Examination of Structural Effect on Thin-Layer Chromatographic Multiple Spot Formation of Sympathomimetic Amines in the Presence of Hydrochloric Acid

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From a number of solutions of sympathomimetic amines in (*a*) water or (*b*) 10 *N* hydrochloric acid, chromatographed on thin layer of alumina, cellulose, or silica gel using a water–hydrochloric acid–phenol solvent system, only two produced multiple spots when the solvent system and an amine solution in excess hydrochloric acid, on cellulose layers, were used. The appearance of multiple spots does not depend upon the chemical structure of the compounds used, since the phenomenon was not reproduced when alumina or silica gel layers were used. The combination of adsorption and partition forces, the presence of the carboxyl groups in prepared cellulose, and the continuity of the thin layer are the main factors leading to the formation of multiple spots.

THE PHENOMENON of multiple spot formation of sympathomimetic amines when paper or cellulose thin layers are used has often been reported. Shepherd and West (1) observed that adrenaline, liberated from tissues using trichloroacetic acid, gave two spots when chromatographed in various solvent systems; they suggested that the multiple spot formation is due to the presence of trichloroacetic acid.

West (2) also observed double spots with trichloroacetic acid and tryptamine derivatives of sympathomimetic amines, and later Beckett *et al.* (3, 4) showed that the spot deformation and multiplication occurs with many bases in the presence of many diverse acids. Robinson and Shepherd (5) considered that the formation of the additional amine spot which was obtained in the presence of trichloroacetic acid is assisted by the formation of loose complexes between the bases, acting as electron donors, and the trichloroacetic acid, acting as electron acceptor by virtue of the marked electronegativity of the three chlorine atoms.

Roberts (6) also observed the phenomenon of multiple spot formation in an attempt to examine some factors which were affecting the R_f values of sympathomimetic amines, but on paper chromatography only. It was found that hydrochloric acid present in the developing solvent converted some of the norepinephrine acid tartrate to norepinephrine hydrochloride. The presence or absence of multiple spots, according to Roberts (7), depends upon the chemical structure of each amine.

The application of thin-layer chromatography for the examination of these phenomena (8–10) indicated that, just as in paper chromatography, it is possible to obtain two amine spots of an amine salt (or pure amine in the presence of one or more equivalents of acid) when a neutral or acidic solvent system is used in thin-layer chromatography involving cellulose as the thin layer.

EXPERIMENTAL

Materials—The amines used were: amphetamine hydrochloride, norepinephrine hydrochloride, normetanephrine hydrochloride, metanephrine hydrochloride, dopamine, ephedrine hydrochloride, isopropylarterenol hydrochloride, nordefrin hydrochloride, dopa, epinephrine, and phenylephrine hydrochloride.

Developing Systems—(*a*) Phenol containing 15% v/v of 0.1 *N* hydrochloric acid in water. (*b*) *n*-Butanol–acetic acid–water (4:1:5 v/v); the liquids were mixed together, left aside overnight, separated, and the organic layer was used.

Detection Methods—The spots were detected using either (*a*) a solution of 0.6 Gm. of potassium ferrocyanide and 0.5 Gm. of sodium hydroxide in 100 ml. of water or (*b*) ninhydrin in butanol (0.2% w/v) and heating the chromatograms at 100° for 3 min.

Thin-Layer Plates—All plates used were 20 × 20 cm.

Using MN 300 G cellulose powder (Machery, Nagel and Co.), Silica Gel G (Merck), and aluminum oxide G (Merck), plates were prepared according to Stahl (11).

The plates were kept in a desiccator using calcium chloride and reactivated at 100° for 5 min. before use.

General Method—The amines, 0.1-μl. quantities, were chromatographed on alumina, cellulose, or silica gel plates from freshly prepared solutions (10 mg./ml.) in distilled water or 10 *N* hydrochloric acid. The solutions were subjected to further chromatographic development at intervals of 1 day, 2 days, 1 week, 3, 6, and 8 weeks.

All chromatograms were carried out at room temperature (22–24°) and until the solvent front

TABLE I—RESULTS USING CELLULOSE THIN-LAYER PLATES

Amines	R_f Values[a] Freshly Prepared Soln.	Aged Soln.
Amphetamine HCl	84 ± 0.05	85 ± 0.05
Normetanephrine HCl	67 ± 0.05	66 ± 0.05
Metanephrine HCl	80 ± 0.03	79 ± 0.05
Dopamine	40 ± 0.02	44 ± 0.03
Arterenol HCl	35 ± 0.05– 12 ± 0.03	36 ± 0.03– 12 ± 0.03
Ephedrine HCl	93 ± 0.02	93 ± 0.02
Isopropylarterenol	76 ± 0.04	76 ± 0.04
Nordefrin HCl	42 ± 0.01	45 ± 0.02
Dopa	43 ± 0.01	36 ± 0.02
Epinephrine	62 ± 0.05– 35 ± 0.02	64 ± 0.01– 36 ± 0.01
Phenylephrine HCl	79 ± 0.02	81 ± 0.01

[a] R_f values and number of spots of various sympathomimetic amines in 10 *N* hydrochloric acid solution, when chromatographed in water–hydrochloric acid–phenol solvent system.

