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Note

Thin-layer chromatographic separation of several chloro- and methyl-substituted 2-aminopyridines

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Chlorinated aminopyridines and a wide range of their derivatives are known to have a variety of biological activities. Chloroaminopyridines are useful as fungicides and pesticides. As intermediates they are also useful in preparing a host of heterocyclic systems with herbicidal properties[1–3]. The availability of many of these substances has been restricted due to the lack of good synthetic methods and convenient separation techniques.

Very few thin-layer chromatography (TLC), gas chromatography (GC), and phosphorescence properties of some aminopyridines have been reported in the literature[4–6].

Recently Kress *et al.*[7] used selective chlorination in the synthesis of some mono- and dichloroaminopyridines. The synthesized compounds were analyzed using published TLC and GC methods as well as other techniques. However, for the resolution, preparation, and identification of the components of mixed isomers a new developing system and preparative TLC were required.

We wish to report our findings on the chromatographic behavior of 2-aminopyridine and 14 related methyl- and chloro-substituted aminopyridines on silica gel. Sensitivity of detection of these compounds after exposure to bromine vapors and after spraying with Fluram™ is documented. The relationship between the chemical structure and chromatographic mobility is presented.

EXPERIMENTAL

Aminopyridines

The following 15 compounds were studied: 2-aminopyridine (I); 2-amino-5-chloropyridine (II); 2-amino-3,5-dichloropyridine (III); 2-amino-4-methylpyridine (IV); 2-amino-3-chloro-4-methylpyridine (V); 2-amino-4-methyl-5-chloropyridine (VI); 2-amino-3,5-dichloro-4-methylpyridine (VII); 2-amino-6-methylpyridine (VIII); 2-amino-3-chloro-6-methylpyridine (IX); 2-amino-5-chloro-6-methylpyridine (X); 2-amino-3,5-dichloro-6-methylpyridine (XI); 2-amino-4,6-dimethylpyridine (XII); 2-amino-3-chloro-4,6-dimethylpyridine (XIII); 2-amino-5-chloro-4,6-dimethylpyridine (XIV); 2-amino-3,5-dichloro-4,6-dimethylpyridine (XV).

Compounds I, IV, VIII, and XII were purchased from Riley Tar and Chemical Company (Indianapolis, Ind., U.S.A.) and used as received. The remaining compounds were prepared in the Lilly Research Laboratories[7].

Solvents

Absolute methanol is used as the sample solvent. Each of the 15 aminopyridines is dissolved at a concentration of 10 mg/ml. The developing solvent is chloroform–dioxane (60:40, v/v).

Visualization

Bromine chamber. An approximately 0.5-cm layer of bromine is poured into a tank like that described below in the equipment section. The plate is placed on the top of two small beakers at the bottom of the tank. The bromine chamber is kept in a well ventilated hood. To minimize disturbing the bromine vapor saturation of the chamber, the lid is not lifted but is slid forward just far enough to introduce the plate into the tank and then slid back to the original position. The plate is exposed to the vapors for 10 min.

Spray reagents. A 0.05 *N* aqueous sodium hydroxide solution, and a 0.5% w/v solution of Fluram™ (fluorescamine; Roche, Nutley, N.J., U.S.A.) in acetone are freshly prepared.

Equipment

The following equipment was used: precoated, 0.25 mm thick, silica gel 60 F_{254} plates of 20 × 20 cm (Merck, Darmstadt, G.F.R.); a rectangular glass tank of 28 × 22 × 8 cm (Brinkmann, Westbury, N.Y., U.S.A.); a chromatographic viewing chamber, "Chromato-Vue", equipped with short (254 nm) and long (366 nm) wavelength UV lamps (Ultra-Violet Products, San Gabriel, Calif., U.S.A.); and 1-μl Microcap pipettes, Drummond type (Ace Glass, Louisville, Ky., U.S.A.).

Procedure

Fifteen points of application (POA) are marked 1 cm apart and 2.5 cm from the bottom edge of the plate. The adsorbent layer is scored across the plate at a distance of 15 cm above the POA. A 10-μg load of each of the 15 aminopyridines is applied on a separate POA by spotting 1 μl of the corresponding solution. Five minutes after the last application, the plate is introduced into the developing tank containing 100 ml of the developing solvent. Approximately 100 min are required for the solvent front to travel to the scored line. The plate is then dried in a ventilated hood and examined under short- and long-wavelength UV light.

To increase the detection sensitivity of the 15 aminopyridines, the dried plate may be exposed to bromine vapors before viewing under short UV light; or it may be sprayed lightly with 0.05 *N* aqueous sodium hydroxide followed by 0.5% Fluram in acetone and viewed under long UV light.

RESULTS AND DISCUSSION

Under short UV light all 15 aminopyridines quench the fluorescent indicator incorporated in the adsorbent layer. They exhibit a weak blue fluorescence under long UV light. The detection sensitivity is improved 20-fold following the exposure of the plate to bromine vapors and viewing it under short UV light where all 15 compounds are detected at a level of 0.5 μg. The spots of the 15 aminopyridines show a yellowish green fluorescence on a dark blue background when the plate is sprayed

with the sodium hydroxide–Fluram reagents and viewed under long UV light. Following the use of this spray the sensitivity of detection is further improved 5- to 10-fold over the bromine vapor treatment. Compounds III, V, VII, IX, XI, XIII, and XV are detected at a concentration of 0.1 μg, while compounds I, II, IV, VI, VIII, X, XII, and XIV are detected at half this concentration, *i.e.*, 0.05 μg per spot.

