TABLE I
INHIBITION ACTIVITY OF AVENACIN ON VARIOUS ORGANISMS

Organisms Inhibited by Avenacin at a Level up to 50 μg/ml[a]	
Organisms Tested	100% Inhibition (at μg/ml)
Mycobacterium tuberculosis	12.5
Helminthosporium sativum	3.13
Pelicularia filamentosa	3.13
Ophiobolus graminis	3.13
Alternaria solani	6.25
Ceratostomella ulmi	6.25
Verticillium albo-atrum	6.25
Neurospora crassa	6.25
Botrytis cinerea	12.5
Polyporus ostreatus	12.5
Trichophyton interdigitale	25
Saccharomyces pastorianus	25
Colletotrichum pisi	50
Endoconidiophora fagacearum	50
Candida albicans	50
Pythium irregulare	50

Organisms not Inhibited by Avenacin at a Level up to 50 μg/ml	
Bacteria	Fungal Plant and Animal Pathogens
Staphylococcus aureus	*Aspergillus niger*
Staphylococcus albus	*Colletotrichum lagenarium*
Bacillus subtilis	*Endoconidiophora fagacearum*
Sarcina lutea	*Fusarium oxysporum lytopersici*
Mycobacterium avium	*Penicillium expansum*
Escherichia coli	*Pullularia sp.*
Proteus vulgaris	*Sclerotinia fructicola*
Pseudomonas aeruginosa	*Trichophyton rubrum*
Aerobacter aerogenes	*Glomerella cingulata*
Klebsiella pneumoniae	*Phoma pigmentovora*
Salmonella enteritidis	
Shigella paradysenteriae	
Trichophyton rubrum	
Brucella bronchiseptica	
Vibrio metschnikovii	
Erwinia amylovora	
Erwinia caratovora	
Xanthomonas phaseoli	
Xanthomonas pruni	

[a] An agar-dilution end-point method was used on enriched media and the concentration of avenacin necessary to prevent growth completely in a given time was measured. Concentrations higher than 50 μg/ml were not tested.

some phytopathogenic fungi but the prospects for use of the compound itself as antibiotic are not very good due to toxic properties. However, as may be noted in the accompanying report on the structure of avenacin, the substance represents a new class of compounds that may be of general interest in studies of biological inhibitors.

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Avenacin, an Antimicrobial Substance Isolated from *Avena sativa*. II. Structure*

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The structure of the antimicrobial substance avenacin accumulated in oat roots has been analyzed. Avenacin can be hydrolyzed by acid and base into five constituents, three of which are carbohydrates, one is *N*-methylanthranilic acid, and one is a triterpene. The terpene is a pentacyclic triterpene belonging in the class of the Δ^{18}-oleanenes. It has six functional groups, five of which are hydroxyl groups, and one is a keto group. Two of the carbohydrate components are glucose, while the third one has not yet been identified. *N*-Methylanthranilic acid is linked through an ester bond to the triterpene and the carbohydrates are apparently linked to each other and to the terpene through glycosidic bonds.

In the accompanying report (Maizel *et al.*, 1964) on the isolation of avenacin from the roots of oat seedlings it was observed that the compound has a molecular weight of about 1100, it is fluorescent in ultraviolet light, and has a characteristic absorption spectrum. Maizel (1960) identified the absorbing-fluorescing component obtained after hydrolysis as *N*-methylanthranilic acid and also demonstrated the presence of sugars and a large nonpolar component among the hydrolytic products. As a basis for presentation of these and additional extensive details that provide a basis for a provisional structure for avenacin a summary of hydrolytic products is given in Figure 1. As shown, complete hydrolysis yields five molecules. The first is a pentoselike compound not yet fully characterized, 2 and 3 are glucose, 4 is a triterpene called avenagenin, since avenacin is a saponin derivative, and 5 is *N*-methylanthranilic acid. Partial hydrolytic products of particular significance at present are avenamine (from acid hydrolysis for removal of the sugar components) and avenagenol (derived by lithium

