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Isolation and Identification of 5-Hydroxyindole-3-acetic Acid and 5-Hydroxytryptophan, Major Allelopathic Aglycons in Quackgrass (*Agropyron repens* L. Beauv.)

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The compounds in quackgrass (*Agropyron repens* L. Beauv.) responsible for inhibiting growth of other plants have been identified as 5-hydroxyindole-3-acetic acid (5-HIAA) and 5-hydroxytryptophan (5-HTP). Identification was accomplished by use of TLC, HPLC, MS, UV, IR, and C,H,N analysis. These compounds accumulate to high levels, throughout the plant, as glucosides attached to the 5-*O*-indolyl moiety in β linkage. 5-HTP is further protected as an N-glucoside on the primary amine. Molecular weights of the glucosides range from 353 [5-(β-D-glucopyranosyloxy)indole-3-acetic acid] to at least 4159. 5-HIAA serves as a growth hormone, and 5-HTP serves as a growth inhibitor in quackgrass. Examples of seedling growth inhibition of corn (*Zea mays* L.) and bean (*Phaseolus vulgaris* L.) are given.

Quackgrass (*Agropyron repens* L. Beauv.), a highly competitive perennial grass weed, has been shown by a number of workers to contain compounds that act as germination and growth inhibitors toward other plants (Ohman and Kommedahl, 1964; Toai and Linscott, 1979; Weston and Putnam, 1986). Most workers have suggested that these compounds are released only from dead quackgrass. They also indicate that these compounds are not phytotoxic unless subjected to some further breakdown, particularly anaerobic degradation in soils (Ohman and Kommedahl, 1964; Toai and Linscott, 1979).

It has been suspected for a long time that plants excrete substances from their roots that are inhibitory to other plant species and may be autotoxic under continuous monoculture. Bonner (1950) reviewed progress in this field of study up through the 1940s. Since that time, a great deal of work has been done and the science of allelopathy has become established. Rice (1984) covered work up to the early 1980s and in his book adopted Molisch's definition of allelopathy, "to refer to biochemical interactions between all types of plants including microorganisms" (Rice, 1984, p 1). Allelopathy refers to stimulatory as well as detrimental effects. This definition of allelopathy will be used in this paper.

Prior to 1987, a number of attempts were made to identify the specific chemicals responsible for the allelopathic effects observed for quackgrass. Although the compounds were not identified, a number of important properties of the compounds were deduced. Notable among these efforts were those of LeFevre and Clagett (1960) and, more recently, Gabor and Veatch (1981). Sikkema and Dekker (1987) noted that although a number of researchers have extracted allelopathic substances from quackgrass, identification of specific compounds and determination of their possible relevance to field observations have not yet been accomplished. They also suggested that there were at least two compounds active in quackgrass, one present throughout the plant suppressing seedling growth (Ohman and Kommedahl, 1960; Gabor and Veatch, 1981) and a second compound, present only in quackgrass leaves, acting as a seed germination inhibitor (Ohman and Kommedahl, 1960). Weston et al. (1987) isolated two flavonoid compounds from dried quackgrass leaves and rhizomes that inhibited radicle elongation in cress *Lepidium sativum* L. Burpee curly. They identified one of these flavonoids as the flavone tricin.

Harborne and Hall (1964) indicated that, while of limited distribution in the plant kingdom, "tricin, in combined form, is a characteristic grass flavone". They also con-

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firmed that tricin, as a glucuronide, was present in alfalfa (*Medicago sativa* L.). Alfalfa seedlings are particularly sensitive to allelopathic compounds from quackgrass. On the basis of the foregoing information, it was felt that the presence of tricin and related flavonoid compounds (while contributory to observed allelopathic effects) was insufficient to explain the accumulated evidence related to quackgrass allelopathy. Quackgrass should differ significantly from other grass species biochemically. It should contain unique compounds or accumulate unusually high levels of compounds, like tricin, relative to other grasses.

The objective of this research was to identify unique compounds in quackgrass that, alone or in combination with flavonoids, could explain observed allelopathic effects.

EXPERIMENTAL SECTION

Reagents and Standards. All reagents, solvents, and standards were analytical grade. Colorimetric reagent chemicals were purchased from Sigma Chemical Co. along with the following standards: indole-3-acetic acid (IAA), anhydrous, crystalline; 5-hydroxyindoleacetic acid, (5-HIAA), 98-100%; 5-hydroxytryptamine hydrochloride 5-HT·HCl; 5-hydroxy-L-tryptophan (L-5-HTP), 99%; L-tryptophan (L-TP), anhydrous, crystalline. DL-5-Hydroxytryptophan (DL-5-HTP) (99%) and tetrabutylammonium hydroxide (40%) were purchased from Aldrich Chemical Co. All solvent operations and derivatization reactions were carried out in a fume hood.

