Two-dimensional thin-layer chromatography of tobacco alkaloids and related compounds*

During studies[1,2] of the metabolism of nicotine and nornicotine in insects feeding on tobacco, the need for a fast, accurate, and simple method for the qualitative analysis of tobacco alkaloids and their possible metabolites became apparent. The method finally adopted is described below.

Following solvent extraction and clean-up by conventional procedures[1,2], chloroform extracts of plant or animal tissues or chloroform solutions were subjected to thin-layer chromatography. Glass plates, 10 × 10 cm, were coated with a layer of silica gel G, 0.25 mm thick, using the Desaga-Brinkmann adjustable spreader, according to the manufacturer's instructions, and activated by heating to 110° for 30 min. The samples were applied using a 10 μl glass pipette and the plates developed first in chloroform–methanol–ammonia (60:10:1), air-dried for 45 min, and then developed in chloroform–methanol–acetic acid (60:10:1) in a direction 90° to the first.

After drying for 45 min the spots were visualized by a modification of the cyanogen bromide method[3]. The plates were sprayed with a 1:1 mixture of 2% *p*-aminobenzoic acid in ethanol and 0.1 *M* phosphate buffer, pH 7.0. After drying for 15 min the plates were placed in a closed container with a few crystals of cyanogen bromide. The spots obtained vary in color from bright red, through brown to yellow. The most discrete spots are obtained when the solvents are used in the above order and R_F values are highly reproducible when the first solvent is freshly mixed before use.

Table I shows the R_F values for a series of tobacco alkaloids, and Fig. 1 shows the same 7 compounds separated on a two-dimensional chromatogram.

TABLE I

R_F VALUES OF TOBACCO ALKALOIDS AND RELATED COMPOUNDS

Alkaloid	*R_F value*	
	$CHCl_3$–MeOH–NH_4OH	*$CHCl_3$–MeOH–CH_3COOH*
Nornicotine	0.34	0.05
Nicotine	0.77	0.08
Nicotyrine	0.87	0.92
Anabasine	0.50	0.06
Nicotine N-oxide	0.08	0.05
Cotinine	0.75	0.76
Norcotinine	0.50	0.51

Although there is a voluminous literature on the thin-layer chromatography of alkaloids other than tobacco alkaloids, there is little information on thin-layer separations of tobacco alkaloids either from each other or from their principal metabolites in animals. There are several reports[4–7] on the separation of nicotine from other unrelated compounds with pharmacological activity and on the use of unidimensional thin-layer chromatography as an adjunct to the gas chromatographic separation

* Contribution from the Entomology Department, North Carolina Agricultural Experiment Station, Raleigh, North Carolina. Published with the approval of the Director of Research.

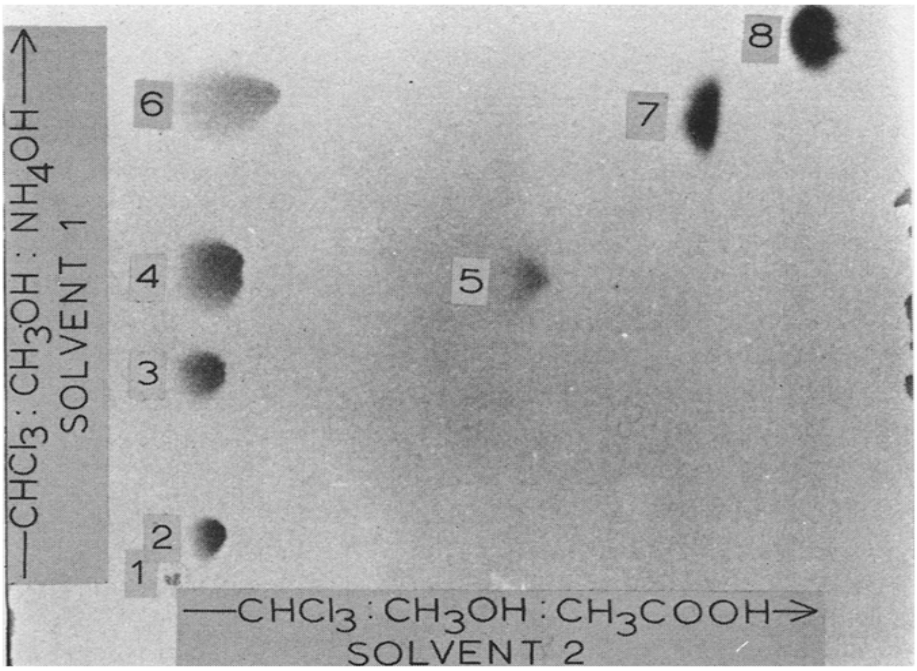

Fig. 1. Two-dimensional chromatograph of tacco obalkaloids and related compounds. (1) Origin; (2) nicotine N-oxide; (3) nornicotine; (4) anabasine; (5) norcotinine; (6) nicotine; (7) cotinine; (8) nicotyrine. 10 μg of each compound were applied in 10 μl of chloroform solution.

of tobacco alkaloids[8,9]. The solvent systems used in the last two reports were: chloroform–methanol[8], chloroform–ethanol[9], and ethanol–0.2 *M* acetate buffer[9]. These solvent systems will not effect the separations described in the present report.

The use of an acidic and a basic solvent in different directions on a single plate gives a flexibility not found in any of the unidimensional systems tested. As the separations are distinct and the method highly reproducible, it should be useful for the separation of a much wider range of related compounds.

This work was supported in part by U.S. Public Health Service Grant No. ES-00044-01A1. The authors are indebted to Dr. H. M. McKennis, Jr. for the authentic sample of norcotinine.

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Received March 29th, 1965