Table II. Residues of NAA and Its Major Conjugates on Field-Treated Zinfandel Grapes

sample	application rate of NAA, ppm	residue,[a] ppm
control	0	<0.05
	0	<0.05
	0	<0.05
	0	<0.05
treated, I	100	0.22
	100	0.23
	100	0.19
	100	0.26
treated, II	200	0.53
	200	0.48
	200	0.44
	200	0.44

[a] HPLC with fluorescence detection; 0.05-ppm sensitivity; recoveries of 0.05, 0.10, and 0.25 ppm were 97.8, 105.0, and 88.9%.

Ten injections of 1 ng of NAA standard were introduced into the HPLC system described above, and the average resulting peak area was 0.29 ± 0.02 in^2 with a coefficient of error at 6.88%.

Four replicate sample treatments of 100 and 200 ppm of NAA treated Zinfandel grapes were analyzed by the described method, and total NAA residues were obtained of 0.20 ± 0.02 and 0.31 ± 0.04 ppm, respectively, with a coefficient of error of 10.0 and 12.9%.

RESULTS AND DISCUSSION

Table I contains recovery data for residues from Zinfandel grapes fortified at levels of 0.05, 0.10, and 0.25 ppm with NAA, NAAsp, and NAGlu standard. Recoveries ranged from 84.0 to 100% for NAA, 80.0% for NAAsp, and 72.0 to 80.0% for NAGlu.

Figure 1 contains HPLC chromatograms for (a) 0.5 ng of NAA standard, (b) the equivalent extractives of 10 mg of untreated Zinfandel grapes processed through the described procedure, (c) the equivalent extractives of 10 mg of untreated Zinfandel grapes fortified with 0.05 ppm of NAA and processed through the described procedure, and (d) the equivalent extractives of 10 mg of Zinfandel grapes field-treated with a 100-ppm solution of NAA which resulted in a residue at harvest (126 days after treatment) of 0.22 ppm of total NAA and major conjugates.

Table II contains residue data on untreated control and 100 and 200 ppm of NAA which were hand-sprayed on Zinfandel grapes in the vineyard. The controls contained <0.05 ppm of total NAA residues while the 100- and 200-ppm treated grapes had residues ranging from 0.19 to 0.26 and 0.44 to 0.53 ppm, respectively.

The described quantitative method for total residues of NAA, NAAsp, and NAGlu on Zinfandel grapes by HPLC is rapid, precise, and accurate and could easily be adapted to other crops and commodities.

Registry No. NAA, 86-87-3; NAAsp, 32667-88-2; NAGlu, 40681-78-5.

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Analysis of 6-Hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline in Alcoholic Beverages and Food

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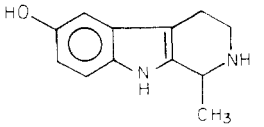
6-Hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline was identified and quantified in various dietary components. The analytical procedure involved the use of a deuterated analogue as the internal standard, extraction with dichloromethane, derivatization with pentafluoropropionic anhydride, and subsequent analysis with glass capillary gas chromatography–mass spectrometry. The compound was found in beer (18–427 nmol/L), wine (0–1.1 nmol/L), fruits (0–1.8 nmol/g), tomatoes (7 pmol/g), and processed cheese (4.5–140 pmol/g).

The tricyclic indole derivative 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline (6OMTHBC, 1) belongs to a group of compounds that has attracted the attention of neurochemists during recent years (Buckholtz, 1980). Tetrahydro-β-carbolines exhibit several physiological effects including monoamine oxidase inhibition (Buckholtz and Boggan, 1977), serotonin (5-hydroxytryptamine) uptake inhibition (Rommelspacher et al., 1978), and stimulation of the voluntary intake of ethanol in rat after intraventricular administration (Melchior and Myers, 1977).



