Oat Phenolics: Avenalumic Acids, a New Group of Bound Phenolic Acids from Oat Groats and Hulls[1]

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ABSTRACT

A new group of phenolic acids was found in aqueous alcoholic extracts of both oat groats and hulls. These acids occurred as conjugates covalently linked to the amine function of several different orthoaminobenzoic acids. One of the conjugates was a pale yellow crystalline solid with a molecular weight of 325 ($C_{18}H_{15}NO_5$). Mass spectral studies revealed an acid composed of $C_{11}H_{10}O_3$ and a molecular weight of 190. Structural analysis (^{1}H-, ^{13}C-nuclear magnetic resonance, ultraviolet, etc.) allowed formulation of the acid as 5-(4′-hydroxyphenyl)-penta-2,4-dienoic acid (i.e., 4′-hydroxycinnamylidene-acetic acid), for which the trivial name avenalumic acid is proposed. Comparison of proton coupling constants of the naturally occurring conjugate with models suggests that the oat constituent is an interconvertible mixture of the 2-*E*,4-*E* and 2-*Z*,4-*E* isomers, with the former isomer predominating. This assignment was confirmed by total synthesis of 2-*E*,4-*E*-avenalumic acid. Two additional derivatives of avenalumic acid were also detected: the 3′-hydroxy- and 3′-methoxy analogues. These acids, which are the ethylenic homologues of the well-known *p*-coumaric, ferulic, and caffeic acids, may be widely distributed in cereal grains.

Since first reported in the late 19th century (Karrer 1958), the hydroxycinnamic acids (e.g., *p*-coumaric, caffeic, and ferulic acids) have been found to be ubiquitous in terrestrial plants. Small amounts of these acids occur in the free form in a variety of tissues, but far larger amounts exist in covalent conjugations. Examples of covalent linkage of hydroxycinnamic acids with representatives of almost every class of plant constituent have been described (see, for example, the review by Harborne 1980). Cereal grains, although not well studied to date, are proving to be no exception, and a variety of low molecular weight soluble and macromolecular insoluble cereal grain components have been shown to contain hydroxycinnamoyl residues (Collins 1986). Physiological activities are known for some of the smaller conjugates, but the dramatic contribution of these phenolic residues, even at low substitution frequency, in the overall functional properties of macromolecules is only now beginning to be realized. For example, they appear to be involved in bridging polysaccharide polymers in cereal cell walls through oxidative dimerization of hydroxycinnamoyl moieties on separate polysaccharide chains (Gubler et al 1985, Ahluwalia and Fry 1986). Smith and Hartley (1983) suggested that cross-linking separate pentosan chains through diferuloyl bridges may modify the pentosan conformation and lead to determining and stabilizing the quaternary structure of cell wall polymers. Besides the potential to undergo oxidative dimerization, the hydroxycinnamate olefinic double bond permits the existence of both *E* (or *trans*-) and *Z* (or *cis*-) stereoisomers. The two isomers differ markedly in molecular dimensions and physicochemical properties and are readily interconvertible in ultraviolet (UV) light, even when covalently attached to high molecular weight compounds (Towers and Abeysekera 1984). Photoisomerization of the olefinic groups in ferulate esters of large molecules (e.g., cell wall polysaccharide-ferulate esters) also produces gross differences in the quaternary structure and physicochemical properties of the macromolecules. This photoreactive property of hydroxycinnamate conjugates has received renewed interest (see review by Towers and Yamamoto 1985) as a basic mechanism for a number of important and diverse physiological and developmental processes (cell wall deposition, phototropism, lignification, disease resistance, etc). Thus, knowledge of the stereoisomeric configuration of bound phenolic acids is a fundamental prerequisite to evaluating their functionality.

Studies have been undertaken in this laboratory to elucidate the chemical structures and functional properties of cereal-grain-conjugated hydroxycinnamoyl derivatives. During these studies it became evident that several novel phenolic acids were present in oat hulls and groats. These acids had not been described either free or in a combined form from plant tissue, but they appeared in the bound form in several avenanthramide alkaloids. This report describes the structural elucidation and physicochemical properties of these newly discovered acids.

MATERIALS AND METHODS

Melting points were obtained on a Fisher-Johns (Fisher Scientific, Ottawa, Canada) apparatus and are uncorrected. All chromatographic separations of extracts and synthetic mixtures were done on Sephadex LH-20 (Pharmacia, Canada) using volume-calibrated glass columns and gravity-flow isocratic elution. Chromatographic mobilities were recorded as relative elution values, K', equal to the ratio V_e/V_b, where V_e is the observed elution volume and V_b is the total volume of the packed gel (i.e., bed volume). Three solvent systems were used for preparative scale column chromatography: A) acetone, water, and acetic acid (30:65:5, v/v); B) acetone, water, and acetic acid (40:55:5, v/v); and C) chloroform, cyclohexane, and methanol:acetic acid (50:35:10:5, v/v). Analytical thin-layer chromatography (TLC) was performed on silica gel plates (Bakerflex, 1B2-F, Canadian Laboratory Supplies Ltd.), using the following solvent systems: D) chloroform and acetic acid (95:5, v/v); E) chloroform, isopropanol, and acetic acid (90:5:5, v/v); and F) acetone, water, and acetic acid (30:65:5, v/v). Phenolics were detected with a UV lamp (≥350 nm, Ultraviolet Products Inc., San Gabriel, CA) before and after spraying with a solution of ethanolamine in isopropanol (5%, v/v). UV spectra were recorded on a Pye Unicam SP 1800 spectrophotometer (Pye Unicam, Cambridge, UK). Electron-impact mass spectra were obtained by direct inlet solid probe analysis using a Finnigan 4500 spectrometer (Finnigan MAT [Canada] Ltd., Mississauga, ON) at 70 eV. ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were recorded at 250 and 62.8 MHz, respectively, on a Bruker WM 250 spectrometer (Bruker Spectrospin [Canada] Ltd., Milton, ON) in Me_2SO-d_6 . Chemical shifts are referenced to Me_2SO-d_6 at 2.49 ppm (^{1}H) and 39.5 ppm (^{13}C) and are reported with respect to Me_4Si.