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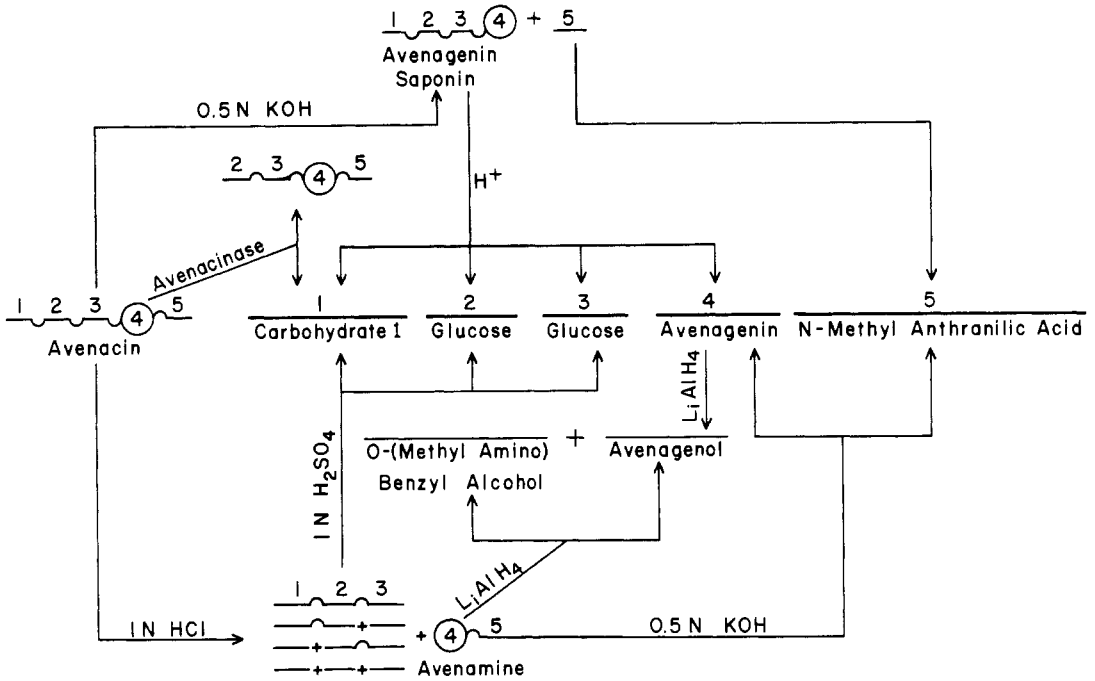


FIG. 1.—Avenacin and its degradation products.

aluminum hydride reduction of either avenamine or avenagenin). Studies of these products and derivatives thereof have provided the most information on structure but other reactions shown in Figure 1 have also been carried out and significant products have been isolated for confirmation and extension of conclusions on structure. Principal emphasis is given here to the triterpene component structure and further details will be considered subsequently.

METHODS

Physical.—Ultraviolet-absorption spectra were obtained with a Cary Model 15 recording spectrophotometer. The instrument was flushed with nitrogen when spectra were recorded below 210 mμ. Infrared spectra were obtained with a Beckman IR7B infrared spectrophotometer. Proton nuclear resonance spectra were recorded with the Varian A-60 high resolution nuclear magnetic resonance spectrometer. Optical rotations were measured with a Bendix automatic polarimeter. Mass spectra were obtained with an Atlas M.A.T. CH4 Model IV, a Model 21-103C (Consolidated Electrodynamics Corp.) and a Perkin-Elmer Hitachi RMU-6A mass spectrometer. The samples were introduced directly into the ionization chamber. Elementary analyses were by the Spang Microanalytical Laboratory, Ann Arbor, Mich. Melting points were determined with a hot-stage microscope and were not corrected.

Chromatography.—Aluminum oxide (active neutral for chromatography, Merck) was used for preparative-column chromatography and the procedure outlined by Neher (1958) was utilized. Column chromatography was greatly facilitated when fluorescent compounds were to be separated, otherwise fractions were tested for the presence of eluted compounds by spotting chromatoplates with 1-μl aliquots and spraying with the cerium sulfate reagent.