6OMTHBC, 1

These compounds are known to be formed from tryptamines and aldehydes under physiological conditions (Whaley and Govindachari, 1951). The in vivo formation of 1-methyltetrahydro-β-carbolines from acetaldehyde and biogenic tryptamines has been implicated in the alcoholic syndrome. It was previously found that 1-methyl-

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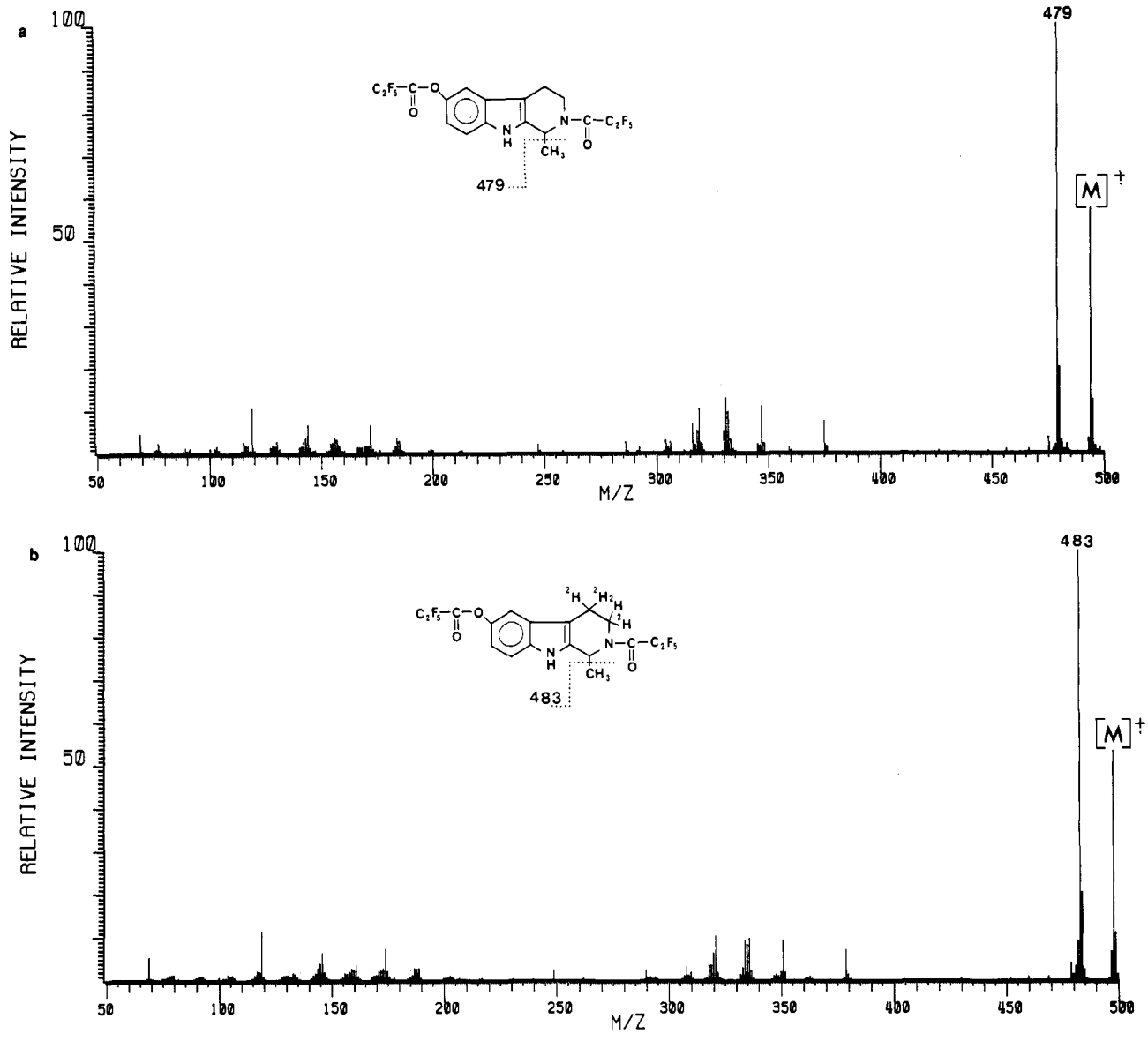

Figure 1. Electron impact mass spectra of the pentafluoropropionyl derivatives of (a) 6OMTHBC and (b) 6OMTHBC-$^2H_4$.