Extraction of Oat Groats and Hulls

All extraction and purification steps were performed under diffuse laboratory light conditions. Since photoisomerization of *E* and *Z* forms might be anticipated even under these conditions,

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no attempt was made to unequivocally establish the natural proportion of *E* and *Z* isomers in these initial structural studies. Oats (*Avena sativa* L. cv. Sentinel) were dehulled, groats and hulls were separately dry ground in a Waring Blendor, and the resulting powders were sieved through a No. 40 U.S. standard mesh screen (particle size ≥425 μm.

The following extraction procedure was used for both groats and hulls. The material was slurried in a fivefold excess of aqueous methanol (80%, v/w) heated to 55°C for 15 min with constant stirring, then cooled overnight at 4°C. The mixture was reslurried and poured while still cold into a volume-calibrated glass column and fitted with a coarse-porosity sintered glass disk. The column was allowed to equilibrate to room temperature (20°C) and settle by gravity to give a packed filter bed and a clear yellow-brown supernatant. The supernatant extract was collected by draining the column and eluting the packed bed with five bed volumes of 80% methanol. The bed was then reslurried and the above extraction procedure repeated twice. The eluates from the three extractions were combined, diluted 25% with isopropanol to reduce foaming and auto-oxidation, and concentrated in vacuo by rotary evaporation at 30°C. The resulting syrup (approximately 13% of the original weight of the groats extracted, 7% for hulls), was dissolved in warm (55°C) aqueous 50% isopropanol, cooled to 4°C, reequilibrated to room temperature, and filtered by gravity through a coarse-porosity sintered glass filter. The resulting clear amber filtered extract was used in all subsequent analyses.

Group Separation of Anionic Phenolics

Group separation of anionic phenolics was achieved by fractionation of the extract using anion-exchange chromatography on Sephadex QAE A-25 in the formate form. The gel was equilibrated in 50% isopropanol in a calibrated column, and the groat or hull extract was added. After washing the column with four bed volumes of 50% isopropanol to remove neutral and cationic constituents, the anionic constituents (i.e., weak acids) were eluted with the acidic solvent—acetone, water, and formic acid (60:35:5). The eluate was concentrated to a syrup by rotary evaporation at 30°C and taken up in 50% isopropanol. Anionic phenolics were then separated from other nonphenolic anions by chromatography on Sephadex LH-20 using isocratic elution with solvent system B. After discarding the first two bed volumes of eluate (i.e., $K' \leq 2.0$), the absorbed phenolics were then eluted with two bed volumes of 80% acetone, evaporated to dryness, and taken up in 50% isopropanol. The anionic phenolic fractions prepared separately from groats and hulls were the source of phenolics described in this study.

Synthesis of Avenalumic Acid

The synthesis of 5-(4′-hydroxyphenyl)-penta-2*E*,4*E*-dienoic acid was done as follows. Zinc dust (0.85 g) in 2.0 ml of tetrahydrofuran (THF) was refluxed with a crystal of I_2 for 15 min. A solution of 1.4 ml of 4-acetoxybenzaldehyde (Aldrich Chemical Co, Montreal) in 6.0 ml of THF was added, followed by a solution of 5.4 ml of ethyl-4-bromocrotonate (Aldrich Chemical Co.) in 6.0 ml of THF. The mixture was refluxed for 2 hr on a sand bath. After cooling, 50 ml of aqueous 0.5*M* H_2SO_4 was gradually added to decompose the reaction product intermediate, and the resultant dispersion was extracted with ethyl acetate (3 × 50 ml). The combined ethyl acetate fraction was evaporated to dryness and the alcohol reaction product dehydrated by refluxing in 200 ml of benzene in a Dean-Stark apparatus for 2 hr. The benzene layer was evaporated to dryness and treated with 20 ml of 1.0*M* NaOH, acidified with HCl, and extracted with ethyl acetate (3 × 20 ml). The ethyl acetate layer was dried to give a pale yellow residue. Column chromatography of this residue using solvents A and B gave 5-(4′-hydroxyphenyl)penta-2*E*,4-*E*-dienoic acid (i.e., avenalumic acid) ($K' = 3.4$–4.9 and $K' = 2.3$–3.8, respectively, 175 mg). Lesser amounts of the 2*E*,4*Z*-; 2*Z*,4*Z*-; and 2*Z*,4*E*-isomers (approximately 40 mg) were recovered as an unresolved mixture ($K' = 1.0$–2.3) using solvent B. The synthesis protocols and stereochemistry of these isomers are summarized in Figure 1.

