a single spot corresponding to I, was further purified by crystallization.

Solvent was removed under vacuum from the ether-soluble fraction (13 g), which on TLC showed two major spots corresponding to I and II. It was then subjected to silica gel 60 (500 g) column chromatography. Elution with dichloromethane with increasing concentration of ethyl acetate yielded fractions containing pure I and pure II, which were further purified by crystallization.

Deoxypodophyllotoxin (I)—This compound was obtained as colorless prisms, mp 167° (ether–dichloromethane). It was identical in all respects with the authentic specimen.

Anal.—Calc. for $C_{22}H_{22}O_7$: C, 66.32; H, 5.57; mol. wt. 398. Found: C, 66.49; H, 5.68; *m/e* 398 (M^+).

5'-Desmethoxydeoxypodophyllotoxin (II)—This compound was obtained as colorless needles, mp 181° (ether–dichloromethane). The IR ($CHCl_3$: 3000, 2900, 2840, 2780, 1775, and 930 cm^{-1}), NMR [$CDCl_3$: δ 2.9 (3H, m), 3.79 (3H, s), 3.86 (3H, s), 3.9 (2H, m), 4.5 (2H, m), 5.90 (2H, s), 6.37 (1H, dd, J = 8 and 2 Hz), 6.49 (1H, s), 6.66 (1H, s), 6.67 (1H, d, J = 8 Hz), and 6.90 (1H, d, J = 2 Hz)], and mass [*m/e* 368 (M^+, base), 353, 340, 338, 337, 323, 309, 308, 253, 230, 212, 185, 151, 138, 95, and 77] spectra were in accord with Structure II. This compound was optically active, $[\alpha]_D^{24}$ − 125° (c 0.014 in chloroform).

Anal.—Calc. for $C_{21}H_{20}O_6$: C, 68.47; H, 5.77; mol. wt. 368. Found: C, 68.47; H, 5.47; *m/e* 368 (M^+).

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Modified Electron-Capture GLC Assay for Salsolinol in Brain Tissue

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Abstract □ A modified assay for a neuroamine-derived tetrahydroisoquinoline, salsolinol, is presented. It combines the ease and rapidity of solvent extraction from brain tissue with the sensitivity of electron-capture GLC. Detection of salsolinol (1,2,3,4-tetrahydro-1-methyl-6,7-isoquinolinediol), the alkaloid derived from condensation of dopamine and acetaldehyde, at levels of 5–10 ng/g of brain tissue is possible. The advantages afforded by the modifications are discussed in relation to existing procedures.

Keyphrases □ Salsolinol—electron-capture GLC analysis, mouse brain tissue □ GLC, electron capture—analysis, salsolinol in mouse brain tissue □ Tetrahydroisoquinolines, substituted—salsolinol, electron-capture GLC analysis in mouse brain tissue □ Alkaloids—salsolinol, electron-capture GLC analysis in mouse brain tissue

The *in vivo* formation of tetrahydroisoquinoline alkaloids from aldehydes and endogenous biogenic amines has been suggested to mediate some effects of alcohol (1, 2). These alkaloids possess a variety of pharmacological actions *in vitro* and *in vivo* (3). Tetrahydropapaveroline (the condensation product of dopamine with dopaldehyde) and salsolinol (1,2,3,4-tetrahydro-1-methyl-6,7-isoquinolinediol) (the condensation product of acetaldehyde with dopamine) were detected in the urine of humans (4) and in rat brain (5, 6). Likewise, tetrahydroprotoberberines (the cyclization derivatives of tetrahydropapaveroline) were recovered from human urine (7).

The analyses used in some of these studies (4, 5, 7) involved costly mass fragmentography. Recently, a sensitive electron-capture GLC assay was reported for salsolinol in rat brain (8). However, the tissue extraction method was time consuming (adsorption onto and elution from alumina, followed by overnight lyophilization) and presented additional problems such as inadequate buffering of the extract prior to alumina adsorption and incomplete removal of the derivatizing reagent prior to injection into the chromatograph. Even with appropriate modifications to eliminate the latter problems, the long extraction procedure still proved undesirable.

The present report describes a more rapid and convenient method for the detection of salsolinol in mouse brain using a simplified solvent extraction and electron-capture GLC system. Detection of salsolinol at levels of 5–10 ng/g of brain tissue [approximately 1% of the endogenous dopamine level (9)] is possible with this method.

