determinations involved three acetylcholine concentrations ranging from 0.004 to 0.08 M, each with a large excess (0.1–1.1 M) of aromatic salt. Usually each series involved ten samples. The association constants were independent of acetylcholine concentration and small changes in ionic strength.

The change in the chemical shift difference between the *N*-methyl and *C*-methyl signals, as defined by

$$\Delta\delta_{obsd} = (\delta_{C\text{-}Me}{}^{N\text{-}Me})_0 - (\delta_{C\text{-}Me}{}^{N\text{-}M})_{arom} \quad (2)$$

is related to an apparent association equilibrium constant K by an expression of the Benesi–Hildebrand type.[14,15]

$$(\Delta\delta_{obsd})^{-1} = (\Delta\delta_{max})^{-1} + (\Delta\delta_{max}K[arom])^{-1} \quad (3)$$

The symbols $(\delta_{C\text{-}Me}{}^{N\text{-}Me})_{arom}$ and $(\delta_{C\text{-}Me}{}^{N\text{-}Me})_0$ represent the chemical shift differences between the *N*-methyl and *C*-methyl signals (as positive numbers) in the presence and absence of added aromatic compound.[9] The term [arom] denotes the concentration of added aromatic and $(\Delta\delta_{max})^{-1}$ is the y-axis intercept of a plot of $(\Delta\delta_{obsd})^{-1}$ vs. [arom]. The value of K can be determined as the ratio of intercept to slope of such a plot.

Strictly speaking eq 3 is valid only if the concentration of the aromatic compound is much larger than that of acetylcholine.[14,15] Although our experimental conditions satisfy this requirement we also calculated K values by the iterative method of Stockton and Martin,[16] which is valid even when the two concentrations are comparable. Usually the association constants obtained by both methods agreed within 10%. The data given in Table II were calculated by the latter method using a least-squares computer analysis.

The NMR samples were prepared in NMR tubes by combining various proportions of freshly prepared stock solutions. High accuracy automatic pipets were used and all concentrations were certain within 5% as confirmed by final solution volumes.

Similar techniques were used in the other NMR spectral studies reported here.

Acetylcholine Hydrolysis Kinetics. The rate of hydrolysis of 0.008 M acetylcholine chloride in 0.05 M NH_3–NH_4Cl buffer (pH 10.3–10.5) with added sodium arenesulfonates was studied by the ferric hydroxamate method of Hestrin.[31] The reactions were initiated by adding an aliquot of acetylcholine chloride solution to a buffered sodium arenesulfonate solution in a volumetric flask suspended in a thermostated water bath (32 ± 0.5 °C). Aliquots (1 mL) were removed at intervals, mixed with an equal volume of freshly prepared solution containing the stoichiometric equivalent of 4 N NaOH and 2 M NH_2OH·HCl, and after 15 min diluted to 4 mL with 0.37 M ferric chloride in 0.1 N HCl. The concentration of unhydrolyzed acetylcholine remaining in the volumetric flask at time t (when the aliquot was taken) was estimated by the absorbance A_t at 540 nm measured on a Beckman Model DB spectrophotometer. The absorbance A_∞ for aliquots removed after complete hydrolysis was always near zero. Rate constants were determined from the slopes of plots of log $(A_t - A_\infty)$ vs. t (s); such plots were defined by more than 20 points and were nicely linear for more than three half-lives. The second-order rate constants were determined by dividing the first-order rate constants by the hydroxide concentration, which was estimated from the pH. All pH measurements were made with a Beckman Zeromatic SS-3 pH meter and a Curtin combination electrode (calomel reference). All rates run with the same arenesulfonate corresponded to the same pH.

