The antifungal factors in barley. IV. Isolation, structure, and synthesis of the hordatines[1,2]

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Careful fractionation of extracts prepared from barley coleoptiles gave small amounts of pure hordatines A and B and substantial amounts of a mixture of their glucosides. The structures of the hordatines were deduced by degradative and spectroscopic studies and by the synthesis of some degradation products. A synthesis of racemic hordatine A analogous to its probable biogenesis was achieved by the oxidative coupling of coumaroylagmatine.
The possible role of the hordatines in lignification is briefly discussed.

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Barley seedlings have been reported (4) to resist invasion by the fungal parasite *Helminthosporium sativum*[3] in the first few days after their emergence. A detailed study implicated chemical resistance factors (4) and led to the isolation (5) of some strongly basic fractions which exhibited antifungal activity. Of these, the largest fraction (referred to, for convenience, as hordatine M) was itself a mixture of several compounds. These could not be separated on a preparative scale, and attempts to obtain crystalline salts were also unsuccessful. On the other hand, the individual components were obviously very closely related. Thus, it seemed both necessary and justifiable to treat the mixture as a molecular entity and to rely on careful degradative work to uncover the differences between the individual constituents.

Hordatine M was normally isolated and studied as the syrupy acetate. It gave a positive Sakaguchi reaction and was therefore a free guanidine. Treatment with aqueous acid on the steam bath liberated substantial amounts of agmatine (I), whereas other low molecular weight bases were not produced in significant quantities.

$$RHN'CH_2CH_2CH_2CH_2NHC(NH_2)NH$$

I R = H
XIX R = *p*-HO·C_6H_4·CHCHCO

Potentiometric titration of hordatine M revealed an approximate equivalent weight of 440, associated with a strongly basic function (pK' 11.2), which could be assigned to the guanidino group of the agmatine moiety. No other function, such as, for example, that which would be expected from a basic amino nitrogen and was clearly evident in the titration curve (Fig. 1) of agmatine itself, could be detected. It followed that N′ of the agmatine residue was neutral and most probably situated in an amide link.

Periodic acid oxidation studies showed that 2 acid equivalents of M consumed 2 moles of periodic acid, concomitant with the liberation of 1 mole of formic acid. Methanolysis gave α-D-methylglucoside, isolated in 80% of the expected yield and unaccompanied by other carbohydrate derivatives. Hordatine M was therefore a D-glucopyranoside, the ring size required by the stoichiometry of the oxidation. It also followed that its molecular weight was of the order of 800 (a simple multiple thereof could not be excluded from consideration but seemed unlikely). Further, to accommodate these results to the potentiometric titration and hydrolysis data, it was necessary to postulate the presence of two agmatine residues, situated in similar environments. Stepwise cleavage, as discussed later, showed that this was the case.

Sufficient data were now available to allow a tentative evaluation of the analytical figures for two hordatine M salts which had been obtained. One of these was a (di)-picrate which was amorphous but could be purified to constant absorption in the

[1]Contribution No. 339 from the Research Institute.
[2]Part of this work has been published as an informal work-in-progress report (1) and in two preliminary communications (2, 3).
[3]This is a commonly used name. The systematic name is *Cochliobolus sativus* (Ito and Kurib.) Drechs. ex Dastur (imperfect state *Bipolaris sorokiniana* (Sacc. in Sorokin) Shoemaker).

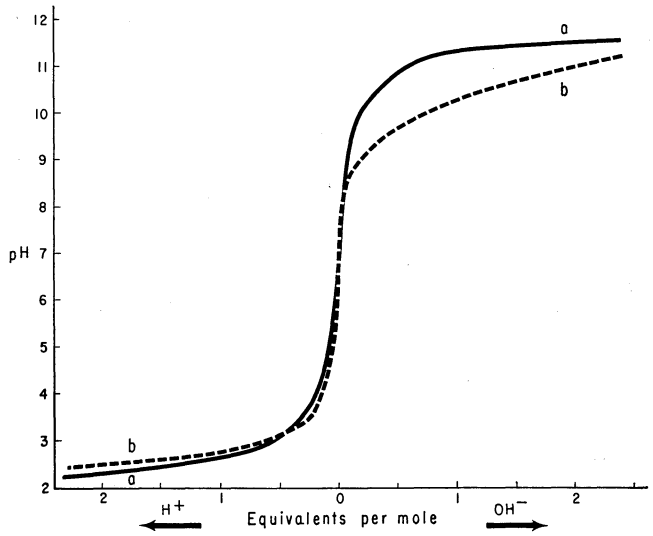
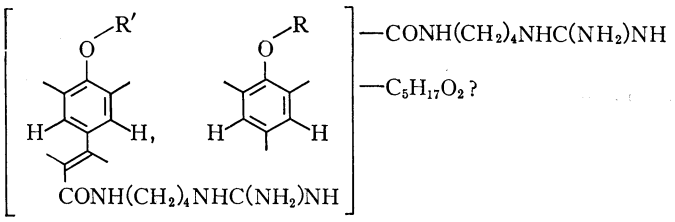


FIG. 1. Titration curves of (*a*) hordatine M (0.0206 mmole in 5 ml of solution) and (*b*) agmatine (0.0235 mmole in 7 ml of solution).

ultraviolet by repeated reprecipitation. The other was a (di)hydrochloride, obtained from the picrate by ion exchange, and not further purified. When considered together, the analytical figures for both salts indicated a $C_{37}H_{58}N_8O_{11}$ formula for hordatine M as the free base. This was accordingly adopted as a working formula with the proviso, at the outset, that it was likely to be only approximately correct.

In its ultraviolet spectrum, hordatine M exhibited a broad band near 300 mμ to which an extinction coefficient of about 20 000 could now be assigned. The chromophore was allocated to a possibly substituted *p*-coumaric acid residue on the following evidence. On irradiation with ultraviolet light or bright daylight, hordatine M suffered smooth hypo- and hypsochromic changes that were typical of the *trans* to *cis* isomerization of a cinnamic acid derivative. Oxidation with nitric acid gave about 1 mole of picric acid. Ozonolysis, followed by hydrolysis, gave oxalic acid in a moderate yield. Lastly, hordatine M could be hydrogenated to a dihydro derivative which, from its ultraviolet spectrum, was an unconjugated phenol ether. Alkali fusion of hordatine M yielded substantial amounts of *p*-hydroxybenzoic acid; this result seemed at first to lend further support to the coumaric acid postulate, but the situation was more complex. A second maximum in the ultraviolet spectrum of hordatine M occurred, as befits a coumaric acid derivative, at about 220 mμ, but its intensity was greater than expected (ϵ 25 000 whereas methoxycoumaroylagmatine had ϵ 14 600). Unusually high extinction coefficients were also observed for dihydrohordatine glucoside and, further, the yield of picric acid from the nitric acid oxidation seemed improbably high if derived from a single aromatic nucleus. The presence of a second, aromatic chromophore was thus indicated, and could be established by considering the aglucones.

These were obtained as the basic product of the mild methanolysis of hordatine M. They were separated by careful countercurrent distributions into fractions of varying degrees of homogeneity, and the resolution, in contrast to that of the glucosides, could be monitored by chromatography and nuclear magnetic resonance (n.m.r.) spectroscopy. The best fractions obtained are known now to have been mostly *trans*-hordatine A, containing only traces of hordatine B but sometimes appreciable amounts of *cis*-hordatine A. In the following discussion, in order to distinguish them from the pure compounds which were

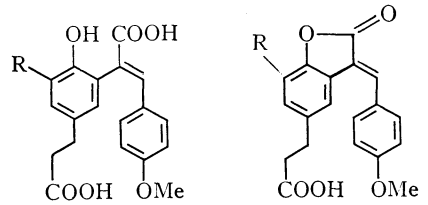


II R = D-glucopyranosyl, R′ ≠ H
III R = H, R′ ≠ H

obtained later, the preparations will be referred to as 'the aglucone'.

The properties of the aglucone resembled those of hordatine M. It gave a picrate which tended to separate from solvents as a gum and, at the time, did not seem to allow further purification. The aglucone was therefore studied as the syrupy diacetate,[4] as obtained from the countercurrent distribution, and elemental analysis was not attempted. The n.m.r. spectrum lacked any bands attributable to a glucose residue, but exhibited all the other characteristic features of the spectrum of hordatine M. The ultraviolet spectrum exhibited bands at 298 mμ (ε 20 000) and at 228 mμ (ε 23 000) in a neutral solution. On basification, the maximum at 298 mμ, which would be expected to move to near 360 mμ if caused by a cinnamic acid residue with a free phenolic hydroxyl, underwent only barely detectable changes. On the other hand, that at 229 mμ suffered a bathochromic shift to 238 mμ. These observations are interpretable if the spectrum results from the superimposition of the chromophores caused by an etherified coumaric acid residue and an unconjugated, free phenol. In accord with this explanation, methylation of the aglucone with dimethyl sulfate gave the expected phenol ether, whose ultraviolet spectrum was insensitive to added base. Provisional partial structures (II and III) could now be written for hordatine M and the aglucone, respectively. The latter was optically active, and therefore contained at least one saturated, asymmetrically substituted carbon atom. Further, both aromatic nuclei were required to produce picric acid on nitric acid oxidation, and thus were unsubstituted in the positions shown. However, it was not known which of the nuclei gave rise to the *p*-hydroxybenzoic acid.

