from 87 ± 3° (16 measurements) to 52 ± 2° (24 measurements)[1]. Application of the hand lotion reduced the mean contact angles of water on seven other subjects by 20–32°, with an average decrease of 28°. The angles were always measured after a 30-min. drying period. The moisturizing effect of the lotion includes the skin surface, possibly because some portion of the lotion was not absorbed by the skin but remained on the surface.

In another experiment, hands were immersed in a 1:1000 benzalkonium chloride solution USP for 10 min. Excess solution was then rinsed off by holding the hands for 5 sec. under running tap water and for 1 sec. under running distilled water. The contact angles of water were measured after drying with towels and in air. On placing drops on the skin, the initial contact angles were near 90°. After about 1 sec., the water drops suddenly began to spread and the contact angles decreased. Evidently, benzalkonium chloride which had been physically adsorbed on the skin was desorbed and dissolved by the drop, reducing its interfacial tension against skin as well as its surface tension. The mean value of the contact angles was lowered from 86 ± 3 to 48 ± 3° (18 measurements).

When hands treated with benzalkonium chloride were washed by 5-min. immersions in running lukewarm tap water, followed by rinsing with distilled water and drying with towels and in air, the average contact angle of water from 24 measurements increased to 84 ± 3°. Within the precision of the measurements, this value does not differ significantly from the initial value of 86 ± 3°, indicating that most if not all of the benzalkonium chloride was removed by immersion in water. Apparently, its adsorption by the skin is largely physical and reversible. This conclusion is made with some reservation, because chemisorption of a monolayer of alkyltrimethylammonium halides by glass, with the headgroups oriented toward the surface and the hydrocarbon tails away from it, produced sizable increases in the contact angles of water (13). Since the contact angles of water on untreated skin are already high, chemisorbed benzalkonium cations might not increase them at all.

[1] The ± sign precedes the standard deviation of the mean.

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Physical Properties of Quaternary Ammonium Salts of Phenothiazine Derivatives

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Abstract □ Physical constants, including melting points and IR and NMR spectra, and analytical data of the quaternary ammonium salts of nine clinically used phenothiazines are presented. Assignments for the spectral peaks were made and are discussed. These compounds were prepared in good yields, so the formation of quaternary ammonium salt may serve as a convenient method for the isolation and identification of these phenothiazines.

Keyphrases □ Phenothiazine derivatives, quaternary ammonium salts—synthesis, physical properties □ Quaternary ammonium salts of phenothiazine derivatives—synthesis, physical properties □ NMR spectroscopy—identity □ IR spectrophotometry—identity

Quaternary ammonium salts of phenothiazine neroleptics have been demonstrated to have a wide variety of biological activities. It was indicated in a previous study (1) that all of the quaternary ammonium salts of these compounds possess antimicrobial activities on Gram-positive and Gram-negative organisms and have a toxicity higher than the corresponding parent compound. Evidence was presented to show that the ^{14}C quaternary ammonium salts of phenothiazines were able to cross the blood–brain barrier to reach the brain tissues (2). The metabolic pathways of the quaternary ammonium salts of phenothiazines (mepazine, promethazine, promazine, chlorpromazine, triflupromazine, prochlorperazine, and trifluoperazine) are quite different from the corresponding hydrochloride salts; the quaternary ammonium salt is found predominantly in the feces (3) while the majority of the hydrochloride salt is excreted through the kidneys (4) and the lungs (5).

Although spectra–structure correlation of tertiary phenothiazines was reported (6), there is paucity of information concerning the physical properties of the quaternary ammonium salts of phenothiazines. The purpose of this paper is to present physical data, including melting points and UV, IR, and NMR spectra, of 11 quaternary ammonium compounds of nine clinically used phenothiazine derivatives: mepazine, promethazine, promazine, chlorpromazine, triflupromazine, prochlorperazine, trifluoperazine, perphenazine, and fluphenazine. Since all of these compounds form

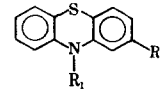
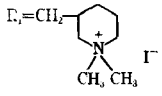
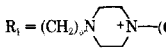
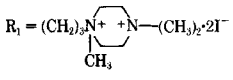


Table I—Physical Constants and Analytical Data of Quaternary Ammonium Salts of Phenothiazine Derivatives