RESULTS AND DISCUSSION

Structure of Avenalumic Acid

The acid was first detected as an unknown product formed during the alkaline hydrolysis of an oat hull anionic phenolic designated AF-8. The AF-8, purified by repeated column chromatography in solvents B and C ($K' = 2.25$–4.0 and $K' = 2.1$–3.5, respectively), was saponified using aqueous 2.5*N* NaOH (55°C, 1 hr). The free acid released from the alkaline hydrolysis was recovered by acidification to pH 3 with HCl and extraction with ethyl acetate. Column chromatography of the ethyl acetate fraction in solvent systems B ($K' = 3.4$–4.9) and C ($K' = 2.0$–3.5) gave a pale yellow microcrystalline solid from aqueous acetone, with a melting point of 217°C. To establish the structure of the free acid, for which the trivial name "avenalumic acid" is proposed, the acid was subjected to mass spectral and NMR spectral analyses.

Electron-impact mass spectral analysis revealed a molecular ion $[M]^{+\cdot}$ at $m/e = 190$ (relative intensity 83%) corresponding to a possible elemental composition of $C_{11}H_{10}O_3$ (Fig. 2). The base peak observed at $m/e = 145$ (100%) corresponded to a loss of COOH from the parent molecule. Further peaks at $m/e = 127$ (55%) and $m/e = 117$ represented a loss of H_2O and CO fragments, respectively, from the base peak. An additional pair of peaks at $m/e = 144$ (37%) and $m/e = 115$ (47%) signified the sequential loss of neutral fragments corresponding to H_2O + CO and CHO from the $[M]^{+\cdot}$. The suggested fragmentation scheme is summarized in Figure 3. These data were compatible with a monohydroxylated phenylpentadienoic or dihydronaphthoic acid structure for avenalumic acid.

Further structural information was obtained from the ^{13}C-NMR spectrum of the compound (Fig. 4). The spectrum contained nine peaks, including five benzenoid ($\delta = 115.8$–129.0 ppm), two in the olefinic region ($\delta = 140.4$–145.2 ppm), and two low-field signals. The low-field peaks were readily attributable to a conjugated carboxylic carbon ($\delta = 167.9$ ppm) and a hydroxyl-substituted phenyl ring carbon ($\delta = 158.7$ ppm). The benzenoid region included a pair of symmetrical peaks at $\delta = 115.8$ and 129.0 ppm (which were approximately double the intensity of the olefinic peaks), and one peak at $\delta = 127.2$ of]—about half

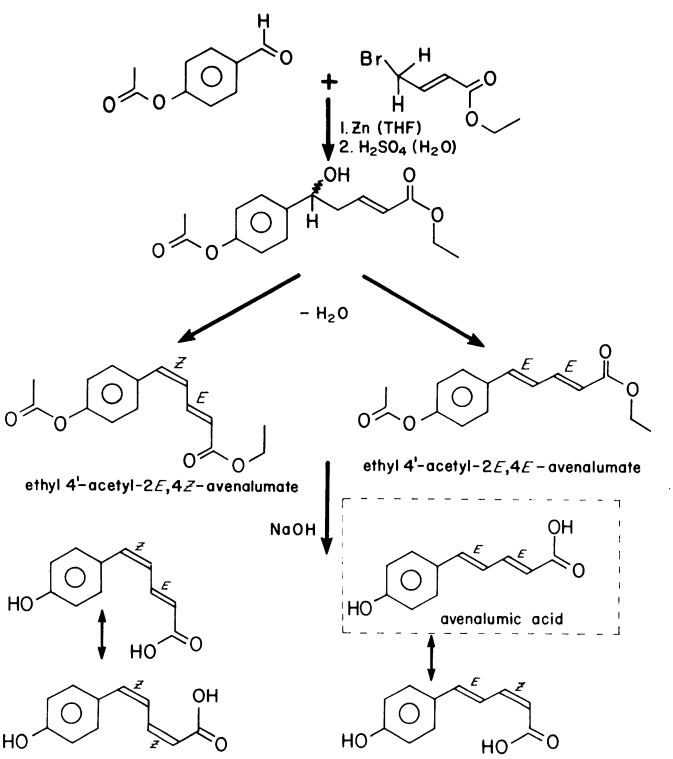

Fig. 1. Protocol for synthesis of avenalumic acid isomers.