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Registry No. Acetylcholine chloride, 60-31-1; acetylcholine bromide, 66-23-9; acetylcholine iodide, 2260-50-6; sodium indole-3-acetate, 6505-45-9; sodium benzenesulfonate, 515-42-4; sodium *p*-toluenesulfonate, 657-84-1; sodium *p*-ethylbenzenesulfonate, 14995-38-1; sodium *p*-isopropylbenzenesulfonate, 15763-76-5; sodium benzoate, 532-32-1; sodium phenylacetate, 114-70-5; sodium 3-phenylpropionate, 114-84-1; sodium *p*-toluate, 17264-54-9; sodium *o*-toluate, 17264-71-0; sodium 3-chlorobenzoate, 17264-88-9; sodium 4-hydroxybenzoate, 114-63-6; sodium 4-methoxybenzoate, 536-45-8; sodium 3,4-dimethoxybenzoate, 34535-88-1; sodium 2-naphthoate, 17273-79-9; disodium phthalate, 15968-01-1; sodium *N*-acetyl-DL-tryptophanate, 62307-74-8; sodium 2-nitrobenzoate, 17264-82-3; sodium 4-nitrobenzoate, 3847-57-2; sodium nicotinate, 54-86-4; phenol, 108-95-2; pyrocatechol, 120-80-9; pyrogallol, 87-66-1; resorcinol, 108-46-3; hydroquinone, 123-31-9.

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Spectroscopic Studies of Hydrophobic Association. Merocyanine Dyes in Cationic and Anionic Micelles[1]

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The effect of the micelle forming surfactants sodium dodecyl sulfate and hexadecyltrimethylammonium bromide on the visible spectra of merocyanine dyes, 1-alkyl-4-[(oxocyclohexadienylidene)ethylidene]-1,4-dihydropyridines, with various length 1-alkyl chains is reported. In all cases the dye spectra were shifted to the red when incorporated into micelles and the magnitude of this shift increases with more hydrophobic dyes. The dependency of the spectral shift on dye chain length also depended on the nature of the surfactant head group.

The solubility of many sparingly soluble solutes in water can be increased by the addition of a surfactant which forms micelles that incorporate the solute. Lawrence[2] first suggested in 1937 that the site of incorporation of solubilized molecules depends on their relative hydrophobic and hydrophilic tendencies but, even after four decades of work, the details of micellar structure and the mechanism of solubilization remain poorly understood and there is still uncertainty over the average site of incorporation. Water penetrates into a micelle[3,4] so that there is a con-

(1) Support of this work by the donors of the Petroleum Research Fund, administered by the American Chemical Society, is gratefully acknowledged.
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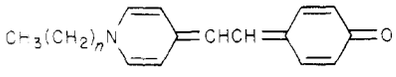
Table I. Properties of *N*-Alkylmerocyanine Dyes[a]

N-alkyl group	mp, °C	% yield	H_2O[b] λ_{max}, nm	pyridine[c] λ_{max}, nm	C/N ratio calcd[d]	C/N ratio found
methyl	220, 260[e]	86.3	442	605	12.1 ± 0.9	11.5
ethyl	265	36.1	444	605	12.9 ± 1.3	13.1
n-propyl	217	52.8	442	605	13.9 ± 1.3	13.2
n-butyl	215	23.1	444	606	14.7 ± 1.6	14.3
n-pentyl	159	20.0	445	607	15.6 ± 1.7	13.9
n-hexyl	144	35.5	446	608	16.5 ± 1.9	16.4
n-octyl	200	27.2	444	607	18.3 ± 2.2	18.1
n-decyl	140	45.5	445	608	20.0 ± 2.7	21.4

[a] 1-Alkyl-4-[(oxocyclohexadienylidene)ethylidene]-1,4-dihydropyridines. [b] Wavelength of maximum absorbance for the lowest frequency peak in 0.02 M NaOH, H_2O. [c] In pyridine. [d] Ratio calculated for sesquihydrated dye. The range was estimated by assuming a 0.5% error in each elemental analysis. [e] Crystal shrinks at 220 °C, melts at 260 °C.

tinuum of environments from the hydrated micelle surface to a nonpolar core. The solubilizate may be adsorbed on the surface, oriented near the surface (short penetration), or buried deeply (deep penetration), or it may be trapped in the hydrocarbon core. Moreover, solubilization is a dynamic equilibrium process and the solubilizate may spend different residence times at different levels between the core and surface.

In order to determine the tradeoff between hydrophobic and hydrophilic forces on the average site of an amphipathic molecule in a micelle, we measured the absorption spectra of a homologous series of merocyanine dyes **1** as



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a function of surfactant concentration. The series represents a systematic change in hydrophobicity as the alkyl group is varied from methyl ($n = 0$) to decyl ($n = 9$). The absorption spectra of merocyanine dyes show characteristic λ_{max} shifts with changes in solvent polarity and the first member of the series ($n = 0$) has been used as a solvent polarity indicator.[5,6] The change in λ_{max} of each dye caused by micelle forming concentrations of surfactant indicates the polarity of its average incorporation site and, for the series, the effect of increasing alkyl chain length on the λ_{max} changes indicates the effect of increasing substrate hydrophobicity on incorporation.

