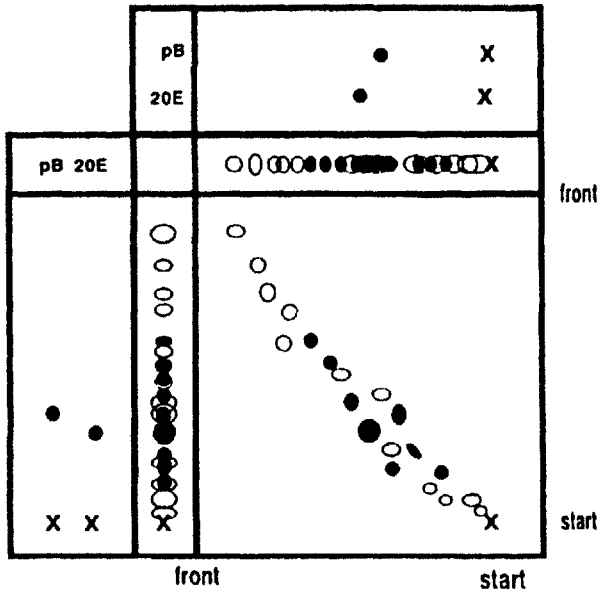


**Figure 3** Result of 2-D TLC on a silica gel F layer of an extract of *Silene italica* spp. *nemoralis*. The same sample and samples of 20-hydroxyecdysone (20E) and polypodine B (pB) were spotted on side tracks and subjected to one-dimensional TLC. Developments in the first (bottom to top) and second (right to left) directions were performed with chloroform–methanol–benzene (25:5:3) and toluene–acetone–96% ethanol–25% aqueous ammonia (100:140:32:9), respectively. The zones were initially detected under 254 nm UV light and then sprayed with vanillin–sulfuric acid reagent and observed in daylight and under 366 nm UV light. Observation of specific violet fluorescence at 366 nm, then black zones when the plate was sprayed, is indicative of ecdysteroids. Open circles denote other components. (Adapted from Ref. 39.)

of planar chromatography written by Sherma in the ACS journal *Analytical Chemistry* (45). Books on physical and chemical detection methods in TLC have been published (46,47) that include descriptions of reagents and methods for steroid visualization.

In their recent article on steroid TLC, Mulja and Indrayanto (3) reviewed the following detection methods. Sulfuric acid is a very widely used detection reagent for steroids; characteristic color and fluorescence response (366 nm UV light) can be produced with a short heating at a relatively low temperature, and permanent black zones appear after longer heating at a higher temperature if layers can withstand the charring reaction (e.g., silica gel G). Other generally applicable, destructive chromogenic and fluorogenic reagents for detection of steroids are antimony trichloride (Carr–Price reagent) (for vitamin D, cardenolides, bufadienolides, triterpenoids); aromatic aldehyde-acids (sapogenin steroids, steroid alkaloids, ketosteroids; PMA (reducing and unsaturated steroids, cholesterol and cholesterol ester, bile acids); chlorosulfonic acid–acetic acid (cardenolides), phosphoric acid (results similar to sulfuric acid) (48); *m*-dinitrobenzene (ketosteroids); and phthalic acid-*p*-phenylenediamine (oxo-steroids). Many steroids absorb UV radiation around 254 nm and can be detected nondestructively by viewing under light of this wavelength on a plate with an F-layer. Iodine and iodine-KI are nondestructive detection reagents that react reversibly with many steroids.

Anisaldehyde (5 mL) in glacial acetic acid (50 mL) and sulfuric acid (97%, 1 mL) was used to detect steroids and terpenes in Baltic amber as deep violet zones after heating to 105°C; resin ethanol extracts were developed on silica gel 60 F plates with *n*-hexane–benzene–methanol (2:6:1) mobile phase (48a).