A Method for the Quantitative Determination of 1-Naphthaleneacetic Acid, 1-Naphthalenylacetylaspartic Acid, and β-D-Glucose 1-(1-Naphthalene)acetate in Grapes

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An analytical method has been developed for the determination of 1-naphthaleneacetic acid (NAA), 1-naphthalenylacetylaspartic acid (NAAsp), and β-D-glucose 1-(1-naphthalene)acetate (NAGlu) in Zinfandel grapes. The sample is blended with ethyl acetate and refluxed for 1 h. An aliquot is evaporated to dryness and hydrolyzed for 90 min in 6 N HCl. The hydrolyzed sample is diluted with water and partitioned into dichloromethane which is removed in vacuo (40–50 °C). The sample is redissolved in diethyl ether and partitioned into 0.2 M K_2HPO_4 which is acidified with 5 N H_2SO_4 and the NAA is partitioned into $CHCl_3$ and analyzed by high-performance liquid chromatography and fluorescence detection. Field-treated grapes (four replications) sprayed with 100 and 200 ppm of NAA had average residues of 0.23 and 0.47 ppm with recoveries ranging from 76.1 to 95.5% at a method sensitivity of 0.05 ppm.

Naphthaleneacetic acid (NAA) has been utilized for chemical thinning of apples and olives, prevention of fruiting of ornamental olives, prevention of preharvest fruit drop in apples and pears, and flower induction of pineapples (E.P.A. Compendium of Registered Pesticides, 1974).

Goren and Bukovac (1973) have shown the formation of 1-naphthalenylacetylaspartic acid (NAAsp) and β-D-glucose 1-(1-naphthalene)acetate (NAGlu) in detached primary leaves of 11–14-day-old cowpea plants. Shindy et al. (1973) found when Kinnow mandarin fruits were dipped in aqueous solutions of [^{14}C]-α-naphthaleneacetic acid and extracts of the tissue were analyzed that a significant amount of the NAA was converted into NAAsp and NAGlu. Several investigators have previously published methods for the analysis of NAA by electron affinity (Bache et al., 1964), ultraviolet absorption (Bache et al., 1962), and high-performance liquid chromatography (Cochrane and Lanouette, 1972; Moye and Wheaton, 1979), but the conjugates NAAsp and NAGlu would not have been detected primarily due to the methods of extraction as well as the cleanup procedures.

The method described presently is a rapid, accurate, and sensitive procedure for the quantitative measurement of NAA including its two major conjugates, NAAsp and NAGlu, by high-performance liquid chromatography.

MATERIALS AND METHODS

Apparatus and Reagents. 1-Naphthaleneacetic acid standard was obtained from Chevron Chemical Co., Ortho Division, Richmond, CA 94804.

All chemicals were reagent grade and all solvents were of high-performance liquid chromatography (HPLC) purity.

1-Naphthalenylacetylaspartic acid was prepared synthetically as follows: NAA (2 g, 0.01 mol) was boiled for 2 h under reflux with a mixture of 10 mL of dry benzene and 2 mL (about 2.5 mol) of thionyl chloride. The solvent and excess thionyl chloride were evaporated off under vacuum at 40 °C, and the residue was transferred to a dropping funnel in 7 mL of benzene. A solution of naphthaleneacetyl chloride was added in 1-mL increments over about 1 h to a magnetically stirred solution of 1.4 g (0.01 mol) of DL-aspartic acid in 40 mL of 1 N aqueous sodium hydroxide solution near 0 °C. After another hour at 0 °C, the mixture was shaken for 3 h as it came to room temperature, acidified with 6 N hydrochloric acid (about 7 mL), and allowed to stand for 30 min. The precipitated crystals were removed by filtration, washed, and dried under vacuum at 40 °C to provide 3.56 g of crude product. Two crystallizations, from absolute ethanol and from methanol, provided 0.55 g of pure 1-naphthalenylacetylaspartic acid, mp 163–165 °C. Zenk (1962) gives a mp of 164 °C.