1,2,3,4-tetrahydro-β-carboline occurs in alcoholic beverages (Beck and Holmstedt, 1981). In the present paper we report the identification and quantification of 6OMTHBC in various beverages and foods.

EXPERIMENTAL SECTION

Apparatus. A computer-controlled LKB 2091 gas chromatograph–mass spectrometer was used for multiple ion detection and recording of mass spectra.

The gas chromatographic separations were achieved with an 18-m SE 52 WCOT glass capillary column (i.d. 0.35 mm) by using helium as a carrier and makeup gas. Splitless injections were carried out by using a "moving needle" device (van den Berg and Cox, 1972). The gas chromatographic conditions were as follows: injector heater 280 °C, column temperature 250 °C, column flow rate ~2 mL/min, and makeup gas flow rate ~12 mL/min. The mass spectrometric conditions were as follows: separator temperature 270 °C, electron energy 70 eV, and trap current 50 μA.

Aliquots (1–2 μL) of the samples were injected, and an initial delay of ~1.2 min in opening the separator valve was used to avoid contamination of the ion source. Under these conditions, the retention time of the bis(pentafluoropropionyl) derivative of 6OMTHBC [6OMTHBC-$(PFP)_2$] and the deuterated analogue [6OMTHBC-$^2H_4$-$(PFP)_2$] was approximately 1.6 min.

Reagents. 5-(Benzyloxy)indole and 5-(benzyloxy)-tryptamine hydrochloride were obtained from Sigma Chemical Co. (St. Louis, MO), lithium aluminum deuteride was from Fluka AG (Buchs, Switzerland), and pentafluoropropionic anhydride (PFPA) was from Reagenta (Uppsala, Sweden). Other chemicals used were of analytical purity.

5-(Benzyloxy)[α,α,β,β-$^2H_4$]tryptamine hydrochloride was synthesized from 5-(benzyloxy)indole, according to Shaw et al. (1976). 6OMTHBC hydrochloride was synthesized from 5-benzyloxytryptamine hydrochloride, according to Taborsky and McIsaac (1964). Similarly, 6-hydroxy-1-methyl[3,3,4,4-$^2H_4$]-1,2,3,4-tetrahydro-β-carboline hydrochloride (6OMTHBC-$^2H_4$) was synthesized from 5-(benzyloxy)[α,α,β,β-$^2H_4$]tryptamine hydrochloride. The mass spectra of 6OMTHBC-$(PFP)_2$ and 6OMTHBC-$^2H_4$-$(PFP)_2$ are shown in parts a and b of Figure 1, respectively.

Sample Preparation. Alcoholic beverages, fruits, vegetables, bread, and dairy products were obtained from local shops and used fresh.

Solid samples (0.1–0.2 g) were homogenized in plastic tubes containing distilled water (1.0 mL), semicarbazide

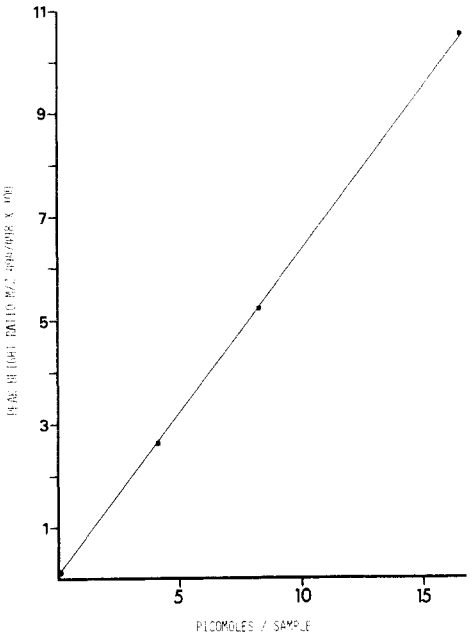

Figure 2. Calibration curve of 6OMTHBC in the low concentration range.