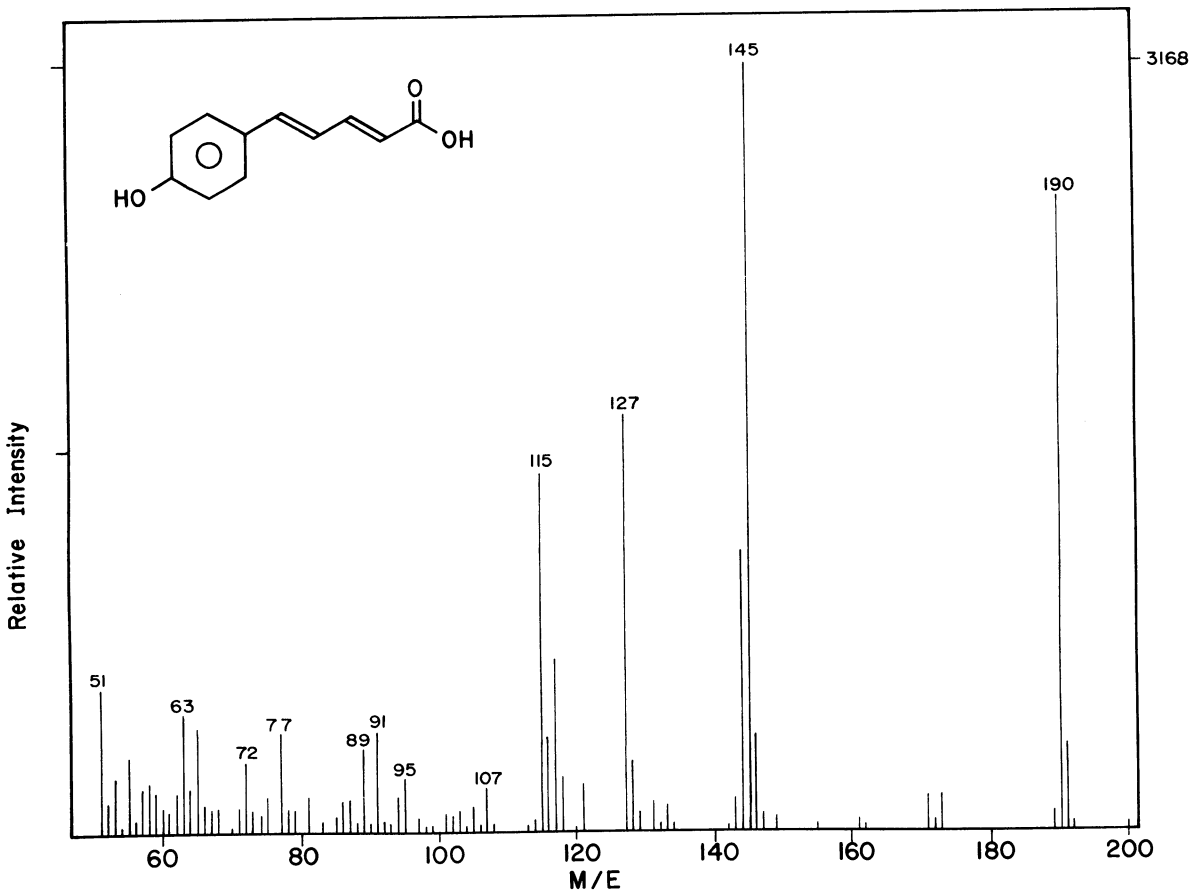

Fig. 2. Mass spectrum of avenalumic acid.

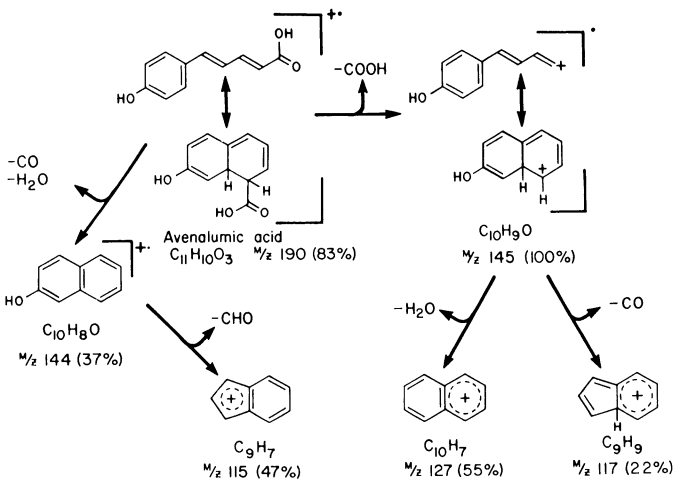

Fig. 3. Mass fragmentation pattern of avenalumic acid showing possible routes to characteristic fragments.

TABLE I
^{13}C- and ^{1}H-Nuclear Magnetic Resonance Spectral Parameters[a] of Avenalumic Acid

Position in Figure 5	^{13}C (ppm)	^{1}H (ppm)	J (Hz)
1	127.2 s	...	...
2	129.0 d	7.38 d	8.6
3	115.8 d	6.77 d	8.5
4	158.7 s	...	...
5	115.8 d	6.77 d	8.5
6	129.0 d	7.38 d	8.6
7	140.4 d	6.93 d	15.5
8	123.4 d	6.83 dd	15.5, 9.7
9	145.2 d	7.30 dd	15.2, 9.7
10	120.3 d	5.90 d	15.2
11	167.9 s	...	...

[a]s = singlet, d = doublet, dd = double doublet.

the intensity of the olefinics, suggesting a 1,4-disubstituted phenyl ring. These findings were supported by the multiplicities obtained from a DEPT (distortionless enhancement by polarization transfer) spectrum of avenalumic acid. The combined spectral data are summarized in Table I and Figure 5. Analysis of peak multiplicities and comparison of the observed and calculated chemical shift values enabled a partial structural assignment of the compound as 5-(4′hydroxyphenyl)-2,4-pentadienoic acid. The stereoisomeric configuration of the four olefinic carbons (at δ = 120.3, 145.2, 123.4, and 140.4 ppm) of the pentadienoic acid moiety could not be obtained unequivocally from this data alone, so H-NMR studies were performed.

