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Chromatography and Electrophoresis of Phenothiazine Drugs

By THEODORE J. MELLINGER and CLYDE E. KEELER

Three separation techniques for various phenothiazine tranquilizers are reported in this paper. Electrophoresis with suitable buffer systems and proper voltage permitted fast and distinctive migration of these phenothiazine bases, but tailing interfered with the usefulness of this technique. Paper chromatography with salt solutions as solvents proved to be a valuable and easy procedure for the separation of this group of drugs. Thin-layer chromatography with silica gel showed the most accurate separation of phenothiazine compounds. Combination of chromatography with color reaction or fluorescence permitted good differentiation of the various phenothiazine tranquilizers.

INCREASING INTEREST in the therapy of mental disorders has led to widespread use of phenothiazine derivatives, that are on the market under various names and chemical modifications. There is an immense clinical literature concerning these drugs but little is known about their separation or identification by means of chromatographic procedures or electrophoresis. Three kinds of techniques have been explored and are reported in this paper: electrophoresis, paper chromatography, and thin-layer chromatography. They were tested upon 23 drugs in the search for well reproducible separation methods with variation in R_f values as well as distinctive spots or zones devoid of tailing.

METHODS

Electrophoresis was carried out with buffer systems proposed by Werum, *et al.* (1). The 23 drugs were applied side by side along the starting line on a strip of Whatman 3 MM paper 30 cm. in width. Several strips were run with each buffer system. A Gordon-Misco apparatus for horizontal paper electrophoresis was used. The average potential difference between the electrodes was 500 to 800 v.

Paper chromatography was carried out with Whatman 3 MM filter paper. The dissolved substances were applied in amounts of 5 to 10 mg. in thin streaks along the starting line and were developed in the ascending direction.

Thin-layer chromatography was run on glass plates 20 × 20 cm. coated with a layer of 500 μ of silica gel G Merck. The coated plates were dried overnight in an incubator at 38°. After spotting the drugs, the chromatograms were developed in the ascending direction.

To locate the drugs, their fluorescence was examined under an ultraviolet lamp with a peak emission at 253 mμ and one at 360 mμ. To produce color reactions, 40% sulfuric acid was sprayed upon the developed chromatograms.

RESULTS

Electrophoresis.—Because all phenothiazine drugs in this study are bases, and therefore positively charged molecules, they migrate toward the negative end of the paper. With appropriate buffer systems and a proper potential difference between the two poles, migration of the drugs was accomplished in 40 to 60 minutes. With the new organic buffers proposed by Werum, *et al.*, the migrating phenothiazine compounds could be followed easily by their fluorescence under ultraviolet light. These new buffers do not quench the fluorescence of the drugs as barbiturate buffers do. When the buffer solutions were modified by leaving out the formamide, the same migration was obtained and the color reactions were more distinctive.

Table I presents the electrophoretic values of 19 phenothiazine compounds and of four other substances closely related to the phenothiazine tranquilizers. The migration values of the drugs are shown in the vertical columns, as they were run on the same paper. However, the values for each substance cannot be compared exactly on the horizontal lines, since they were not run together. In spite of this, there can be seen a general trend in most of the substances to decrease the speed of migration with increasing pH, possibly due to decreasing ionization at higher pH. This is less apparent for the three sulfoxidized phenothiazines, imipramine, and the two thypendyl compounds. It cannot be ascertained from these experiments whether the deviations of the latter compounds are merely due to physico-chemical differences, or are

Received March 12, 1962, from the Research Division, Milledgeville State Hospital, Milledgeville, Ga.
Accepted for publication April 23, 1962.

TABLE I.—PAPER ELECTROPHORESIS OF 19 PHENOTHIAZINE COMPOUNDS AND FOUR PHENOTHIAZINE-LIKE DRUGS AT DIFFERENT pH (MIGRATION IN CM., COMPARABLE VERTICALLY)

