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*Communication*

# Preparation of Benzalkonium Salts Differing in the Length of a Side Alkyl Chain

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**Abstract:** Benzalkonium salts are widely used as disinfectants, biocides and detergents, among a variety of other applications. The cationic surface-activity of these salts determines their potential to act as a biocide on both target and non-target organisms. In this study, a quick synthesis of a complete set of the benzalkonium salts differing in the length of an alkylating chain (from $C_2$ to $C_{20}$) is described. Moreover, their $^1$H-NMR, HPLC-MS, TLC and HPLC analysis were recorded.

**Keywords:** Benzalkonium salt, detergent, surfactant, biocide, HPLC, TLC.

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## Introduction

Quaternary compounds, including the benzalkonium salts, constitute an economically important class of industrial chemicals that are widely distributed among a diverse array of products and users

from an industrial to the household sector. Because of their strong cationic surface activity, quaternary compounds are used primarily as disinfectants, biocides, and detergents, but also as anti-electrostatics, and as phase transfer catalysts [1].

Very important features of benzalkonium salts are their bactericidal and antimicrobial properties. The antimicrobial activity depends on a changing length of the side *n*-alkyl chain. It is well known that the $C_{12}$-homolog is most effective against yeast and fungi, the $C_{14}$-homologue against gram-positive bacteria and $C_{16}$-homolog against gram-negative bacteria [2]. For these reasons they are widely used as preservatives for ophthalmic, nasal and parenteral products. They are also used as topical antiseptics and disinfectants for medical equipment [3-6].

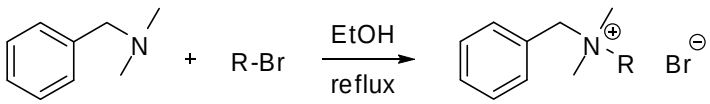
These compounds are not generally used as single components, but rather as mixtures composed of two or three benzalkonium members differing only in the length of the alkyl chains [7-8]. Such mixtures are produced on a large-scale in industry. However, a targeted synthesis of pure individuals could be of interest because of the above-mentioned specificity of each salt against different pathogens.

Preparation of these compounds has been described previously [9-12], but a general synthesis applicable to the whole series of benzalkonium salts is lacking. The synthesis of long chain derivatives ($C_8$-$C_{20}$) was described earlier by our group [13]. To provide a synthetic route to such compounds, the preparation of the whole set of these compounds ($C_2$-$C_{20}$) is now shown. A new set of the short chain benzalkonium salts was developed and the preparation of the long chain benzalkonium salts was improved.

## Results and Discussion

The *N*-benzyl-*N*,*N*-dimethylalkyl bromides **3-12** were prepared by the general method shown in Scheme 1.

**Scheme 1.** Preparation of benzalkonium salts.




1 2 3 - 11

Yields of all reactions, together with the melting points of the prepared salts, are shown in Table 1. Thin-Layer Chromatography (TLC) retention factors and High Pressure Liquid Chromatography (HPLC) retention times for of all the salts are also listed in this table. The yields achieved in this study were superior to those obtained earlier for $C_8$-$C_{20}$ derivatives [13]. Moreover, the $C_2$-$C_6$ derivatives were added to complete the whole $C_2$-$C_{20}$ set. Yields achieved for those compounds were also adequate. We have to point out that use of our method (mixture of the pure compounds) might conceivably replace the synthetic “all in one” mixtures now used in industry. For example, Ajatine® (a $C_{12}$ analogue used as disinfectant) is generally a mixture of the parent substances (*N*-benzyl-*N*,*N*-

dimethylamine, $C_{12}$ alkylating chain and solvent) and a reaction product (the appropriate monoquaternary salt). Our pure products (without parent substances) could be used in more sophisticated applications, where potential adverse effects of the parent substances would no longer be a factor.