From the stereochemical standpoint, there are four possible isomeric configurations for the 2,4-pentadienoic function: both *E*; both *Z*; 2*E*,4*Z*; and 2*Z*,4*E*. Disregarding possible weak, long-range couplings, all configurations would give rise to ^{1}H spectra containing two doublets (corresponding to protons at the 2 and 5 positions) and two double doublets (corresponding to the inner pair on carbons 3 and 4). However, the four configurations would each give distinctly different chemical shifts and coupling constants, reflecting different environments and *E* or *Z* orientations of proton pairs on the unsaturated carbons. Thus, knowledge of the location, multiplicity, and coupling constants for these protons would enable stereoisomeric assignment of the double bonds in avenalumic acid. The ^{1}H-NMR spectrum of avenalumic acid contained a prominent pair of symmetrical two-proton doublets readily attributable to the AA′BB′ spin system of the 4′-hydroxyphenyl ring moiety.

The rest of the spectrum, corresponding to the four olefinic protons of the pentadienoic moiety, consisted of a clearly resolved doublet at relatively high field, a partially resolved low-field double

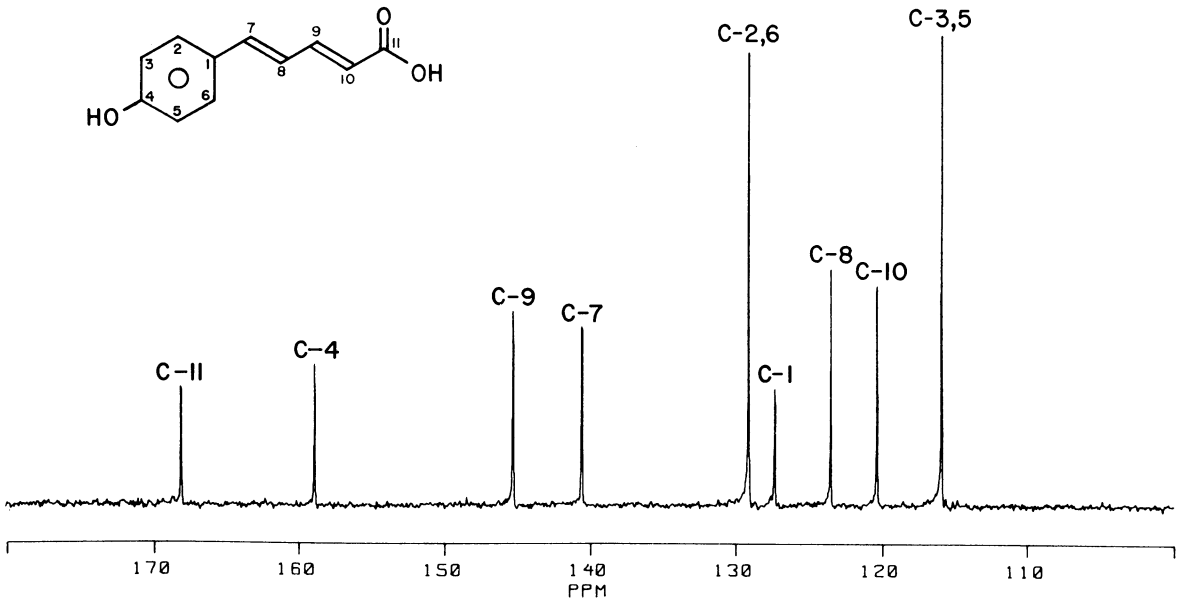

Fig. 4. ^{13}C-nuclear magnetic resonance spectrum of avenalumic acid in Me_2SO-d_6.

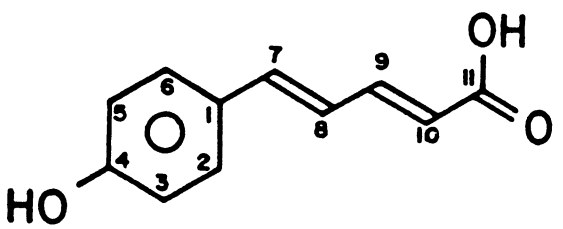

Fig. 5. Structure of avenalumic acid.

doublet, and a poorly resolved two-proton multiplet between the two. However, when the spectrum was recorded at 44°C (Fig. 6), the overlapping two-proton multiplet was clearly resolved into a doublet and a double doublet, both of which exhibited strongly asymmetric intensities. The high-field doublet at $\delta = 5.90$ ppm could now be assigned to the H-10 proton, and its large coupling constant ($J_{10,9} = 15.2$ Hz) was consistent with an *E* orientation with respect to the H-9 proton. Homonuclear decoupling of the H-10 allowed location of the H-9 as the low-field double doublet centered at $\delta = 7.30$ ppm.