(3.6 μmol), and 6OMTHBC-$^2H_4$ (92.7 pmol) by using an Ultra-Turrax homogenizer and centrifuged at 4000*g* (4 °C) for 15 min. The supernatant was transferred to clean acid-washed (dichromate–sulfuric acid) and silanized glass tubes containing NaCl (0.4 g) and 0.1 mL of 5 M K_2CO_3.

Dichloromethane (CH_2Cl_2) (5 mL) was then added to the tubes, and they were shaken for 10 min and centrifuged at 1000*g* for 5 min. The organic layers were transferred to new tubes and evaporated to dryness under nitrogen. The residues were treated with 50 μL of PFPA for 20 min at 60 °C. After being cooled, the excess reagent was evaporated under a stream of nitrogen, and the residues were dissolved in 25 μL of EtOAc.

Beverage samples (1.0 mL) were added to glass tubes containing 6OMTHBC-$^2H_4$ semicarbazide, NaCl, and K_2CO_3 (same amounts as above). The samples were extracted and derivatized as described above.

Quantification. Standard samples were prepared by adding varying amounts (0–328 pmol) of 6OMTHBC HCl to glass tubes containing 1.0 mL of water and 6OMTHBC-$^2H_4$ (92.7 pmol). These samples were prepared and analyzed as described above. Calibration curves were constructed by plotting the peak height ratios (*m/z* 494/498) of the standard samples against the 6OMTHBC concentration (Figure 2). The 6OMTHBC levels were determined from the peak height ratios of each sample by reference to the calibration curve.

RESULTS AND DISCUSSION

The electron impact mass spectra of the pentafluoropropionyl derivatives of 6OMTHBC and 6OMTHBC-$^2H_4$ (Figure 1) showed abundant molecular ions at *m/z* 494 and 498 and base peaks at *m/z* 479 and 483. The multiple ion detection was performed by monitoring ion intensities at these four mass numbers.

The possibility of artifactual formation of 6OMTHBC during the workup procedure was considered and studied by adding serotonin-$^2H_4$ oxalate (4.5 μmol) to the samples instead of 6OMTHBC-$^2H_4$. Subsequent monitoring for artifactually formed 6OMTHBC-$^2H_4$ in the extracts assured that no artifactual formation occurred. It was previously noted that the addition of semicarbazide is essential

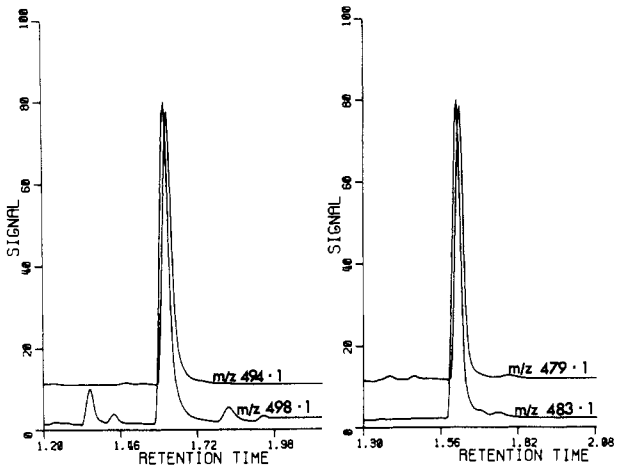

Figure 3. Chromatograms obtained in the analysis of a beer sample (Pilsner). Mass numbers and relative gain amplification factors are indicated in the figure.

Table I. Levels of 6OMTHBC in Beer

beer	alcohol content, % (w/w)	nmol/L
Bass	4.5	258
Guiness	4.5	427
Falcon	4.5	87
Three Town	4.5	157
Porter	2.8	107
Pilsner	2.8	57
Tuborg	2.8	88
Three Town	2.8	32
Coop Dark	1.8	36
Coop Light	1.8	57
Pripps Blå	1.8	18
Three Town	1.8	22

in order to avoid artifactual formation (Beck and Holmstedt, 1981).