There are several articles which deal with HPLC analysis of benzalkonium derivatives [14-16]. None of these, however, describe HPLC analysis of the whole benzalkonium set. In this study, our previously developed HPLC method was modified for a determination of each benzalkonium salt with an aim of distinguishing them [17]. Such a method could be applicable not only for the benzalkonium salts but also for other long chain quaternary compounds. For quick laboratory analysis of prepared benzalkonium salts, a TLC method was also developed to distinguish each benzalkonium salt. Such a method could be of interest in the laboratories not equipped by special expensive equipment (HPLC).

**Table 1.** Yields, melting points, retention factors and retention times of prepared bezalkonium salts.

| Compound | Yield (%) | m.p. (°C) | TLC Rf | HPLC Rt |
|---|---|---|---|---|
| **3** | 94 | 120.5-121.5 | 0.083 | 4.76 |
| **4** | 44 | 150.0-152.0 | 0.260 | 5.84 |
| **5** | 54 | 122.0-123.5 | 0.420 | 7.13 |
| **6** | 61 | 53.0-56.0 | 0.512 | 8.47 |
| **7** | 61 | 34.0-37.5 | 0.558 | 9.92 |
| **8** | 80 | 37.0-40.0 | 0.592 | 11.55 |
| **9** | 71 | 43.5-47.5 | 0.612 | 13.43 |
| **10** | 58 | 50.0-53.0 | 0.634 | 15.60 |
| **11** | 71 | 81.0-83.0 | 0.651 | 18.15 |
| **12** | 64 | 85.0-88.0 | 0.664 | 21.25 |

## Conclusions

A quick and easy method for the preparation of pure benzalkonium salts in adequate yields was described. Their purities were analyzed by $^1$H-NMR, HPLC-MS, TLC and HPLC techniques.

## Experimental

### *General*

All chemicals used in this study were obtained from Sigma-Aldrich (Czech Republic). Purity of all products was tested by determination of their melting points (Boetius block) and were uncorrected, TLC (Kieselgel Merck; mobile phase *n*-BuOH/$CH_3COOH$/$H_2O$ (5:1:2); Detection: $UV_{254}$, Dragendorff´s reagent); HPLC-MS (HP1100 HPLC system - Agilent Technologies (Waldbronn,

Germany); quadrupole mass spectrometer MSD1456 VL; data were collected in positive ion mode with an ESI probe voltage of 4000 V) and NMR analysis (Varian Gemini 300, 300 MHz, DMSO-$d_6$).

*Synthesis*

Briefly *N,N*-dimethylbenzylamine (**1**; 7.4 mmol) in dry ethanol (25 ml) was mixed with an equimolar amount of the appropriate 1-bromo-alkane (**2**; 10.4 mmol) and the mixture was refluxed for 28 hours. Solvent was evaporated and the crude *N*-benzyl-*N,N*-dimethylalkyl-1-ammonium bromides (**3-12**) were recrystalized from acetone, washed with ether and allowed to dry at r.t. The corresponding yields and melting points are summarized in Table 1. Spectral data ($^1$H-NMR and MS spectra) are listed below:

*N-benzyl-N,N-dimethylethyl-1-ammonium bromide* (**3**). $^1$H-NMR: $\delta$ 1.35 (t, 3H, $CH_3CH_2N^+$); 2.93 (s, 6H, $(CH_3)_2N^+$); 3.35 (t, $J$ = 6.33 Hz, 2H, $CH_2N^+$); 4.52 (s, 2H, $PhCH_2N^+$); 7.53 (bs, 5H, Ph); ESI-MS: m/z 164.2 [$M^+$] (calc. for $[C_{11}H_{18}N]^+$ 164.27).

*N-benzyl-N,N-dimethylbutyl-1-ammonium bromide* (**4**). $^1$H-NMR: $\delta$ 0.90 (t, $J$ = 6.47 Hz, 3H, $CH_3$); 1.28 (m, 2H, $(CH_2)_3$); 1.78 (m, 2H, $CH_2CH_2N^+$); 2.96 (s, 6H, $(CH_3)_2N^+$); 3.24 (t, $J$ = 6.33 Hz, 2H, $CH_2N^+$); 4.59 (s, 2H, $PhCH_2N^+$); 7.56 (bs, 5H, Ph); ESI-MS: m/z 192.2 [$M^+$] (calc. for $[C_{13}H_{22}N]^+$ 192.32).