Thin-layer chromatography (Stahl, 1962) was performed on silica gel G (Merck) coated plates for all compounds with exception of the carbohydrates, which were analyzed on kieselguhr (Stahl and Kaltenbach, 1961). Detection of components was by fluorescence (Mineralight Model SL 3660, Ultraviolet Products, Inc.) for all compounds containing *N*-methylanthranilic acid. Carbohydrates were detected by spraying the dried chromatoplates with a mixture of 9 ml ethanol, 0.5 ml concd H_2SO_4 and 0.5 ml anisaldehyde followed by heating to 120° for 10–20 minutes. Compounds with a 1,2-glycol configuration were visualized with the periodate-benzidine reagent (Stahl, 1962). All other compounds were detected by spraying with a 1% solution of $CeSO_4$ in 1 N H_2SO_4 and heating for 20 minutes at 120°.

Molecular Weight Determinations.—Molecular weights were obtained by mass spectrometry, the Rast camphor method, or calculated from the $E^{1\%}_{1cm}$ values obtained at 223 mμ for those components containing the *N*-methylanthranilic acid chromophore. $E^{1\%}_{1cm}$ found for *N*-methylanthranilic acid (mw 151) is 1720. The calculations are based on the assumption that compounds derived from avenacin which do not contain *N*-methylanthranilic acid do not absorb at 223 mμ. Avenagenol exhibited a strong peak at 193 mμ but there was no absorption at 223 mμ.

Determination of Carbohydrates.—Glucostat reagent was used for the detection of glucose (Worthington Biochemical Corp., Freehold, N. J). Instructions (Method 1-A) were followed and the reagent was used to spray on chromatoplates on which carbohydrates 1 and 2 were separated. The method used by Chargaff and Davidson (1956) was applied for quantitative simultaneous determination of hexoses and pentoses.

EXPERIMENTAL

Avenacin.—Avenacin isolated from roots of oat seedlings (yield 155 mg/kg of moist roots, Maizel *et al.*, 1964) has the following properties: $E^{1\%}_{1cm}$ at 223 mμ = 238; mp 248–250° decomp; $[\alpha]^{24°}_{D}$ = +35.7° (*c* 1, H_2O).

Anal. Calcd. for $C_{55}H_{83}O_{21}N$ (1094); C, 60.4; H, 7.7; N, 1.3. Found: (1100 Rast; 1091 UV) C, 60.5; H, 7.6; N, 1.4.

The infrared spectrum of avenacin in Nujol shows broad absorption bands at 3400 and 1070 cm^{-1} and characteristic peaks at 1719, 1680, 1610, 1582, 1522, and 1245 cm^{-1}.

N-Methylanthranilic Acid.—Avenacin (110 mg) was suspended in 0.25 N KOH (10 ml) and refluxed for 2.5 hours, after which the clear reaction mixture was cooled and acidified to *p*H 2.3 with 1 N HCl. Five extractions with 12-ml portions of ether removed all the fluorescence from the water phase. After evaporation of the ether extract the product (12.8 mg) was dried over P_2O_5 *in vacuo* and sublimed, mp 178–180°; mixed mp 178–179°. The ultraviolet-absorption spectrum between 210 and 400 mμ (Maizel *et al.*, 1964) was the same as that obtained for avenacin and avenamine ($E^{1\%}_{1cm}$ 1720). Commercial *N*-methylanthranilic acid gave an identical spectrum and the same extinction coefficient. The infrared spectra of *N*-methylanthranilic acid and the isolated compound were identical, with significant bands at 1660 cm^{-1} (carbonyl) and 3380 cm^{-1} (secondary amine).