Bioassay. Ten seeds of alfalfa (*M. sativa* L. Saranac AR), corn (*Zea mays* L. PAG SX155), or beans (*Phaseolus vulgaris* L. Redkloud) were placed in each Petri dish fitted with two layers of filter paper. The paper was moistened with 10 mL of an aqueous solution of the isolate or pure chemical for corn or beans (5 mL for alfalfa). Treatments, including an untreated control, were replicated five times. The dishes were placed in a dark incubator for 3 days at 20 °C. Growth of shoots and roots was measured to the nearest millimeter. Quackgrass (*A. repens* L. Beauv.) rhizome tests were performed on single node segments of rhizomes harvested in April 1987. At this time two-thirds of the buds had broken dormancy; these were discarded and only 15-mm segments of healthy rhizomes containing one dormant bud each were utilized (to minimize internal hormone effects). Five segments were used per Petri dish, along with two filter papers and 10 mL of pure chemical solution. As above, each treatment was replicated five times. The test was run at 20 °C in a dark incubator for 8 days. Measurements were the same as for other species on shoot and root growth. In addition, the number of buds breaking dormancy were counted.

Growth measurement data were subjected to logarithmic transformation prior to statistical analysis. Treatment and control means were reconverted for graphic representation. Where appropriate, an analysis of variance and Duncan's multiple-range test were performed to test significance. Linear regression analysis was performed where different concentrations of one chemical were compared to control values. Alfalfa, the most sensitive species tested, was used to track allelopathic activity through purification steps only, and associated growth values relating to pure chemicals will be presented elsewhere.

Materials. Quackgrass roots and rhizome (roots) as well as stems and leaves (tops) were collected from an established quackgrass sod in Tompkins County, NY, in July 1985. The plants had just completed pollination at the time of harvest. The harvest site had been left in a natural state, and no herbicides had been used there for over 3 years. Tops were separated from roots. Roots were washed several times with tap water, rinsed with distilled water, and blotted dry with absorbent paper. Both tops and roots were subdivided, a portion was stored in plastic bags in a freezer set at −20 °C (hereafter referred to as fresh plant material), and the remainder was oven-dried at 60–70 °C. Dried plant material was ground in a Wiley mill, to pass a 40-mesh screen, and stored in glass bottles in the freezer at −20 °C until extraction. Initially, all identification attempts were made with dried root material.

Sample Extraction and Purification. Plant material (whether fresh or dry) was extracted successively with two solvent systems: first with 80% methanol/20% water (v/v) and second with 80% 2-propanol/20% water. Each successive solvent was added at a ratio of 10 mL of solvent to 1 g of plant material. The sample/solvent mix was blended for 5 min in a Pyrex jar with a Tekmar Super Dispax ultrasonic blender equipped with a 45-mm head and Teflon bearings. Following the first solvent blend, the solvent was filtered off in a Buchner funnel employing VWR No. 613 filter paper. The residue plus filter was mixed with the second solvent and the mixture reblended for an additional 5 min, followed by filtration. This method, combined with an alfalfa bioassay test, was found to maximize the extraction of growth inhibitors while minimizing the level of more polar solutes. The alfalfa bioassay was an adaptation of the method employed by Toai and Linscott (1979).

The two extracts were combined and reduced to dryness in a rotary vacuum evaporator and a water bath set at 50 °C (all solvent evaporations were performed this way). The sample was weighed and dissolved in distilled water, and the pH was adjusted to 7.5 with 5% $NaHCO_3$ in water (w/v). This extract, with appropriate solvent rinsings, was transferred to a separatory funnel and extracted twice with hexane. This treatment, labeled lipids, removed most pigments, lipids, and waxes. Alfalfa bioassay indicated that there was no allelopathic activity in the lipid fraction and it was discarded in further work. The remaining water solution was acidified to pH 2 with 0.01 N HCl and extracted twice with diethyl ether (which had passed through activated alumina to remove possible peroxides). The residue from the ether fraction was deemed to contain organic acids and contained no allelopathic activity toward alfalfa seedlings; it, too, was discarded. The remaining water fraction was adjusted to pH 7.5 with 5% $NaHCO_3$, and remaining traces of diethyl ether were removed in the rotary vacuum evaporator. All allelopathic activity toward alfalfa seedlings resided in the water fraction, inhibiting both root and shoot growth. No alfalfa seed germination effects were found in any of the extracts or fractions from fresh or dried roots or shoots.

The water extract was passed through a 25-mm diameter × 100-cm height glass column, packed with Amberlite XAD-2 resin, analytical grade. The adsorbed material was washed with five column void volumes of distilled water. All allelopathic activity toward alfalfa seedlings remained on column. The column eluate contained amino acids, salts, and sugars (principally triticin; Arni and Percival, 1951). This was labeled as water soluble and discarded.

The mixture adsorbed to XAD-2 was eluted with 70% methanol/30% H_2O (v/v). All measurable allelopathic activity toward alfalfa seedlings was removed with this solvent. The eluted material was evaporated to dryness in the rotary vacuum evaporator and weighed. The resulting dried mixture was crystalline and brown. It was soluble in H_2O/methanol/2-propanol, nearly insoluble in 1-butanol, and insoluble in diethyl ether and less polar solvents. This material was labeled as phenolic glycosides.

Carbohydrate Determinations. Initially, samples of phenolic glycosides (200 mg) were mixed with 20 mL of 7% HCl in water (w/v), sealed in Teflon-capped Pyrex reaction vessels, and heated at 100 °C for 1 h. The cooled hydrolysis mixture was extracted three times with 1-butanol, which removed the released aglycons. The remaining aqueous solution was neutralized to pH 7.0 with 5% $NaHCO_3$. Traces of 1-butanol were removed by evaporation. The remaining aqueous solution was analyzed for sugar content by gas chromatography, using the method of Sweeley et al. (1963).