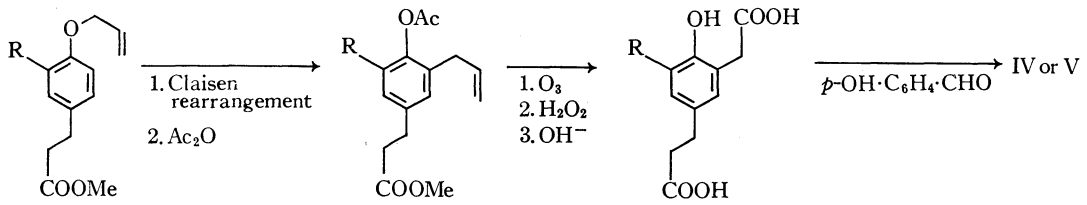
Hydrogenation of the aglucone, or methanolysis of the dihydroglucoside, provided the dihydrohordatines as the syrupy acetates, ranging in composition from almost pure dihydrohordatine A to mixtures of dihydrohordatines A and B in almost equal proportions. The substantially pure A component was optically active, and its ultraviolet spectrum in an alkaline solution clearly showed the presence of both a free phenol (λ_{max} 287 mμ (ε 4 600)) and a phenol ether (λ_{sh} 281 mμ (ε 4 000)). Methylation of the dihydrohordatines, followed by vigorous alkaline hydrolysis, gave two crystalline acids (IV, $C_{19}H_{18}O_6$, and V, $C_{20}H_{20}O_7$) in proportions which depended on the relative amounts of the A and B components in the starting material. The evidence leading to the structural assignments (stereochemistry not implied) for IV and V and for their bright-yellow lactones VI and VII has been discussed in sufficient detail (ref. 2 and Experimental) and was confirmed by synthesis as shown in Scheme 1.



IV R = H
($\lambda\lambda_{max}$ 298 and 218 mμ)
V R = OMe
($\lambda\lambda_{max}$ 305 and 220 mμ)

VI R = H
($\lambda\lambda_{max}$ 381 and 250 mμ)
VII R = OMe
($\lambda\lambda_{max}$ 380 and 250 mμ)

[4]On one occasion a fraction partially crystallized. The hand-picked product could not be recovered in a crystalline form from solvents, and elemental analysis was therefore impracticable. The infrared spectrum of the crystals was not informative; the ultraviolet and n.m.r. spectra were indistinguishable from those of the syrupy acetate.



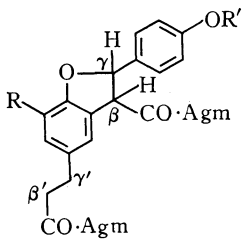
SCHEME 1. Synthesis of IV and V.

It was readily apparent that a representation of dihydrohordatine A as VIII would lead to IV by elimination and hydrolysis. Corresponding structures for dihydrohordatine B, hordatine A, and hordatine B would then be given by IX, X, and XI, respectively, and hordatine M would be represented as a mixture of glucosides XII and XIII. In practice, compounds X to XIII would occur in admixture with their double-bond *cis* isomers.

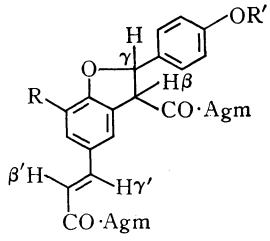
Evidence requiring an analogous interpretation was also available as the result of the acidic degradation of hordatine. Vigorous methanolysis of the aglucone liberated about 1 mole of agmatine, traces of other small fragments, substantial amounts of a 'compound B', and some yellow material (λ_{max} 388 mμ) which was probably closely related to compound B. After extensive purification, B was obtained as an almost homogeneous, colorless, syrupy hydrochloride which, significantly, was optically inactive. It comprised one agmatine residue, which could be liberated by vigorous hydrolysis, and a conjugated stilbene system, for which the evidence was as follows.

Compound B had absorption maxima at 309 and 229 mμ, that at 309 mμ being the more intense (in contrast to hordatine). Over platinum oxide in acetic acid, it absorbed 1 mole of hydrogen, but the dihydro derivative still contained a conjugated aromatic system. This was not due to a coumaric acid residue, because dihydro B was stable to irradiation under conditions in which compound B suffered *trans* to *cis* isomerization. The n.m.r. spectrum of dihydro B showed the presence of a phenylpropionic acid side chain and of a methoxycarbonyl group. Further, it lacked the signals attributable to H_β and H_γ in VIII; instead, a singlet appeared at τ 2.1, reminiscent of that arising from the deshielded olefinic proton in IV (τ 2.15). In its ultraviolet spectrum, dihydro B exhibited changes on basification which were very sensitive to the conditions (see Experimental). An interpretation, together with reasonable structures for compound B and dihydro B, is given in Scheme 2.

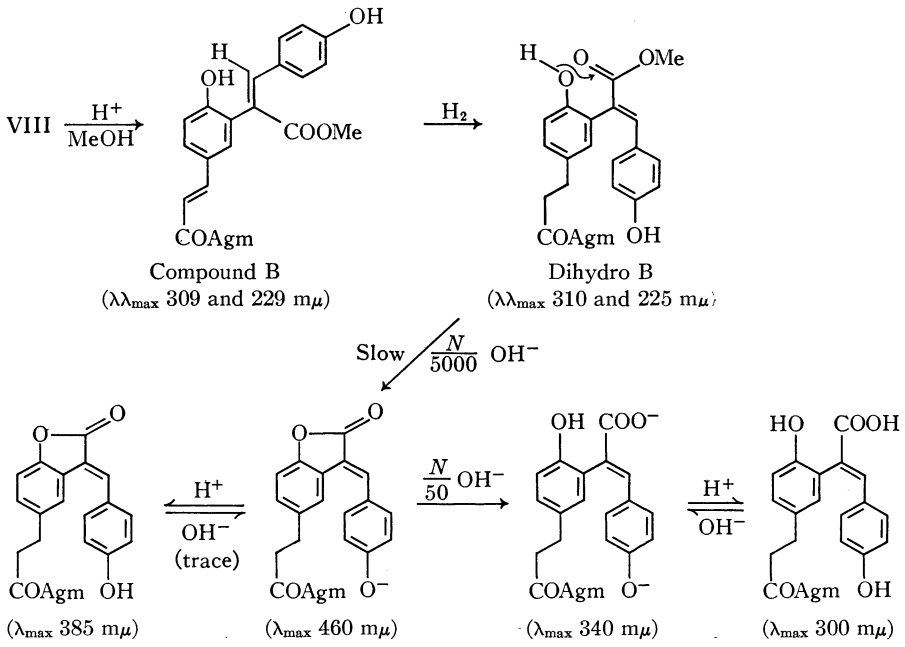
These structural proposals accounted for all the available evidence except for the tentative empirical formula for hordatine M, which exceeds the requirements of XII by $C_3H_{10}O_2$. No degradative evidence for the presence of these atoms had been obtained despite careful search. On the other hand, recalculation of the original analytical data showed that those for M dipicrate were, in fact, in very good agreement with



VIII R = H, R′ = H
IX R = OMe, R′ = H
XIV R = H, R′ = α-D-glucopyranosyl
XV R = OMe, R′ = α-D-glucopyranosyl



X R = H, R′ = H
XI R = OMe, R′ = H
XII R = H, R′ = α-D-glucopyranosyl
XIII R = OMe, R′ = α-D-glucopyranosyl



SCHEME 2. Compound B and its transformation products.

the composition $C_{34}H_{48}N_8O_9 \cdot 2C_6H_3N_3O_7$, required by hordatine A glucoside (XII) (the absence of the B component in this particular sample was evident from a negative test for methoxyl). Similar agreement was shown by the analytical figures for hordatine M dihydrochloride, provided that the presence of solvent was assumed. This interpretation of the analytical data could eventually be substantiated by other analyses. As work progressed, it became clear that the aglucones should be co-metabolites of hordatine M; this was easily verified by chromatography. Extensive refractionation of some barley extracts then culminated in the isolation of pure specimens of both hordatine A and hordatine B. The optically active diacetates of the substances were characterized by ultraviolet and n.m.r. spectroscopy, and both yielded dipicrates which gave elemental analyses in excellent agreement with structures X and XI, respectively. The dipicrate of naturally occurring hordatine A was also identical, as shown by its infrared spectrum, with that of the methanolysis product.

Unambiguous support for XII and its derived structures was obtained from the n.m.r. spectra (Figs. 2–4 and Table I). These were invariably broad, but the results obtained for all compounds, generally determined in both deuterium oxide and acetic acid-d_4, were consistent. Thus, all but two of the bands could be assigned, qualitatively and quantitatively, to protons which had already been identified by other means. The remaining signals arose from only two hydrogen atoms, in excellent agreement with the empirical formulae of XII and its derivatives. The positions and nature of the signals, which occurred as a pair of doublets (J_{AB} ~7.5 c.p.s.), also agreed with the structural requirements of XII, as evidenced by published n.m.r. studies (6) of dehydrodiconiferyl alcohol (XVI) and its analogues. The same studies (6) assigned a *cis* relation to H_β and H_γ in XVI, but were careful to stress the tentative nature of this conclusion; nevertheless, it was provisionally extended (2) to the hordatines. Recently it has become even more apparent (7, 8)[5] that n.m.r. data alone may not suffice to define the configuration of coumarans. The stereochemistry of H_β

[5]Reference 7 quotes coupling constants (6–7 c.p.s.) for relevant protons in *both cis* and *trans* relationships.