Avenamine.—Avenacin (5 g) was dissolved in 0.75 N HCl (150 ml) and heated on a steam bath for 1 hour. The mixture was cooled, distilled water (500 ml) was added, and the precipitate was filtered off, washed with water, and dried (2.79 g). This material was taken up in benzene (30 ml), the undissolved material was separated and dissolved in a mixture of chloroform (2 ml) and methanol (2 ml), and the two solutions were combined and applied to a 2.8 × 42–cm column filled with aluminum oxide, activity IV. Elution was started with pure benzene followed by benzene-ether mixtures (1, 2, 5, 10, 20 . . . 100% ether; Neher, 1958). Approximately 250–500 ml of each solvent was passed through the column before the addition of the next mixture or pure solvent. Avenamine was eluted in the 40% ether–60% benzene mixture. Other minor components were obtained by the same type of succession of mixtures of ether and methanol. After elution the solvents were evaporated and the residue was recrystallized first from methanol-water and then from benzene–petroleum ether (bp 60–70°) to yield 723 mg avenamine; mp 208–210°.

Anal. Calcd. for $C_{38}H_{55}O_7N \cdot CH_3OH$ (670): C, 69.9; H, 8.9; N, 2.1. Found: (682, UV) C, 70.0; H, 8.7; N, 2.0.

MW Calcd. for $C_{38}H_{55}O_7N$: 638. Found: 638 (mass spectrometry).

The ultraviolet spectrum of avenamine is identical with that of its *N*-methylanthranilic acid component in the range of 210–400 mμ; $E^{1\%}_{1cm}$ at 223 mμ for avenamine is 382 compared to 1720 for *N*-methylanthranilic acid. The molecular weight determined on this basis is 682 for avenamine. A mass spectrogram of avenamine (obtained through the courtesy of the Applied Physics Corp. with the Atlas MAT CH4 Model IV mass spectrometer) shows three *m/e* peaks of principal interest: 638 (M^+), 486 (*M*-*N*-methylanthranilic acid), and 151 (*N*-methylanthranilic acid). Avenamine dissolved in $CDCl_3$ exhibits in its proton nuclear magnetic resonance spectrogram one proton at 5.05 ppm and further shows 7 protons adjacent to oxygen. The infrared spectrum of avenamine in chloroform shows peaks of principal interest at 1703 and 1428 cm^{-1}. The R_F value in ethylacetate-hexane (3:1, v/v) is 0.34.

Avenamine Tetraacetate.—Avenamine (295 mg) was dissolved in pyridine (3 ml), acetic anhydride (3 ml) was added, and the mixture was agitated at room temperature for 18 hours. Water (30 ml) was added and the precipitate was washed with water and dried. This crude product (150 mg), which contained two fluorescent components, was dissolved in a minimum amount of methanol (about 1 ml) and applied to the origin of three 20 × 20–cm chromatoplates (1 mm) and developed in ethylacetate-hexane (37:63 v/v). Two fluorescent zones were extracted separately and the solvents were evaporated leaving 3 mg of the minor and 133 mg of the major component. The major component was recrystallized from benzene-hexane; mp 203–205°.

Anal. Calcd. for $C_{46}H_{63}O_{11}N$ (806.0): C, 68.5; H, 7.9; N, 1.7. Found: (787, UV) C, 68.5; H, 8.0; N, 1.5.

The infrared spectrum of avenamine acetate in chloroform as solvent shows no hydroxyl absorption at 3480 cm^{-1}.

Avenagenol.—Avenamine (400 mg) was dissolved in dry tetrahydrofurane (150 ml), lithium aluminum hydride (200 mg) was added, and the reaction mixture was stirred for about 3 hours at room temperature, after which excess lithium aluminum hydride was slowly decomposed by addition of saturated aqueous sodium sulfate solution (2 ml). The reaction mixture was stirred for another 3 hours after which anhydrous sodium sulfate (2 g) was added and the solids separated from the supernatant solution. The solids were thoroughly washed with tetrahydrofurane and supernatant and washings were evaporated to dryness yielding 394 mg solid. This material was dissolved in a minimum amount of methanol (1–2 ml) and adsorbed on approximately 2 g of aluminum oxide which was then dried, suspended in chloroform, and layered on top of a 1.4 × 23–cm column filled with aluminum oxide, activity IV. Elution was started with carbon tetrachloride and successively followed by ether, ethyl acetate, and methanol as described previously for avenamine. *o*-Aminomethyl benzyl alcohol was eluted first in an eluate of 50% ether and 50% carbon tetrachloride while avenagenol emerged from the column in an eluate containing 1% methanol–99% ethyl acetate. After evaporation of this fraction, avenagenol (193 mg) remained and was recrystallized several times from methanol-water; mp 322–324°.