*N-benzyl-N,N-dimethylhexyl-1-ammonium bromide* (**5**). $^1$H-NMR: $\delta$ 0.88 (t, $J$ = 6.47 Hz, 3H, $CH_3$); 1.26 (bs, 6H, $(CH_2)_3$); 1.79 (m, 2H, $CH_2CH_2N^+$); 2.95 (s, 6H, $(CH_3)_2N^+$); 3.22 (t, $J$=6.33Hz, 2H, $CH_2N^+$); 4.58 (s, 2H, $PhCH_2N^+$); 7.55 (bs, 5H, Ph); ESI-MS: m/z 220.2 [$M^+$] (calc. for $[C_{15}H_{26}N]^+$ 220.38).

*N-benzyl-N,N-dimethyloctyl-1-ammonium bromide* (**6**). $^1$H-NMR: $\delta$ 0.86 (t, $J$ = 6.47 Hz, 3H, $CH_3$); 1.29 (bs, 10H, $(CH_2)_5$); 1.78 (m, 2H, $CH_2CH_2N^+$); 2.95 (s, 6H, $(CH_3)_2N^+$); 3.24 (t, $J$=6.33Hz, 2H, $CH_2N^+$); 4.56 (s, 2H, $PhCH_2N^+$); 7.56 (bs, 5H, Ph); ESI-MS: m/z 248.2 [$M^+$] (calc. for $[C_{17}H_{30}N]^+$ 248.44).

*N-benzyl-N,N-dimethyldecyl-1-ammonium bromide* (**7**). $^1$H-NMR: $\delta$ 0.83 (t, $J$ = 6.60 Hz, 3H, $CH_3$); 1.23 (bs, 14H, $(CH_2)_7$); 1.77 (m, 2H, $CH_2CH_2N^+$); 2.92 (s, 6H, $(CH_3)_2N^+$); 3.24 (t, J=7.15Hz, 2H, $CH_2N^+$); 4.54 (s, 2H, $PhCH_2N^+$); 7.53 (bs, 5H, Ph); ESI-MS: m/z 276.3 [$M^+$] (calc. for $[C_{19}H_{34}N]^+$ 276.50).

*N-benzyl-N,N-dimethyldodecyl-1-ammonium bromide* (**8**). $^1$H-NMR: $\delta$ 0.83 (t, $J$= 6.60 Hz, 3H, $CH_3$); 1.22 (bs, 18H, $(CH_2)_9$); 1.77 (m, 2H, $CH_2CH_2N^+$); 2.94 (s, 6H, $(CH_3)_2N^+$); 3.24 (t, $J$=6.32Hz, 2H, $CH_2N^+$); 4.55 (s, 2H, $PhCH_2N^+$); 7.52 (m, 5H, Ph); ESI-MS: m/z 304.3 [$M^+$] (calc. for $[C_{21}H_{38}N]^+$ 304.55).

*N-benzyl-N,N-dimethyltetradecyl-1-ammonium bromide* (**9**). $^1$H-NMR: $\delta$ 0.83 (t, $J$ = 6.60 Hz, 3H, $CH_3$); 1.22 (bs, 22H, $(CH_2)_{11}$); 1.78 (m, 2H, $CH_2CH_2N^+$); 2.95 (s, 6H, $(CH_3)_2N^+$); 3.23 (t, $J$=7.71Hz, 2H, $CH_2N^+$); 4.54 (s, 2H, $PhCH_2N^+$); 7.52 (m, 5H, Ph); ESI-MS: m/z 332.3 [$M^+$] (calc. for $[C_{23}H_{42}N]^+$ 332.61).