EXPERIMENTAL

Extraction—The extraction method from tissue is essentially that of Maruyama and Takemori (9) with some modifications. A single mouse brain was added to an all-glass grinding vessel[1] containing 1 ml of 0.05 *N* oxalic acid, saturated with sodium chloride, and 3.5 ml of 25% 1-butanol in 2-propanol and was homogenized. Standards were added at this point and mixed by passing the pestle through the homogenate.

The homogenate was transferred to a conical test tube and centrifuged at 3000×*g* for 5 min in a clinical centrifuge. The lower phase was discarded, and a 3-ml aliquot of the upper solvent phase was removed. Hexane, 3 ml, and 0.5 ml of 0.5 *M* sodium phosphate buffer (pH 6.5) were added to this aliquot, and the mixture was shaken for 5 min, followed by a 5-min centrifugation. The upper phase was discarded, and a 0.5-ml

[1] Size 22, Kontes Glass Co., Vineland, N.J.

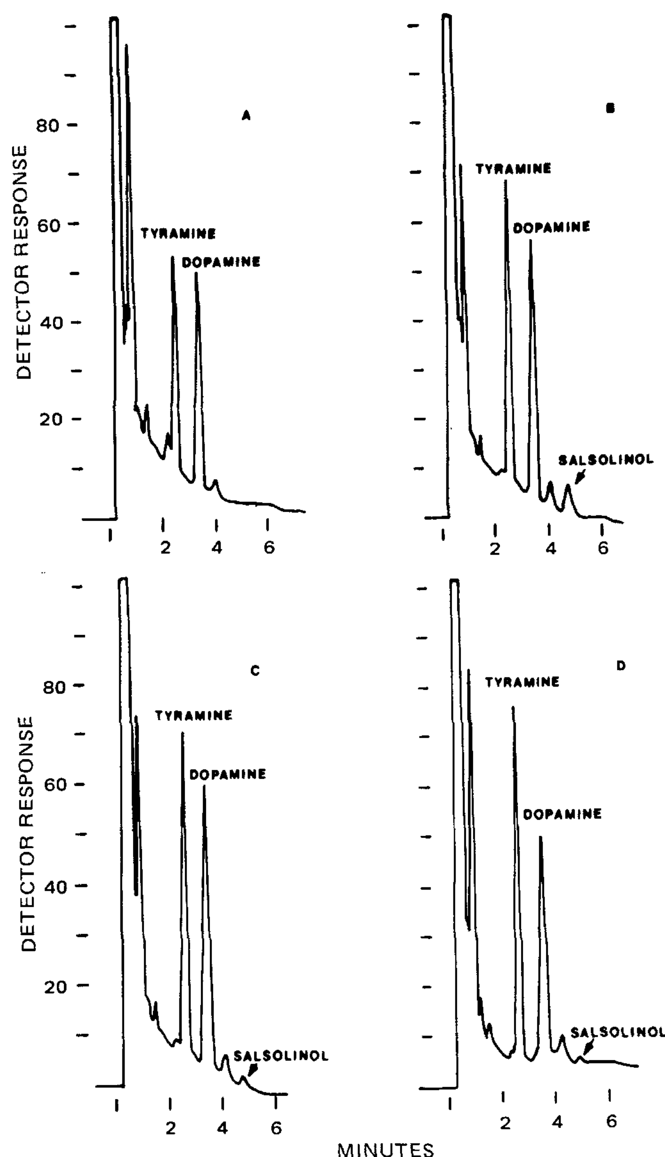

Figure 1—*Chromatograms of mouse brain extracts. Key: A, single mouse brain with 500 ng of tyramine added; B, single mouse brain with 500 ng of tyramine and 40 ng of salsolinol added; C, single mouse brain with 500 ng of tyramine and 10 ng of salsolinol added; and D, single mouse brain with 500 ng of tyramine and 4 ng of salsolinol added. Samples of 5 µl were injected at attenuation 512; attenuation was changed to 256 after tyramine appeared. See text for conditions. The peak immediately preceding tyramine corresponds to norepinephrine.*

aliquot of the aqueous lower phase was saturated with sodium chloride and reextracted with 0.5 ml of 25% 1-butanol in 2-propanol. The lower phase was discarded, and 0.5 ml of the upper solvent phase was transferred to another tube and evaporated to dryness under nitrogen; the residue was used for derivatization.