Stereoconfiguration of the glucosides was determined by subjecting 1-g samples of the phenolic glucosides to hydrolysis by either α- or β-glucosidase with methods of Sumner and Somers (1953) and continuous liquid/liquid extractor with 1-butanol as the extractant for released aglycons. The hydrolysis was conducted for 72 h. The hydrolytic system was tested with *p*-nitrophenyl β-D-glucopyranoside prior to attempting to hydrolyze quackgrass phenolic glucosides. β-Glucosidase released *p*-nitrophenol while α-glucosidase did not.

Aglycon Isolation from Phenolic Glucosides. Phenolic glucosides isolated from dried plant material proved to be extremely complex although they appeared to be based on aglycons absorbing UV light at a maximum at 278–280 nm. Further, isolated aglycons proved to be unstable, converting to a number of other compounds in either water or alcohol solvents. A hydrolytic method was devised that worked well with phenolic glucosides isolated from fresh plant material. One gram of isolated phenolic glucoside was mixed with 50 mL of 0.1 N HCl, placed

in a 50-mL sample bottle, flushed with N_2, and sealed with a Teflon cap. The bottle was stored in a refrigerator set at 5 °C for 72 h. It was shaken at least three times in this period. After 72 h the hydrolysate was extracted three times with 1-butanol. The combined 1-butanol fractions were extracted in a separatory funnel with distilled water to remove excess HCl. Either isolated aglycons were used directly or the 1-butanol (after drying over $MgSO_4$) was removed in the rotary vacuum evaporator and the remaining aglycons were stored in the dry state.

Fractionation of Phenolic Glucosides by Molecular Size. Phenolic glucosides were split into five column fractions on a 45-mm diameter × 55-cm height glass column packed with Sephadex G-25, medium size. The sample was dissolved in a small volume of distilled water, applied to the column, and eluted with distilled water. Fractions were made, based on visible color or UV absorption intensity at 280 nm. All fractions eluted in about five column void volumes. Fraction 1 included material beyond the exclusion limit of Sephadex G-25 (>5000 MW). Fractions 4 and 5 included monoglucosides of cinnamic acids (~310 MW). All fractions showed allelopathic activity toward alfalfa seedlings, but activity was concentrated in fractions 4 and 5.

Fractionation of Dried Quackgrass Root Phenolic Glucoside Aglycons by Molecular Size. The hydrolytic method employed in carbohydrate determinations was used to provide a free, dry aglycon fraction from the total phenolic glucoside isolate. The isolated aglycons were dissolved in 50/50 (v/v) methanol/H_2O, and applied to a XAD-2 column. The column was then flushed with five column void volumes of H_2O to remove traces of salts and sugars. The adsorbed aglycons were removed from the column with 70% methanol/30% H_2O (v/v) and concentrated to dryness. Aglycons were fractionated on a 25-mm diameter × 100-cm height glass column packed with Sephadex LH-20. Aglycons were dissolved in a small volume of methanol and eluted with methanol. Fractions were separated on the basis of visible color or UV absorption at 280 nm. Seven fractions were produced in about five column void volumes. Fraction 1 occupied the first column void volume. It still displayed UV absorption at 278–279 nm, indicating that hydrolysis of the total phenolic glucoside isolate had been incomplete. Fractions 1–5 showed allelopathic activity toward alfalfa seedlings with activity concentrated in fractions 4 and 5. Fractions 6 and 7 showed no measurable allelopathic activity. Colored material gradually built up on both Sephadex columns, and they were periodically cleaned with 0.1 N HCl followed by a distilled water rinse. There was no measurable allelopathic activity in the cleanup fractions.

TLC and HPLC of Aglycons. The most useful TLC system for quackgrass phenolic aglycons employed silica gel G plates (0.25-mm-thick layer) and a solvent mixture of 1-butanol/acetic acid/H_2O, 4/1/1 (v/v/v). Visualization reagents were prepared and used as found in the *Handbook of Chromatography* (1972). Sulfuric acid and charring was used for general visualization. Ferric chloride–ferricyanide reagent was used to visualize phenolic compounds. Modified Ehrlich reagent [(*p*-dimethylamino)-cinnamaldehyde] was used to visualize nitrogen heterocyclic compounds. Ninhydrin was used to visualize primary amines. The specific reagent, nitrosonaphthol–nitrous acid was used to visualize 5-hydroxyindoles. Long-wave (360-nm) UV light was used to expose fluorescent compounds. TLC systems and listed R_F values listed by Jepson (1969) for indoles and related compounds were used for confirmation of identities of 5-HIAA and 5-HTP.