Anal. Calcd. for $C_{30}H_{50}O_6 \cdot 2CH_3OH$ (570.8): C, 67.3; H, 10.2. Found: C, 67.4; H, 10.1.

The R_F value in ethyl acetate was 0.20. A mass spectrogram of avenagenol (obtained through the courtesy of the Perkin-Elmer Co. with the Hitachi RMU-6A mass spectrometer) shows three principal peaks at *m/e* 161, 175, and 189.

The infrared spectrum of avenagenol in Nujol exhibits strong peaks at 3380, 1645, 1020, and 1042 cm^{-1}. There is no peak between 1400 and 1445 cm^{-1}. The ultraviolet-absorption spectrum of avenagenol in 2% aqueous ethanol shows an intense peak at 193 mμ. Avenagenol does not react with periodate (Stahl, 1962).

Avenagenol Hexaacetate.—Avenagenol (37 mg) was dissolved in pyridine (0.5 ml) and acetic anhydride (0.5 ml). After 20 hours at room temperature water (5 ml) was added to the reaction mixture, and the precipitate was washed with water twice, dried, and recrystallized from ether-pentane yielding 42 mg of hexaacetate, mp 226–228°.

Anal. Calcd. for $C_{42}H_{62}O_{12}$ (759.0): C, 66.4; H, 8.2; acetate, 29.6. Found: (760, extrapolated from mass spectroscopy) C, 66.2; H, 8.2; acetate, 29.8.

A mass spectrogram (resolution good only to 700) of avenagenol acetate shows peaks at *m/e* 400, 460, 520, 580, 640, and 700. The proton nuclear magnetic resonance spectrum of avenagenol acetate obtained in carbon tetrachloride is presented in Figure 2. The R_F value of the acetate in ethyl acetate–hexane (45:55, v/v) is 0.75.

Carbohydrates.—The aqueous phase of the hydrolysis mixture from the avenamine preparation was neutralized and desalted (Thompson and Wolfram, 1962)

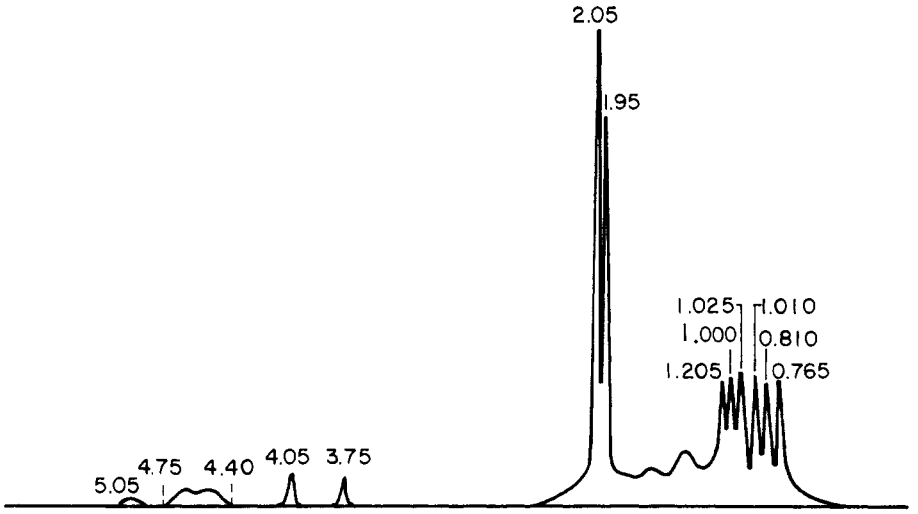


FIG. 2.—Schematic magnetic resonance spectrum of avenagenol acetate in carbon tetrachloride. Values given for the chemical shifts are in parts per million.

leaving 2.11 g of solid (98% recovery). This material was used directly and, after complete hydrolysis in 1 N H_2SO_4 at 100° for 6 hours, for chromatographic separations. The R_F values found in solvent I (Stahl and Kaltenbach, 1961) were: fructose, 0.60; arabinose, 0.70; glucose, 0.40; carbohydrate 1, 0.66; carbohydrate 2, 0.40.