*N-benzyl-N,N-dimethylhexadecyl-1-ammonium bromide* (**10**) 1H-NMR: $\delta$ 0.84 (t, $J$ = 6.60 Hz, 3H, $CH_3$); 1.22 (bs, 26H, $(CH_2)_{13}$); 1.78 (m, 2H, $CH_2CH_2N^+$); 2.94 (s, 6H, $(CH_3)_2N^+$); 3.22 (t, $J$=8.39 Hz, 2H, $CH_2N^+$); 4.52 (s, 2H, $PhCH_2N^+$); 7.52 (bs, 5H, Ph); ESI-MS: m/z 360.4 [$M^+$] (calc. for $[C_{25}H_{46}N]^+$ 360.64).

*N-benzyl-N,N-dimethyloctadecyl-1-ammonium bromide* (**11**). $^1$H-NMR: $\delta$ 0.84 (t, $J$ = 6.47 Hz, 3H, $CH_3$); 1.24 (bs, 30H, $(CH_2)_{15}$); 1.75 (m, 2H, $CH_2CH_2N^+$); 2.93 (s, 6H, $(CH_3)_2N^+$); 3.25 (t, $J$=8.25Hz, 2H,$CH_2N^+$); 4.52 (s, 2H, $PhCH_2N^+$); 7.53 (bs, 5H, Ph); ESI-MS: m/z 388.4 [$M^+$] (calc. for $[C_{27}H_{50}N]^+$ 388.71).

*N-benzyl-N,N-dimethyleicosyl-1-ammonium bromide* (**12**). $^1$H-NMR: $\delta$ 0.83 (t, $J$ = 6.47 Hz, 3H, $CH_3$); 1.24 (bs, 34H, $(CH_2)_{17}$); 1.74 (m, 2H, $CH_2CH_2N^+$); 2.94 (s, 6H, $(CH_3)_2N^+$); 3.24 (t, $J$ = 8.26 Hz, 2H, $CH_2N^+$); 4.54 (s, 2H, $PhCH_2N^+$); 7.52 (m, 5H, Ph); ESI-MS: m/z 416.4 [$M^+$] (calc. for $[C_{27}H_{50}N]^+$ 416.78).

Subsequently, TLC and HPLC separations of all prepared compounds were developed (Table 1). The HPLC system consisted of a P200 gradient pump (Spectra-Physics Analytical, Fremont, USA), a 7125 injection valve – 10 μΛ loop (Rheodyne, Cotati, USA), a UV1000 detector (Spectra-Physics Analytical, Fremont, USA) and a CSW Chromatography Station 1.5 software (Data Apex, Prague, Czech Republic). For analyses a 250 × 4.6 mm I.D. Waters Spherisorb Cyano (5 μm) column was used (Supelco Inc.). The mobile phase contained 45% acetonitrile and 55 % water. This mixture was prepared as 0.1 M sodium acetate solution. Finally the pH was adjusted with acetic acid to 5.0. It was delivered isocratically at a flow-rate of 1 mL/min. The absorbance was measured at 263 nm [18]. TLC was performed on 140 mm × 140 mm plates coated with a 0.2 mm layer of silica gel 60 F254 (Merck, Darmstadt, Germany). Plates was streaked with a CAMAG Linomat IV automatic applicator (Camag, Berlin, Germany) and developed with a methanol/chloroform/acetic acid (25:5:0.5 v/v)) mobile phase in a twin trough chamber with a stainless steel lid (Camag, Berlin, Germany). Compounds were dissolved in acetonitrile/water (45:55 v/v) as 0.1 M acetate buffer, pH 5.0. Samples (5 μL from 2.5 mM solutions) were applied 1 cm from the bottom edge of the plate. Development was stopped when the mobile phase front reached 1 cm from the top of the plate.