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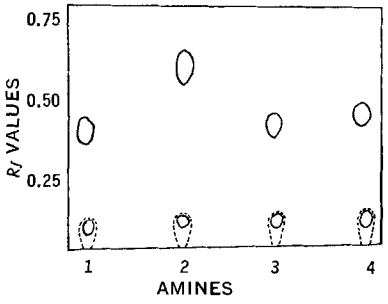

Fig. 1—TLC of certain amines freshly prepared in 10 N *HCl, on cellulose, when* n*-butanol–acetic acid–water solvent system is used. Continuous line indicates amines. Dotted line indicates acid. Key: 1, arterenol HCl; 2, isopropylarterenol HCl; 3, dopa; 4, epinephrine.*

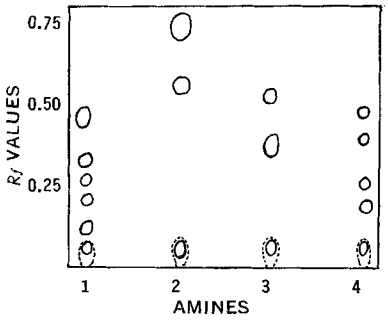

Fig. 2—TLC of certain amines as aged solutions in 10 N *HCl, on cellulose, when* n*-butanol–acetic acid–water solvent system is used. Continuous line indicates amines. Dotted line indicates acid. Key: 1, arterenol HCl; 2, isopropylarterenol HCl; 3, dopa; 4, epinephrine.*

was advanced 10 cm. (65 to 75 min.). The chromatoplates were removed from the solvent, air dried, and the detecting reagents were used to locate the spots.

The R_f values were measured from the center of the spots.

RESULTS

Using silica gel thin-layer plates, freshly prepared aqueous solutions of all amines used produced one spot. The spots, with the exception of arterenol (R_f value 0.75) and dopa (R_f value 0.81), were moved with the solvent front. A similar pattern was observed for the same solutions when chromatographed in intervals of up to an 8-week period, with the exception of dopa which gave an elongated spot, when an 8-week-old solution was chromatographed.

In the presence of 10 *N* hydrochloric acid, the amine spots were completely separated from the acid spots and moved with the solvent front, with the exception of arterenol and dopa, where the spots were moved faster (about 0.8 cm.) than those of the free amines. Hydrochloric acid, in all cases, gave elongated spots, having an R_f value of 0.53 ± 0.03.

However, when an 8-week-old solution was chromatographed, only dopa gave an elongated amine spot having the same R_f value as that of the free base.

In all cases where alumina thin-layer plates were employed, no movement of the amine spots was observed.

The results when cellulose thin-layer plates were used are summarized in Table I.

These results indicated that only arterenol and epinephrine appeared to produce a second spot when the 10 *N* hydrochloric acid solution was used. The second spot was observed in a freshly prepared as well as in an aged solution.

Using *n*-butanol–acetic acid–water solvent system and the cellulose thin layer, arterenol, isopropylarterenol, dopa, and epinephrine gave two discrete spots, when a fresh solution of the amines in 10 *N* hydrochloric acid was chromatographed (Fig. 1). However, when an 8-week-old solution of the amines in 10 *N* hydrochloric acid was used, isopropylarterenol and dopa gave three spots, while epinephrine gave five; six spots were observed during the development of arterenol (Fig. 2).

On alumina or silica gel thin layers and the above solvent system, only elongated spots were detected.

DISCUSSION

From the results obtained, it is noted that when the water–hydrochloric acid–phenol solvent system and alumina thin layers were used, only one spot of the utilized amines was always produced in the presence of 10 *N* hydrochloric acid. The amine spots remained at the starting line, which indicates that the spots were unaffected by the presence of even an excess of acid.

In thin-layer chromatograms with alumina, adsorption forces are operating exclusively.

The results, using silica gel thin layers and the same solvent system as above, showed that the amine spots moved faster than when alumina layers were used, and that most of them followed the solvent front. However, single spots of the amines are obtained, with the exception of dopa, which in an 8-week-old solution, either in water or 10 *N* hydrochloric acid, gave an elongated spot.

With silica gel thin layers, partition effect will in general be more important than adsorption forces.

In a thin layer of cellulose, both partition and adsorption forces exist and their relative role depends upon the conditions (8–10).

Since the added acid has a negligible effect on the movement of the amines on alumina, the effect of single adsorption forces does not account for the multiple spots of amines in cellulose (arterenol and epinephrine). On silica gel plates, the added acid has an effect on the movement of the amines and it seems probable that the added acid, as it moves along the plate, has a substantial effect on the partition of the amines between the mobile solvent and the stationary phase, but two spots were not produced on silica gel layers.