Enzyme Experiments.—Avenacinase was isolated from *Ophiobulus graminis* var. *avenae* as described by Turner (1961). The medium used for growing the fungus was Vogel's medium (Vogel, 1956) supplemented with 2% sucrose, 0.1% peptone, 0.02% liver extract, 0.02% yeast extract, and 1 mg thiamine per liter of medium. Purified enzyme solution (100 ml; prepared as described by Turner, 1961) derived from 3 liters of culture medium (10 Fernbach culture flasks with 300 ml medium each) was incubated with 2 mg of avenacin (added as 0.2 ml of a 10 mg/ml solution in 80% ethanol) at 35° for 24 hours and lyophilized. The remaining solid was subsequently extracted with three 8-ml portions of chloroform and three 8-ml portions of ethanol, and the residue was dissolved in water. All the fluorescence was in the chloroform fraction which yielded only one compound when chromatographed in water-saturated methyl ethyl ketone on silica-gel covered chromatoplates (R_F 0.35; avenacin 0.05; avenamine 0.95). Carbohydrate 1 was found in the alcohol fraction while free glucose could not be detected in any of the three fractions.

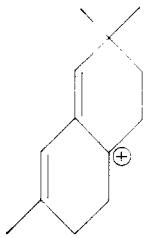
DISCUSSION

From the evidence now available it is well established that avenacin is a triterpenoidal saponin having a carbohydrate chain of three units and an aromatic acid bound to the triterpene. The aromatic acid component is bound as an ester and the pure substance obtained from hydrolysis of avenacin is identical in all respects with synthetic *N*-methylanthranilic acid. Criteria utilized were melting temperatures, mixed-melting temperature, and ultraviolet and infrared absorption spectra. The *N*-methylanthranilic acid component accounts for all of the absorption in the ultraviolet shown by avenacin between 210 and 400 mμ and it also accounts for all of the bright blue fluorescence of the saponin.

The total structure of avenagenin, the triterpene constituent of avenacin, is less firmly established than that of the aromatic acid component but the data presented give strong support to its formulation as a pentacyclic triterpene containing one keto group, five hydroxyl groups, and a single ethylenic bond. A good deal of this support comes from data concerning the properties of reduced avenagenin, avenagenol, and its hexaacetate. Mass spectrographic analysis on the hexaacetate yielded six *m/e* peaks with mass differences of 60 each, starting at 400. Since acetyl groups split off as acetic acid (*m/e* 60) on electron impact the six peaks account for 5 acetyl groups up to the peak at 700. However, six acetyl groups are present in avenagenol acetate as established by direct acetate determinations and by nuclear magnetic resonance data which show 18 methyl protons of acetate groups. On this basis, and since no peaks were observed below 400, one further peak at 760 can be predicted. This is above the usable resolution of the spectrometer and was not observed, but if the extrapolation is made to give the precise molecular weight for avenagenol hexaacetate as 760 then avenagenol has a molecular weight of 508 and the parent hydrocarbon (as an oleanen, $C_{30}H_{50}$, mw 411) would have the value 412. If one assumes the series of *m/e* peaks obtained for avenagenol acetate to be "M + 1" peaks (Biemann, 1962), these values are in excellent agreement with the analytical data for avenagenol hexaacetate (for $C_{42}H_{62}O_{12}$, mw 759), for avenamine (for $C_{38}H_{55}O_7N_1$, mw 638), and are completely consistent with all of the other analyses as well. Furthermore the nuclear magnetic resonance spectrum of avenagenol hexaacetate (Fig. 2) shows all the eight methyl groups characteristic of the triterpenes (six as angular methyls and two as primary alcohols).