	pH					
	3.3	4.7	6.0	7.2	8.0	9.3
Chlorpromazine	5.4	6.2	5.2	4.9	5.4	1.8
Perphenazine	6.0	5.4	4.4	4.3	3.9	2.5
Prochlorperazine	6.4	5.5	4.6	4.0	3.8	1.8
Pipamazine	5.0	4.9	4.6	3.3	5.2	2.4
Thiopropazate	5.1	4.9	4.0	4.1	2.3	1.7
Acetophenazine	5.7	5.0	4.6	4.4	5.1	2.8
Proketazine	5.5	4.2	4.3	4.2	4.5	4.2
Butyrylperazine	5.8	4.2	3.6	2.8	3.3	4.1
Fluphenazine	6.8	3.7	4.3	4.5	2.7	1.2
Trifluoperazine	7.0	4.4	4.7	4.7	3.2	1.7
Triflupromazine	5.9	4.8	5.3	5.5	4.6	1.8
Promazine	6.5	7.0	6.7	6.0	7.7	7.5
Mepazine	6.0	6.3	5.6	5.2	6.2	4.6
Methoxypromazine	6.1	6.3	6.1	5.4	7.3	5.9
Thioridazine	4.9	4.5	5.4	4.6	4.1	1.9
Thiethylperazine	4.2	6.0	4.9	4.1	1.9	1.5
Chlorpromazine-sulfoxide	6.4	7.1	6.1	6.3	8.2	8.3
Thioridazine-sulfoxide	5.7	6.0	5.9	5.2	7.1	6.6
Trifluoperazine-sulfoxide	7.2	6.2	5.8	5.4	7.4	5.9
Phenothiazine-like drugs:						
Imipramine	6.4	6.9	6.1	5.8	8.7	7.3
Chlorprothixine	5.5	5.3	5.2	4.2	1.1	1.7
Isothypendyl	6.4	6.7	7.1	6.3	8.4	7.1
Prothypendyl	6.0	6.2	5.8	5.5	7.9	7.4

the result of chemical interaction with some of the organic buffer substances used.

With most buffer systems, the drugs migrated at very similar speeds when compared vertically in Table I. Buffer pH 8 permitted the best separation. A drawback of paper electrophoresis was excessive tailing of most compounds, except phenothiazine sulfoxides, when concentrations over 10 mg. per spot were applied.

Paper Chromatography.—When the conventional solvent systems of lower alcohols with water or acidic or basic solutions are used, all phenothiazine drugs run with or near the front, and form tails. Low affinity of the drugs for the stationary phase is probably the cause. Although this paper chromatographic technique is so unsatisfactory, it is the most frequently cited.

When the lipid solvents were omitted and salt solutions were used as solvents, the picture changed, and a satisfactory separation of all phenothiazine drugs could be carried out on paper chromatography. Small amounts of acids or lower alcohols added to these salt mixtures, not exceeding 10% of the total volume, permitted a further variation of R_f values. Table II shows the solvents used and the wide variety of R_f values for the phenothiazine compounds. When these solvent systems were studied, they appeared to be similar to "reverse phase techniques" since substances with higher lipid solubility showed lower R_f values.

Another method using mineral oil treated paper with methanol as the solvent gave very satisfactory separations without tailing, especially when used in circular chromatography to distinguish the original phenothiazine drugs from their metabolites in urine or bile. When the differentiation of one original phenothiazine derivative from another is concerned, it was not as suitable since the drugs developed nearly the same R_f of about 0.60 to 0.70.

Thin-layer Chromatography.—Although several techniques for the separation of lipids similar to paper chromatographic procedures had been described before (2–4), the introduction of thin-layer chromatography by Stahl (5–7), constituted real progress in the separation of lipid soluble substances. Reproducible standard conditions of a uniformly distributed layer of the absorbent is attained. Several substances or mixtures together with reference compounds can be developed and compared on the same chromatogram. We tested this technique for the separation of phenothiazine drugs that are all nitrogenous bases with relatively high fat solubility resembling, in this property, many of the alkaloids, antihistaminics, and synthetic narcotics.

When silica gel G Merck was used as the thin layer, all phenothiazine derivatives showed a strong absorption to silica gel. Chloroform, ether, hexane, or other lipid solvents did not move the substances from their starting line even after many hours of running the chromatogram. However, when an appropriate mixture of lipid solvent and water was used, the drugs migrated with a variety of quite distinctive R_f values. This is shown in Table III. The average time of development required in the ascending direction was 2 hours. This separation method was superior to that obtained by paper chromatography with salt solutions where a spreading out of the spots often takes place. The sensitivity or detectability of the spots moving in sharply defined zones was increased. Figure 1 shows the sharp separation of a thin-layer chromatogram compared with the spots of electrophoresis. Even when some of the drugs have nearly similar R_f values with one solvent system they can be distinguished by their fluorescences, their color reactions, or by a two-dimensional chromatogram. This property makes the method especially suitable for testing the purity or decomposition of phenothiazine drugs. The silica gel thin-layer technique proved to be, by far, the best procedure for any phenothiazine drug.

