Thin-Layer Chromatography in Drug Analysis II. Procedure for the Identification of Various N^4-Substituted Sulfonamides

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A thin-layer chromatographic procedure using the step technique is described for the separation and identification of various N^4-acylated and N^4-aroylated sulfonamides. The test has been successfully applied to mixtures of (*a*) phthalylsulfacetamide, phthalylsulfathiazole, and succinylsulfathiazole and (*b*) phthalylsulfanilamide, succinylsulfacetamide, and succinylsulfanilamide. Detection was accomplished by use of ultraviolet observation and an 0.05% alcoholic bromcresol purple solution. The procedure can be completed within two and one-half hours.

A METHOD for the detection of N^4-acylated and N^4-aroylated sulfonamides alone or when present in mixtures along with N^4-unsubstituted compounds has not yet been reported in the thin-layer chromatography (TLC) literature. Sophoulis (1) has reported a paper chromatographic procedure for various sulfonamides including phthalylsulfacetamide, phthalylsulfathiazole, and succinylsulfathiazole, which required 6 hours for development. The procedure gave R_f values which were too close to provide separation of phthalylsulfacetamide and succinylsulfathiazole

The usual color reactions for the detection of the unsubstituted sulfonamide spots are based on the reaction of the free aromatic NH_2 group. The standard procedure for the detection of N^4-substituted sulfonamides on paper is by use of acid-base indicators (2). In developing the TLC procedure described in this paper, it was found that for certain substituted compounds, bromcresol purple gave the best results when silica gel G was used. For others, detection by ultraviolet fluorescence was necessary.

This paper deals with the separation and identification, by TLC, of the N^4-acylated and N^4-aroylated sulfonamides together or in combination with N^4-unsubstituted sulfonamides. A successful procedure was obtained using the step technique (3). This necessitated the use of two chambers. One of the two was lined with filter paper that was saturated with the solvent system. This was necessary to avoid the edge phenomenon (3). Unsubstituted sulfonamides are detected on the same plates using the Bratton-Marshall reagent. The identification of these was accomplished according to a previously described method (4).

The procedure is useful in the identification of the N^4-substituted sulfonamides present in the U.S.P. XVI (5) and N.F. XI (6). It also provides a means for the detection of the corresponding unsubstituted sulfonamides, if present.

EXPERIMENTAL

Apparatus.—Glass plates (200 × 200 mm.). An unlined tank ($8^1/_2$ in. × 4 in. × $8^1/_2$ in.) for solvent A. A second tank similar to A but containing solvent B and lined with solvent-saturated filter paper.

Preparation of Plates.—The plates were coated according to the procedure described in the previous paper (4).

Solvent A.—Methanol-ethanol (1:1). Prepared by mixing 60 ml. each of anhydrous ethanol and A.C.S. reagent methanol.

Solvent B.—*n*-Propanol–0.05 *N* hydrochloric acid (4:1). Prepared by mixing 100 ml. of *n*-propanol, 25 ml. of water, and 0.1 ml. of concentrated hydrochloric acid.

Adsorbant.—Silica gel G.

Standards.—Twenty-five mg. dissolved in 50 ml. acetone. These were freshly prepared. The compounds used were: phthalylsulfacetamide (I),[1] phthalylsulfathiazole (II), succinylsulfathiazole (III), phthalylsulfanilamide (IV),[1] succinylsulfanilamide (V),[1] and succinylsulfacetamide (VI)[1].

Preparation of Samples.—An equivalent of 25 mg. of sulfonamide was extracted with 50 ml. of acetone. For suspensions, 1 ml. of water was added before extraction. If necessary, the solutions were centrifuged and the clear supernatant liquid used.

Observation.—Short wave U.V. lamp (254 mμ)[2] and 0.05% alcoholic solution of bromcresol purple.

Quantity of Sulfonamide Applied.—One mcg. for compounds I to IV inclusive, 3 mcg. for compounds V and VI.

Using the Desaga apparatus,[3] the adsorbant was applied to the plates as described in the previously published paper (4). The tanks were allowed to equilibrate overnight. In addition to a finish line at 100 mm. from the start, a small notation was made at the 50-mm. level.

Samples (2 μl.) and standard (2 μl.) solutions representing compounds I to IV inclusive were applied on the plates along an imaginary line 15 mm. from the starting edge. For compounds V and VI, 6 μl. was applied. Two microliters of a standard solution containing 1 mg. per 2 ml. and representing a possible unsubstituted hydrolysis product were also applied.

Development was started in tank A. When the front reached 50 mm., the plates were removed, dried at 100° for 5 minutes, allowed to cool, and then placed into tank B. When final development to 100 mm. was completed, the plates were removed and again dried at 100° for 5 minutes. Observation was begun using a short wave U.V. lamp.

Phthalylsulfacetamide (I), Phthalylsulfathiazole

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[1] Obtained from K and K Laboratories, Jamaica 33, N. Y.