Experimental Section

The visible absorption spectra of 10^{-5} M dye solutions containing surfactant in the concentration range 5×10^{-4} to 1×10^{-1} M were taken on a Varian Techtron Model 635 UV/vis spectrometer. Spectra at surfactant concentrations near the cmc and in dry pyridine were determined on a Cary 219 spectrophotometer. All spectra for any one dye were taken as close together in time as possible using the same stock solutions. The final concentration of NaOH in the solution was 0.02 M so that the dye molecules were not in the conjugate acid form. The reproducibility of duplicate λ_{max} determinations was ±1 nm.

Sodium dodecyl sulfate (NaLS) was purified by recrystallization from 95% ethanol[8] and hexadecyltrimethylammonium bromide (CTABr) was washed repeatedly with anhydrous ether and recrystallized three to five times from absolute alcohol.

The merocyanine dyes were prepared by N-alkylation of 4-methylpyridine with the appropriate *n*-alkyl bromide, followed by condensation of the resulting pyridinium salt with 4-hydroxybenzaldehyde in ethanol–piperidine. The resulting benzyl alcohol was dehydrated and deprotonated by gentle heating in aqueous KOH. Cooling the solution yielded blue–red crystals which were recrystallized several times from hot water. The *N*-methylmerocyanine dye was reported previously[5,7,9] and our synthesis represents no major departure from the published procedure.[10]

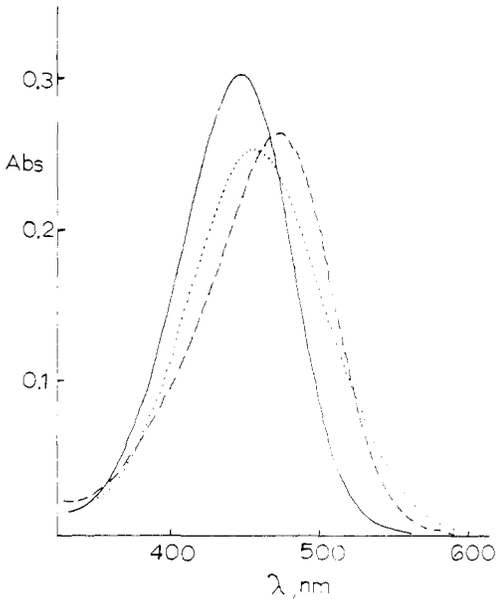

Figure 1. The absorption spectra of *N*-pentyl dye in 0.02 M NaOH with: — no added surfactant, ---- 0.035 M NaLS, ···· 0.072 M CTaBr.

These dyes have proven difficult to characterize in the classical sense. The reported melting points for *N*-methylmerocyanine are 195, 208, and 220 °C, while in our work a phase change (crystals shrink) at 220 °C and an apparent melting point at 260 °C were observed. Freshly recrystallized samples are blue–black but dry to a bright red in air within 30 min. This variability suggests crystalline modifications differing by the number of water molecules incorporated into the crystal.[11] Analysis results of different samples indicate different hydration states. For example three recrystallizations of the *N*-methyl dye and drying in a desiccator (P_2O_5) give: calcd (for $C_{14}H_{13}NO \cdot H_2O$) C 73.36, H 6.59, N 6.11; found C 74.96, H 7.37, N 6.69. Six recrystallizations followed by air drying gives: calcd (for $C_{14}H_{13}NO \cdot 1^1/_2H_2O$) C 70.56, H 6.77, N 5.88; found C 70.76, H 7.37, N 6.13. Davidson and Jencks[9] report that a sample dried under vacuum in the dark for 1 week gave an analysis corresponding to $C_{14}H_{13}NO \cdot {}^1/_2H_2O$. A microscopic examination of dye prepared by us in this way indicated an amorphous powder, whereas the more hydrated forms

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(6) In pure pyridine 1 ($n = 0$) has a λ_{max} 605 nm which is decreased to 444 nm in pure water (ref 7).
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(11) An X-ray crystallographic study of *N*-methylmerocyanin is being undertaken by R. P. Dodge. Only air-dried crystals were intact enough for crystal study.