Location of the Drugs.—Since all phenothiazine

TABLE II.—R_f VALUES OF 19 PHENOTHIAZINE COMPOUNDS AND FOUR PHENOTHIAZINE-LIKE DRUGS WITH PAPER CHROMATOGRAPHY (NUMBERS REPRESENT $R_f \times 100$)

	1*N* Sodium formate	1*N* Sodium formate 90 *n*-Propanol 10	1*N* Sodium formate 90 1*N* Ammonia 10	1*N* Sodium formate 97 90% Formic acid 3	1*N* Sodium acetate	1*N* Sodium acetate 90 *n*-Propanol 10	10% Sodium chloride 92 *n*-Propanol 8
Time in minutes, ascending	40″	50″	110″	35″	30″	120″	100″
Chlorpromazine	34	32	11	43	30	54	70
Perphenazine	31	41	17	52	25	49	36
Prochlorperazine	18	20	11	58	18	43	44
Pipamazine	35	48	13	40	27	51	56
Thiopropazate	20	14	12	56	09	28	53
Acetophenazine	26	50	29	50	22	65	71
Proketazine	34	56	28	53	33	60	61
Butyrylperazine	19	35	18	42	17	48	45
Fluphenazine	23	32	19	51	15	45	47
Trifluoperazine	27	25	25	60	17	35	55
Triflupromazine	41	50	12	59	35	59	76
Promazine	58	61	20	63	56	88	80
Mepazine	41	50	13	53	38	69	71
Methoxypromazine	44	55	17	53	42	69	70
Thioridazine	24	40	09	37	24	45	52
Thiethylperazine	14	23	37	45	13	33	28
Chlorpromazine-sulfoxide	69	77	67	70	67	89	85
Thioridazine-sulfoxide	56	64	46	62	54	78	76
Trifluoperazine-sulfoxide	74	78	70	80	70	96	91
Phenothiazine-like drugs:							
Imipramine	65	78	19	68	62	84	83
Chlorprothixine	26	31	12	41	23	40	28
Isothypendyl	61	67	35	67	60	82	80
Prothypendyl	54	61	28	60	52	75	74

TABLE III.—R_f VALUES AND SOLVENT SYSTEMS FOR THIN-LAYER CHROMATOGRAPHY WITH SILICA GEL FOR 23 PHENOTHIAZINE DRUGS OR SIMILAR COMPOUNDS (NUMBERS REPRESENT $R_f \times 100$)

	tert-Butyl Alcohol 90 1*N* Ammonia 10	*n*-Propanol 88 1*N* Ammonia 12	Ether, Satd. with Water, Overrun	70% Methanol	85% *n*-Propanol	*n*-Butanol, Satd. with 1*N* Ammonia
Chlorpromazine	37	44	28	14	23	66
Perphenazine	28	57	07	48	24	53
Prochlorperazine	10	31	06	24	08	55
Pipamazine	38	71	..	41	37	79
Thiopropazine	64	79	49	65	53	81
Acetophenazine	12	36	04	52	18	38
Proketazine	19	45	06	53	25	44
Butyrylperazine	08	31	06	28	10	45
Fluphenazine	37	56	09	68	27	57
Trifluoperazine	18	33	09	34	12	51
Triflupromazine	36	50	48	22	22	72
Promazine	16	31	12	11	11	50
Mepazine	29	46	13	13	13	62
Methoxypromazine	15	26	12	12	09	45
Thioridazine	24	39	14	24	15	64
Thietylperazine	14	50	..	23	13	61
Chlorpromazine-sulfoxide	05	10	01	09	05	27
Thioridazine-sulfoxide	03	13	01	09	03	33
Trifluoperazine-sulfoxide	03	11	02	17	03	31
Phenothiazine-like drugs:						
Imipramine	45	55	24	13	16	66
Chlorprothixine	34	78	..	20	36	88
Isothypendyl	26	39	24	15	11	61
Prothypendyl	16	21	11	11	08	44