Parent Compound	Quaternary Methiodide	IR, μ			Melting Point	Formula	Analysis, % Calc.		Found	NMR, τ
Mepazine	R = H 	3.45	7.80	9.60	298–301° dec.	$C_{20}H_{25}IN_2S$	C	53.09	53.21	2.70–3.10 (m, 8H, aromatic)
		6.25	7.95	10.00			H	5.53	5.45	6.13 (d, 2H)
		6.35	8.10	10.47			N	6.19	5.99	6.50–6.80 (m, 4H)
		6.80	8.53	10.75			S	7.08	7.06	6.90 (3, 3H)
		7.45	8.90	11.55						7.06 (3, 3H)
		7.62	9.10	13.10						7.80–8.00 (m, 1H)
										8.00–9.35 (m, 4H)
Promethazine	R = H $R_1 = CH_2CH(CH_3)\overset{+}{N}(CH_3)_3 \cdot I^-$	3.35	8.08	11.20	225–226° dec.	$C_{18}H_{23}IN_2S$	C	50.70	50.80	2.61–3.18 (m, 8H, aromatic)
		6.20	8.33	11.75			H	5.40	5.51	5.83 (d, 2H)
		6.35	8.85	12.05			N	6.57	6.68	6.59 (s, 9H)
		6.45	9.25	13.15			S	7.51	7.50	7.83 (m, H)
		6.97	9.70	13.40						8.42 (d, 3H, J = 6 Hz.)
		7.60	10.65	13.85						
Promazine	R = H $R_1 = (CH_2)_3\overset{+}{N}(CH_3)_3 \cdot I^-$	3.42	8.00	10.86	255–257°	$C_{18}H_{23}IN_2S$	C	50.70	50.80	2.75–3.13 (m, 8H, aromatic)
		6.28	8.35	11.62			H	5.40	5.50	6.07 (t, 2H, J = 7 Hz.)
		6.88	8.90	13.10			N	6.57	6.70	6.33–6.87 (m, 2H)
		7.49	9.63	13.68			S	7.51	7.49	6.92 (s, 9H)
		7.80	10.37							7.67–8.15 (m, 2H)
Chlorpromazine	R = Cl $R_1 = (CH_2)_3\overset{+}{N}(CH_3)_3 \cdot I^-$	3.33	8.03	10.35	145–147°	$C_{18}H_{22}ClIN_2S$	C	46.91	47.23	2.63–2.90 (m, 7H, aromatic)
		6.40	8.37	10.47			H	4.78	5.11	5.73–(t, 2H, J = 7 Hz.)
		6.87	8.67	11.35			N	6.08	5.96	6.00–6.35 (m, 2H)
		7.15	8.90	11.53			S	6.95	6.74	6.60 (s, 9H)
		7.60	9.10	12.50						7.17–7.82 (m, 2H)
		7.85	9.70	13.07						
Trifluprom azine	R = CF_3 $R_1 = (CH_2)_3\overset{+}{N}(CH_3)_3 \cdot I^-$	3.25	8.01	11.30	169–170°	$C_{19}H_{22}F_3IN_2S$	C	46.16	46.33	2.53–2.92 (m, 7H, aromatic)
		6.22	8.54	12.08			H	4.49	4.48	5.39 (t, 2H, J = 7 Hz.)
		6.82	8.90	13.08			N	5.67	5.71	6.25–6.53 (m, H)
		7.01	9.25	13.78			S	6.49	6.62	6.87(s, 9H)
		7.50	10.65							7.68–7.95 (m, 2H)
Prochlorperazine (I)	R = Cl 	3.43	8.38	11.05	165–167°	$C_{21}H_{27}ClIN_3S$	C	48.92	49.37	2.92–3.25 (m, 7H, aromatic)
		6.38	8.86	11.95			H	5.23	5.40	6.10 (t, 2H, J = 7 Hz.)
		6.85	9.02	12.50			N	8.13	8.17	6.50–6.75 (m, 4H)
		7.13	9.60	13.30			S	6.90	7.04	6.90 (s, 6H)
		7.43	9.90							7.20–7.65 (m, 6H)
		8.05	10.78							8.02–8.35 (m, 2H)
Prochlorperazine (II)	R = Cl 	9.30	9.23	11.86	224–225°	$C_{22}H_{30}ClI_2N_3S$	C	40.17	40.34	2.50–3.00 (m, 7H, aromatic)
		6.60	9.55	12.23			H	4.60	4.68	5.99 (s, 12H)
		7.10	10.13	12.58			N	6.39	6.24	6.51 (s, 6H)
		7.45	11.00	13.50			S	4.87	4.69	6.65 (s, 3H)
		8.40	11.16							7.65–8.00 (m, 2H)

crystalline quaternary ammonium salts in good yields (approximately 80%), quaternary ammonium salt formation may serve as a convenient method for the isolation of the parent compound.