Based on the general chromatographic mobilities and separations (see Fig. 1) of the 15 aminopyridines studied, they may be classified into three groups, *viz.* the non-polar group, exhibiting the highest R_F values represented by compounds III, VII, XI, and XV; an intermediate-mobility group consisting of compounds II, V, VI, IX, X, XIII, and XIV; and a polar group, with slow mobility, including compounds I, IV, VIII, and XII.

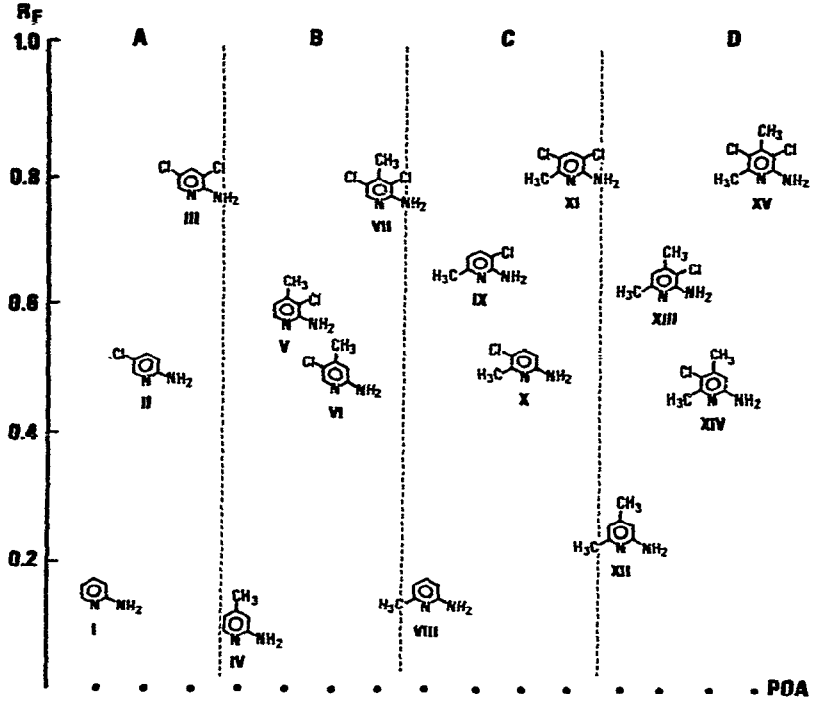

Fig. 1. Thin-layer chromatogram of aminopyridines on silica gel 60 F_{254} developed with chloroform–dioxane (60:40), 10 μg/spot. The Roman numerals refer to the compounds identified in Experimental. The circle in the pyridine represents both the unsaturation structure of the ring and the actual position of the developed compound. POA = Point of application.

The relative mobilities of compounds IV and VIII are similar to those of the γ- and α-picolines, previously reported by Petrowitz *et al.*[8], where a higher R_F is observed with the α-substituted compounds.

Substituting a hydrogen by a less polar[9] chlorine atom increases the mobility of the chloro compounds as follows: compound II > I; V, VI > IV; IX, X > VIII; and XIII, XIV > XII. A similar effect was previously observed for the pesticide DDT and some related compounds[10].

The effect of the chlorine atom to enhance the mobility is dramatically demon-

strated in the case of the 3,5-dichloro derivatives, compounds III, VII, XI, and XV. The latter four compounds have the fastest moving spots in columns A, B, C, and D, respectively.

Examination of Fig. 1 reveals that in every case of the studied isomeric pairs of the monochloro derivatives V, VI, IX, X, XIII, and XIV, the *ortho*-chloro (relative to the 2-amino group of the pyridine ring) compounds V, IX, and XIII move faster, *i.e.*, are less strongly adsorbed than the *para* isomers VI, X, and XIV. This may be explained on the assumption that one of the amine hydrogens, necessary for effective adsorption to the silica layer, is ineffective, probably due to an intramolecular bond formation, and/or steric hindrance[11]. Although the adsorption characteristics of the *para* isomer are explained by the same factors (steric hindrance, inductive effects, solvent effect and spatial arrangement of the substituent groups on the ring) affecting the behavior of the *ortho* isomers, the difference in mobility is due to the possibility of the latter isomer forming internal bonds[12]. Similarly, *ortho*-disubstituted benzenes such as the fluoro-, chloro-, bromo-, and methoxyanilines as well as the chloro-, methoxy-, and hydroxyphenols, are adsorbed less strongly and hence have faster mobility on silica, than their corresponding *para* isomers[13–17]. The possibility of the hydrogen bonding between the adjacent groups of the *ortho* isomers in the above-mentioned examples results in rendering the adsorption energy of the bonded compound less than that of the slow-moving isomeric compound in which the intramolecular bonding is absent[18]. The decreasing mobilities of the 2-, 3-, and 4-aminopyridines on silica gel are reported to be in reverse order of the strength of hydrogen bonding between the isomeric (pyridine N and NH_2 groups) and the SiOH groups on the surface of the adsorbent[5].

Five of the compounds studied, V, VI, VII, IX and XIII, were separated by preparative TLC, using the system reported here, and their melting point, proton chemical shifts, and elemental analyses were determined to establish their identities[7].

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