The recovery of 6OMTHBC in the extraction procedure was estimated to 70–80% by comparing the peak height of extracted and nonextracted standard samples. The yield of the derivatisation reaction was estimated to be >90%. The reproducibility of individual 6OMTHBC assays was estimated by analyzing a diluted beer sample repeatedly ($n = 9$). The mean level was 24.9 ± 0.7 (SD) pmol/sample, indicating an experimental error of <6%.

The identificaton of 6OMTHBC in the samples was based on the presence of peaks at both *m/z* 479 and *m/z* 494 at the same retention time as authentic 6OMTHBC and with the same ion intensity ratio. As an example of an identification, the results from the analysis of a beer sample are depicted in Figure 3. In cases where 6OMTHBC was present in low amounts (<5 pmol/g or mL) the identification was based only on the ion intensity recorded at *m/z* 494 (e.g., the wine samples). This is due to interference at high gain of the $M^+ - 19$ (*m/z* 479) from 6OMTHBC-$^2H_4$-$(PFP)_2$ at *m/z* 479 (see Figure 1). In banana, the identification of 6OMTHBC was verified by the recording of a complete mass spectrum (Figure 4).

Different types of beverages and food were analyzed and 6OMTHBC was quantified by using the ion intensity ratio recorded at *m/z* 494/498. In beer, 6OMTHBC was present in substantial amounts (Table I). The results indicate a relationship between the 6OMTHBC concentration and the alcohol content. The levels of this compound in beer are markedly higher than those previously found for 1-methyl-1,2,3,4-tetrahydro-β-carboline (Beck and Holmstedt, 1981). The levels of 6OMTHBC in wine and sherry

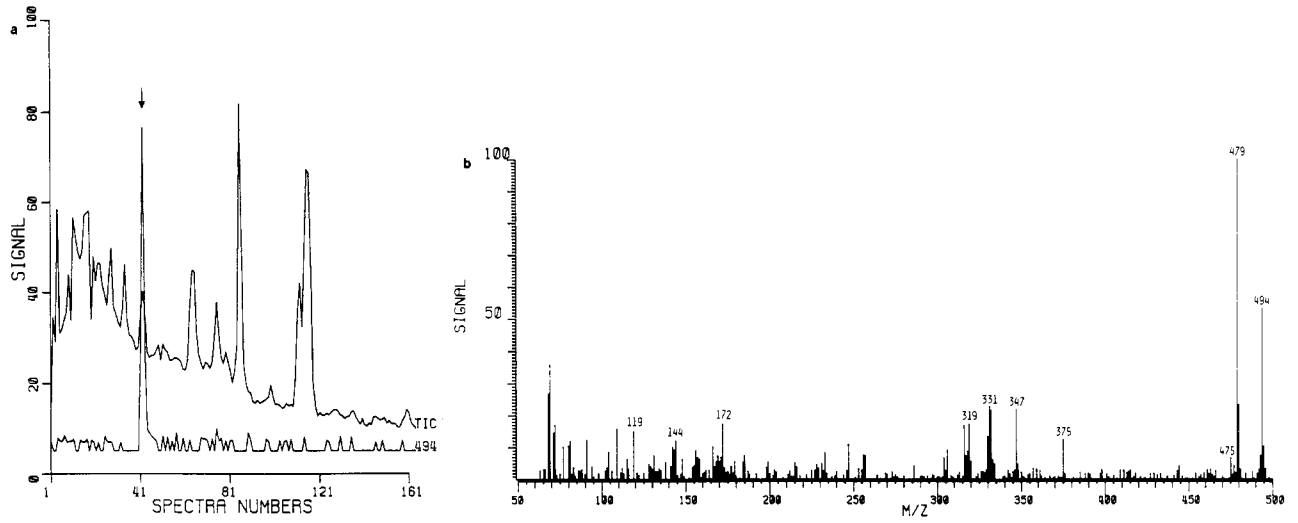

Figure 4. Results of the analysis of a banana extract obtained by recording mass spectra repeatedly. The ion trace chromatograms (a) showed a peak in the total ion chromatogram (TIC) at the same retention time as 6OMTHBC-$(PFP)_2$ (arrow) and a peak at m/z 494 at the same retention time. The mass spectrum of the peak (b) was identical with that of derivatized 6OMTHBC (Figure 1a).