EXPERIMENTAL[1]

Preparation of Quaternary Ammonium Salts—The hydrochloride salt (0.5 g.) of each phenothiazine derivative was dissolved in water (or in aqueous methanol in case of less soluble salts), and the solution was adjusted to pH > 10 by adding potassium carbonate solution. The oily precipitate which occurred was extracted several times with chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate, and then the solvent was evaporated under reduced pressure to yield 0.45–0.46 g. of viscous oil. The free base of each compound obtained was dissolved in acetone, and methyl iodide (1.05 mole equivalents for the monomethiodides and more than 2.2 mole equivalents for the dimethiodides of piperazinyl derivatives) dissolved in acetone was added. The reaction was allowed to proceed at room temperature for 1 hr. (promazine, trifluoperazine, and perphenazine) to 18 hr. (triflupromazine, mepazine, promethazine, and chlorpromazine).

In some cases (mepazine, promazine, trifluoperazine, and perphenazine), the quaternary ammonium salt precipitated as such; in others (promethazine, chlorpromazine, triflupromazine, and prochlorperazine), addition of ether or benzene was necessary to precipitate the product. The crude product was collected and recrystallized from acetone or acetone–ether to yield the final product (80–85%). The physical constants, including melting points and UV and IR absorption peaks, and elemental analyses are shown in Table I.

DISCUSSION

IR Spectra—The IR spectra of quaternary ammonium salts are in general similar with those of the corresponding parent compounds (Table I). The IR spectra of quaternary ammonium salts derived from the 10-substituted phenothiazine have a strong peak in the region of 13.1–13.3 μ, which is assigned to the out-of-plane bending vibrations of 1,2-disubstituted benzene (7). In the case of quaternary ammonium salts derived from 2,10-disubstituted phenothiazines, besides a band at 13.1–13.3 μ (assignable to the out-of-plane bending vibration of 1,2-disubstitution of ring A), at least three more bands of varying intensity are present in the regions of 10.6–10.9, 11.3–11.6, and 12.3–12.5 μ, which are due to the substitution in position 2 of the phenothiazine ring system and to 1,2,4-trisubstitution of ring B. Besides the two characteristic bands at 6.3 and 6.4 μ, spectra typical of the aromatic ring system were observed in all compounds.

NMR Spectra—Table I shows the important NMR signals of these compounds. In all the compounds, the signals of the aromatic protons appeared as a multiplet in the range of 2.48–3.25 τ. In all the monomethiodide derivatives, the spectrum of the two protons on the carbon adjacent to the ring nitrogen was shown as a triplet in the region of 5.39–6.10 τ, with a coupling constant of 6–7 Hz. The signals of the methylene protons on the second carbon of the propyl group (except mepazine and promethazine) were recorded as a multiplet at 7.17–8.35 τ. The spectrum of the methylene protons on the first carbon of the propyl group of promazine, chlorpromazine, and triflupromazine appeared as a multiplet in the region of 6.00–6.87 τ. However, in the monomethiodides of prochlorperazine and trifluoperazine, these two protons combined with four protons on carbons at positions 3 and 5 of the piperazinyl ring system to form a multiplet in the region of 7.20–7.68 τ. The protons on two carbons attached to the nitrogen at position 1 of the piperazinyl ring system were shown at 6.50–6.53 τ as a multiplet. Those methyl protons on the quaternary nitrogen of all the monomethiodides appeared as a sharp singlet between 6.59 and 7.25 τ. In the mepazine methiodide, the doublet signal at 6.09 τ was due to the protons on the carbon at position 2, and the multiplet signal