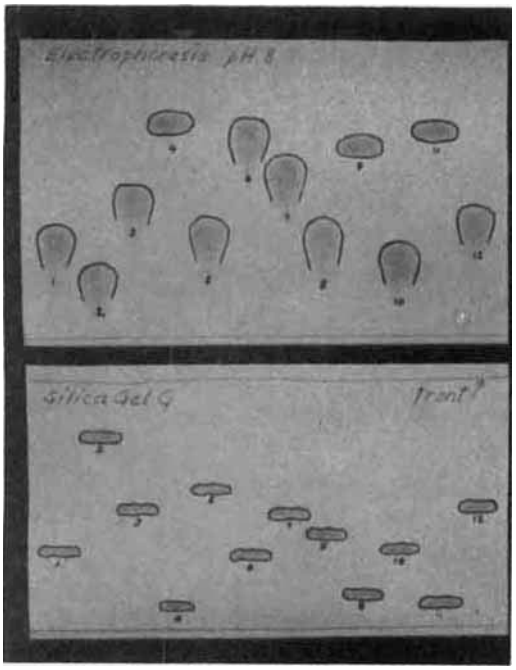

Fig. 1.—Comparable migration with paper electrophoresis buffer pH 8 (above), and thin-layer chromatography with silica gel, *n*-propanol 88 and 1 *N* ammonia 12 (below). 1, prochlorperazine; 2, thiopropazate; 3, chlorpromazine; 4, chlorpromazine-sulfoxide; 5, perphenazine; 6, promazine; 7, mepazine; 8, thioridazine; 9, thioridazine-sulfoxide; 10, trifluoperazine; 11, trifluoperazine-sulfoxide; 12, triflupromazine.

derivatives of this study showed a visible fluorescence of varying intensity under ultraviolet irradiation, this locating method was very satisfactory. An ultraviolet lamp having a maximum intensity at 263 mμ was superior to one with 360 mμ for this purpose. The sensitivity of this procedure was in many cases higher than with color reactions. Compounds having a chlorine on position 2 of the phenothiazine nucleus, such as chlorpromazine, perphenazine or perchlorperazine, or agents without substitution on this position, such as promazine and mepazine showed the least fluorescence or appeared often as quenched zones on the slightly fluorescent background of the paper or silica gel. This happened especially in solvent systems containing acids. It was found that the combination of acid solvent and ultraviolet or daylight easily decomposed the phenothiazine drugs, usually decreasing their visible fluorescence. Acidic solvent systems had to be used in light-protected tanks and avoided whenever possible.

When the phenothiazine drugs were run together on the same chromatogram, it could be easily observed that each emitted a fluorescent color according to its substitution on position 2 on the phenothiazine nucleus. For instance, drugs with a trifluomethyl group, as triflupromazine, trifluoperazine, or fluphenazine, emit the same bluish-yellow fluorescence. They could not be differentiated from each other by this method, but they could be distinguished easily from acylated compounds like acetophenazine, proketazine, or butyrylperazine, that showed an orange-to-brick-red fluorescence. Thioridazine showed a blue fluorescence which was strongly intensified by potassium permanganate spray. The fluorescent colors of the various phenothiazine drugs are shown in Table IV together with their color reactions.

All phenothiazine drugs responded to 40% sulfuric acid spray with various color reactions that distinguish them according to their radicals on position 2. As with the fluorescence, this color test did not permit any further differentiation between drugs with different radicals on position 10. When ferric chloride or ferric nitrate was added to 40% sulfuric acid spray, no difference was observed in color variation or intensity except the velocity of color development. These catalysts are useful in colorimetry of phenothiazines but superfluous in spray reagents for chromatograms. Adding 1% *p*-dimethylaminobenzaldehyde in 25% sulfuric acid did not show any further color difference, but later

Table IV.—Locating Methods for Phenothiazine Compounds and Phenothiazine-Like Drugs after Chromatography or Electrophoresis