TABLE I

Nuclear magnetic resonance spectra*

	Hordatine M diacetate	Dihydrohordatine M diacetate	Hordatine A diacetate	Hordatine B diacetate	Dihydrohordatine A diacetate	Crude aglucone methyl ether†‡	Crude dihydroaglucone methyl ether†‡
H_{Ar}, $H_{\beta'}$, $H_{\gamma'}$	τ 1.9–3.5 (9)§ m‖	τ 2.0–2.9 (7) m	τ 1.9–3.5 (9) m	τ 1.9–3.5 (8) m	τ 2.2–3.0 (7) m	τ 1.9–3.7 m	τ 2.4–3.4 m
H_{γ}	τ 3.66 (1) d [7]¶	τ 3.67 (1) d [7]	τ 3.81 (1) d [8]	τ 3.81 (1) d [7]	τ 3.80 (1) d [7.5]	τ 3.92 m	τ 4.02 d [7]
$H_{\alpha a}$	τ 4.50 (1) d [4]	τ 4.46 (1) d [5]	—	—	—	—	—
H_{β}	τ 5.67 (1) d [7]	τ 5.50 (1) d [7]	τ 5.43 (1) d [8]	τ 5.3–5.5 (?) m	τ 5.35 (1) d [7.5]	τ 5.63 m	τ 5.71 d [7]
H_{Glu}, H_{OMe}	τ 5.5–6.1** (6.5) m	τ 5.5–6.1 (6) m	—	τ 5.89 (3) broad s?	—	τ 6.05 s	τ 6.20 s
N—CH_2—	τ 6.1**–6.8 (8) m	τ 6.1–6.7** (8) m	τ 6.0–6.8 (8) m	τ 6.1–6.8 (8) m	τ 6.2–6.8** (8) m	τ 6.3–7.0 m	τ 6.3–6.9** m
Ar—CH_2—CO	—	τ 6.6**–7.3 (4) m	—	—	τ ~6.6**–7.3 (4) m	—	τ 6.9**–7.7 m
CH_3—COO-	τ 7.60 (6) s	τ 7.60 (6) s	τ 7.63 (6) s	τ 7.63 (6) s	τ 7.63 (6) s	—	—
N—CH_2—CH_3—	τ 7.75–8.3 (8) m	τ 7.85–8.5 (8) m	τ 7.75–8.4 (8) m	τ 7.75–8.4 (8) m	τ 7.8–8.6 (?) m	τ 8.1–8.7 m	τ 8.1–8.8 m

*Determined on a Varian A60 spectrometer in deuterium oxide solutions with tetramethylsilane as an internal reference (unless otherwise stated).
†Determined in acetic acid-d4 solutions with tetramethylsilane as an external reference.
‡These spectra contained additional bands obviously arising from impurities.
§Values in parentheses refer to the relative area.
‖Multiplicity (m, multiplet; d, doublet; and s, singlet).
¶Values in brackets refer to J_{AB} (c.p.s.) (estimated to the nearest 0.5 c.p.s.).
**Bands overlapped in this region.

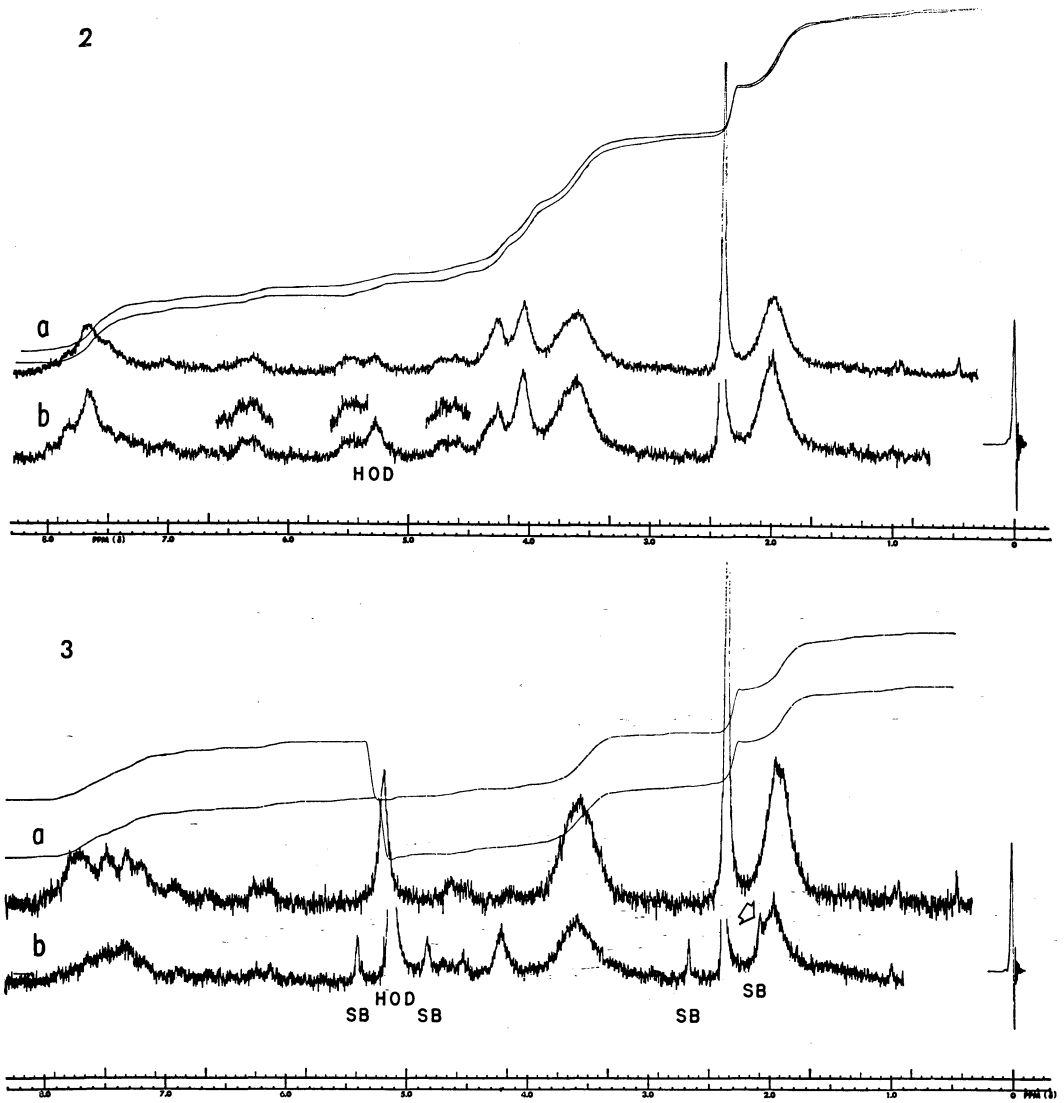


FIG. 2. The n.m.r. spectra of hordatine M diacetate in deuterium oxide (*a*, fraction B; *b*, fraction C).
FIG. 3. The n.m.r. spectra in deuterium oxide of (*a*) hordatine A diacetate and (*b*) hordatine B diacetate.

and H_γ must therefore remain unassigned until further work has been done.

The anomeric protons of hordatine M (Fig. 2) and of the dihydrohordatine glucosides (2) absorb at relatively low field, where they appear as poorly resolved doublets. The magnitudes of the associated coupling constants can only be estimated roughly, but appear to be near 4 c.p.s. and certainly do not exceed 5 c.p.s. These observations favor an α stereochemistry (9). However, it should be noted that almost all naturally occurring, phenolic glucosides have the β configuration (10). Unfortunately, attempts to hydrolyze hordatine M with stereospecific enzymes have so far been unsuccessful.