HPLC was conducted on a Micromeretics Model 7000 high-pressure liquid chromatograph. A stainless steel column, 4.6-mm i.d. × 25-cm length packed with 5-μm Spherisorb ODS (obtained from Sulpelco), was used for separations. The solvent system employed was 70% pH 7.5 phosphate buffer (containing 0.01 M tetrabutylammonium hydroxide as an ion pair reagent)/30% methanol. The flow rate was set at 0.15 mL/min and temperature at 20 °C. The detector employed was fixed UV emitting at 280 nm. Under these conditions 5-HIAA eluted at ~23, 5-HTP at ~25, TP at ~29, and IAA at ~33 min, respectively. Column retention times increased over a matter of months. Quantitation of the above listed compounds was achieved by measurement of recorded peak areas and comparison to standard curves of known standards. Under the conditions of analysis employed, IAA could be detected at 0.01 ppm relative to dry weight of plant material.

UV, IR, Mass Spectral, C,H,N,O, and Elemental Analysis. UV/vis spectrophotometry was performed with use of a double-beam spectrophotometer (Beckman Model DB). It was operated in the spectral range 220–700 nm, utilizing 1-cm quartz cells maintained at 20 °C.

IR spectrophotometry was performed with a double-beam infrared spectrophotometer (Perkin-Elmer Model 783) operating in the frequency range 4000–200 cm^{-1}. KBr pellets were used to contain samples.

C,H,N analyses were performed utilizing a carbon, hydrogen, nitrogen analyzer (Hewlett-Packard, Model 185). Oxygen was determined by difference since other elements were not present. Samples of 0.7 mg were used in the analysis.

Qualitative elemental analysis was performed according to methods of Pasto and Johnson (1969).

Mass spectral analysis was performed at the Mass Spectrometer Facility, Chemistry Department, Cornell University, Ithaca, NY 14853-0144. Isolated aglycons and phenolic glucosides as well as standards were subjected to both EI and CI mass spectrometry. Chemical ionization mass spectrometry employed methane as the reagent gas. Spectra were recorded as the samples were heated from ambient temperature to 500 °C. The presence and identification of cinnamic acid derivatives in phenolic aglycon fractions were determined by EI GC–MS of TMS derivatives. A 3% OV 101 liquid phase was employed and temperature programmed from 125 to 250 °C. Peak identification was accomplished by a PBM and STIRS Version 4.0 computerized mass spectral identification program. Mass spectral interpretation, as required, was performed by R.D.H.

RESULTS AND DISCUSSION

Initial attempts to characterize the active allelopathic compounds extracted from dried quackgrass roots (using the alfalfa bioassay to test allelopathic activity, TLC, and various color reagents) indicated that active compounds were phenolic and contained sugar. Color tests indicated that the sugar was either glucose or galactose. The materials were soluble in H_2O or methanol but poorly soluble or insoluble in less polar solvents. Attempts to hydrolize materials remaining in H_2O solution, after lipids and organic acids were removed by solvent partition, produced active aglycons, but they could not be isolated by conventional techniques and left streaks on paper chromatograms and TLC plates.

Allelopathic activity appeared to be concentrated, in these chromatograms, in areas of high fluorescence under UV irradiation. This activity, along with the location of fluorescent spots, changed during the time that aglycons remained in solution (whether in H_2O or methanol) and indicated that the released aglycons were unstable.

Active phenolic glycosides, isolated from quackgrass extracts, comprised 16.5 mg/g dw (dry weight) of dried roots and 20.4 mg/g dw of dried tops. UV analysis of this material from either roots or tops produced a spectrum with a maximum absorption at 278–280 nm with a shoulder at 310–320 nm. Infrared spectral analysis, using the Sadtler Chemical Class Spec-Finder (1966) for spectral band comparison, suggested that the glycosides contained mixed aromatic–heterocyclic compounds with three functional groups. The presence of aromatic –OH was inferred from the phenolic nature of the glycosides. Elemental and C,H,N analyses indicated the presence of only C, H, N, and O. IR absorption peaks at 1740 and 1600 cm^{-1} suggested carboxyl carbonyl and C–N stretches, respectively, accounting for the other functional groups. The phenolic glucosides did not respond to ninhydrin, inferring that the nitrogen function was, at least secondary, and probably existed in the heterocyclic ring.

Acid hydrolysis released most of the aglycons from the sugars. The released sugar was proven to be solely glucose by GLC chromatography of TMS sugar derivatives. The aglycons that could be hydrolyzed were shown to be in β

configuration with attached glucose by subjecting the phenolic glycosides to hydrolysis with either α- or β-glucosidase. Only β-glucosidase released aglycons from the glucosides.

Column chromatography of the phenolic glucosides of dried quackgrass tops, on Sephadex G-25, produced five fractions labeled 1–5. Of an original 22.7 mg/g dw, of plant material, a total of 20.4 mg/g dw (90%) was recovered. The following column fraction yields were produced (mg/g dw): 1, 1.25; 2, 6.35; 3, 12.37; 4, 0.35; 5, 0.05.

Column chromatography of the phenolic glucosides, of dried quackgrass roots, on Sephadex G-25, produced five fractions labeled 1–5, corresponding to fractions 1–5 from dried quackgrass tops. The following fraction yields (mg/g dw) were produced: 1, 7.39; 2, 4.43; 3, 1.10; 4, 1.79; 5, 0.53. A total of 15.2 mg/g dw (92%) of the original material was recovered. Further analytical work on dried quackgrass was concentrated on phenolic glucosides from dried roots.

