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-

tion curve containing 100 mcg. of salicylic acid and the others did not.

EXPERIMENTAL

Aliquots of aspirin in ethylene dichloride containing 2.5, 5.0, 10.0, 20.0, 30.0, and 40.0 mcg. as salicylic acid, respectively, were transferred to snap-cap bottles. The volumes of each were adjusted to 50 ml. with ethylene dichloride. Three milliliters of 0.01 *N* acetic acid and 0.05 ml. of an aqueous solution of 40% ceric ammonium nitrate were added. The bottles were capped and shaken on a reciprocal shaker at approximately 200 c.p.m. for 15 min. The contents of each bottle were transferred to 25 × 150-mm. screw-cap culture tubes. (The screw-cap culture tubes hold almost the entire volume.) The tubes were capped with Teflon-lined screw caps and centrifuged 10 min. at a relative centrifugal force (RCF) of approximately 1000 to separate the two phases.

The aqueous phase was removed by aspiration, and 30.0 ml. of the ethylene dichloride phase of each was transferred to other snap-cap bottles. Six-milliliter aliquots of 1% $NaHCO_3$ were added, and the bottles were capped and shaken at approximately 200 c.p.m. for 10 min. The contents of each bottle were transferred to clean 25 × 150-mm. screw-cap culture tubes and centrifuged 5 min. at approximately 1000 RCF. Four-milliliter aliquots of the $NaHCO_3$ (upper) phase were transferred to 19 × 150-mm. test tubes. One milliliter of 6 *N* NaOH was added and thoroughly mixed with the $NaHCO_3$. The fluorescence was measured after 10 min. using a fluorometer[1] with a general-purpose lamp and primary filters 7-54(2) and 34A, sandwich arrangement, and secondary filter 5-58, which are equivalent to 325 and 400 nm., respectively. This procedure was repeated three times, adding 25, 100, and 200 mcg. of salicylic acid in ethylene dichloride, respectively, before adjusting the volumes to 50 ml. with ethylene dichloride. Equivalent sets of aspirin solutions with and without added salicylic acid were prepared, treated with 5.9 ml. of water and 0.1 ml. of 40% ceric ammonium nitrate, and shaken at approximately 200 c.p.m. for 1 hr. as described by Cotty and Ederma (1). The procedure was continued as already described.

A final set of aspirin solutions in ethylene dichloride, equivalent to the two sets without added salicylic acid, was prepared as the control. Thirty-milliliter aliquots were transferred to other snap-cap bottles, 6.0 ml. of 1% $NaHCO_3$ were added to each, and the procedure was continued as described. Appropriate blanks were run.

The data in Fig. 1, recording the effect of adding salicylic acid on the recovery of aspirin by both the modified and the reference methods, show the significant reduction in the range of aspirin standardization curves from 18.9 to 3.4%, and the improvement in the recovery of aspirin from 57 to 93% of theoretical using the modification described by the authors.

A number of experiments, using different concentrations of acetic acid and shaking periods that varied from 1 to 60 min., were performed to determine the concentration of acetic acid at which: (*a*) most interference of salicylic acid was eliminated, (*b*) net recovery of aspirin was the same whether or not salicylic acid was present, and (*c*) the least amount of shaking time was required.

The data presented in Table I are a pertinent summary of these experiments in which 10 mcg. of aspirin was used to evaluate recovery of aspirin and 200 mcg. salicylic acid was used to evaluate efficiency of elimination of interference of salicylic acid. Both aspirin and salicylic acid were prepared in ethylene dichloride. Per group of three, one contained 10 mcg. aspirin and 200 mcg. salicylic acid, another contained 200 mcg. salicylic acid only, and the third contained 10 mcg. aspirin only. The first evaluated recovery of aspirin in the presence of salicylic acid, the second was a blank for the salicylic acid, and the third was a control for the aspirin. All were brought to a volume of 30 ml. with the appropriate amount of ethylene dichloride before treating with acetic acid and ceric ammonium nitrate. The data show that for a range of acetic acid from 0.005 to 0.015 *N*, the net results were the same. Therefore, 0.01 *N* was selected as optimum. At higher concentrations of acetic acid, 0.02 *N* for example, 30 min. of shaking time instead of 15 was required to obtain a minimum blank value.