The structure (X) proposed for hordatine A implied its ready accessibility in the laboratory. In vitro syntheses, based on biogenetic considerations, of the phenylcoumarans XVI and XVII have been

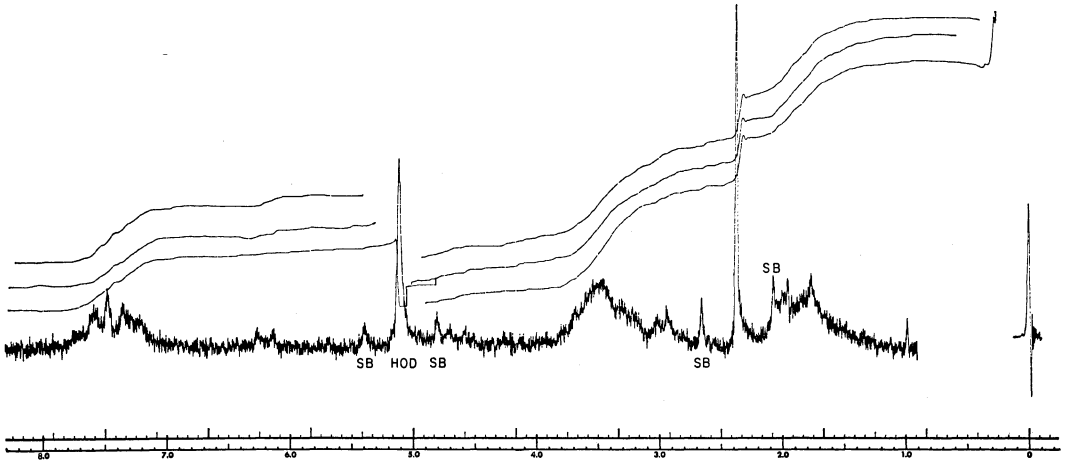
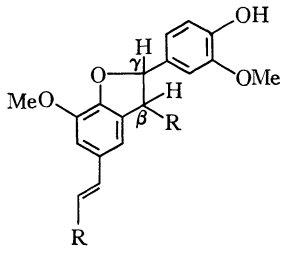


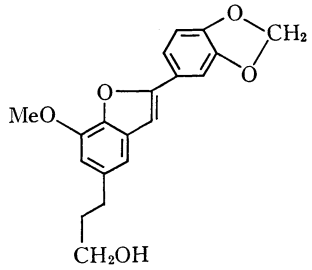
FIG. 4. The n.m.r. spectrum of dihydrohordatine A diacetate (containing a small amount of dihydrohordatine B) in deuterium oxide.

achieved by Freudenberg (11, 12), and XVIII has been known since 1908 (13). There could be little doubt that X was biogenetically derived from the oxidative phenol coupling (14) of coumaric acid and subsequent amidation, or more directly from coumaroylagmatine (XIX), which had already been isolated (15) as a co-metabolite of the hordatines. In the plant, the direct route would be the more economical in enzyme requirements and, hence, the more probable; it was also the more attractive for the proposed in vitro synthesis. When tested, coumaroylagmatine reacted readily with very dilute hydrogen peroxide in the presence of horseradish peroxidase (12). The most prominent component, formed in about a 34% yield, of the somewhat complex product was chromatographically indistinguishable from hordatine A and could be isolated in a pure state by countercurrent distribution. The substance was optically inactive, but in all other respects was identical, both as the diacetate and dipicrate, with the corresponding salts of hordatine A. It also gave the expected derivative on hydrogenation, and could be degraded further to the crystalline lactone VI.

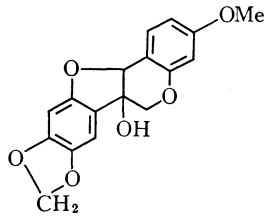
It was of some interest to determine whether the dimerization of coumaroylagmatine, itself accessible by synthesis, could also be induced by purely chemical means. After a number of reagents were tested, limited success was achieved with alkaline ferricyanide. The reaction was slow and incomplete, but the very complex product appeared (on chromatographic evidence) to contain hordatine A. After fractionation, it yielded a picrate which was identical with hordatine A dipicrate (as shown by ultraviolet and infrared spectra), but too little



XVI R = CH_2OH
XVII R = $COOC_2H_5$
XVIII R = CH_3



XX



XXI

was obtained to allow further characterization.

Phenylpropanoid dimers with a phenylcoumaran skeleton are important in theories of lignin biosynthesis (16), but evidence for their natural occurrence has been more than sparse. The only claim concerns the detection of traces of dehydrodiconiferyl alcohol in spruce sap by chromatographic means (17), and even this has been criticized (18) as being possibly caused by an artifact. On the other hand, a known compound whose significance in this connection appears to have been overlooked is egonol (XX), which was isolated from Styrax species (19). Its structure suggests that it may have been derived by oxidative decarboxylation of a 3-carboxy-2-phenylcoumaran precursor. Hydroxycinnamic acids and esters have been implicated (20) in the lignification processes of the *Gramineae*, and the syntheses reported here and by Freudenberg (12) have demonstrated that oxidative coupling of phenylpropanoids to phenylcoumarans can be a favored process. The possible role of the hordatines in lignin biosynthesis, as either intermediates or shunts, therefore deserves investigation. It may be relevant that the hordatines have solubility properties which would alleviate the problems (21) of translocation in the plant. On the other hand, they are optically active, whereas no optical activity has ever been detected in lignin (21).

Under defined conditions, the hordatines have marked antifungal properties which will be discussed elsewhere. In passing, however, one may note the points of structural similarity to the natural antifungal agents exemplified by pisatin (XXI) (22).

EXPERIMENTAL

Microanalyses were performed by Dr. C. Daesslé, Montreal. Melting points were determined on the Kofler block, optical rotations on 1% solutions in water, and infrared spectra on KBr disks with a Perkin–Elmer 21 spectrophotometer. Ultraviolet spectral constants are for solutions in 96% ethanol. Nuclear magnetic resonance spectra were determined on a Varian A60 instrument, with tetramethylsilane as an external reference for solutions in D_2O, and as an internal reference for solutions in other solvents. Analytical and preparative thin-layer chromatography (t.l.c.) was carried out on a microcrystalline cellulose (FMC Corporation, American Viscose Division, Newark, Delaware), with the upper phase of *n*-butanol – acetic acid – water (4:1:5) as irrigant and diazotized nitroaniline, bromocresol green, or the Sakaguchi reagent as indicator. Countercurrent distributions were performed by the single-withdrawal procedure (for terminology and symbols, see ref. 23), with 100 elements (25 ml each phase) and the above solvent system. Distributions and other lengthy processes on dilute solutions were carried out in the dark or in orange light.

Isolation of Metabolites

In a typical run, the basic material (5.7 g) obtained as previously described (5), by ion-exchange of the hot-water extract of 6-day-old barley coleoptiles (4.3 kg fresh weight), was fractionated by countercurrent distribution (300 transfers). After analysis (ultraviolet and t.l.c.), suitable fractions were combined (Table II), evaporated to dryness *in vacuo*, dissolved in 96% ethanol, and separated from insoluble material by filtration after several days.

TABLE II

Countercurrent distribution of metabolites

r fractions	*ρ* fractions	Combined fraction	Weight (mg)
39–44	—	A	632
45–51	—	B	610
52–56	—	C	411
57–64	—	D	126
65–99	199–0	E	582

Fraction A contained hordatine glucosides together with salts (mainly inorganic), and was used only for exploratory purposes.

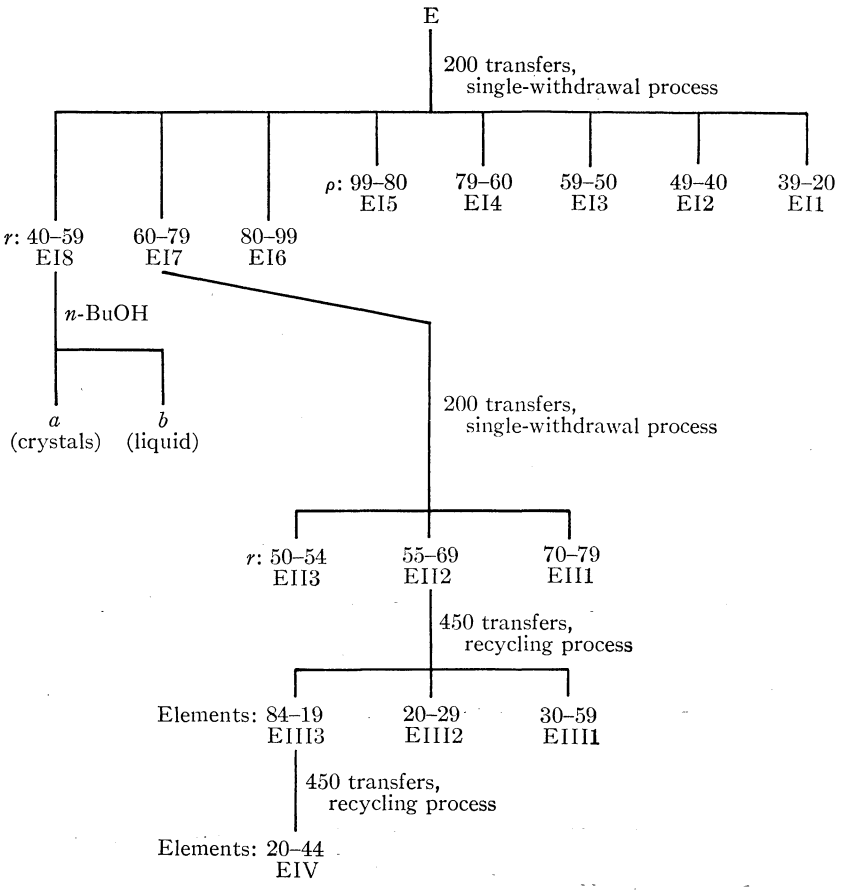
Fractions B and C (hordatine M) consisted of mixtures of the glucosides of *trans*- and *cis*-hordatines A and B. Attempts at further resolution by repeated countercurrent distribution or preparative t.l.c. were unsuccessful. For the constants quoted in Table III,

TABLE III

Physical constants of hordatine M fractions

Fraction	$\lambda\lambda_{max}$ (H_2O)*	$E^{1\%}_{1\,cm}$	$[\alpha]_D^{23}$	R_f†
B	312, 300, 223	218, 214, 300	+53°	0.28 (minor), **0.33**, 0.37
C	308, 297, 223	231, 234, 289	+60°	0.28 (minor), 0.33, **0.37**

*Unchanged by the addition of alkali.
†Low concentration (⩾0.01 mg/spot) is required for resolution.