Derivatization—Derivatization of the compounds was carried out using a modification of the method of Bigdeli and Collins (8). Acetonitrile, 0.2 ml, and 0.05 ml of heptafluorobutyric anhydride[2] were added to the nitrogen-dried residue and allowed to react at room temperature for 30 min. The samples were evaporated to dryness under nitrogen, and the residue was dissolved in 1 ml of benzene. Immediately prior to assay, each sample was washed with 5 ml of a saturated solution of sodium borate (10) by shaking, followed by a brief centrifugation to separate the aqueous and organic phases. A portion (2–5 µl) of the upper benzene layer was injected into the chromatograph.

Table I—Retention Times and Recoveries for Tyramine, Dopamine, and Salsolinol

Compound	Retention Time, min	Recovery[a], % ± *SEM*
Tyramine	2.6	38.8 ± 1.13[b]
Dopamine	3.5	68.5 ± 5.07[c]
Salsolinol	4.8	62.3 ± 2.14[d]

[a] Mean of six to 16 determinations. [b] Recovery was calculated from 500 ng of added tyramine. [c] Dopamine recovery was estimated on the basis of an average whole brain content of 350 ng (9). [d] Recovery was calculated from 10–100 ng of added salsolinol. Recovery was linear over the range of 4–400 ng of added salsolinol.

Instrumentation—A gas chromatograph[3] with a ^{63}Ni-electron-capture detector was used for the analyses. A 1.83-m (6-ft) glass column (2 mm i.d.) packed with 3% OV-17 on 100–120-mesh Gas Chrom Q[4] was used. Temperatures were: injector, 250°; oven, 150°; and detector, 300°. The mobile phase was argon–methane (95:5) at a flow rate of 20 ml/min.

Quantitation—The peak height ratio method (11) was employed to estimate quantities of salsolinol. Tyramine[2] (500 ng) was added to each homogenate as the internal standard; various amounts of authentic salsolinol[5] (4–400 ng) were also added. A standard curve was constructed by plotting the ratio of the relative peak height of salsolinol to the relative peak height of tyramine *versus* the quantity of authentic salsolinol in the sample. Regression lines were fitted by computer analysis using the method of least squares.

RESULTS AND DISCUSSION

Chromatograms obtained are shown in Fig. 1. Figure 1A represents a brain extracted as described under *Experimental* but with no salsolinol added. Figures 1B, 1C, and 1D represent single brains with 40, 10, and 4 ng of salsolinol added, respectively. The on-column lower limit of detection sensitivity for salsolinol under the conditions of the assay (attenuation = 256) was approximately 10 pg. Identity of the peaks was confirmed by comparison of retention times of derivatized authentic standards or by addition of derivatized authentic standards to the injection solution.

Recoveries and retention times for tyramine, dopamine, and salsolinol are shown in Table I; recoveries were slightly higher than those reported by Bigdeli and Collins (8) for dopamine and salsolinol. Regression analysis indicated that the method was linear over the tested range of 4–400 ng of added salsolinol. Norepinephrine was neither well extracted nor sensitive to detection by this method.

The assay described in this report differs in several respects from the extraction and assay procedures previously reported (8, 9). Maruyama and Takemori (9) suggested adjusting the aqueous phase (after separation from hexane) to pH 2 with hydrochloric acid prior to saturation with sodium chloride and extraction with 25% 1-butanol in 2-propanol. However, preliminary experiments using this method gave low recoveries. Furthermore, adding no acid gave the maximum recovery for salsolinol and dopamine, the compounds of major interest for *in vivo* studies conducted in this laboratory (12). The entire extraction procedure described here takes approximately 3 hr to bring as many as 16 samples to the derivatizing step; the alumina adsorption method of Bigdeli and Collins (8) involves an extensive time period, even including overnight lyophilization to reach this point.

Bigdeli and Collins (8) suggested a ratio of 0.2 ml of acetonitrile to 0.4 ml of heptafluorobutyric anhydride during derivatization. In the present method, the quantity of heptafluorobutyric anhydride can be reduced to 0.05 ml without any decrease in efficiency while derivatizing as much as 1 mg of any of the amines. Therefore, a significant saving of an expensive reagent is realized, and the amount of unreacted heptafluorobutyric anhydride that must be removed prior to the electron-capture GLC assay is substantially reduced.

In this respect, the use of benzene as a solvent [rather than ethyl acetate (8)] allows a significant improvement. Any traces of unreacted heptafluorobutyric anhydride left in the GLC injection solution make it virtually impossible to obtain meaningful results. "Washing" the sample with a saturated solution of sodium borate (see *Experimental*) removes

[2] Eastman Kodak Co., Rochester, N.Y.