Other data, not recorded, showed that with 3 ml. of 0.01 *N* acetic acid and 0.05 ml. of 40% ceric ammonium nitrate, shaking more than 15 min. did not further reduce the blank value due to salicylic acid but did continue to reduce the recovery of aspirin slightly but measurably. These data also showed that 25% of the 36% increase in recovery of aspirin was due to changing from water to 0.01 *N* acetic acid, and the remainder was due to the reduction of the volume from 6 to 3 ml.

[1] Turner, model 110.

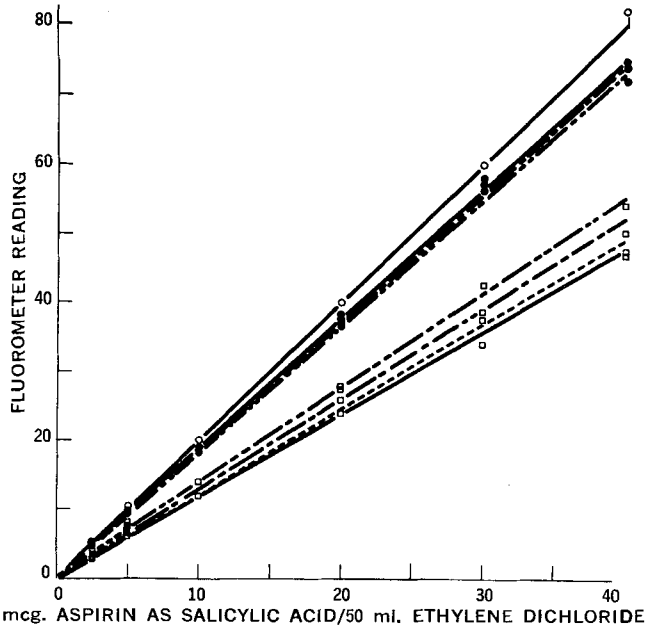

Figure 1—*Effect of ceric ammonium nitrate treatment on the recovery of aspirin. Key:* ○, *control not treated with ceric ammonium nitrate;* ●, *modified method, 3 ml. of 0.01* N *acetic acid and 0.05 ml. of 40% ceric ammonium nitrate;* □, *reference method (1), 5.9 ml. of water and 0.1 ml. of 40% ceric ammonium nitrate;* ——, *zero salicylic acid;* - - -, *with 25 mcg. salicylic acid;* — — —, *with 100 mcg. salicylic acid; and* - - — - -, *with 200 mcg. salicylic acid.*

DISCUSSION

In the method of Cotty and Ederma (1) showing recovery of aspirin added to pooled whole blood, no evaluation of the effect of ceric ammonium nitrate treatment on aspirin was presented. Observation shows that the "protection" against the effect of ceric ammonium nitrate attributed to acetylation of the phenolic group of salicylic acid is apparently not as complete as expected under the conditions described (1). Use of a very dilute solution of acetic acid

Table I—Effect of Acetic Acid Concentration on Recovery of Aspirin and Elimination of Interference of Salicylic Acid

Aspirin, mcg.	Salicylic Acid, mcg.	Acetic acid, 3 ml.	Fluorometric —Reading 3×[a]— Total	Net	Ceric Ammonium Nitrate Treatment[b], min.
10	200	0.005 *N*	34	30	15
0	200		4		
10	0		29.5	29	
10	200	0.01 *N*	34	29.5	15
0	200		4.5		
10	0		29	28.5	
10	200	0.015 *N*	33.5	28.5	15
0	200		5		
10	0		28.8	28.3	
10	200	0.02 *N*	34	29	30
0	200		5		
10	0		28.5	28	

[a] Fluorometric reading at the 3× slit. [b] Each contained 0.05 ml. of 40% ceric ammonium nitrate.

