must be followed by a desalting procedure, with consequent loss of material, if the pure compound is required. We have found that this difficulty can be obviated by the use of the completely volatile solvent system IV, *tert.*-amyl alcohol–pyridine–acetic acid–water (110:1.0:0.08:25). Descending development on washed Whatman No. 3MM paper for 30 h gave good resolution, without tailing, of DNP-amino acids with the exception of the DNP-glycine and DNP-serine pair (Table I).

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Spray detection of bile acids on thin-layer chromatograms

The detection of bile acids in thin-layer chromatography (TLC) has been accomplished by means of spray reagents: namely, phosphomolybdic acid[1], antimony trichloride[2] or concentrated sulfuric acid[3]. But the usefulness of color detection in bile acid identification has been demonstrated by KRITCHEVSKY *et al.*[4] and by ANTHONY AND BEHER[5]. The present study expands on the use of color detection, thus aiding in the identification of bile acids.

Materials and methods

The bile acids were obtained from Applied Science Laboratories, Inc., Pa., and isooctane, isopropyl alcohol and acetic acid were all Baker Analysed Reagent grade. A thin-layer plate precoated to a thickness of 0.5 mm with Silica Gel F_{254} (Merck) and a commercial chromatographic chamber (Gelman Instrument Co., Ann Arbor, Mich.) were used. The plates were heated at 100° for 1 h and the chamber was saturated with the solvent isooctane–isopropyl alcohol–acetic acid (40:10:1) prior to the chromatogramphy at room temperature (23–25°). When chromatograms were run, 20 × 20 cm plates were used and the solvent front was permitted to rise 135 mm from the origin. The chromatoplate was then removed from the chamber, air dried for several minutes and then dried thoroughly at 100° for 10 min. After cooling, the plate was exposed to iodine vapor for 15 min, sprayed with tap water and the color was

noted. This color was found to disappear within 10–15 min when left exposed to air or dried at 100° for 5 min. The plate was next sprayed with the benzoic acid spray reagent (0.15 g of benzoic acid, 0.15 ml of concentrated sulfuric acid and 20 ml of acetic acid) and placed in an oven at 100° for 15–20 min and the color was noted. Five to twenty micrograms of each acid in ethanol were sufficient for color detection.

Results and discussion

Color reactions of the bile acids are shown in Table I. The methyl ester of cholic acid (not shown in Table I) gives a navy blue color with iodine water. The special interest of benzoic acid spray reagent is that it gives a different color with chenodeoxy- and deoxycholic acids, two compounds whose R_F values do not always permit

TABLE I

COLOR REACTIONS OF BILE ACIDS (10 μg)

Bile acids	*Detecting reagents*[a]	
	A	*B*
Cholic acid	Brown	Brownish yellow
Chenodeoxycholic acid	Yellow	Brown
Deoxycholic acid	Yellow	Deep yellow
Hyocholic acid	Light yellow	Light brown
Lithocholic acid	Light yellow	Light brown

[a] A = Exposed to iodine vapor for 15 min and sprayed with water. B = Benzoic acid spray reagent (0.15 g of benzoic acid, 15 ml of concentrated sulfuric acid and 20 ml of acetic acid).

unequivocal identification. One other distinct advantage is the lack of background color. The reagent reacts only with the test compounds and the resultant color stands out clearly from the white background. Lastly it is suggested that each investigator should prepare his own color standards.

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