	Visible Color After 40% H_2SO_4 Spray	Fluorescence Under U.V. Light
Chlorpromazine	violet	quenching[a] or faint blue
Perphenazine	violet	quenching or faint blue
Prochlorperazine	violet	quenching or faint blue
Pipamazine	violet	quenching or faint blue
Thiopropazate	violet	quenching or faint blue
Acetophenazine	orange-pink	orange-brick red
Proketazine	orange-pink	orange-brick red
Butyrylperazine	orange-pink	orange-brick red
Fluphenazine	orange	bluish-yellow
Trifluoperazine	orange	bluish-yellow
Triflupromazine	orange	bluish-yellow
Promazine	orange-pink	quenching or faint blue
Mepazine	orange-pink	quenching or faint blue
Methoxypromazine	purple-blue	quenching or faint blue
Thioridazine	blue	blue
Thiethylperazine	blue	blue
Chlorpromazine-sulfoxide	violet-pink	quenching
Thioridazine-sulfoxide	blue	blue
Trifluoperazine-sulfoxide	orange	blue
Phenothiazine-like drugs:		
Imipramine		quenching or faint blue[c]
Chlorprothixine	orange	[d]
Isothypendyl	yellow	bluish-yellow
Prothypendyl	yellow	bluish-yellow

[a] Dark zone on slightly fluorescent background. [b] Visible blue after adding $KMnO_4$ or bromine. [c] Increased blue-green fluorescence after 40% H_2SO_4 spray. [d] Brick fluorescence after 40% H_2SO_4 spray.

the spots turned to a green-blue tint. The palladium chloride reaction (8), despite its good sensitivity, showed the least color differentiation.

COMMENTS

The described procedures have revealed that the phenothiazine compounds examined in our experiments cannot be recognized completely by their color reactions or fluorescent properties. Only chromatography and electrophoresis made it possible to distinguish between substances giving the same color reactions or fluorescence but with a different radical on position 10 of the phenothiazine nucleus. This combination of chromatographic techniques with color reactions was of great help in many analyses in our laboratory concerned with the identification of phenothiazine drugs in toxicological investigations, in drug metabolism studies, in purification procedures, and in purity tests of commercial preparations.

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New 3-Thiophene Derivatives as Sedative Agents

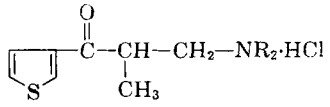
By HEINO A. LUTS† and W. LEWIS NOBLES

A group of compounds prepared from 3-propionylthiophene has been synthesized for pharmacological evaluation. Preliminary studies indicate that some of these agents combine sedative and stimulating properties in an unusual manner.

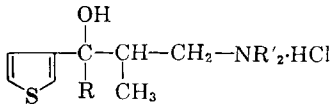
IN A SEARCH for compounds possessing possible analgetic activity, a program designed to produce certain Mannich bases and their derivatives from 3-propionylthiophene has been carried out. It has been noted that in many cases the 3-thienyl analog often has greater activity than the corresponding 2-thienyl compound (1–5). In fact, Campaigne (5) has suggested that, based on available data, it is impossible to predict the activity of the 3-thienyl isomer from a knowledge of the activity of the corresponding 2-isomer. Furthermore, Campaigne suggests that in any program in medicinal chemistry involving thiophene analogs, the 3-isomer should always be included since there is a high probability that it will be active and a good chance that it will be more active than the 2-isomer.

Previously, we (6) reported on the preparation of 2-substituted analogs of *d*-propoxyphene. In line with this and the above statements of Campaigne, it appeared quite logical to extend this work to the preparation of the corresponding 3-isomers.

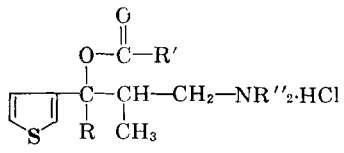
In our work, three general types of compounds were prepared. These are



Type I



Type II



Type III

The compound designated as Type I was prepared using the standard conditions normally employed in the Mannich reaction (7). Type II compounds were formed by the conventional Grignard reaction on the preceding Mannich base; also, the Mannich base was reduced to the

Received March 27, 1962, from the School of Pharmacy, University of Mississippi, University.
Accepted for publication June 5, 1962.
Abstracted in part from a thesis submitted by Heino A. Luts to the Graduate School of the University of Mississippi in partial fulfillment of the requirements of the degree of Master of Science.
Presented to the Scientific Section, A.PH.A., Las Vegas meeting, March 1962.
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