Table II. Levels of 6OMTHBC in Wine

beverage	country of origin	nmol/L
Chianti Rosso	Italy	1.1
Bourgogne Vieux	France	0.9
Medoc	France	0.45
Parador	Spain	0.6
Zeller Schwartze Katze	Germany	0.35
Beyaz	Turkey	n.d.[a]
Maritime	France	n.d.[a]
Manzanilla la Capitana	Spain	0.4
Liebfraumilch	Germany	0.35
Sherry Manzanilla	Spain	0.25
Val de Loire	France	0.35
Aquavite	Sweden	n.d.[a]

[a] Not detectable: <0.15 nmol/L.

Table III. Levels of 6OMTHBC in Various Fruits and Vegetables

specimen	pmol/g
banana peel	325
banana meat	550
banana seeds	1850
grapefruit	9
plum, yellow	1000
plum, red	950
pineapple	26
cherry	n.d.[a]
orange	9
apple, red	n.d.
apple, green	n.d.
pear	n.d.
grape, red	n.d.
lemon	n.d.
strawberry	n.d.
peach	n.d.
potato	n.d.
raddish	n.d.
onion	n.d.
cabbage	n.d.
tomato	7
green bell pepper	n.d.
carrot	n.d.
cucumber	n.d.

[a] Not detectable: <1 pmol/g.

were low (Table II), in contrast to what was found for 1-methyl-1,2,3,4-tetrahydro-β-carboline (Beck and Holmstedt, 1981), and may reflect differences in the concentration of the precursor tryptamines in wine and beer.

Table IV. Levels of 6OMTHBC in Some Dairy Products

specimen	pmol/g or mL
blue cheese	140
hard cheese[a]	<1
Lantbrie	9.5
Camembert	4.5
milk	<0.2
yogurt	<0.2

[a] Four types tested.

Considerable quantities of 6OMTHBC were detected in banana and plum (Table III). In addition, grapefruit, pineapple, orange, and tomato contained 6OMTHBC. Of these, banana, pineapple (juice), plum, and tomato are known to contain significant levels of serotonin, the precursor amine (Rice et al., 1976). In banana, the reaction product between dopamine and acetaldehyde, salsolinol, has previously been identified (Riggin et al., 1976). Of the dairy products tested (Table IV), 6OMTHBC was found to be present only in processed cheese.

The origin of 6OMTHBC in food and beverages is unknown; however, it is most likely formed by a Pictet-Spengler condensation of serotonin and acetaldehyde. In addition, the presence of 6OMTHBC in foods and beverages may in part explain its presence in human urine (Beck et al., 1981). Since it has been suggested that tetrahydro-β-carbolines function as neurotransmittors and/or neuromodulators (Buckholtz, 1980), their presence in foodstuffs may have behavioral or toxicological implications for the consumer.

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Radioimmunoassay for Diethylstilbestrol and the Monoglucuronide Metabolite in Bovine Liver

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The complex matrix in liver causes difficulties in development of a radioimmunoassay (RIA) to quantify compounds bound in liver. An RIA method was developed for diethylstilbestrol (DES) in bovine liver and employs a purification procedure to circumvent these problems; the procedure includes liquid–liquid partitioning, Sephadex LH-20 chromatography, and quantitative enzymatic hydrolysis of the principal metabolite, DES glucuronide. Assay background due to liver matrix was 0.05 ppb ($1/10^9$). Average recovery of both DES and its monoglucuronide from fortified liver was 43% by RIA. Tritiated DES used as an internal standard had a higher apparent recovery (67.7%) by liquid scintillation counting. With an in vivo contaminated liver, accuracy was confirmed by a gas chromatography–mass spectrometry method. By repeating the extraction and RIA without enzymatic hydrolysis on this same liver, the free DES was calculated to be 7.8%. The limit of determination for this method with 95% confidence limits is 0.3 ppb.