SCHEME 3. Refractionation of fraction E.

the fractions were precipitated as the picrates, washed with water, and reconverted into the acetate form by ion exchange (Dowex 2X10 (Cl^-) followed by Dowex 2X7.5 (OAc^-)).

The n.m.r. spectra (Fig. 2) of the fractions did not differ significantly from those of material not purified through the picrates, and the latter was used for most of the degradative work.

In another run, the picrate was precipitated repeatedly by slow concentration of its solution in 96% ethanol. The waxy solid (slow liquefaction above 115 °C) had $\lambda\lambda_{max}$ 357, 320, and 224 mμ (ε 29 400, 31 500, and 48 800).

Anal. Calcd. for $C_{34}H_{48}N_8O_9 \cdot 2C_6H_6N_3O_7$: C, 47.19; H, 4.65; N, 16.75; O, 31.43; OCH_3, 0. Calcd. for $C_{37}H_{58}N_8O_{11} \cdot 2C_6H_3N_3O_7$: C, 47.11; H, 5.16; N, 15.70; O, 32.02. Found: C, 47.29; H, 5.17; N, 16.23; O, 31.64; OCH_3, 0.0.

Hordatine M dipicrate was filtered through Dowex 1X10 (Cl^-) in 80% ethanol. The product obtained on evaporation was precipitated from water by the addition of acetone and, after trituration with more acetone, was obtained as a colorless, amorphous solid which was dried at 56° *in vacuo*.

Anal. Calcd. for $C_{34}H_{48}N_8O_9 \cdot 2HCl \cdot CH_3COCH_3 \cdot 2H_2O$: C, 50.51; H, 6.87; N, 12.74; O, 21.82; Cl, 8.06. Calcd. for $C_{34}H_{47}N_8O_9(OCH_3) \cdot 2HCl \cdot CH_3COCH_3 \cdot H_2O$: C, 51.21; H, 6.79; N, 12.57; O, 21.53; Cl, 7.95. Calcd. for $C_{37}H_{58}N_8O_{11} \cdot 2HCl$: C, 51.44; H, 7.00; N, 12.97; O, 20.37; Cl, 8.29. Found: C, 51.31, 51.42; H, 6.80, 6.86; N, 12.53; O, 21.92; Cl, 8.09.

Fraction D, frequently containing small amounts of faster travelling components, was used only for relays.

Fraction E

Material derived from six runs was combined and refractionated according to Scheme 3. All final fractions, except EI8*a*, were purified via their picrates as described above.

Fraction EIII1 (hordatine A, 99 mg, R_f 0.54) had $[\alpha]_D^{23}$ +69° and $\lambda\lambda_{max}$ 307, 298, and 229 mμ (ε 18 400, 18 400, and 20 700) in a neutral solution and 305, 297, and 235 mμ (ε 18 700, 19 400, and 17 000) in an alkaline solution. The dipicrate, reprecipitated by careful concentration of its solution in warm methanol, was microcrystalline, m.p. 127–128 °C, $\lambda\lambda_{max}$ 358, 319, and 229 mμ (ε 29 200, 30 200, and 45 500).

Anal. Calcd. for $C_{28}H_{38}N_8O_4 \cdot 2C_6H_3N_3O_7$: C, 47.62; H, 4.40; N, 19.44. Found: C, 47.79; H, 4.57; N, 19.34.

Fraction EII1 (44 mg) was essentially identical with fraction EIII1.

Fraction EIV (hordatine B, 22 mg, R_f 0.53) had $[\alpha]_D^{23}$ +54° and $\lambda\lambda_{max}$ (H_2O) 316, 301, and 224 mμ (ε 16 200, 15 500, and 22 000). The n.m.r. spectrum (Fig. 3) was very similar to that of hordatine A, except for a band (ca. 3H) at τ 5.79. The dipicrate, recrystallized as above, had m.p. 132–135 °C and $\lambda\lambda_{max}$ 355, 332, and 228 mμ (ε 31 000, 35 000, and 52 000).

Anal. Calcd. for $C_{29}H_{40}N_8O_5 \cdot 2C_6H_3N_3O_7$: C, 47.50; H, 4.47; N, 18.92; O, 29.33. Found: C, 47.53; H, 4.70; N, 18.88; O, 29.01.

Fraction EI4 (63 mg, R_f 0.70) was almost pure coumaroylagmatine (14).

Fraction EI8*a* (92 mg) was recrystallized from aqueous alcohol and identified as adenine by comparison with an authentic specimen (ultraviolet, infrared, ferric chloride, t.l.c.).

Photoisomerizations

An aqueous solution (1.8%) of hordatine M was irradiated in a Pyrex flask with light from an 85 W lamp (GE CH-3). The ultraviolet spectra, determined at intervals, appeared as a family of curves (1) with isosbestic points at 272 and 254 mμ. Reaction was complete after ca. 40 min (λ_{max} 277 mμ ($E^{1\%}_{1cm}$ 125)). The R_f values of the product were unchanged, but the intensities of the two principal spots were reversed. Similar changes were observed for free hordatine, compound B (see below), and *p*-methoxycinnamoylagmatine (14).

Acetylation of Hordatine M

A solution of crude glucoside in acetic anhydride, kept at room temperature for 3 days, was evaporated to dryness *in vacuo*. The product, isolated as a glass by countercurrent distribution (100 transfers, elements 46–52) was soluble in chloroform when freshly prepared. It had $\lambda\lambda_{max}$ 313 and 298 mμ ($E^{1\%}_{1cm}$ 176 and 168), and $\nu_{max}^{CHCl_3}$ 3 300 (NH), 1 755 (CH_3COO—), 1 663 (C=N), and 1 555 and 1 410 (CH_3COO^-) cm^{-1}.

Anal. Calcd. for $C_{34}H_{44}N_8O_5(OCOCH_3)_4 \cdot 2CH_3COOH$: CH_3CO, 25.8; equiv. wt. 500.5. Found: CH_3CO, 25.6; equiv. wt. (titration with mineral acid) 510. The elemental analysis was unsatisfactory.

Hydrogenation of Hordatine M

The substance (567 mg), in acetic acid (5 ml), was shaken with hydrogen over platinum oxide (30 mg) for 1 h. After this essential step (no absorption by sample), the solution was filtered and hydrogenated over fresh catalyst (H_2 absorbed, 15.5 ml; calculated, 15.3 ml/mole equivalent).

The product, dihydrohordatine glucoside diacetate, obtained by evaporation, had $\lambda\lambda_{max}$ 282 and 220 mμ (ε 2 600 and 18 000), stable to dilute base.

Potentiometric Titrations

Hordatine M diacetate (114 mg) in water (2 ml) was percolated through a column (3.5 × 0.7 cm) of Amberlite IRA 400 (OH^-) and the column washed with water until neutral. An aliquot (4 ml) of the combined effluents (25 ml) was acidified (1.00 ml of 0.091 *N* H_2SO_4) and titrated at the potentiometer with 0.100 *N* NaOH in an inert atmosphere. Found: p*K'* 11.2; equiv. wt. 444. Calculated for $C_{34}H_{48}N_8O_9 \cdot 2CH_3COOH$: equiv. wt. 416.5. The data from this and a similar experiment with agmatine disulfate were plotted in a manner suitable for comparison (Fig. 1).

Hydrolysis of Hordatine M to Agmatine

The substance (61 mg) was kept on the steam bath in hydrochloric acid (6 *N*, 1.2 ml) for 2.5 h, the solvent was removed *in vacuo*, and the residue, in water, was filtered through columns of Rexyn CG 51 (H^+) and Dowex 1 (OH^-). After neutralization with dilute hydrochloric acid, the product (0.65 mole/mole) was obtained by evaporation and recrystallization from methanol.

Anal. Calcd. for $C_5H_{14}N_4 \cdot 2HCl$: C, 29.55; H, 7.93; N, 27.60; Cl, 34.93; equiv. wt. 103. Found: C, 29.96; H, 7.62; N, 27.83; Cl, 34.85; equiv. wt. (potentiometer) 103.

The substance was shown to be identical with authentic agmatine dihydrochloride by melting point, mixture melting point, and infrared spectrum, and was further characterized as the dipicrate (crystallized from water) by melting point, mixture melting point, ultraviolet spectrum, and elemental analysis.

Anal. Calcd. for $C_5H_{14}N_4 \cdot 2C_6H_3N_3O_7$: C, 34.70; H, 3.43; N, 23.81. Found: C, 34.51; H, 3.66; N, 23.84.

Vigorous alkaline hydrolysis of further samples of the degradation product gave a readily volatile base (ammonia) and putrescine. The latter was isolated as the dihydrochloride and dipicrate salts, shown to be identical with authentic specimens by melting point and mixture melting point, respectively.