[1] UV absorption spectra were taken in a Perkin-Elmer model 202 spectrophotometer. IR absorption spectra were recorded in a Perkin-Elmer Infracord model 137E, using KBr pellets as mulls. NMR spectra were taken in a 10–20% $(CD_3)_2SO$ or $(CD_3)_2CO$ solution in a Varian A-60 spectrometer, using tetramethylsilane as an internal standard.

in the range of 7.40–7.68 τ came from protons on the carbon at position 5 of the piperidyl ring system. Five protons from the carbons at positions 3, 4, and 5 formed a multiplet in the range of 8.07–8.83 τ. The single proton on the first carbon of the isopropyl group in promethazine methiodide had a multiplet at 7.83 τ, and the methyl protons on the side chain appeared as a doublet at 8.42 τ, with a coupling constant of 6 Hz.

In the dimethiodide derivatives of prochlorperazine and trifluoperazine, a broad peak observed at 5.99 and 6.08 τ, respectively, represented 12 protons, including four protons on the carbons at positions 1 and 3 of the propyl group and eight protons on the piperazinyl ring system. In these two compounds, the methyl protons on the nitrogen at position 4 of the piperazinyl ring system had a singlet at 6.65 and 6.75 τ, respectively. The dimethiodide of perphenazine and fluphenazine had a broad singletlike peak at 6.03 and 6.00 τ, respectively, representing 17 protons from the first and third carbons of the propyl, piperazinyl, and hydroxyethyl groups. The methyl protons adjacent to the nitrogen at position 1 of the piperazinyl ring system of perphenazine and fluphenazine showed a single peak at 6.72 and 6.67 τ, respectively, while the methyl protons adjacent to the nitrogen at position 4 exhibited a singlet signal at 6.60 and 6.52 τ, respectively.

In all cases, quaternization of the terminal nitrogen in the side chain of phenothiazine derivatives appeared to shift the spectrum of the methyl protons on the terminal nitrogen downfield by 0.58–1.24 τ. The presence of a substituent group of Cl and CF_3 on the ring system shifted the aromatic peaks downfield. This effect was more prominent with the presence of CF_3 in position 2 of the phenothiazine ring system.

SUMMARY

Physical properties, including melting points and IR and NMR spectral data, of quaternary ammonium salts of some important phenothiazine derivatives have been presented. All these compounds gave crystalline products with a good yield (80–85%), so formation of a quaternary ammonium salt may serve as a convenient method for the isolation and identification of these clinically used phenothiazines.

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Direct Measurement of Aspirin

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Abstract □ A fluorometric procedure for the direct measurement of aspirin in which interference due to salicylic acid and its conjugates was eliminated by reaction with ceric ammonium nitrate was modified, improving recovery of aspirin from 57 to 93%.

Keyphrases □ Aspirin—fluorometric determination, elimination of interference by salicylic acid □ Fluorometry—determination of aspirin without interference of salicylic acid

A direct determination of aspirin in human blood samples by fluorometry was described previously (1). In this method, an ethylene dichloride extract of salicylates is treated with an aqueous solution of ceric ammonium nitrate, which reacts with salicylic acid and its conjugates, leaving only aspirin in the organic phase. In this laboratory, it was found that the recovery of aspirin from an ethylene dichloride solution after treatment with an aqueous solution of ceric ammonium nitrate was only 57% and that the determination of aspirin was affected by the amount of salicylic acid present. For amounts of salicylic acid of 25, 100, and 200 mcg., the slopes of aspirin standardization curves varied 4.3, 8.6, and 18.9%, respectively, from the slope of an aspirin standardization curve without salicylic acid. The difference between each slope was statistically significant from the others. Improvement of recovery of aspirin to 93% was obtained by using 3 ml. of 0.01 *N* acetic acid and 0.05 ml. of ceric ammonium nitrate instead of 5.9 ml. of water and 0.10 ml. of ceric ammonium nitrate, respectively.

Although the determination of aspirin was still affected by the amount of salicylic acid present, the variation of the slopes of equivalent standardization curves was reduced to 0.7, 0.7, and 3.4%, respectively, from the slope of an aspirin standardization curve without salicylic acid. While the slope of the aspirin standardization curve containing 200 mcg. of salicylic acid differed statistically from the slopes of aspirin standardization curves containing 25 mcg. and zero salicylic acid, respectively, the slope of the aspirin standardiza-