First-order calculation of the coupling constants indicated that the H-9, H-8 coupling was 9.7 Hz. By elimination, the remaining asymmetric doublet at $\delta = 6.93$ ppm had to be the H-7 proton, and its large coupling constant ($J_{7,8} = 15.5$ Hz) enabled assignment of the *E* stereochemical configuration as well for the H-7 and H-8 olefinic protons. Thus, avenalumic acid must have the *E,E* orientation in the olefinic side chain, and its complete structure is 5-(4′hydroxyphenyl)-2*E*,4*E*-pentadienoic acid. The ^{1}H-NMR parameters are summarized in Table I and Figure 5. That this assigned structure for avenalumic acid was correct was further confirmed by NMR spectral comparison with authentic standards of *E,E*-cinnamylideneactic acid and 5-(3′,4′-methylenedioxyphenyl)2*E*,4*E*-pentadienoic acid (piperic acid). Finally, avenalumic acid was synthesized (as outlined in the materials and methods section and summarized in Figure 1), and its parameters (melting point; UV spectra; and ^{1}H-NMR, ^{13}C-NMR, and mass spectral analyses) proved to be identical with those of the isolated compound.

Physicochemical Properties of Avenalumic Acid

In the free form, avenalumic acid is a pale yellow substance, with a melting point of 217°C. It is soluble in aqueous alkali, from which it can be regenerated on acidification. It appears pale yellow on TLC plates and dark blue-green in UV light. In the presence of alkali, it appears bright yellow in visible light and green-blue fluorescent in UV light. On exposure to daylight or UV radiation, it undergoes photoisomerization, presumably to a mixture with the 2*Z*,4*E*-; 2*Z*,4*Z*-; and 2*E*,4*Z*-oriented isomers (Fig. 1). Of the four isomers, the 2*E*,4*E* is the least soluble in aqueous acetone, and this isomer can be recovered pure by fractional recrystallization. It is unstable on prolonged exposure to air and/or daylight, even in the solid form, and gradually darkens to amber and dark brown decomposition products. On TLC, the *E,E*-isomer runs between *p*-coumaric and ferulic acid in the solvents used in this study.

As shown in Figure 7A, it exhibits two maxima in UV at 236 and 328 nm. These maxima show both hyperchromic shifts on addition of strong alkali as well as bathochromic shifts of the two maxima by 10 and 32 nm, respectively. In comparison with *p*-coumaric acid (Fig. 7B), the widely distributed hydroxycinnamic acid to which it is structurally related, avenalumic acid exhibits remarkably similar spectral characteristics. The similar maxima and alkali shifts make it doubtful that they could be differentiated based on UV spectral properties alone. In fact, these properties suggest that further analysis may sometimes be required when attempting to identify and/or quantitate covalently bound hydroxycinnamic acids in hydrolysates solely by spectral (absorption or fluorescence) techniques.

Structure of Bound Avenalumic Acid Derivatives

Covalently bound avenalumic acid and two additional homologues have been found in oat groats and hulls. The conjugates were discovered during studies on the structure of the avenanthramide alkaloids (Collins 1989). The first conjugate, designated AF-8, contained avenalumic acid in amide conjugation with the rare aminophenolic, 4-hydroxy-2-aminobenzoic. The structure elucidation of the conjugate was based on hydrolytic, chromatographic, mass spectral and NMR data, and comparison with synthetic analogues. The ^{1}H-NMR data for this conjugate is summarized in Table II and Figure 8. This compound was found in hull extracts but was absent or in much lower concentration in groat extracts.

Two additional conjugates, designated AF-12 and AF-14, were isolated from groat extracts but also occur in the hulls in trace amounts. AF-12, purified by repeated column chromatography (solvent A, $K' = 6.2$–8.5; solvent B, $K' = 2.0$–3.5; and solvent C, $K' = 4.0$–5.8), gave pale yellow needles from aqueous acetone (mp 225°C). AF-14, purified by the same procedure (solvent A, $K' = 11.5$–15.0; solvent B, $K' = 3.5$–4.6; and solvent C, $K' = 7.9$–10.2) gave an amorphous yellow solid. Their structures (Table II and Figure 8) were elucidated from chromatographic, hydrolytic, mass spectral, and NMR data. The ^{1}H-NMR chemical