As mentioned previously all of the ultraviolet absorption of avenacin above 210 mμ is accounted for by the *N*-methylanthranilic acid component but below 200 mμ (at 193 mμ) avenagenol gives an intense peak of absorption. This corresponds to the behavior of an isolated ethylenic bond (Ellington and Meakins, 1960) and evidence as to its location is provided by nuclear magnetic resonance and infrared spectral data. The proton at 5.05 ppm in the nuclear magnetic resonance spectrogram of avenagenol acetate is characteristic for trisubstituted ethylenic bonds. Trisubstituted double bonds give rise to C-H stretching and bending vibrations in the infrared but are not found in avenagenol. Trisubstituted double bonds that do not give rise to these vibrations have as yet only been found in Δ^{18}-oleanenes (Cole and Thornton, 1957). Further-

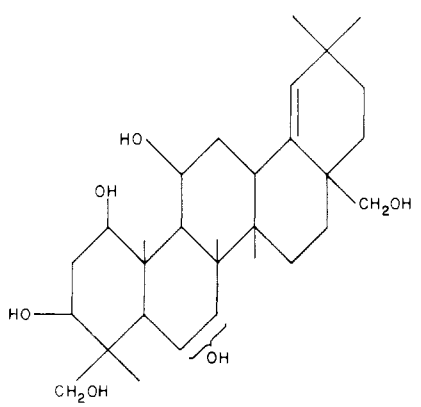
more a Δ^{18}-oleanene ring system for avenagenol is also favored by the molecular fragmentation pattern obtained by mass spectrography of avenagenol with its 3 principal peaks at *m/e* 161, 175, and 189. It is suggested that the peak at *m/e* 175 is represented by the ion shown in formula I. If this is correct the



I

ready loss of the C-17 substituent found to be a characteristic feature of the mass spectra of Δ^{18}-oleanenes (Djerassi *et al.*, 1962) is indicated.

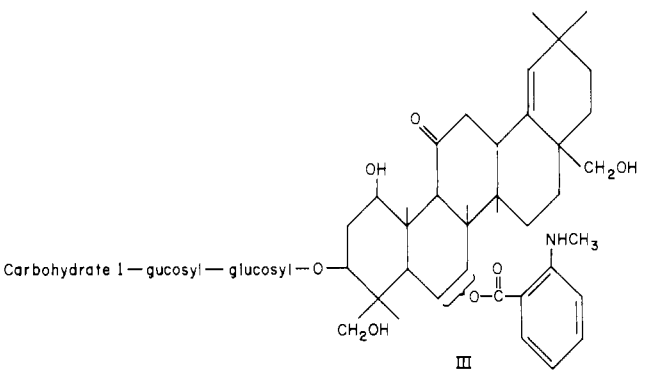
Avenagenin and avenagenol have a total of 6 oxygen functions. This was concluded from elemental analyses and the nuclear magnetic resonance spectrum of avenagenol acetate which shows 18 methyl protons of acetate groups (Fig. 2). One of these functions as found in avenagenin and avenamine represents a keto group. Its absorption in the infrared at 1703 cm^{-1} as well as an absorption at 1428 cm^{-1} may be used to assign the keto group to the C-11 position (Cole and Thornton, 1956). Two of the oxygen functions represent primary alcohol groups, since the nuclear magnetic resonance spectrogram shows two unsplit protons at 3.75 and 4.05 ppm. In analogy to the values published for protons alpha to primary acetoxyl functions by Shamma *et al.* (1962) one might assume that the two primary alcohol groups of avenagenin occupy the C-23 and C-28 positions. The remaining three oxygen functions can be accounted for as secondary hydroxyls because avenamine shows a total of 7 protons adjacent to oxygen. If the formulation of the *m/e* 175 peak (I) is correct, none of the 3 secondary alcohol functions of avenagenin would be present in rings D and E and would have to be assigned to positions in rings A, B, and C. Further assignment is also possible since avenagenol does not react with periodate and it can be assumed that avenagenol does not have a glycol configuration. From these considerations it is reasonable to assign to avenagenol the structure in formula II.