C,H,N analysis of dried root, fraction 1, produced an empirical formula of $C_{156}H_{285}NO_{124}$. Assuming only one compound, this fraction would have a molecular weight of 4159. C,H,N analysis of column fraction 5 produced an empirical formula of $C_{17}H_{22}N_2O_8$, molecular weight 382. This compound responded negatively to ninhydrin, indicating that there was no available primary amine function. The compound showed a UV maximum at 279 nm and an IR spectrum closely matching that of total phenolic glucosides. Assuming that the compound was a monoglucoside, and subtracting $C_6H_{10}O_5$, an aglycon with the formula $C_{11}H_{12}N_2O_3$ was left, which matched the formula for 5-hydroxytryptophan (5-HTP). The lack of activity with ninhydrin supported the view that the compound in fraction 5 was the N-glucoside of 5-HTP.

All five Sephadex G-25 column fractions, subjected to EI mass spectrometry, produced spectra with a prominent peak, often the base peak, at m/z 146, characteristic of 5-hydroxyindole compounds (Jamieson and Hutzinger, 1970). This was confirmed by CI MS. A prominent fragment, in Sephadex column fraction 4, released at a probe temperature of 400–440 °C, at an intensity of 33% of the base peak, occurred at m/z 353, which corresponded to the monoglucoside of 5-hydroxyindoleacetic acid. A detectable peak at m/z 382, in fractions 4 and 5 corresponded to the monoglucoside of 5-HTP. CI mass spectrometry of fractions 1 and 2 showed strong peaks at m/z 130, typical of indoles (Jamieson and Hutzinger, 1970), and at m/z 133, in the spectrum of DL-5-hydroxytryptophan, as well as 5-hydroxyindoleacetic acid (Base peak 146).

An additional high molecular weight peak was detectable in all five fractions at m/z 577, which corresponded to $C_{20}H_{36}NO_{18}$ and represented the side chain of 5-HTP or TP cleaved β to nitrogen with nitrogen attached to a chain of three glucosyl units (m/z 162). The m/z 577 peak represented a fragment of higher N-glucosides. All diglucosides and higher glucosides had one molecule of H_2O strongly associated.

Sephadex G-25 fractions 1–5 responded positively when reacted on TLC plates with 1-nitroso-2-naphthol–nitrous acid, a specific color reagent for 5-hydroxyindole compounds.

Aglycon Characterization. The aglycon mixture, isolated from dried quackgrass roots, proved to be much more active than the phenolic glucosides as growth inhibitors toward alfalfa seedlings. UV/vis spectrophotometry of dried quackgrass root phenolic aglycons, in methanol solution, displayed an absorption maximum at 278–280 nm and a shoulder in the region between 310 and 320 nm. The shoulder changed with time (days) in solution. The solutions became darker (yellow to brown) over time.

The aglycon mixture was separated by molecular size on a Sephadex LH-20 column with methanol as the eluant. Seven fractions were isolated, indicating that hydrolysis was incomplete. The first fraction eluted with the column void volume. As with Sephadex G-25, fractions 4 and 5 contained most of the allelopathic activity toward alfalfa seedlings. Mass spectrometry using EI and CI indicated the same or similar results found with phenolic glucoside fractions. GC–MS of TMS derivatives of the seven fractions showed that fractions 4–7 contained low levels of cinnamic acid derivatives. These were not quantitated, but it was estimated that they totaled no more than 5 ppm/g dw of root material. They were estimated in relative concentration as ferulic > *p*-hydroxybenzoic > vanillic > *o*-coumaric > sinapic > caffeic acids. These compounds accounted for the UV absorption shoulder at 310–320 nm found in both phenolic glucosides and aglycon fractions.

TLC of aglycons (Jepson, 1969) confirmed the presence of both 5-HIAA and 5-HTP in the aglycon mixture. With this information, it became increasingly apparent that dried plant material could contain any or all of the potential degradation or addition products of these compounds. Undenfriend et al. (1956) indicated that 5-HIAA was unstable following both base and acid hydrolysis of its conjugates. Amadori addition and Maillard browning products may result from reaction of TP or 5-HTP with glucose. Production of these products was confirmed for 5-HTP by the methods of Lee et al. (1979) for TP. Reaction of 5-HTP with glucose produced the analogous reaction products Lee and co-workers found for the reaction of TP with glucose. The reaction products of 5-HTP and glucose had an $R_f \times 100$ of 2 units less than TP + glucose products. further, it was obvious that the fluorescence observed, related to allelopathic compounds, was largely due to these addition products. These products, by themselves, may have allelopathic activity, but this line of investigation was not pursued. Lee and co-workers (1979) indicated that TP–glucose addition products were physiologically active in mammalian systems.

Attempting to assay the multitude of 5-HTP addition compounds proved difficult. It was decided to estimate total 5-hydroxyindole levels in dried plant materials by UV analysis of methanolic solutions of total isolated phenolic glucosides. Analysis was accomplished by absorption measurement at 279 nm with a standard curve for 5-HTP. This method avoided further alterations caused by hydrolytic steps. Dried tops and dried roots contained 2270 and 550 μg/g dw, respectively (as 5-HTP equivalent).