The application of radioimmunoassay (RIA) to drug residue analysis in animal tissues has been limited despite the proven sensitivity and specificity of RIA below the parts per billion (ppb, $1/10^9$) range in biological fluids (Hoffmann, 1978). Complex tissues such as liver necessitate involved purification procedures. Hoffmann (1978) developed such purification procedures with RIA for both natural and synthetic steroids in muscle, liver, kidney, and fat.

Diethylstilbestrol (DES) is difficult to quantitate below 1.0 ppb in bovine liver, which is among the last tissues to contain DES after cattle are withdrawn from the drug (Donoho et al., 1973; Aschbacher, 1976). Methods to measure DES in bovine liver include gas chromatography (GC) (Donoho et al., 1973), GC with mass spectrometry (GC–MS) (Day et al., 1975), and liquid chromatography (Kenyhercz and Kissinger, 1978), but quantitation below 1.0 ppb has not been documented for any of these methods. RIA is capable of greater sensitivity.

Several researchers have developed RIA techniques for DES (Hoffmann, 1978; Gutierrez-Cernosek and Cernosek, 1977; Richoubac et al., 1977), but none was suitable for use with liver. Hoffmann and Laschutza (1980) and Vogt (1980) developed assays with extensive purification procedures for DES in bovine tissues, including liver; Hoffmann and Laschutza used both silica gel chromatography and liquid–liquid partition while Vogt relied entirely on liquid–liquid partition.

In this study, we investigated the use of a simplified purification procedure combined with RIA for detecting and quantifying DES in bovine liver. The method developed was simpler and easier to perform than the above RIA methods.

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EXPERIMENTAL SECTION

Materials. The DES antiserum (No. 40A-16) was obtained from R. M. Gutierrez-Cernosek and S. F. Cernosek and was previously described (Gutierrez-Cernosek and Cernosek, 1977). The antigenic conjugate was prepared by the method of Rombauts et al. (1973) in which one phenolic group is covalently bonded through an ether linkage to the free amines of bovine serum albumin. The second antibody was lyophilized anti-rabbit γ-globulin goat serum obtained from Micromedic Systems, Horsham, PA. Reagent-grade crystalline DES was purchased from J. T. Baker Chemical Co., Phillipsburg, NJ. Monoethyl-tritiated DES ([^{3}H]DES), with a specific activity of 81 Ci/mmol, was purchased from Amersham Corp., Arlington Heights, IL. After storage for more than 1 year at 4 °C in benzene at a concentration of 3 μCi/mL, the [^{3}H]DES required purification by Sephadex LH-20 chromatography, as described below. DES monoglucuronide (DES-MG) was purchased in crystalline form, preweighed in glass ampules, from Aldrich Chemical Co., Milwaukee, WI. β-Glucuronidase from bovine liver was purchased from Calbiochem Corp., San Diego, CA. Absolute ethanol was used, and all other organic solvents were glass distilled. All other chemicals were reagent grade.

RIA Buffers. The phosphate-buffered saline (PBS) was that described by England et al. (1974) but containing 0.02% (w/v) merthiolate. The 0.1% gel–PBS included 0.1% (w/v) gelatin, and the concentrated 0.1% gel–PBS was 1.11 times more concentrated. The antiserum buffer was PBS with 0.25% (v/v) normal rabbit serum and 0.05 M disodium ethylenediaminetetraacetate. DES antiserum was diluted 1:12000 with the antiserum buffer. The second antibody was reconstituted with 25 mL of 0.1% gel–PBS. Tracer solution was [^{3}H]DES in 0.1% gel–PBS (3300 dpm/200 μL). Eight DES standards (0.1–2.0 ng of DES/mL) for the RIA standard curve were prepared in 10% ethanol–0.1% gel–PBS.

Sephadex LH-20 Column. The column for purifying DES was packed with 1.00 g (dry weight) of Sephadex