II

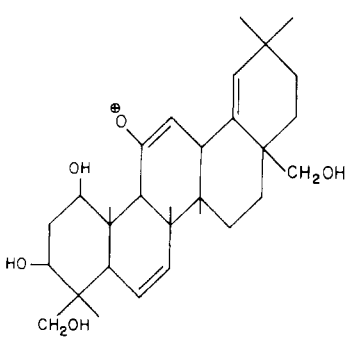
The carbohydrate part of the avenacin molecule is a trisaccharide consisting of two moles of glucose and one mole of an as yet unidentified sugar. Chromatographic examinations of the carbohydrate fraction from acid hydrolysis of avenacin (amounting to 40% in weight) showed that it contained a mixture of tri-, di-, and monosaccharides, but after complete hydrolysis only two spots were detectable by paper and thin-layer chromatography in several solvent systems (Stahl and Kaltenbach, 1961). One compound behaved in a manner identical with glucose and was proved to be glucose by means of the glucose oxidase reaction performed on the chromatogram with the glucostat reagent. The other component (carbohydrate 1 in Fig. 1) was similar in R_F value to arabinose and gave a pentose reaction with *o*-aminobiphenyl reagent. With this reagent hexoses and pentoses can be estimated simultaneously in mixture by measuring the absorbance at two different wavelengths (Chargaff and Davidson, 1956). Application of the method yielded a 2:1 molar ratio of hexose to carbohydrate 1, with glucose and ribose as standards. Carbohydrate 1, though it behaves like a pentose in the orcinol test, shows a hexose reaction with cysteine–sulfuric acid reagent and a keto-hexose reaction with carbazole sulfuric acid (Dische, 1962). Carbohydrate 1 was compared with 32 monosaccharides or derivatives thereof by chromatography and found not to be identical with any of them. It does not show methyl or methyl-ether protons in the nuclear magnetic resonance spectrum and is therefore believed to represent an unknown or rare monosaccharide, the structure of which is presently under investigation.

As discussed earlier, some aspects of the structure of avenacin remain for further study but present knowledge permits the presentation of a provisional formula as shown at III. In this structure it is es-



III

tablished that *N*-methylanthranilic acid is linked to avenagenin in an ester linkage as shown by the base lability and the carbonyl-stretching vibration at 1719 cm^{-1} in the infrared. However, data are not yet available which permit assignment of the ester linkage to a specific position in the hexahydroxy-Δ^{18}-oleanene ring system. Mass spectrographic data, however, permit the assignment of the ester linkage to a secondary OH-group of avenagenin. The mass spectrogram of avenamine gives rise to an *m/e* peak at 486, presumably representing the ion shown in formula IV (*M-N-*



IV

methylanthranilic acid) and an m/e peak at 151 (*N*-methylanthranilic acid), indicating clearly that only a secondary alcohol group is involved in the ester linkage. As to the sugars, it seems likely that they are linked to each other and to the rest of the molecule by a single glycosidic linkage since short-time hydrolysis liberated only about one reducing group per molecule of avenacin. The glycosidic linkage, by analogy to other saponins, can be assumed to occupy the C-3 position, but this is not proved. It is glucose which links the carbohydrate chain to avenagenin since avenacinase (Turner, 1961) splits off only carbohydrate 1 and does not release glucose. Thus the enzyme preparation, though not highly purified, is free of relevant glucosidases. Carbohydrate 1 therefore must occupy the "outside" position (Reichstein, 1962). There is no proof yet showing whether the oligosaccharide moiety is branched or built up linearly.

No extensive studies have yet been carried out to establish which portions of the avenacin molecule are responsible for its inhibitory action on fungi but it is already clear that the two terminal components, *N*-methylanthranilic acid and carbohydrate 1 are essential. The growth of the fungus *O. graminis* var. *avenae* on oats is evidently due to its production of avenacinase, the enzyme which removes carbohydrate 1 from avenacin. The same results would be expected if a parasite were able to produce an esterase that would hydrolyze off the *N*-methylanthranilic acid. It is also of interest to examine whether the triterpene is essential for antifungal activity since it may serve only to link the carbohydrate and the aromatic acid.

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