Further work was carried out with fresh plant material. No IAA was detected in any of the isolated fractions. Levels of 5-HIAA, 5-HTP (both inhibitory to alfalfa seedling roots), and the precursor TP are presented in Table I. 5-Hydroxytryptamine (5-HT), a potential intermediate between 5-HTP and 5-HIAA, which has been shown to possess auxin activity (Grosse, 1982), was not found in any of the extracts from either fresh or dry plant material. Sosa (1980) reported that TP is strongly retarded by Sephadex gels, and Schneider et al. (1972) reported that 5-HT was strongly absorbed on Sephadex G-25 at neutral pH. Tests on acidic column cleanup fractions from XAD-2, Sephadex G-25, or Sephadex LH-20 were negative for 5-HT. Pure 5-HT had no significant effects on quackgrass rhizome segments, corn, bean, or alfalfa seedlings in the range 10^{-11}–10^{-5} M, and the results of these tests are not shown.

Table I. Phenolic Glucoside Yield and Active (5-HIAA, 5-HTP) and Precursor (TP) Aglycon Contents of Fresh Quackgrass Tops and Roots

sample	yield, mg/g dw	μg/g dw equiv 5-HIAA	5-HTP	TP	aglycon, % total glucosides
		Fresh Tops			
total phenolic glucosides	16.7				
column fraction 1	1.33	2	<0.1	<0.1	0.1
column fraction 2	2.00	14	150	<0.1	8.2
column fraction 3	2.33	42	203	<0.1	10.5
column fraction 4	7.33	200	1900	<0.1	28.6
column fraction 5	3.67	660	1320	506	67.7
total recovered	16.7	918	3593	506	
% recovery	100				
		Fresh Roots			
total phenolic glucosides	92.0				
column fraction 1	25.67	903	<0.1	663	6.1
column fraction 2	41.67	1750	<0.1	511	5.4
column fraction 3	11.67	550	333	<0.1	7.6
column fraction 4	0.83	94	14	<0.1	12.9
column fraction 5	0.83	121	20	<0.1	16.9
total recovered	80.7	3418	367	1174	
% recovery	88				

Summarizing identification work, 5-HIAA and 5-HTP were found to be the active allelopathic aglycons isolated from quackgrass, whether from fresh or dried plant material. UV and IR spectra of the pure compounds accounted for most of the representative spectral absorption peaks in phenolic glucosides not accounted for by glucose. The remaining peaks were accounted for by cinnamic acid derivatives present in amounts too low to account for observed allelopathic activity. N-Glucosylation was confirmed by colorimetric tests and mass spectrometry. β configuration of aglycon–glucose linkage was shown by enzymatic analysis. Glucose proved to be the sole sugar in the glucosides, as indicated by colorimetric tests and GLC. D or L configuration of 5-HTP was not proven, but IR spectra of 5-HTP, isolated from dried quack grass roots, most closely matched the spectrum of DL 5-HTP. Mass spectrometry of phenolic glucosides indicated that glucose was attached to the 5-*o*-indolyl moiety of 5-HIAA and 5-HTP. Some evidence by IR and MS suggested that the carboxyl groups of these aglycons existed in glucose ester linkage. Finally, the isolated aglycons cochromatographed with authentic standards in both the TLC and HPLC systems.

Examining Table I, the following observations were made. Considering fresh plant material, roots accumulated phenolic glucosides rich in 5-HIAA with little 5-HTP (~ 10/1 ratio). Substantial amounts of TP were stored in higher glucosides. Tops were rich in lower phenolic glucosides containing ~2× as much 5-HTP as 5-HIAA. Only fraction 5 in fresh tops (smallest glucosides) contained substantial amounts of TP. The aglycon percent in fraction 5 indicated that some of the aglycons existed in the free state (probably 5-HIAA since fraction 5 did not respond to ninhydrin, suggesting a masking of primary amine by glucose).

From the results given, it would appear that quackgrass is a hyperaccumulator of 5-HIAA and 5-HTP as their glucosides. 5-HIAA and 5-HTP levels of 918 and 3593 μg/g dw, respectively, were found in fresh tops, as well as 3418 and 367 μg/g dw, respectively, in fresh roots.

TP also accumulated as glucosides, particularly in roots. Both TP and 5-HTP may serve as nitrogen reservoirs for amino acid synthesis. Glucoside formation with these compounds should prevent anthranilate synthetase feedback inhibition, as discussed by Gross (1982), and thereby give these compounds a role in ammonia detoxification.

Comparing dried to fresh plant material (Table I), drying increased phenolic glucoside content in top material (from 16.7 to 22.7 mg/g dw). The increase in glucoside content occurred as total aglycon content (5-HIAA + 5-HTP + TP) fell from 5017 to 2270 μg/g dw (5-HTP equiv), a 55% loss. In roots, drying decreased phenolic glucoside content from 92 mg/g dw in fresh material to 16.5 mg/g dw, an 82% loss. Total aglycon content decreased from 4959 μg/g dw (5-HIAA + 5-HTP + TP) to 550 μg/g dw, an 89% loss.