However, using the *n*-butanol–acetic acid–water solvent system and cellulose thin layers, most of the amines chromatographed developed a second spot when in a freshly prepared 10 *N* hydrochloric acid solution; moreover, the number of the spots was increased with the age of the acid solution, presumably due to the oxidation products of the amines which were developed during the prolonged presence of the acid of the solution, leading to the formation of the additional spots.

It is suggested that the formation of double spots does not depend upon the structure of the amine,

since amines giving two spots in one acidic solvent (*i.e.*, *n*-butanol–acetic acid–water) give only one spot in another (*i.e.*, water–hydrochloric acid–phenol). Moreover, amines which appeared to give six or more spots when chromatographed from freshly prepared solutions in 10 *N* hydrochloric acid on cellulose paper (7) gave only two spots when chromatographed from the same freshly prepared solution on cellulose thin layer. In the former, a gradual decrease, while in the latter, a gradual increase in the number of the spots with the age of the solution was observed.

It is therefore concluded that the formation of two amine spots, when cellulose thin layers are used, results from the following factors: (*a*) a continuity of adsorption forces along the direction of the mobile solvent on cellulose thin layers (9, 10), (*b*) a combination of adsorption and partition forces, and (*c*) the presence of the carboxyl groups in the prepared cellulose. That the presence of the carboxyl groups in the prepared cellulose is leading to the formation of double spots, has been previously discussed (10) and therefore further discussion here is unnecessary.

This investigation shows that the presence of more than one spot in cellulose chromatograms, using extracted biological material, does not necessarily indicate that two or more amines are present.

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Modified Synthesis of *dl-N*-Norarmepavine and *dl*-Armepavine

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The synthesis of *dl-N*-norarmepavine and *dl*-armepavine, utilizing the carboethoxy protecting group, is described.

THE POTENTIAL of *dl*-1-(4-hydroxybenzyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (V*a*) as an intermediate to medicinal agents prompted an investigation of its synthesis. The levorotatory base (V*a*) was first isolated by Kupchan and coworkers (1, 2) from *Nelumbo lutea* and named (−)-*N*-norarmepavine. The alkaloid subsequently was isolated by Tomita, Yang, and Lu (3), and by Yang (4).

A total synthesis of V*a* *via* the Bischler-Napieralski reaction has been reported by Yamaguchi and Nakano (5). The benzyl group was utilized for the protection of the phenolic hydroxyl group. This report describes the use of the carboethoxy protecting group in the preparation of V*a* and V*b*.

The requisite amide (III*b*) for the Bischler-Napieralski reaction was prepared either from III*a* or by the condensation of I with the acid chloride (6) of II*b*. The cyclization of III*b* to IV*a* was accomplished with phosphorus oxychloride. The low pressure reduction of IV*a* followed by hydrolysis gave V*a*. Alternately, V*a* was prepared by the hydrolysis of IV*a* to IV*b*, followed by sodium borohydride reduction. It was observed also that sodium borohydride converts IV*a* directly to V*a*. Treatment of V*a* with formic acid and formaldehyde provided *dl*-armepavine (V*b*). Since V*a* was converted to *dl*-armepavine (V*b*), as described above, this sequence also comprises a total synthesis of the alkaloid (Scheme I.)

The nuclear magnetic resonance (NMR) spectrum of V*a* in deuteriochloroform showed six aromatic protons (τ = 3.0–3.4), two labile protons (τ = 5.4, by D_2O exchange), one tertiary proton (quadruplet centered at τ 5.85), and six methoxyl protons (broad doublet centered at τ 7.05), which is in good agreement with the reported NMR spectrum of (−)-*N*-norarmepavine (2).

Sufficient work has not been done to make a critical comparison of the various methods of protecting the phenolic hydroxyl group in the preparation of *dl-N*-norarmepavine. The use of sodium borohydride in the reduction of IV*a* with attendant hydrolysis of the carboethoxy group, however, provides a means of removing the protecting group under alkaline conditions.

EXPERIMENTAL[1]

N-β-(3,4-Dimethoxyphenethyl)-4-hydroxyphenylacetamide (IIIa)—A mixture of 3.6 Gm. (0.02 mole) of homoveratrylamine, 3.0 Gm. (0.02 mole) of *p*-hydroxyphenylacetic acid, 10 ml. of dry decalin, and 10 ml. of tetralin was refluxed at 180–185° for 8 hr. The solvent was decanted from the semisolid; the last traces of solvent were removed by steam distillation. The residual solid was washed successively with 10% sodium bicarbonate, water,

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[1] Melting points were taken in open glass capillaries using a Thomas-Hoover Uni-Melt apparatus and are corrected. Infrared spectra were determined on a Perkin-Elmer model 137 G Infracord spectrophotometer. The NMR spectrum of *dl-N* norarmepavine (V*a*) was obtained by means of the Varian A-60A spectrometer using tetramethylsilane (TMS) as the internal standard and deuteriochloroform as the solvent. The shifts were measured on the τ-scale relative to the internal standard TMS (τ 10.0). Assignment of protons in a certain area is based on correct integral information from the NMR spectrum.