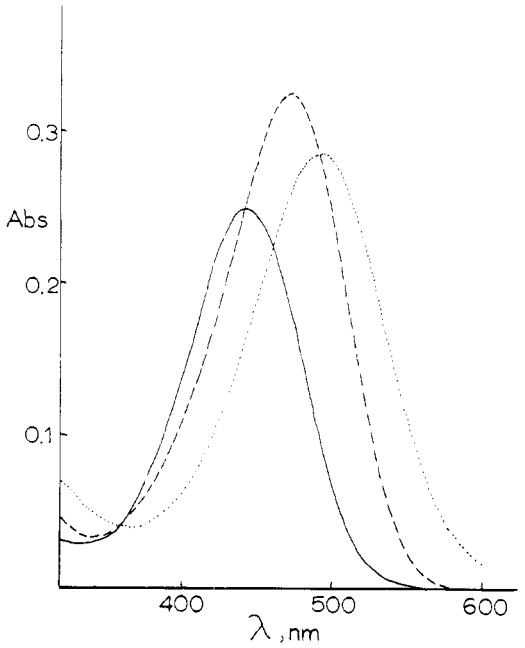

Figure 2. The absorption spectra of *N*-decyl dye in 0.02 M NaOH with: — no added surfactant, - - - - 0.034 M NaLS, ···· 0.026 M CTABr.

Table II. Maximum Spectral Changes Caused by Surfactants

N-alkyl group	NaLS[a] λ_{max}, nm	NaLS $10^{19}\Delta E_T$, J	CTABr[c] λ_{max}, nm	CTABr[c] $10^{19}\Delta E_T$, J
methyl	466	−0.23	447	−0.05
ethyl	470	−0.24	447	−0.05
n-propyl	471	−0.27	450	−0.08
n-butyl	475	−0.29	460	−0.15
n-pentyl	473	−0.27	482	−0.34
n-hexyl	473	−0.25	490	−0.41
n-octyl	476	−0.30	495	−0.46
n-decyl	475	−0.28	494	−0.44

[a] Wavelength of maximum absorbance for the lowest frequency peak observed for 0.1 M NaLS in 0.02 M NaOH. [b] The electronic transition energy observed for 0.1 M NaLS, 0.02 M NaOH relative to that for 0.02 M NaOH alone. [c] In 0.1 M CTABr, 0.02 M NaOH.

were crystalline. The higher homologues also had multiple hydration states, but the elemental analyses of all the dyes gave C/N ratios consistent with the assigned structures. The dyes were too insoluble for NMR studies. The visible spectra of *N*-methylmerocyanine in water and diverse solvents agree with those reported in the literature[5] and all higher homologues have virtually identical spectra in water and exhibit the same λ_{max} changes with solvent polarity (Table I).[10,12] All the dyes give C/N ratios consistent with the assigned structures. The IR spectra of all compounds (in Nujol) give the same transition at 1580 cm^{-1} tentatively assigned to the C=O stretch. All compounds were found to contain no mobile components when subjected to silica gel thin-layer chromatography with ethanol or methanol.

Results and Discussion

Surfactants cause a red shift in the spectra of merocyanine dyes. The spectra for the *N*-pentyl and *N*-decyl dyes (Figures 1 and 2) are typical. The effect of various concentrations of NaLS and CTABr on the visible spectra of all eight dyes are given in Figures 3 and 4 respectively and the maximum spectral changes caused by both surfactants are in Table II. At surfactant concentrations near its critical micelle concentration (cmc), the entire visible spectrum shifts continuously to a lower frequency as increasing numbers of dye molecules are taken into the micelles formed by increasing the surfactant concentration. There is no isobestic wavelength. At surfactant concentrations well above its cmc, the spectrum no longer changes