After more vigorous hydrolysis of hordatine M (6 *N* hydrochloric acid, sealed tube, 110 °C, 20 h), the yield of agmatine was estimated by titration as 1.23 moles/mole.

Periodate Oxidation of Hordatine M

(*a*) Aliquots (4 ml) of a solution (25 ml) containing hordatine M disulfate (0.069 mmole, prepared as above) and periodic acid (1.00 ml of 0.20 *M* solution) were titrated at intervals (*i*) with 0.0100 *N* NaOH after the addition (24) of ethylene glycol (1 ml), and (*ii*) by the arsenious oxide – iodine method (25). Appropriate blanks were determined in the absence of glucoside. The results (Table IV) were calculated as the organic acid produced and the periodic acid consumed.

(*b*) The product of a similar oxidation of hordatine M disulfate (1.05 mmoles) was distilled *in vacuo* below room temperature. The distillate, collected at −70 °C, was neutralized (1.04 ml of 0.10 *N* NaOH consumed). Recrystallized from methanol, the resultant salt was identified as sodium formate by melting point, mixture melting point, and infrared spectrum.

TABLE IV
Periodate titrations

Reaction time (h)	Organic acid (equivalent/mole)	Periodic acid (moles/mole)
1.5	0.36	0.70
2.5	0.63	1.25
18.0	0.93	1.99

Nitric Acid Oxidation

Hordatine M (51 mg) was heated on the steam bath for 1 h with concentrated nitric acid (2.5 ml). Chromatography over silica gel (0.4 g) of the residue obtained on evaporation gave picric acid (12 mg), shown to be identical with an authentic specimen by t.l.c., melting point, mixture melting point, and ultraviolet and infrared spectra. In a similar experiment, the yield of picric acid was estimated spectrophotometrically as 0.99 mole/mole of substrate.

Alkali Fusion

Hordatine M (68 mg) was added in small portions to a melt of sodium and potassium hydroxides (4 g, 1:1) at 230 °C. After 1 h under nitrogen (frequent stirring), the mixture was cooled, dissolved in water, acidified, and continuously extracted with ether. The acidic components (20 mg) of the extract, on preparative t.l.c. (silica, *n*-butanol – chloroform – acetic acid (12.5:250:0.5)), gave *p*-hydroxybenzoic acid as the principal product, identified by melting point, mixture melting point, ultraviolet and infrared spectra, and chromatographic behavior. Treatment with diazomethane gave methyl anisate, identified by vapor-phase chromatography. A similar fusion of salicylic acid did not lead to detectable amounts of the *p* isomer.

Ozonolysis of Hordatine M

The acetylated glucoside (131 mg) was ozonized in water at room temperature until the ultraviolet spectrum was constant (λ_{max} 288 mμ). Hydrogen peroxide (1 ml, 30%) was added and the temperature of the solution raised to the boiling point. After the addition of hydrochloric acid (3 ml), the solution was refluxed overnight, extracted with ether (extracts discarded), and evaporated *in vacuo*. Ion exchange of the residue and extraction of the solid acidic products with ether gave substantially pure (t.l.c.) oxalic acid (4.2 mg, 32 mole %), which was sublimed and characterized by melting point and mixture melting point.

Methanolysis of Hordatine M

The glucoside (860 mg) was refluxed gently for 1.5 h in methanol (86 ml) containing HCl (2% w/v). The product was evaporated to dryness *in vacuo*, dissolved in water, and filtered through Dowex 2X8 (OAc^-). The filtrate and washings were concentrated to ca. 2 ml and adsorbed on Rexyn CG 51 (H^+) (25 ml). Elution with water gave α-D-methylglucopyranoside (160 mg) contaminated with a trace of only glucose (t.l.c.). After recrystallization from isopropanol, it had $[\alpha]_D^{24}$ +151° (*c*, 1.56) and did not depress the melting point of an authentic specimen. The infrared spectra were identical.

Further elution of the Rexyn column with acetic acid (1 *N* in 80% methanol) gave a basic fraction which contained the aglucones.

Isolation of Hordatines A and B as Methanolysis Products

(*a*) The basic fractions from two such runs were combined and fractionated by countercurrent distribution (400 transfers) in *n*-butanol–water. Fractions *r* 61–55 (95 mg) partially crystallized on evaporation. Hand-picked and dried on porous tile, the crystals of almost pure hordatine A diacetate had $\lambda\lambda_{max}$ 305, 298, and 229 mμ (ε 19 800, 19 800, and 22 900) (neutral) and 305, 297, and 238 mμ (ε 22 600, 23 300, and 20 300) (alkaline), and gave 100% inhibition of *Monilinia fructicola* at 5 p.p.m. (standard spore-drop assay). The n.m.r. spectrum showed only a trace of absorption attributable to methoxyl. Attempts to recrystallize the substance for analysis were unsuccessful, and the isolation of crystalline material could not be repeated on subsequent occasions.

(*b*) In other runs, hordatine A, virtually free of hordatine B but containing a considerable proportion of the *cis* isomer, was obtained by the more convenient countercurrent distribution (200 transfers) in *n*-butanol – water – acetic acid. The required fractions (*r* 84–70), after evaporation and re-solution in water, were extracted with small volumes of *n*-butanol and ether (extracts discarded) and then precipitated with picric acid. After being washed with water, the salt had $\lambda\lambda_{max}$ 359, 320, and 228 mμ (ε 30 600, 29 100, and 48 000). The infrared spectrum was indistinguishable from that of natural hordatine A dipicrate (metabolite fraction EII1). After reconversion into the acetate form, the product had $[\alpha]_D^{23}$ +68° and $\lambda\lambda_{max}$ 297 and 229 mμ (ε 15 400 and 20 600) with a shoulder at 285 mμ (*cis* isomer). The n.m.r. spectra of the three preparations showed only the expected, slight differences in the vinyl and aromatic proton regions.

A similar purification of fractions *r* 69–60 yielded material apparently rich in hordatine B, with $[\alpha]_D^{23}$ +55° and $\lambda\lambda_{max}$ 315, 300, and 229 mμ (ε 15 100, 15 300, and 22 000). In the n.m.r., a broad singlet at τ 5.81 corresponded to ca. 2H.

Methylation of the Aglucone

The aglucone (200 mg) was refluxed for 40 min in methanol (20 ml) containing methyl sulfate (0.6 ml) and potassium carbonate (800 mg). The solution was filtered from inorganic salts and the product isolated by countercurrent distribution (100 transfers, *r* 49–35, 124 mg). It had $\lambda\lambda_{max}$ 297 and 227 mμ (ε 16 700 and 23 100), stable to base. Potentiometric titration (as for the glucoside above) gave p*K'* 11.25 and equiv. wt. 380 (calculated for $C_{28}H_{37}N_5O_3$-$(OCH_3)\cdot 2CH_3COOH$: equiv. wt. 342).

Methanolysis of the Aglucone

The aglucone (300 mg) was refluxed for 4 h in methanol (60 ml) containing HCl (10% w/v). No carbon dioxide was evolved (barium hydroxide trap).

The product was converted into the acetate form and adsorbed on Rexyn CG 51 (H^+) as described above. Elution with water (50 ml) followed by methanol (20 ml) gave a syrup (6 mg) containing *p*-hydroxybenzaldehyde as the main component (t.l.c.) and characterized, after sublimation, by melting point and mixture melting point and, similarly, as the 2,4-dinitrophenylhydrazone.

Continued elution of the column with 1 *N* aqueous acetic acid gave agmatine diacetate (105 mg, 0.95 mole/mole), characterized by t.l.c. and conversion into the hydrochloride.

Further elution of the column with acetic acid (1 *N* in 80% methanol) gave a syrup (221 mg) which, on repeated preparative t.l.c. (methanol containing 0.1% hydrochloric acid), yielded a colorless, syrupy compound B and yellow material with intense absorption at 388 mμ.

Compound B

After further chromatography on cellulose, compound B (as the hydrochloride) was optically inactive (Hg and Na lines) and had λ_{inf} 390 mμ ($E^{1\%}_{1cm}$ 5.5, impurity) and $\lambda\lambda_{max}$ 309 and 229 mμ ($E^{1\%}_{1cm}$ 563 and 409). The addition of alkali (final concentration 0.0002 *N*) produced a weak maximum near 470 mμ (increased intensity on standing) and a pronounced inflexion near 365 mμ (rapid decrease on standing).

The substance (56 mg) was refluxed on the steam bath in 6 *N* hydrochloric acid for 24 h. Tarry material was removed by filtration, and the filtrate was washed with ether and evaporated. Preparative t.l.c. of the product gave crude agmatine dihydrochloride (23 mg, m.p. 150–170 °C), readily purified as the dipicrate (melting point and mixture melting point).