β-D-Glucose 1-(1-naphthalene)acetate standard was utilized as the 1-*O*-(naphthalenylacetyl)-4,6-*O*-benzylidene-β-D-glucopyranose, since the glucoside itself was very unstable. The preparation of the 1-*O*-(naphthalenylacetyl)-4,6-*O*-benzylidene-β-D-glucopyranose was as follows: 10 g of 4,6-*O*-benzylidene-D-glucopyranose (Zenk, 1962) was dissolved in 200 mL of ethanol, the solution was cooled to −5 °C, and 36 mL of 1.0 M sodium methoxide was added. The mixture was allowed to stand at −5 °C for 15 min. The precipitated sodium salt was filtered and washed with absolute ethanol and diethyl ether. The salt was dried under reduced pressure and then suspended in 100 mL of dichloromethane. Naphthaleneacetic anhydride (12.7 g) prepared according to the procedure of Ferrari (1970) was added, and the mixture was stirred overnight. The crude product was filtered and dissolved in 20 mL of boiling 1:1 (v/v) acetone–water. The hot solution was filtered, 80 mL of water was added to the filtrate, and the product was allowed to crystallize at 0 °C. The crystals were filtered, washed with water, and dried at room temperature, yielding 2.5 g of product, which was identified by NMR and IR analysis.

High-Performance Liquid Chromatography Apparatus and Conditions. Components of the HPLC system were Model 995 chromatographic pump (Tracor, Austin, TX), a Rheodyne sample injector valve, Model 7120, with a fixed 20-μL loop, a Fluorichrom fluorescence detector equipped with a deuterium light source (Varian, Palo Alto, CA) and containing an excitation filter (220-nm interference filter, Varian No. 220 I) and a 360-nm band-pass emission filter (Varian No. 7-54 and 7-60 in combination), and a 25 cm x 4.6 mm i.d. stainless steel column packed with Partisil 10 (Whatman, Clifton, NJ). The mobile phase was 0.1% acetic acid, 2.1% 2-propanol, and 97.8% hexane (v/v) at a flow rate of 1.2 mL/min. The detector output was connected to a HP 3390 A reporting integrator set at an attenuation of 2^3 (Hewlett-Packard, Santa Clara, CA 95050), and the chart speed was 0.5 cm/min.

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Table I. Recovery of Residues from Grapes Fortified with NAA and Its Major Conjugates

fortification level, ppm	NAA[a]		NAAsp[a]		NAGlu benzylidine[a]	
	total ppm found	recovery, %	total ppm found	recovery, %	total ppm found	recovery, %
0.05	0.05 ± 0.01	100.0	0.04 ± 0.01	80.0	0.04 ± 0.01	80.0
0.10	0.09 ± 0.02	90.0	0.08 ± 0.01	80.0	0.08 ± 0.01	80.0
0.25	0.21 ± 0.04	84.0	0.20 ± 0.04	80.0	0.18 ± 0.02	72.0

[a] Each value represents the average of five replicate analyses by HPLC with a method sensitivity of 0.04 ppm.

Plant Material and Experimental Design. Plant material consisted of mature cordon-trained grapevines of *Vitis vinifera* L. cv. Zinfandel grown in an irrigated vineyard located in California. The experimental design was that of a randomized complete block with treatments being replicated 4 times. A buffer vine was between each experimental unit. Sodium 1-naphthaleneacetate (NAA, 3.5% active ingredient) was applied in mid-June during the first bloom (0–15% calyptras off) of second crop flowers. Treatments consisted of 0, 100, and 200 ppm of NAA. A wetting agent (Triton B-1956) in the amount of 50 ppm was included with the treatments, and the vines were sprayed to runoff (approximately 1.3 L/vine) with a hand-held Hudson sprayer. Grapes were harvested in October.

Extraction of Samples. Grapes (25 g) were placed in a 1-qt stainless steel jar with 100 mL of ethyl acetate. The mixture was blended for 1 min at medium speed and then transferred to a 500-mL boiling flask. The flask was attached to a condenser, placed on a heating mantle, and allowed to reflux for 1 h. The sample was cooled in an ice bath, and the ethyl acetate was decanted through Whatman No. 1 filter paper into a clean 500-mL round-bottomed flask. The blender jar was rinsed with 100 mL of fresh ethyl acetate, which was then added to the solids remaining in the boiling flask. The mixture was heated under reflux for 1 h, cooled, and filtered. The blender jar was rinsed with 100 mL of ethyl acetate, which was added to the solids and refluxed for 1 h. After the ethyl acetate was decanted the solids were discarded and the pooled extractives were concentrated in vacuo at 50 °C to about 1 mL. Ethyl acetate was used to rinse the flask, the sample was quantitatively transferred to a 10-mL volumetric flask, and it was adjusted to volume.