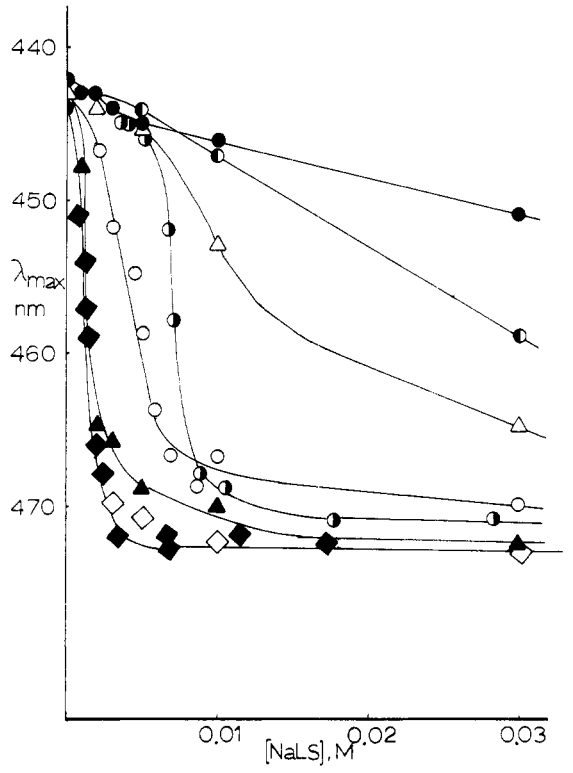

Figure 3. Effect of NaLS on the spectra of various merocyanine dyes in 0.02 M NaOH: ● *N*-methyl, ◐ *N*-ethyl, △ *N*-propyl, ○ *N*-butyl, ◑ *N*-pentyl, ▲ *N*-hexyl, ◇ *N*-octyl, and ◆ *N*-decyl dyes.

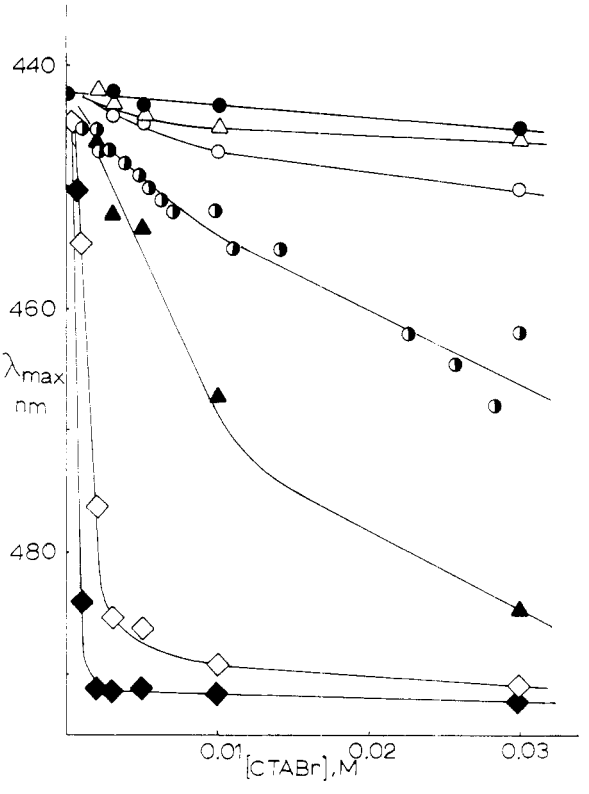

Figure 4. Effect of CTABr on the spectra of various merocyanine dyes in 0.02 M NaOH. The symbols are the same as in Figure 3.

(12) Both the *N*-methyl and *N*-decyl dyes give the same λ_{max} changes with changes in solvent composition in *t*-BuOH–H_2O and Me_2SO–H_2O mixtures; cf. Anand Kapur, unpublished results.

with increasing amounts of surfactant because all the dye molecules are taken into micelles. Higher concentrations of CTABr are needed to take in all the dye than is required for NaLS.

There are informative differences in the effects observed with different dyes.

(1) The more hydrophobic dyes exhibit pronounced red shifts at surfactant concentrations well below the generally observed cmc. The minimum surfactant concentration causing a spectral change decreases with increasing dye hydrophobicity.

(2) The maximum spectral change observed at high surfactant concentration increases with dye hydrophobicity.

(3) The dependency of the spectral shift on dye chain length also depended on the nature of the surfactant head group. For example the red shift observed with less hydrophobic dyes (e.g. methyl) is more pronounced in solutions of anionic surfactants ($\Delta\lambda$ = 24 nm) than in cationic micelles ($\Delta\lambda$ = 5 nm), whereas more hydrophobic dyes (e.g. decyl) give more pronounced red shifts in cationic micelles ($\Delta\lambda$ = 49 nm) than in anionic ones ($\Delta\lambda$ = 30 nm).