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**References and Notes**


1. Grillitsch, B.; Gans, O.; Kreuzinger, N.; Scharf, S.; Uhl, M.; Fuerhacker, M. Environmental risk assessment for quaternary ammonium compounds: a case study from Austria. *Wat. Sci. Technol.* **2006**, *54*, 111-118.
2. Daoud, N.N.; Dickinson, N.A.; Gilbert, P. Determination of benzalkonium chloride by chemical ionization mass spectroscopy. *Microbios* **1983**, *37*, 73-85.
3. Debreceni, G.; Meggyesi, R.; Mestyan, G. Efficacy of spray disinfection with a 2-propanol and benzalkonium chloride containing solution before epidural catheter insertion--a prospective, randomized, clinical trial. *Br. J. Anaesth.* **2007**, *98*, 131-135.
4. Chung, S.H.; Lee, S.K.; Cristol, S.M.; Lee, E.S.; Lee D.W.; Seo, K.Y.; Kim, E.K. Impact of short-term exposure of commercial eyedrops preserved with benzalkonium chloride on precorneal mucin. *Mol. Vis.* **2006**, *12*, 415-421.
5. Brill, F.; Goroncy-Bermes, P.; Sand, W. Influence of growth media on the sensitivity of Staphylococcus aureus and Pseudomonas aeruginosa to cationic biocides. *Int. J. Hyg. Environ. Health.* **2006**, *209*, 89-95.
6. Rabenau, H.F.; Kampf, G.; Cinatl, J.; Doerr, H.W. Efficacy of various disinfectants against SARS coronavirus. *J. Hosp. Infect.* **2005**, *61*, 107-111.
7. Labranche, L.P.; Dumont, S.N.; Levesque, S.; Carrier, A. Rapid determination of total benzalkonium chloride content in ophthalmic formulation. *J. Pharm. Biomed. Anal.* **2007**, *43*, 989-993.
8. Cabal, J.; Kassa, J.; Severa, J. A comparison of the decontamination efficacy of foam-making blends based on cationic and nonionic tensides against organophosphorus compounds determined in vitro and in vivo. *Hum. Experim. Toxicol.* **2003**, *22*, 507-514.
9. Rudakova, A.W.; Popova, L.P. Study of reaction capability of lygnoceryl alcohol (In Russian). *Z. Obs. Chim.* **1966**, *36*, 242-244.
10. Novaki, L.P.; El Soud, O.A. Solvatochromism in aqueous micellar solutions: effects of the molecular structures of solvatochromic probes and cationic surfactants. *Phys. Chem. Chem. Phys.* **1999**, *1*, 1957-1964.
11. Stepanenko, B.N.; Ulámána, T.S.; Zelenkova, V.V. Synthesis of some qauternary salts of ammonium compounds (In Russian). *Chim. Farm. Z.* **1974**, *8*, 21-24.
12. Echols, L.; Maier, V.P.; Poling, S.M.; Sterling, P.R. New bioregulators of gibberellin biosynthesis in Gibberella fujikuroi. *Phytochemistry* **1981**, *20*, 433-438.
13. Kuca, K.; Kivala, M.; Dohnal, V. A general method for the quaternization of N,N-dimethyl benzylamines with long chain n-alkylbromides. *J. Appl. Biomed.*. **2004**, *2*, 195-198.
14. Prince, S.J.; McLaury, H.J.; Allen, L.V.; McLaury, P. Analysis of benzalkonium chloride and its homologs: HPLC versus HPCE. *J. Pharm. Biomed. Anal.* **1999**, *19*, 877-882.
15. Elrod Jr., L.; Golich, T.G.; Morley, J.A. Determination of benzalkonium chloride in eye care products by high-performance liquid chromatography and solid-phase extraction or on-line column switching. *J. Chromatogr.* **1992**, *625*, 362-367.

16. Ambrus, G.; Takahashi, L.T.; Marty, P.A. Direct determination of benzalkonium chloride in ophthalmic systems by reversed-phase high-performance liquid chromatography. *J. Pharm. Sci.* **1987**, *76*, 174-176.
17. Kuca, K.; Dohnal, V.; Bielavska, M.; Cabal, J. Determination of benzalkonium bromide homologues in desinfection products using high-performance liquid chromatography. *Anal. Lett.* **2005**, *38*, 673-682.
18. *European Pharmacopoeia 5 – Directorate for the Quality of Medicines of the Council of Europe*: Council of Europe: Aubin (France), **2005**; *Vol. 1*, pp. 1068-1070.

*Sample Availability:* Samples of all prepared compounds are available from the authors.