The Enzymatic Oxidation of Phenolic Tetrahydroisoquinoline-1-carboxylic Acids[1]

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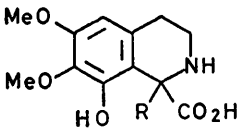
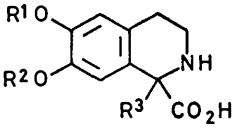
Tetrahydroisoquinoline-1-carboxylic acids with a 6- or 7-hydroxy substituent undergo oxidative decarboxylation on treatment with horseradish peroxidase or fungal laccase to give high yields of the corresponding 3,4-dihydroisoquinolines. Attempts to apply this reaction to the formation of an aporphine were unsuccessful.

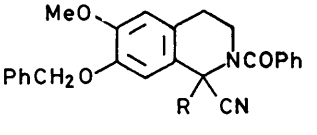
THE suggestion by Hahn[2] that the carbon atom at position 1 of isoquinoline alkaloids and any substituent thereon are biogenetically derived from the appropriate α-keto-acid *via* a Pictet–Spengler ring closure reaction has received much recent support. Peyoxylic (1) and peyoruvic (2) acids have been detected[3,4] in peyote cacti, and have been shown[5] to be precursors of the quinolines by anodic oxidation at low potential[11] or by prolonged aerial oxidation[12] in basic media. In both reactions, the presence of a free phenolic group at position 6 or 7 of the isoquinoline system is essential, and these are considered to be decarboxylations induced by oxidation of the phenolic hydroxy group. If so, then at least some *in vivo* decarboxylations of tetra-



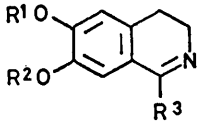
(1) R = H
(2) R = Me



(3) R^1 = H, R^2 = R^3 = Me
(4) R^1 = R^2 = H, R^3 = 3,4-$(OH)_2C_6H_3CH_2$
(5) R^1 = R^3 = H, R^2 = Me
(6) R^1 = H, R^2 = Me, R^3 = $PhCH_2$
(7) R^1 = R^2 = Me, R^3 = H
(8) R^1 = R^2 = R^3 = Me
(9) R^1 = R^2 = Me, R^3 = $PhCH_2$
(10) R^1 = Me, R^2 = H, R^3 = $PhCH_2$
(11) R^1 = H, R^2 = Me, R^3 = 4-$MeOC_6H_4CH_2$



(12) R = H
(13) R = Me
(14) R = $PhCH_2$



(15) R^1 = R^3 = H, R^2 = Me
(16) R^1 = H, R^2 = R^3 = Me
(17) R^1 = H, R^2 = Me, R^3 = $PhCH_2$
(18) R^1 = H, R^2 = Me, R^3 = 3,4,5-$(MeO)_3C_6H_2CH_2$
(19) R^1 = Me, R^2 = H, R^3 = $PhCH_2$
(35) R^1 = Me, R^2 = $PhCH_2$, R^3 = 3,4,5-$(MeO)_3C_6H_2CH_2$

alkaloids anhalonidine and anhalamine. The isoquinoline-1-carboxylic acid (3) is a precursor[6] of salsolidine, while acid (4) is incorporated into norlaudanosoline,[7] morphine,[8] and reticuline.[9]

The original rejection of the Hahn hypothesis was due to failure[10] to decarboxylate isoquinaldic acids under mild ('physiological') conditions. However, it has recently been demonstrated that these acids can readily be decarboxylated to the corresponding 3,4-dihydroisoquinoline-1-carboxylic acids might be catalysed by phenol oxidases. Kapadia and his co-workers[5] established that incubation of (2) with fresh slices of peyote cactus gives the corresponding dihydroisoquinoline, but no study of the reaction of isoquinaldic acids with specific oxidases has so far been reported.