Dihydro B

Compound B (45 mg) was hydrogenated over pre-reduced platinum oxide (15 mg) in acetic acid (absorbed: 2.1 ml of H_2; calculated for $C_{24}H_{28}N_4O_5 \cdot HCl$: 2.06 ml of H_2/double bond). The dihydro derivative, isolated as the syrupy hydrochloride, had $\lambda\lambda_{max}$ 310 and 225 mμ ($E^{1\%}_{1cm}$ 258 and 295) and λ_{sh} 290 mμ ($E^{1\%}_{1cm}$ 225). When the spectroscopic solution was made 0.0002 *N* in sodium hydroxide, the ensuing reaction, complete in about 1 h, gave rise to a family of curves with an isosbestic point at 400 mμ and $\lambda\lambda_{max}$ 460 and 365 mμ ($E^{1\%}_{1cm}$ 65 and 220 (2 min) and 258 and 85 (1 h), respectively). Acidification gave, reversibly, $\lambda\lambda_{max}$ 385, 312, and 295 mμ ($E^{1\%}_{1cm}$ 160, 128, and 128).

When a spectroscopic solution of dihydro B was made 0.02 *N* in sodium hydroxide, the instantly appearing absorption at 465 mμ was rapidly destroyed. After 24 h, the system absorbed maximally at 340 mμ in an alkaline solution and at 300 mμ in an acidified solution.

Dihydrohordatines

(a) From Dihydrohordatine Glucoside

The dihydroglucoside (600 mg) was methanolyzed (2% HCl) as described above. α-Methylglucoside (90 mg) was isolated as the neutral product. The basic portion was fractionated by countercurrent distribution (300 transfers), and fractions *r* 84–75 were purified through the picrate and reconverted into the acetate form as described earlier. The product (22 mg) had $\lambda\lambda_{max}$ 282 and 228 mμ (ε 3 750 and 16 400) (neutral) and 287 and 248 mμ (ε 4 600 and 14 000) (alkaline), and $[\alpha]_D^{23}$ +49°. The n.m.r. spectrum (weak) did not show a detectable signal arising from methoxyl.

A similar work-up of fractions *r* 74–65 gave a similar product (28 mg) which exhibited methoxyl absorption (ca. 1H) in the n.m.r.

(b) From Hordatine A

Naturally occurring hordatine A (40 mg) was hydrogenated in acetic acid (2 ml) over pre-reduced platinum oxide (14 mg, 83%). One mole equivalent of H_2 was absorbed. The product obtained on evaporation was shown to be identical with the above by n.m.r. and ultraviolet spectra, but had $[\alpha]_D^{23}$ +44°.

(c) From the Aglucone

A similar hydrogenation of crude aglucone (84 mg) followed by countercurrent distribution (700 transfers) gave dihydrohordatine (t.l.c., ultraviolet spectrum) containing appreciable amounts of the B component (n.m.r.).

Alkaline Degradation of Dihydrohordatine

Dihydrohordatine (155 mg, n.m.r. spectrum in Fig. 4), obtained by methanolysis of dihydroglucoside and located in fractions *r* 36–26 of a 150-transfer countercurrent distribution, was methylated as described for the aglucone. The crude product was refluxed for 18 h in potassium hydroxide solution (20% w/w, 10 ml) at 125 °C under nitrogen. Extraction of the cooled and acidified solution with ethyl acetate and preparative t.l.c. (benzene – acetic acid – water (6:7:3)) of the product (59 mg) gave substantially pure acids IV and V (40 and 3 mg, respectively). In the same manner, dihydrohordatine (120 mg), obtained from fractions 25–16 of the above countercurrent distribution, gave acids IV and V (53 mg) in the approximate ratio of 4:3.

Acid IV

The compound crystallized on trituration with chloroform and was recrystallized from ether – light petrol, m.p. 164–166 °C. The spectral data have been given in detail (2).

Anal. Calcd. for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30; O, 28.04. Found: C, 66.70, 66.83; H, 5.40, 5.54; O, 27.22.

The acid was held in a bath at 168 °C for 5 min. *Lactone* VI crystallized from ethyl acetate or methanol as yellow needles, m.p. 155–158 °C, or as a mixture of crystalline forms melting from about 140 °C. It dissolved in aqueous 0.1 *N* sodium hydroxide, acidification giving acid IV.

Anal. Calcd. for $C_{19}H_{16}O_5$: C, 70.36; H, 4.97. Found: C, 70.81; H, 4.99.

IV (10.3 mg) was ozonized in ethyl acetate at −70 °C until ultraviolet absorption became constant.

The solution was briefly boiled with a few drops of 30% hydrogen peroxide and washed with water. Concentration gave crystals (ca. 2 mg) of an acid, m.p. 218–221 °C, $\lambda\lambda_{max}$ 312 and 236 mμ ($E^{1\%}_{1cm}$ 157 and 212) (neutral) and 306 and 230 mμ ($E^{1\%}_{1cm}$ 157 and 238 (alkaline), ν_{max} 1 660 and 1 645 cm^{-1}. It gave an intense violet color with methanolic ferric chloride.

Acid V

The substance crystallized on trituration with benzene and was recrystallized from aqueous methanol. It had m.p. 180–184 °C; $\lambda\lambda_{max}$ 305 and 220 mμ (ϵ 19 900 and 24 000) (50% ethanol), λ_{max} 290 mμ (ϵ 21 000) (0.025 *N* sodium hydroxide, 50% ethanol), $\lambda\lambda_{max}$ 310 and 220 mμ (ϵ 26 000 and 28 000) (acidified 50% ethanol); ν_{max} 1 700 and 1 687 cm^{-1}; τ (in dimethyl sulfoxide-d_6) 2.32 (s, 1H), 2.7–3.7 (A_2B_2 system (4H) approximating to a pair of doublets centered at 2.87 and 3.23, and superimposed on signals arising from two or three other aromatic protons), 6.18 (s, 3H), 6.28 (s, 3H), and 7.1–7.8 (multiplet partly submerged under the solvent peak). When dried at 56 °C, the acid became slightly yellow and probably contained some lactone.

Anal. Calcd. for $C_{20}H_{20}O_7$; C, 64.51; H, 5.41; O, 30.1. Found: C, 65.22; H, 5.54; O, 28.95.

At the melting point, acid V formed the corresponding yellow lactone VII, characterized by $\lambda\lambda_{max}$ 380 and 250 mμ and ν_{max} 1 757 and 1 705 cm^{-1}.

Synthesis of 2-(5-(2-Carboxyethyl)-2-hydroxyphenyl)-3-(4-methoxyphenyl)acrylic Acid (IV)

β-4-Allyloxyphenylpropionic Acid Methyl Ester

Phloretic acid methyl ester (35 g) was refluxed for 4 h in acetone (40 g) containing allyl bromide (25 g) and potassium carbonate (28 g). The filtered solution was diluted with ether and rapidly extracted with cold sodium hydroxide solution (1 *N*, 50 ml) and water. Evaporation of the ether layer gave an almost pure product (32 g). The pure ester had b.p. 103 °C at 0.2 mm Hg, $n_D^{21.5}$ 1.5157, $\lambda\lambda_{max}$ 282 and 276 mμ (ϵ 1 590 and 1 280), and ν_{max}^{liquid} 1 732 cm^{-1}. The corresponding acid, obtained by hydrolysis in 0.05 *N* sodium hydroxide, crystallized from ether – light petrol, m.p. 89–90 °C, $\lambda\lambda_{max}$ 282 and 276 mμ (ϵ 1 655 and 1 340).

Anal. Calcd. for $C_{12}H_{14}O_3$: C, 69.92; H, 6.85; equiv. wt. 206. Found: C, 69.97; H, 6.63; equiv. wt. 210.

β-(3-Carboxymethyl-4-hydroxyphenyl)propionic Acid

The allyloxyphenyl ester was refluxed under nitrogen in a bath at 210 °C until absorption at ν_{max} 3 430 cm^{-1} was maximal (3.5 h). The product distilled, mainly at 104 °C and 0.003 mm Hg, and contained about 10% of unrearranged ester (n.m.r.). It was acetylated (acetic anhydride – pyridine, room temperature, overnight), and a portion (513 mg) of the crude derivative was ozonized in chloroform (5 ml) at 0 °C until olefinic absorption had disappeared from the infrared spectrum. The solution, diluted with chloroform (5 ml), was shaken at room temperature for 64 h with 0.01 *N* sulfuric acid (25 ml) and 30% hydrogen peroxide (0.5 ml). The mixture was extracted with ether (2 × 50 ml), and the combined extracts were washed with dilute ferrous sulfate solution and water. The solvent was removed and the product refluxed for 1 h in 1 *N* sodium hydroxide (10 ml) and methanol (10 ml). The solution was washed with ether (discarded), acidified, and extracted with more ether. Elution of the extract from silica gel with benzene containing ethyl acetate (30–60%) gave the desired acid (220 mg), m.p. 155–157 °C after recrystallization from ether–chloroform; λ_{max} 279 mμ (ϵ 2 200) (neutral), $\lambda\lambda_{max}$ 298 and 241 mμ (ϵ 4 300 and 11 500) (alkaline); ν_{max} 3 480, 1 700, and 1 687 cm^{-1}.

Anal. Calcd. for $C_{11}H_{12}O_5$: C, 58.95; H, 5.40. Found: C, 58.75; H, 5.34.