Clearly, drying caused substantial losses of allelopathic compounds. Extracts of tops were more allelopathic than those of roots because there was a higher level of lower glucosides and free aglycons (of 5-HIAA and 5-HTP) in tops relative to roots. Additionally, there was much less degradation of allelopathic compounds in the course of drying top material. Phenolic glucosides from dried plant material were more stable toward hydrolysis than those from fresh plant material. It appeared that drying of plant material increased resistance of remaining phenolic glucosides toward degradation in the environment.

Thimann (1958) listed 5-HIAA as a weak auxin, only 0.01 as active as IAA in avena coleoptile and split pea stem curvature tests. Fellows and Bell (1970) found 5-HTP and 5-HIAA in a West African legume *Griffonia simplicifolia* reported to have physiological effects on humans. They speculated that 5-HIAA might serve as a growth hormone in this plant.

Pure solutions of 5-HIAA and 5-HTP were tested on quackgrass rhizome bud segments, germinating seed of corn or beans. Data points presented in Figures 1–3 are all significantly different at the 5% level. There was no significant effect of either compound on germination, and the data are not presented here. Levels of compounds tested were varied from 10^{-11} to 10^{-5} M, including a distilled H_2O control. This range of concentrations was chosen to encompass the effective concentration range of IAA on roots and buds in other plants.

Tests of 5-HIAA (Figure 1A) and 5-HTP (Figure 1B) on quackgrass rhizome segments indicated that 5-HIAA acted as a growth hormone and 5-HTP acted as a growth inhibitor in quackgrass. 5-HIAA was generally as active in quackgrass as Thimann (1958) found IAA to be in other plants. 5-HIAA showed maximum activity in the concentration range between 10^{-9} and 10^{-8} M for both root and shoot growth, with some stimulation of bud development.

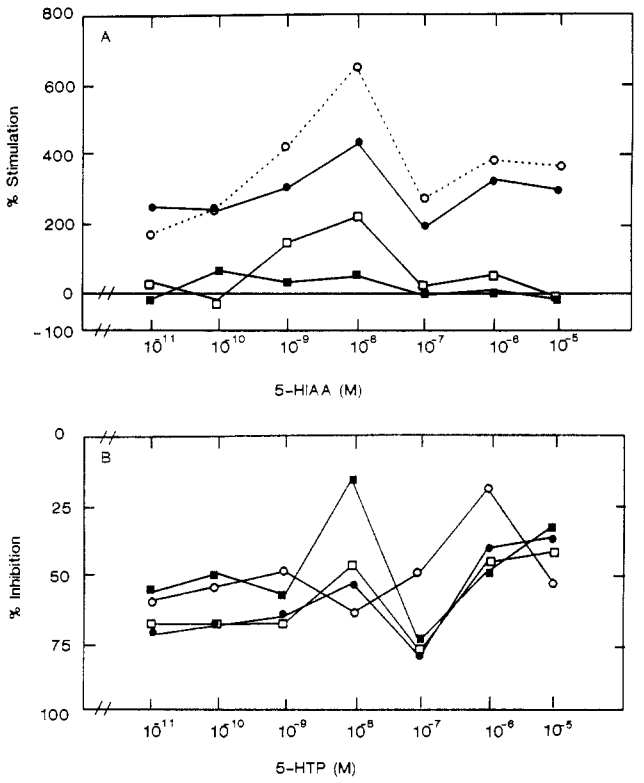

Figure 1. Quackgrass rhizome bud activation and growth relative to 5-HIAA (A) or 5-HTP (B) concentration. Dose–response curves relative to control (0 on ordinate scale): root numbers (○), total root length (●), total shoot length (□), number of developing buds (■). Control values: root numbers, 6; total root length, 124 mm; total shoot length, 87 mm; number of developing buds, 6 of 25 possible.

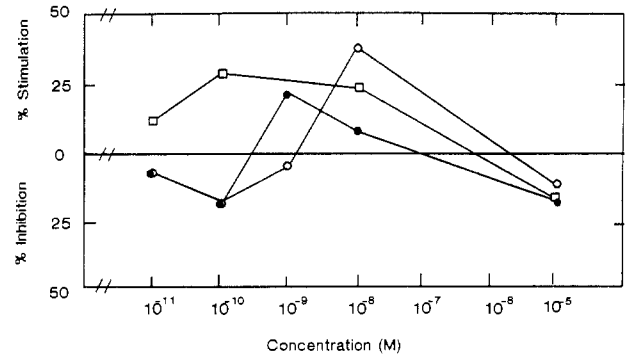

Figure 2. Dose–response curves for corn roots and shoots relative to 5-HIAA or 5-HTP: 5-HTP effect on root growth (○), 5-HTP effect on shoot growth (●), 5-HIAA effect on root growth (□). There was no significant effect of 5-HIAA on shoot growth. Control values: root growth, 64 ± 1 mm; shoot growth, 64 ± 1 mm.

No IAA had been previously detected in extracts of untreated field grown quackgrass, by either TLC or HPLC. The detection limit of the HPLC analytical system used was 10 ng/g fresh weight of plant material for both IAA and 5-HIAA. Standard methods for isolating free or bound IAA in plant material were tried without success. This work indicated that 5-HIAA could be an active growth hormone in some species.