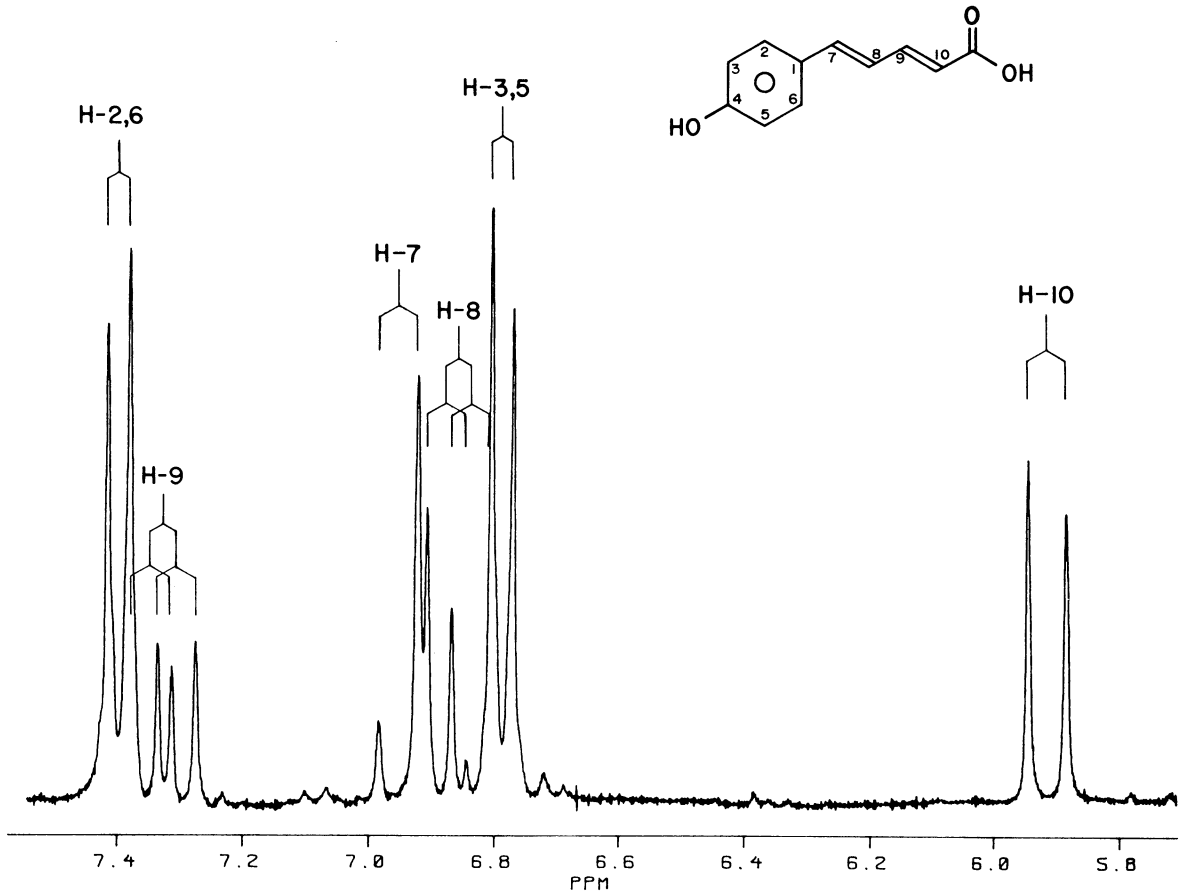

Fig. 6. ^{1}H-nuclear magnetic resonance spectrum of avenalumic acid in Me_2SO-d_6 recorded at 44°C.

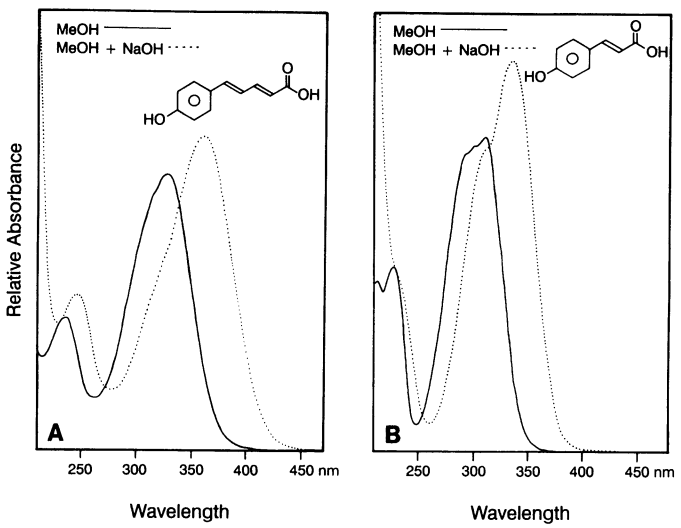

Fig. 7. Ultraviolet spectrum of avenalumic acid (**A**) and *p*-coumaric acid in neutral and alkaline methanol (**B**).

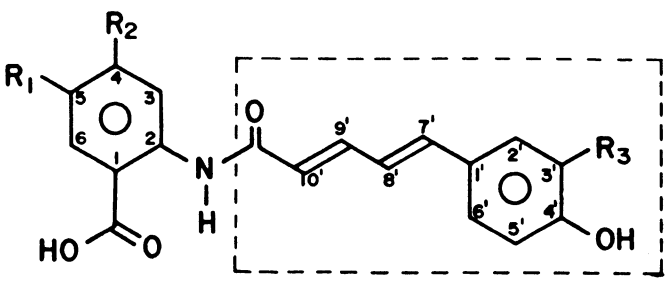

Fig. 8. Structure of bound avenalumic acid.

shifts, multiplicities, and coupling constants are shown to allow comparison with free avenalumic acid values (Table I and Figure 5). For conjugate AF-12, the data in the table indicate that the bound acid is in fact 3′-methoxyavenalumic, since a methoxy residue at the 3′-position appears as a singlet at 3.80, and the olefinic protons (H-7′, 8′, 9′, and 10′) have coupling constants indicative of the *E,E* stereoisomer. Similarly, the acid in conjugated AF-14 is 3′-hydroxyavenalumic. In each case, having established the stereochemistry of free avenalumic acid as *E,E*, assignment of the structures for the two homologues can readily be made from the ^{1}H-NMR data alone. Relative to free avenalumic acid, the protons adjacent to the amide carbonyl (i.e., H-10′) appear approximately 0.25–0.30 ppm farther downfield, and the coupling constants are about 0.5 Hz smaller in the conjugates.

