These peaks are not fully resolved at 60 mc/sec. Analysis should be possible by using spectra obtained at 100 mc/sec.

$$\text{Mole \% X} = \frac{A_x}{A_x + A_y} \times 100$$

$$\text{Mole \% Y} = 100 - \text{Mole \% X}$$

where Mole % X is the mole percentage of component X in mixture with component Y, A_x is the average area of the analysis peak(s) of component X, and A_y is the average area of the analysis peak(s) of component Y. Expressions for A_x and A_y for each mixture are given in Table II. These expressions include all the possible analysis peaks for each system and may be modified when interferences are detected. If preferred, results may also be expressed on a weight percentage basis. The precision and accuracy of the procedure are typical for NMR measurements and are estimated to be within 3% absolute if two or more integral traces are obtained for each peak.

This procedure can also be used to determine the absolute concentration of bacteriostats by using the usual NMR techniques for sample molarity. Alternatively, combination NMR-IR or NMR-UV procedures can be employed for certain mixtures.

No attempt was made to extend this technique to three-component bacteriostat mixtures. However visual comparison of the spectra of the individual bacteriostats indicates that analysis of selected three-component systems is possible.

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