[2] Chromato-Vue C-3 marketed by Black Light Eastern Corporation, 4 Manhasset Ave., Port Washington, L. I. N. Y., or the equivalent.

[3] Obtainable from C. Desaga, Heidlberg, Germany, through Brinkmann Instruments, Inc., Great Neck, L. I., N. Y.

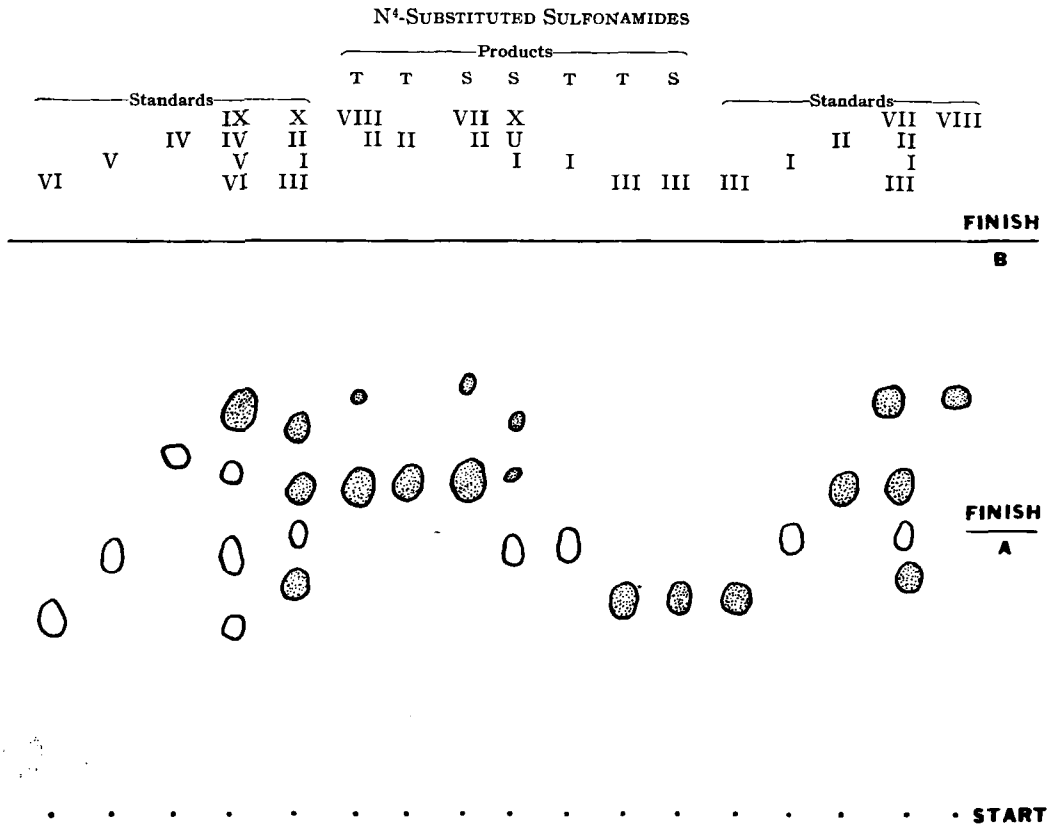

Fig. 1.—I = Phthalylsulfacetamide; II = phthalylsulfathiazole; III = succinylsulfathiazole; IV = phthalylsulfanilamide; V = succinylsulfanilamide; VI = succinylsulfacetamide; VII = sulfathiazole; VIII = sulfamethazine; IX = sulfanilamide; and X = sulfacetamide. U, unknown; A, solvent A; B, solvent B; T, tablet; and S, suspension. The clear spots refer to areas detected by use of ultraviolet lamp. The shaded spots refer to areas detected by use of bromcresol purple indicator.

(II), and Succinylsulfathiazole (III).—Compound I appeared as a bright blue fluorescent area which was marked, if present. Compounds II and III sometimes appeared faintly blue. Spraying with 0.05% alcoholic solution of bromcresol purple showed blue or purple spots on a yellow background in the presence of II and III or any unsubstituted sulfonamide produced by hydrolysis. Compound I appeared blue when first sprayed, but the color faded with either continued spraying or within a short period after the spraying was completed. On first application, the entire plate appeared blue. The background rapidly faded, leaving only areas of II, III, and the unsubstituted sulfonamides visible.

A reproduction of a developed plate is shown in Fig. 1. The copy was obtained using a Xerox 914 copier and a modified procedure of Hilton and Hall (7).

Phthalylsulfanilamide (IV), Succinylsulfanilamide (V), and Succinylsulfacetamide (VI).—The sensitivity of the U.V. detection method for observing V and VI is less than for any of the other four compounds tested. A 3 mcg. sample was the smallest amount easily detected. Compounds V and VI appeared as blue fluorescent areas after exposure of the plates to the ultraviolet lamp for a period of two or three minutes. Spraying with the indicator gave rapidly fading blue or purple spots.