5-HTP was generally inhibitory at all concentrations tested on quackgrass rhizome segments (note the difference in scale between parts A and B of Figure 1). There was a minimum inhibition of bud development at 10^{-8} M. Free 5-HTP appeared to be inhibitory at extremely low levels (10^{-11} M in this case). Developing buds, total shoot length, and total root length showed a maximum inhibition at 10^{-7} M. It was probable that 5-HTP was being converted to 5-HIAA with maximum activity at 10^{-8} M 5-HTP. The nature of inhibition was unclear. 5-HTP may have acted as a growth hormone, a growth inhibitor, or a competitive inhibitor for processes requiring TP as reported (Last and Fink, 1988) for the inhibitory properties of 5-methylanthranilic acid in the plant biosynthesis of TP. The general trend toward less inhibition at higher concentrations was consistent with the hypothesis that 5-HTP could act as an anti-auxin (Åberg, 1957). More specifically, in this instance, 5-HTP appeared to act as a competitive auxin antagonist. Since 5-HIAA (applied to quackgrass rhizome segments at 10^{-11} M concentration) stimulated development, it could be assumed that native auxin was present at suboptimal concentrations. At this level a competitive auxin antagonist would express root growth inhibition. If 5-HTP was converted to 5-HIAA in quackgrass rhizome segments, then increasing levels of 5-HTP should lead to increasing levels of 5-HIAA. At some point 5-HIAA levels should become optimal. Further additions of 5-HTP would lead to accelerated growth at some concentration beyond the 5-HIAA optimum. Testing of this premise will be the subject of further research. See Åberg (1957, p 174) for additional discussion of competitive auxin antagonists.

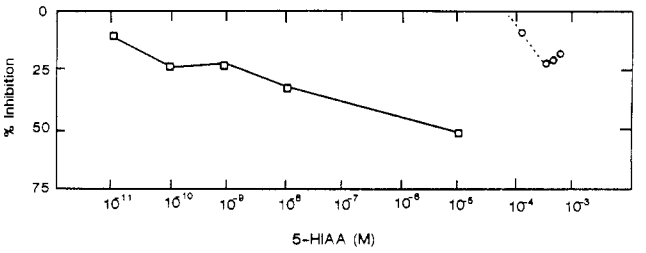

Figure 3. Dose–response curves for red kidney bean roots and shoots relative to 5-HIAA concentration: roots (□), shoots (○). There was no significant effect of 5-HIAA on shoot growth below 10^{-4} M. There was no significant effect of 5-HTP on either root or shoot growth. Control value: root length, 74 ± 1 mm.

The effects of 5-HIAA and 5-HTP on corn (Figure 2) and kidney bean (Figure 3) seedlings demonstrate the range of effects these compounds have on various plant species. In Figure 2 it is shown that 5-HIAA stimulated corn root growth at levels below 10^{-6} M, with a maximum between 10^{-8} and 10^{-10} M. Seiler (1951) estimated the native auxin content of corn seedling roots as equivalent to approximately 10^{-9} M IAA. There was no effect of 5-HIAA on shoot growth, and the results are not shown. 5-HTP stimulated root growth with a maximum at 10^{-8} M and shoot growth with a maximum at 10^{-9} M. This effect must have been independent of conversion to 5-HIAA in shoots since 5-HIAA was without effect on shoot growth. These results suggest that 5-HTP has auxin or anti-auxin (Åberg, 1957) activity in corn and that 5-HIAA can act as a root hormone in this plant. 5-HTP was not tested at concentrations greater than 10^{-5} M since a number of authors have indicated that TP was inhibitory to cress root growth in concentrations of about 5×10^{-4} M (Åberg, 1957).

In Figure 3, 5-HIAA had no effect on kidney bean shoot growth in the range 10^{-11}–10^{-5} M (not shown) but became inhibitory above 10^{-4} M. 5-HIAA inhibited root growth at all levels tested. Endogeneous auxins may exist in bean roots at supraoptimal levels, and therefore all added dosages would be increasingly inhibitory (Åberg, 1957). 5-HTP had no significant effect on either shoots or roots, and the results are not shown.

The root growth inhibitory effects shown with 5-HIAA

and 5-HTP are typical of root growth inhibition caused by excessive ethylene production and suggest that both of these compounds either act as auxins or affect auxin–ethylene interactions (Chadwick and Berg, 1970).

The aglycon tricin, identified by Weston et al. (1987) as an allelopathic compound in quackgrass, was not found in significant levels in this study. If present as such, it would have been isolated in the lipid or organic acid fractions during liquid/liquid partition of quackgrass extracts. It is probable that flavonoid compounds in quackgrass exist as glycosides resistant to hydrolysis (Harborne and Hall, 1964) and, as glycosides, are inactive as growth inhibitors. The relatively slow, low-temperature plant-drying methods employed by Weston et al. (1987) probably contributed to hydrolysis of flavonoid glycosides while the methods employed in this study limited their hydrolysis.

Phenolic compounds such as tricin are known to inhibit auxin oxidases occurring in plant roots. It is therefore conceivable that these flavonoid compounds act in concert with 5-HIAA and 5-HTP to produce supraoptimal levels of growth hormones in seedlings of susceptable species, thereby leading to growth inhibition.

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