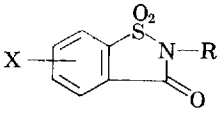
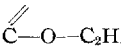
TABLE 1.—SACCHARIN DERIVATIVES



Compd.	X	R	M.p., °C.[a]	Ref.
IV	6-SO_2NH_2	CH_3	230–232	(8)
V	6-Cl	CH_3	180–181	This paper
1	6-NO_2	*n*-C_3H_7	120–121	(9)
2	6-NO_2	*i*-C_3H_7	148	(14)
3	4-NO_2	C_2H_5	184.5–185.5	(10)
4	4-NO_2	*n*-C_3H_7	138–139.5	(10)
5	H	*i*-C_3H_7	62–64	(14, 15)
6	H		136	(11, 13)
7	H	$OCH(CH_3)_2$	136–137	(14, 15)
8	H	$N(C_2H_5)_2$	206–207	(11)

[a] Melting points were determined either with a Fisher-Johns melting point apparatus or by the capillary tube method and are uncorrected.

from acetone–water gave white needle crystals, m.p. 180–181°.

Anal.[6]—Calcd. for $C_8H_6ClNO_3S$: C, 41.50; H, 2.61. Found: C, 41.60; H, 2.73.

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[6] Analyses were performed by Elek Microanalytical Laboratories, Torrance, Calif.

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Thin-Layer Chromatography of Cardiac Glycosides

By EUGENE J. JOHNSTON and ALLEN L. JACOBS

A rapid thin-layer chromatographic procedure for the separation and identification of the common cardiac glycosides is presented. A benzene–ethanol solvent is used for development and a perchloric acid spray for visualization.

THIN-LAYER chromatography has proven to be more rapid and sensitive than paper chromatography for the identification and purity determination of many drugs. A number of solvent systems and sprays useful for cardiac glycosides have been published (1–4). This paper reports the development of a relatively simple technique which has certain advantages over these approaches.

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EXPERIMENTAL

Thin-Layer Plates.—A 0.25-mm. layer of Silica Gel G (E. Merck, Darmstadt) is applied to the plates. The plates are air dried for 10 min., then heated in an oven for 45 min. at 120°. The plates are stored in a desiccator and used without further activation.

Solvent System.—Benzene–95% ethanol (7:3 v/v).

Spray Reagent.—Fifteen milliliters of 70% perchloric acid added to 100 ml. of water.

Preparation of Samples.—The substances are dissolved in a suitable solvent, usually methanol. For purity studies, 100 mcg. of substance is spotted on

TABLE I.—CHROMATOGRAPHIC DATA

Substance	R_f	Fluorescence
Acetyl digitoxin	0.82	Red
Digitoxin	0.72	Red
Digoxin	0.62	Blue
Lanatoside A	0.52	Red
Lanatoside B	0.41	Red
Lanatoside C	0.36	Blue
Desacetyl lanatoside C	0.27	Blue
Ouabain	0.09	Yellow-green

the plate, although for simple detection 1 mcg. is sufficient.

Visualization.—The plates are sprayed, then placed in a 100° oven for a few minutes. The perchloric acid produces a charring effect and a fluorescence which is visible under long-wave ultraviolet light (366 mμ.).

DISCUSSION

The sensitivity of detection by fluorescence is augmented by the visualization of the spots in ordinary light. Good separation of the common cardiac glycosides can be obtained (Table I). The presence of certain contaminants is easily detected. The system has the advantages of simplicity, speed (approximately 0.5 hr.), efficiency of separation, and avoids the use of hazardous spray reagents, such as antimony trichloride.

Good results can also be obtained using a spray consisting of 0.5 ml. of *p*-anisaldehyde in 50 ml. of glacial acetic acid and 1 ml. of concentrated sulfuric acid (5). After heating the plate for a few minutes at 100°, the glycosides appear as blue spots with the exception of ouabain which appears as a yellow spot.

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Determination of Organically-Bound Iodine in Pharmaceuticals

By GERALD J. YAKATAN* and MURRAY M. TUCKERMAN

Eight official iodinated compounds with iodine contents from 50 to 66 per cent were assayed by the methods official in U.S.P. XVI and N.F. XI, by reduction with zinc in alkali, and by a standard procedure using oxygen flask combustion. An additional four compounds, formerly official, with iodine content as low as 23 per cent were also examined. The results indicate that the suggested standard procedure is equal or superior to the other methods in recovery of iodine and generally equivalent in reproducibility. The alkaline zinc reduction method is suggested for those compounds having an electronegative substituent *ortho* or *para* to the iodine atoms on the aromatic ring. The oxygen flask combustion is suggested for all other iodinated compounds and as a general method. The suggested standard procedure consists of combustion by the oxygen flask method, absorption of the combustion products in an alkaline sulfite solution, and titration of the acidified solution of iodide with standard silver nitrate using a silver-calomel electrode pair for potentiometric determination of the end point.

THE QUANTITATIVE determination of iodine in organic compounds has long proved a source of difficulty for the analytical chemist. This is reflected in the numerous proposals for the determination of iodine in organic substances. An assay procedure for the determination of iodine in an organic compound actually involves two problems. First, the organic compound must be decomposed to liberate the iodine, and then the iodine must be quantitatively determined. By far the more difficult of these problems is finding an efficient method for the decomposition of the organic compound which will not result in the loss of any of the iodine present.

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EXPERIMENTAL

Reagents.—All reagents employed were reagent grade chemicals. All standard solutions employed were prepared and standardized according to the official compendia.

Methods.—*A. Parr Bomb Method* (*1–3*).—The organic matter is oxidized by fusion with sodium peroxide in a bomb and the halide present is converted to sodium halide.

B. Alkaline Permanganate Method (*4*, *5*).—This assay is based on the conversion of organically-bound iodine to iodide by the action of permanganate in alkali and reduction with bisulfite.

C. Zinc–Sodium Hydroxide Method (*6*).—In this method the organic iodine is replaced with hydrogen generated in the nascent form by the reaction of zinc and sodium hydroxide.