2-(5-(2-Carboxyethyl)-2-hydroxyphenyl)-3-(4-methoxyphenyl)acrylic Acid

The acid from above (120 mg) was refluxed with anisaldehyde (0.25 ml) and pyridine (0.25 ml) for 18 h in a bath at 120 °C. The product was dissolved in dilute sodium hydrogen carbonate solution, extracted with ether (discarded), and extracted with ethyl acetate after acidification. The desired lactone (123 mg), m.p. 117–145 °C, was eluted from silica gel with 20% ethyl acetate in benzene. Recrystallized from ethyl acetate, the compound was shown to be identical with lactone VI by melting point, mixture melting point, and infrared and ultraviolet spectra. Solution in 0.3 *N* sodium hydroxide followed by acidification gave the corresponding acid, shown to be identical with acid IV by melting point, mixture melting point, and infrared and ultraviolet spectra.

Synthesis of 2-(5-(2-Carboxyethyl)-2-hydroxy-3-methoxyphenyl)-3-(4-methoxyphenyl)acrylic Acid (V)

An analogous series of reactions, with methyl dihydroferulate as the starting material, gave the following characterized intermediates.

β-4-Allyloxy-3-methoxyphenylpropionic acid methyl ester, b.p. 104–106° C at 0.005 mm Hg, ν_{max}^{liquid} 1 735 cm^{-1}, characterized as the corresponding acid, m.p. 96–102 °C (crystallized from methanol or benzene), $\lambda\lambda_{max}$ 318, 280, and 228 mμ (ϵ 1 100, 3 500, and 8 400), ν_{max} 1 689 cm^{-1}.

Anal. Calcd. for $C_{13}H_{16}O_4$: C, 66.09; H, 6.83; O, 27.09. Found: C, 65.74; H, 6.60; O, 26.76.

β-(3-Carboxymethyl-4-hydroxy-5-methoxyphenyl)-propionic acid was isolated by preparative t.l.c. and crystallized from ethyl acetate. Repeated recrystallization from aqueous alcohol or acetone gave the pure compound, m.p. 165–167 °C, λ_{max} 281 mμ (ϵ 2 800), ν_{max} 1 707 cm^{-1}.

Anal. Calcd. for $C_{12}H_{14}O_6$: C, 56.69; H, 5.55. Found: C, 56.88; H, 5.54.

Condensation of the above acid (56 mg) with anisaldehyde in the presence of pyridine gave crude lactone VII (t.l.c.), which was hydrolyzed to the corresponding acid by dilute base. After purification by preparative t.l.c., the product (6 mg) crystallized from aqueous methanol and was shown to be identical with V by melting point, mixture melting point, and ultraviolet and infrared spectra.

TABLE V

Antifungal activities

Concentration (p.p.m.)*	80	40	20	10	5	2.5
(±)-Hordatine A	100	100	100	100	80	0
(±)-Hordatine A, after irradiation with sunlight	100	100	100	0	0	0
Hordatine A, natural	100	100	100	100	46	0
Hordatine A, by methanolysis†	100	100	100	100	11	0
Coumaroylagmatine	0	0	0	0	0	0
Agmatine	11	0	0	0	0	0

*All compounds in the acetate form.
†Contained some *cis* isomer (λ_{sh} 280 mμ).

Synthesis of (±)-Hordatine A

(a) With Peroxidase

A stirred solution of coumaroylagmatine acetate (200 mg) in water (80 ml) at 30–35 °C, containing 0.002 ml of a 1% solution of horseradish peroxidase in 2.8 *M* ammonium sulfate (Calbiochem, Los Angeles, California, grade A), was treated dropwise during 16 h with hydrogen peroxide solution (0.022%, 70 ml). The solvent was removed *in vacuo* (⩾40 °C) and the residue subjected to countercurrent distribution (200 transfers). Fractions *r* 85–54 contained the desired product (68.5 mg, 34 mole %, estimated spectrophotometrically), optically inactive but identical with hordatine A in chromatographic behavior (cellulose, *n*-butanol – water – acetic acid (4:5:1), R_f 0.62; cellulose, water-saturated *n*-butanol, R_f 0.39; silica gel (Camag), concentrated hydrochloric acid – methanol (1:100), R_f 0.70; silica gel, acetic acid – methanol (5:95), R_f 0.39).

The dipicrate precipitated from water as an amorphous solid, whose infrared spectrum was almost identical with that of hordatine A dipicrate (photographs of the spectra have been published (3)). On careful concentration (room temperature), it separated from methanol as an oil which solidified on trituration, m.p. 128–130 °C, $\lambda\lambda_{max}$ 359, 318, and 229 mμ (ε 29 300, 31 300, and 47 300).

Anal. Found: C, 47.34; H, 4.47; N, 19.26.

The diacetate, recovered by ion exchange of the dipicrate, had $\lambda\lambda_{max}$ 307, 298, and 229 mμ (ε 19 600, 19 700, and 22 100) (neutral) and 305 (sh), 297, and 238 mμ (ε 20 000, 20 700, and 20 000) (alkaline). The n.m.r. spectrum was identical with that of hordatine A within experimental error. The inhibition data, with *Monilinia fructicola* as the test organism in the standard spore-drop assay, are given in Table V.

The substance (21.4 mg) was hydrogenated (1 mole equivalent of H_2 absorbed) as described for hordatine A. The product, indistinguishable from dihydrohordatine A by ultraviolet and n.m.r. spectra, was methylated and hydrolyzed with 20% potassium hydroxide as described earlier. The acidic product (6 mg), on preparative t.l.c., sublimation, and recrystallization, gave lactone VI, identified by melting point, mixture melting point, and t.l.c.

(b) With Ferricyanide

A solution of coumaroylagmatine acetate (98 mg) in potassium ferricyanide (0.005 *M*, 55 ml) was adjusted to pH 10 with 20% sodium carbonate. After 48 h at room temperature, it was placed in the first two elements of the Craig machine and fractionated (100 transfers). Fractions *r* 79–52 contained unchanged starting material (t.l.c., approximately 45 mg spectrophotometrically). The residue obtained when fractions *r* 46–39 were evaporated was dissolved in water and treated with picric acid. The precipitated salt was purified as described above; the product (7 mg, m.p. 123–126 °C, mixture m.p. 124–129 °C) was shown to be indistinguishable from hordatine A dipicrate by t.l.c. and ultraviolet and infrared spectra.

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REFERENCES

1. A. Stoessl. Advan. Chem. Ser. **53**, 80 (1966).
2. A. Stoessl. Tetrahedron Letters, 2287 (1966).
3. A. Stoessl. Tetrahedron Letters, 2849 (1966).
4. R. A. Ludwig, E. Y. Spencer, and C. H. Unwin. Can. J. Botany, **38**, 21 (1960).
5. K. Koshimizu, E. Y. Spencer, and A. Stoessl. Can. J. Botany, **41**, 744 (1963).
6. C. H. Ludwig, B. J. Nist, and U. L. McCarthy. J. Am. Chem. Soc. **86**, 1186 (1964).
7. C. A. Anirudhan, D. W. Mathieson, and W. B. Whalley. J. Chem. Soc., C, 634 (1966). H. Suginome and T. Iwadare. Bull. Chem. Soc. Japan, **39** (7), 1535 (1966). M. Nakazaki, Y. Hirose, and K. Ikematsu. Tetrahedron Letters, 4735 (1966).
8. C. H. Ludwig. Private communication.
9. J. M. van der Veen. J. Org. Chem. **28**, 564 (1963).
10. Lord Todd. Chem. Brit. **2**, 428 (1966).
11. K. Freudenberg and H. Richtzenhain. Ber. **76**, 997 (1943).
12. H. Nimz, K. Naya, and K. Freudenberg. Ber. **96**, 2086 (1963).

13. H. COUSIN and H. HERRISSEY. Bull. Soc. Chim. France, (4), **3**, 1070 (1908). H. ERDTMAN. Ann. **503**, 283 (1933).
14. A. I. SCOTT. Quart. Rev. London, **19**, 1 (1965).
15. A. STOESSL. Phytochemistry, **4**, 973 (1965).
16. K. FREUDENBERG. Science, **148**, 595 (1965).
17. K. FREUDENBERG and J. M. HARKIN. Phytochemistry, **2**, 189 (1963).
18. F. F. NORD and W. J. SCHUBERT. *In* Biogenesis of natural compounds. P. Barnfeld (*Editor*). The Macmillan Co., New York. 1963. p. 722.
19. S. KAWAI and N. SUGIYAMA. Ber. **72**, 1146 (1939).
20. S. A. BROWN. *In* Biochemistry of phenolic compounds. J. B. Harborne (*Editor*). Academic Press, Inc., London. 1964. p. 361.
21. S. A. BROWN. Science, **134**, 305 (1961).
22. D. R. PERRIN and W. BOTTOMLEY. J. Am. Chem. Soc. **84**, 1919 (1962). A. J. BIRCH. Chem. Ind. London, 1173 (1966).
23. E. HECKER. Verteilungsverfahren im Laboratorium. Verlag Chemie, Weinheim. 1955.
24. T. G. HALSALL, E. L. HIRST, and J. K. N. JONES. J. Chem. Soc. 1399 (1947); 1427 (1947).
25. I. M. KOLTHOFF and R. BELCHER. Volumetric analysis. Vol. III. Interscience Publishers, Inc., New York. 1957. p. 273.