Hydrolysis. A 1-mL aliquot of the ethyl acetate extract was placed in a 500-mL round-bottomed flask; the sample was dried under a stream of dry nitrogen, 50 mL of 6 N hydrochloric acid was added, and the sample was heated under reflux for 90 min. After the sample had cooled slightly, the sides of the reflux condenser were washed with 25 mL of deionized water. The sample was then further cooled in an ice bath. The cold sample was transferred to a 500-mL separatory funnel, and the flask was rinsed with 100 mL of deionized water followed by 50 mL of dichloromethane. The mixture was shaken for 1 min and allowed to separate. The organic lower layer was filtered through Whatman No. 1 filter paper into a 500-mL round-bottomed flask and the aqueous layer was partitioned with two more 50-mL portions of dichloromethane. The combined dichloromethane layers were concentrated in vacuo (40–50 °C) to about 1 mL and transferred to a 15 × 130-mm glass-stoppered test tube with dichloromethane.

Cleanup. The hydrolyzed extract was dried under a stream of nitrogen and then redissolved in 5 mL of ethyl ether. The sample was partitioned twice with 4 mL of 0.2 M dibasic potassium phosphate. The ether was discarded, and the pooled aqueous layers were partitioned twice with 2 mL of chloroform. The organic layers were discarded, and the aqueous layer was acidified with 1 mL of 5 N sulfuric acid (Moye and Wheaton, 1979). The NAA was partitioned twice with 3 mL of chloroform and analyzed by HPLC.

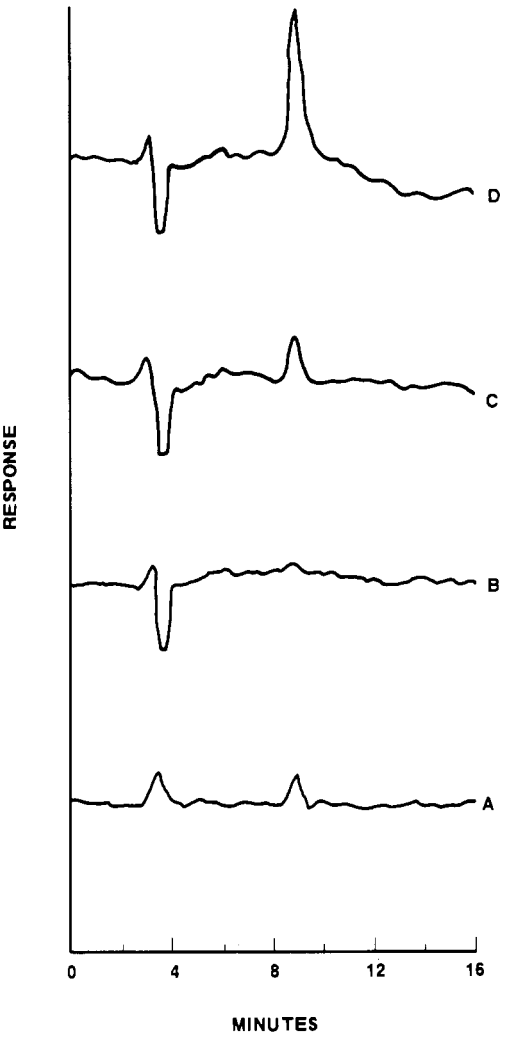

Figure 1. Actual high-performance liquid chromatograms of (A) 0.5 ng of 1-naphthaleneacetic acid standard, (B) the equivalent extractives of 10 mg of untreated Zinfandel grapes, (C) the equivalent extractives of 10 mg of untreated Zinfandel grapes fortified with 0.05 ppm of 1-naphthaleneacetic acid, (D) the equivalent extractives of 10 mg of Zinfandel grapes field-treated with a 100-ppm solution of 1-naphthaleneacetic acid which resulted in a residue at harvest (126 days after treatment) of 0.22 ppm of total 1-naphthaleneacetic acid and its major conjugates.

High-Performance Liquid Chromatographic Analysis. All samples were analyzed by HPLC as total NAA. Quantitation was performed by comparing the peak areas from crop extractives ranging from 0.2 to 20 mg in 20 μL of chloroform to the peak area of 1 ng of NAA standard in 20 μL of chloroform. The response of the detector was linear within an investigated range of 0–2 ng. Greater concentrations of NAA resulted in an off-scale response. The retention time for NAA on the HPLC column was 8.5 min. Control samples had no peaks at this time period.

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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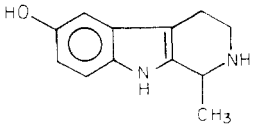
6-Hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline was identified and quantified in various dietary components. The analytical procedure involved the use of a deuterated analogue as the internal standard, extraction with dichloromethane, derivatization with pentafluoropropionic anhydride, and subsequent 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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