A summary of approximate R_f values for the various sulfonamides tested is shown in Table I.

TABLE I.—APPROXIMATE R_f VALUES OBTAINED FOR VARIOUS N⁴-SUBSTITUTED SULFONAMIDES

Sulfonamide	Av. R_f[a]	Observation
Succinylsulfacetamide	0.37	UV
Succinylsulfathiazole	0.43	Indicator
Succinylsulfanilamide	0.49	UV
Phthalylsulfacetamide	0.51	UV
Phthalylsulfathiazole	0.61	Indicator
Phthalylsulfanilamide	0.66	UV

[a] Data obtained from seven or more plates.

RESULTS AND DISCUSSION

For the samples tested, separation and identification of the N⁴-substituted sulfonamides was accomplished by use of the step technique (3). The substituted compounds were easily separated from the free sulfonamides. Verification of the presence of any unsubstituted sulfonamides can be made using diazotization and the Bratton-Marshall reagent. For identifying the unsubstituted sulfon-

amide, the method described in the previously published paper (4) was used.

The R_f values for the N^4-substituted sulfonamide official in the U.S.P. XVI (5) and N.F. XI (6), namely I, II, and III were sufficiently different to permit identification

The R_f values for IV, V, and VI were also sufficiently different to allow separation from each other. Of the six compounds tested, all can be separated either by R_f values or fluorescent observation, except I and V. At equal concentrations of 1 mcg. per spot, V is not visible. Though II and IV give close R_f values, II changes the indicator color to blue or purple whereas IV does not. Compound IV gives a bright blue fluorescence and II does not.

After two days the standard solutions showed the presence of the free form.

Due to the uncertainty of reproducing R_f values from one plate to another, it is important to use standards along with the samples.

The total development time is approximately two and one-half hours.

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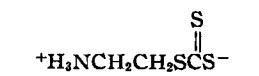
Communications

Structure of the Carbon Disulfide Adduct of β-Mercaptoethylamine

Sir:

We have recently reported (1, 2) the preparation of a compound from the reaction of β-mercaptoethylamine (MEA) and carbon disulfide, in either aqueous or alcoholic ammonia, which gave good protection to animals against an otherwise lethal dose of X-irradiation. This compound was believed to be 2-mercaptoethyldithiocarbamic acid on the basis of elemental analysis, its reaction with acylating agents, and a characteristic dithiocarbamate absorption peak (3) in the ultraviolet at 248 mμ (log ε max. = 3.03) (C_2H_5OH). Other characteristic absorption peaks for dithiocarbamates at 290–310 mμ and 340–360 mμ were absent, however, and further examination of the ultraviolet absorption characteristics of dithiocarbamate and trithiocarbonate zwitterions has shown the product in question to be a trithiocarbonate zwitterion I. A previous example of a trithiocarbonate zwitterion has not appeared in the literature.



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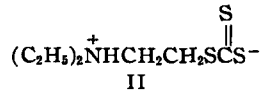


II

Trithiocarbonate zwitterion II of unquestioned structure was prepared from 2-diethylaminoethanethiol hydrochloride (Evans Chemetics, Inc.) and carbon disulfide in ammoniacal solution, analogously to the preparation of I, m.p. 114–115° with effervescence, (90% yield).

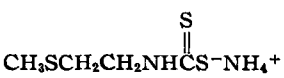
Anal.—Calcd. for $C_7H_{15}NS_3$: C, 40.16; H, 7.22; N, 6.70; S, 45.94. Found: C, 40.74; H, 7.20 N, 6.96; S, 46.13.

The compound reduced iodine, and it showed definite trithiocarbonate absorption peaks (4) in the ultraviolet at 226 mμ (log ε max. = 4.07) and 303 mμ (log ε max. = 4.18) in alkaline C_2H_5OH. Compound I, quite similar in color and physical appearance, showed ultraviolet absorption peaks at 226 mμ (log ε max. = 3.96) and 304 mμ (log ε max. = 4.15), when observed in the same solvent.

Furthermore, compound III, possessing definite dithiocarbamate absorption characteristics in the ultraviolet, was prepared from the S-methyl ether of MEA [prepared by the procedure of Gonick (5)] and carbon disulfide in ammoniacal solution, and was isolated as an unstable ammonium salt, m.p. 104–106°, (70% yield).

Anal.—Calcd. for $C_4H_{12}N_2S_3$; N, 15.27; S, 52.17. Found: N, 15.06; S, 51.38.

Loss of ammonia and carbon disulfide was evident within 24 hours. Characteristic dithiocarbamate absorption was observed at 257 mμ (log ε max. = 3.92), 292 mμ (log ε max. = 4.06), and 345 mμ (log ε max. = 1.95) in alkaline C_2H_5OH.



III

Because of the remarkable agreement in physical properties and ultraviolet absorption between the carbon disulfide adduct I of MEA and a similarly constituted trithiocarbonate