The more hydrophobic dyes induce micellization indicating a strong interaction between the dye alkyl group and the surfactant hydrocarbon chain. Hydrophobic solutes have been shown to induce micellization before,[13,14] and this accounts for the problems associated with the dye method of cmc determination.[13] We did not vary the dye concentrations used in this work, and the surfactant cmc values cannot be determined with certainty from the observed spectral changes. However it can be seen from the data in Figure 1 that the concentration of NaLS necessary to cause one-half the maximum red shift is cut in half every time the dye chain length is increased by one methylene group. All dyes more hydrophobic than the *n*-butyl one give appreciable spectral change below the cmc of NaLS (8.2×10^{-3} M).[13,15]

The magnitude of the maximum red shift caused by surfactants increases with increasing dye hydrophobicity implying that the average environment of an incorporated dye molecule depends upon its hydrophobicity. The spectrum of a merocyanine dye is insensitive to changes in chain length unless they lead to changes in the molecular environment immediately around the aromatic chromophore group. Dye hydrophobicity does not influence the spectra observed in mixed aqueous organic solvents.[12] Therefore the greater spectral changes observed with the more hydrophobic dyes is significant.

Surprisingly, *the observed shifts indicate a highly aqueous environment for even the most hydrophobic dye.* In all cases the red shifts are much *smaller* than that found upon changing the solvent from water to methanol (λ_{max} 509 nm).[10] This forces us to conclude that, although the more hydrophobic dyes bind more "tightly" to micelles, the chromophore is still oriented near the surface and still partially hydrated even if the *N*-alkyl group is highly hydrophobic. This contradicts the view that a very hydrophobic group "pulls" a solubilized molecule deeply into the micelle.[16,17] We suggest that the dye alkyl chain interacts by van der Waals' forces with adjacent surfactant chains increasing the average time spent at the short penetration distance at the expense of some interaction of the quinoid chromophore with the hydrated head group. Merocyanine dyes give a blue shift in the presence of hydrophilic salts because of 1:1 complexation with the ions[9] and this interaction would probably also occur between the quinoid chromophore and the ionic surfactant head groups if they were in close proximity. The red shift, with increasing concentration of surfactant, for methyl, ethyl, propyl, and butyl dyes is more pronounced in solutions of anionic surfactant (NaLS) than in cationic surfactant (CTABr). This observation suggests that these dyes are on a time average penetrating more deeply into the anionic micelles. On the other hand, the more hydrophobic dyes undergo a more pronounced red shift in CTABr solutions indicating a deeper penetration into cationic micelles than into anionic ones. The CTABr head group tightly associates with aromatic rings, especially phenoxides.[18,19] Therefore the dye does not penetrate very deeply into CTABr micelles unless the dye hydrophobicity is high enough to overcome interaction with the head group. The interaction between more hydrophobic dyes and surfactant side chains is stronger with CTABr because its chains are longer and the surfactant has greater solubilizing power. The loss of hydrogen bonding plays a key role in the red shifts observed with merocyanines[20] and, since intramicellar water has a reduced tendency to hydrogen bond with organic molecules in CTABr micelles,[21] this could also account for the larger red shifts observed with CTABr.

Registry No. 1 (n = 0), 23302-83-2; 1 (n = 1), 70850-52-1; 1 (n = 2), 70850-53-2; 1 (n = 3), 70850-54-3; 1 (n = 4), 70850-55-4; 1 (n = 5), 70879-10-6; 1 (n = 7), 70850-56-5; 1 (n = 9), 70850-57-6; 4-methylpyridine, 108-89-4; 4-hydroxybenzaldehyde, 123-08-0; methyl bromide, 74-83-9; ethyl bromide, 74-96-4; propyl bromide, 106-94-5; butyl bromide, 109-65-9; pentyl bromide, 110-53-2; hexyl bromide, 111-25-1; octyl bromide, 111-83-1; decyl bromide, 112-29-8; 1-methyl-4-methylpyridinium bromide, 60199-17-9; 1-ethyl-4-methylpyridinium bromide, 32353-49-4; 1-propyl-4-methylpyridinium bromide, 70850-58-7; 1-butyl-4-methylpyridinium bromide, 65350-59-6; 1-pentyl-4-methylpyridinium bromide, 70850-59-8; 1-hexyl-4-methylpyridinium bromide, 70850-60-1; 1-octyl-4-methylpyridinium bromide, 70850-61-2; 1-decyl-4-methylpyridinium bromide, 70850-62-3.

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