The three avenalumic acid conjugates elucidated above are not the sole representatives of this new type of phenolic acid in oat groats and hulls. At least six additional conjugates containing avenalumic acids have been detected and partially characterized. The complexity of the mixture has made quantitation of individual components difficult, and no attempt has been made to determine the yields of the three avenalumic acid derivatives described in this study. However, efforts are being made to develop an anylsis system using high-performance liquid chromatography-diode array spectrophotometric analysis system to address this problem.

CONCLUSIONS

Three new phenolic acids have been identified in oat groats and hulls. The first, designated avenalumic acid, was identified as 5-(4′-hydroxyphenyl)-2*E*-4*E*-pentadienoic acid, and its identity was confirmed by total synthesis. The other two consisted of the 3′-hydroxy and 3′-methoxy derivatives of avenalumic acid. As a group, they constitute the *E*-ethylenic (i.e., *trans*-ethylenic) homologues of the widely distributed *E*-isomers of *p*-coumaric,

TABLE II
[1]H-Nuclear Magnetic Resonance Spectral Parameters[a] of Bound Avenalumic Acids in Oats

Position in Figure 8	Conjugate AF-8 (R_1 R_2 R_3 = H OH H)	Conjugate AF-12 (R_1 R_2 R_3 = OH H OCH_3)	Conjugate AF-14 (R_1 R_2 R_3 = OH H OH)
3	8.19 d $J_{3,5}$ = 2.4 Hz	8.36 d $J_{3,4}$ = 9.0 Hz	8.33 d $J_{3,4}$ = 9.0 Hz
4	... ...	6.98 dd $J_{4,3}$ = 9.0 Hz	6.93 dd $J_{4,3}$ = 9.0 Hz
		$J_{4,6}$ = 2.9 Hz	$J_{4,6}$ = 3.0 Hz
5	6.45 d $J_{5,3}$ = 2.4 Hz	... ...	... ...
	$J_{5,6}$ = 8.8 Hz		
6	7.84 d $J_{6,5}$ = 8.8 Hz	7.37 d $J_{6,4}$ = 2.9 Hz	7.36 d $J_{6,4}$ = 3.0 Hz
2′	7.40 d $J_{2',3'}$ = 8.6 Hz	7.16 d $J_{2',6'}$ = 1.6 Hz	6.92 d $J_{2',6'}$ = 2.0 Hz
3′	6.77 d $J_{3',2'}$ = 8.6 Hz	... ...	... ...
5′	6.77 d $J_{5',6'}$ = 8.6 Hz	6.76 d $J_{5',6'}$ = 8.2 Hz	6.70 d $J_{5',6'}$ = 8.1 Hz
6′	7.40 d $J_{6',5'}$ = 8.6 Hz	6.96 dd $J_{6',5'}$ = 8.2 Hz	6.83 dd $J_{6',5'}$ = 8.1 Hz
		$J_{6',2'}$ = 1.6 Hz	$J_{6',2'}$ = 2.0 Hz
7′	6.93 d $J_{7',8'}$ = 15.7 Hz	6.98 d $J_{7',8'}$ = 15.5 Hz	6.79 d $J_{7',8'}$ = 15.7 Hz
8′	6.99 dd $J_{8',7'}$ = 15.7 Hz	7.01 dd $J_{8',7'}$ = 15.5 Hz	6.95 dd $J_{8',7'}$ = 15.7 Hz
	$J_{8',9'}$ = 10.3 Hz	$J_{8',9'}$ = 10.2 Hz	$J_{8',9'}$ = 9.9 Hz
9′	7.34 dd $J_{9',8'}$ = 10.3 Hz	7.30 dd $J_{9',8'}$ = 10.2 Hz	7.28 dd $J_{9',8'}$ = 9.9 Hz
	$J_{9',10'}$ = 14.7 Hz	$J_{9',10'}$ = 14.7 Hz	$J_{9',10'}$ = 14.7 Hz
10′	6.17 d $J_{10',9'}$ = 14.7 Hz	6.18 d $J_{10',9'}$ = 14.7 Hz	6.14 d $J_{10',9'}$ = 14.7 Hz
OCH_3	... ...	3.80 s	... ...

[a]d = doublet, dd = double doublet, s = singlet.

caffeic, and ferulic acids. In the oat grain, they occur in the bound form, covalently linked to the amine group of hydroxy-substituted aminobenzoic acids; the structures of three of these conjugates have been elucidated.

The physicochemical properties of these acids closely parallel those of the more commonly encountered hydroxycinnamic acids. Like the latter, the avenalumic acids are capable of undergoing photoisomerization, a process that has been shown to elicit gross changes in the physicochemical properties and functionality of molecules to which they are conjugated. Unlike the hydroxycinnamic acids, they can acquire four stereoisomeric configurations rather than two, and this photochemical process may be of profound importance if the avenalumic acids are conjugated to structural macromolecules in cereal cells. Their occurrence in alkaloid conjugates and their presumably close biosynthetic relationship to the hydroxycinnamates suggest that this may indeed be so. It would be of great interest to know if these acids are widely distributed in cereals and in different classes of cereal grain components.

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