Table II—Statistical Analysis of Aspirin Standardization Curves Modified and *Reference 1* Methods

Salicylic Acid Added, mcg.	Slope: Average	Slope: Statistical	*SD*, %	*CV*[a], %	Ratio to Control, %
Control					
0	2.02	2.04	±0.020	±0.98	
Ceric Ammonium Nitrate Treatment According to Modified Method					
0	1.90	1.89	±0.019	±1.0	93
25	1.82	1.88	±0.019	±1.0	92
100	1.87	1.88	±0.026	±1.4	92
200	1.84	1.83	±0.034	±1.8	90
Ceric Ammonium Nitrate Treatment According to Reference Method					
0	1.16	1.16	±0.020	±1.70	57
25	1.24	1.21	±0.030	±2.50	59
100	1.32	1.26	±0.022	±1.75	61
200	1.38	1.38	±0.024	±1.74	68

[a] Coefficient of variation.

enhances this "protection" considerably. Premixing of ceric ammonium nitrate and acetic acid is not recommended. The effectiveness of the ceric ammonium nitrate deteriorates rapidly under these conditions. Because the original work with ceric salts and phenols used HNO_3 (2), the latter was also tried. With 2 *N* HNO_3, recovery was almost as good as with acetic acid, but net recovery of aspirin was not the same whether or not salicylic acid was present.

Precision was evaluated by comparing the averages of the coefficients of variation of each of the four standardization curves obtained by each method (Table II). Averages and standard deviations were 1.30 ± 0.38 and 1.92 ± 0.39% for the modified and reference methods, respectively, yielding a difference between averages that was statistically significant at the 1% level (3). Reliable values by both the modified and original methods require knowledge of the salicylic acid content because blank values and aspirin standardization curves vary with salicylic acid content. While an average blank obtained with 50 mcg. salicylic acid may be used for amounts of salicylic acid up to 100 mcg. for both methods, reducing accuracy only slightly, the modified method permits using the aspirin standardization curve (calibrated with 100 mcg. salicylic acid) for a range of 0–200 mcg. salicylic acid, which is a definite advantage over the original method which requires using aspirin standardization curves appropriate to the amount of salicylic acid present (Fig. 1) (4). The modification also improves sensitivity approximately 30%.

A modification for developing fluorescence with aspirin is also presented. At a NaOH concentration of 1.2 *N*, heat was not required to convert aspirin to salicylic acid, and the fluorescence of equivalent amounts of aspirin and salicylic acid was found to be equal.

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Reduced Side Effects following Administration of a New Anorectic Agent, 4′-Chloro-2-(ethylamino)propiophenone, in Timed-Release Form

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Abstract □ Studies representing several parts of a development program for the anorexigenic SK&F 70948 are summarized. *In vitro* release rates and the results of an anorectic study in dogs demonstrated the timed-release characteristics of a 25:75 blend (25% nontimed-release pellets and 75% wax–lipid-coated pellets). The global efficacy, weight loss, and side-effect incidence of a 75- and 150-mg. timed-release formulation were studied in a multiinvestigator clinical trial; a parallel multiinvestigator clinical trial included 25- and 50-mg. t.i.d tablet regimens. In each comparison, the incidence of side effects seen with the timed-release formulation was significantly less than that seen with the t.i.d tablets ($p < 0.01$). However, the global efficacy and anorectic activity of the timed-release and t.i.d regimens did not differ significantly. These results suggested that the lower incidence of side effects following timed-release medication was related to the constant and sustained body levels of drug seen with the timed-release formulation.

Keyphrases □ 4′-Chloro-2-(ethylamino)propiophenone—reduction of side effects using timed-release form □ Timed-release dosage forms—reduction of side effects of 4′-chloro-2-(ethylamino)-propiophenone □ Anorectic agent, 4′-chloro-2-(ethylamino)propiophenone—reduction of side effects found using timed-release dosage form

During pharmaceutical development, one often attempts to modify a drug's activity in order to increase its usefulness in the treatment of disease. One such modification—prolongation of drug action—has been attempted over the years using a number of different methods with varying degrees of success. From studies comparing prolonged-action preparations with other dosage forms, it has become clear that each drug in-