[3] Model 5713A, Hewlett-Packard, Palo Alto, Calif.
[4] Applied Science Laboratories, State College, Pa.
[5] Aldrich Chemical Co., Milwaukee, Wis.

any traces of the derivatizing reagent (10), thus removing the interfering contaminant; however, this washing is not possible with ethyl acetate as the injection solvent (8), since the latter is miscible with the sodium borate solution. Therefore, benzene was selected since the derivatized compounds are freely soluble in it, it is not miscible with aqueous solutions, and no significant loss occurs from this organic layer by washing with the saturated solution of sodium borate.

The sensitivity achieved for salsolinol in the present method is comparable to that for tetrahydropapaveroline using GC–mass spectrometry (5). Sandler *et al.* (4) also used a GC–mass spectrometric method for salsolinol, but they did not report the absolute sensitivity of their method.

Preliminary *in vivo* experiments utilizing this method to investigate the role of salsolinol in the effects of alcohol were reported elsewhere (12).

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Quantitative Determinations of Codeine Phosphate, Guaifenesin, Pheniramine Maleate, Phenylpropanolamine Hydrochloride, and Pyrilamine Maleate in an Expectorant by High-Pressure Liquid Chromatography

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Abstract □ The quantitative determinations of codeine phosphate, guaifenesin, pheniramine maleate, phenylpropanolamine hydrochloride, and pyrilamine maleate in a liquid dosage form are described. All active and inactive ingredients (sodium benzoate and FD&C Yellow No. 5 dye) can be separated with high-pressure liquid chromatography except the two antihistamines, pheniramine maleate and pyrilamine maleate. Pheniramine maleate is determined colorimetrically, and pyrilamine maleate is determined either by difference or spectrophotometrically. The methods are simple, short, accurate, and precise. The standard deviations are reported.

Keyphrases □ Codeine phosphate—high-pressure liquid chromatographic analysis, cough syrup □ Guaifenesin—high-pressure liquid chromatographic analysis, cough syrup □ Phenylpropanolamine hydrochloride—high-pressure liquid chromatographic analysis, cough syrup □ Pheniramine maleate—colorimetric analysis, cough syrup □ Pyrilamine maleate—spectrophotometric analysis, cough syrup □ High-pressure liquid chromatography—analyses, codeine phosphate, guaifenesin, and phenylpropanolamine hydrochloride, cough syrup □ Colorimetry—analysis, pheniramine maleate, cough syrup □ Spectrophotometry—analysis, pyrilamine maleate, cough syrup □ Antitussives—cough syrup ingredients analyzed by high-pressure liquid chromatography, colorimetry, and spectrophotometry

A commonly used cough syrup contains an antitussive agent, codeine phosphate, an expectorant, guaifenesin, two antihistamines, pheniramine maleate and pyrilamine maleate, and a decongestant, phenylpropanolamine hydrochloride. In addition to active ingredients, most cough syrups contain dye(s), preservative(s), flavor(s), and sweetening agent(s). Due to interferences, no easy methods are available for the quantitative determinations of various ingredients in such a cough syrup. The purpose of these investigations was to develop easy, simple, short, and accurate methods for the quantitative determinations of various ingredients in such a cough syrup by high-pressure liquid chromatography (HPLC).

EXPERIMENTAL

Chemicals and Reagents—All chemicals and reagents including codeine phosphate (I), guaifenesin (II), pheniramine maleate (III), phenylpropanolamine hydrochloride (IV), and pyrilamine maleate (V) were ACS, USP, or NF grade and were used without further purification.

Apparatus—A high-pressure liquid chromatograph[1] equipped with a fixed wavelength (254 nm) detector and recorder[2] was used.

Column—A nonpolar column[3] consisting of a monomolecular layer of octadecyltrichlorosilane permanently bonded to Si–C (30 cm long and 4 mm i.d.) was used.

Chromatographic Conditions—The chromatographic solvent was 0.05 M KH_2PO_4 in water containing 13% (v/v) methanol. The temperature was ambient, and the solvent flow rate was 2.0 ml/min (at an inlet

[1] Waters ALC 202 equipped with a U6K universal injector.

[2] Omniscribe 5213-12 equipped with an integrator.

[3] Waters μ-Bondapak/C_{18}, Catalog No. 27324 (prepacked column).