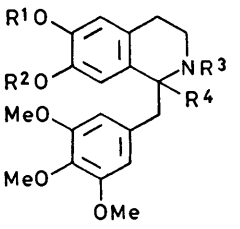
The oxidation of monophenols is catalysed by the tyrosinase, peroxidase, and laccase groups of enzymes. The tyrosinases[13a] operate by preliminary hydroxylation

of monophenols to *o*-diphenols, and their use was considered undesirable in an investigation in which the hydroxylation pattern of the isoquinoline might be important. Although peroxidases may also occasionally induce aromatic hydroxylation, the ready availability of crystalline enzyme from horseradish roots made its use attractive. Most of the preliminary decarboxylation studies, however, were carried out using a crude preparation of fungal laccase from *Polyporus versicolor*. Fungal laccases have been used only rarely in synthetic chemistry [14] but their lack of hydroxylating action, together with their possible ability to act as one- [13b] or two-electron [15] oxidants suggested their suitability in the present investigation.

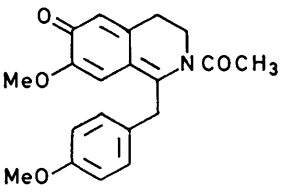
The substrate isoquinoline-1-carboxylic acids paralleled those used in the previous electrochemical study [11] to facilitate comparison of results. Acids (3), (5), (6), and (21), having a 6-hydroxy substituent, were readily prepared by Pictet–Spengler condensation of 3-hydroxy-4-methoxyphenethylamine with the appropriate α-keto-acids; the dimethoxy acids (7)—(9) were obtained by acid hydrolysis of the corresponding Reissert compounds. However, attempts to hydrolyse 7-benzyloxy Reissert compounds (12)—(14) under a variety of conditions to 7-hydroxy-substituted acids were unsuccessful, and only acid (10) was available from the electrochemical work.[11] The 3,4-dihydroisoquinolines (15)—(19) corresponding to the acids investigated were prepared by standard Bischler–Napieralski cyclisation reactions.

In preliminary experiments the 6-hydroxy acid (3) was incubated in phosphate buffer with a crude laccase preparation and 6-hydroxy-7-methoxy-1-methyl-3,4-dihydroisoquinoline (16) was isolated in low yield by continuous solvent extraction. However, because of the difficulty of recovering phenolic dihydroisoquinolines quantitatively from aqueous solutions, subsequent decarboxylations were followed by u.v. spectrophotometry; the decarboxylation products all have maxima above 350 nm, a region in which the carboxylic acids show little absorption. Using calibrated laccase solutions, it proved possible to carry out reproducible rate measurements on the decarboxylation reaction with the 6-hydroxy-acids (3), (5), (6), and (21) and compound (10). In most cases, decarboxylation was complete in a few minutes, with yields of dihydroisoquinoline >80%, whereas <5% reaction occurred when the acids were left in phosphate buffer for 24 h without laccase present. No laccase-induced reaction of the non-phenolic acids (7)—(9) was observed, precluding the possibility that the crude laccase preparation used co-incidentally contained an isoquinaldic acid decarboxylase.

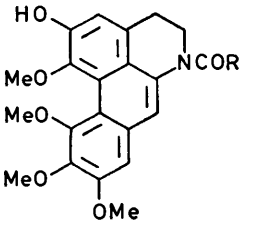
The values of K_M and V for the various substrates, calculated on the assumption that the decarboxylation reactions follow simple Michaelis–Menten kinetics are shown in the Table. Although these results must be treated with caution, as some evidence was obtained of a slower further oxidation of the dihydroisoquinolines, it seems that the nature of the substituent on C-1 of the acids had an effect both on the formation and on the breakdown of the enzyme–substrate complex.



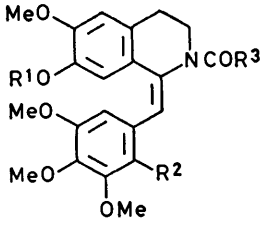
(20) $R^1 = H, R^2 = Me, R^3 = CO_2H$
(21) $R^1 = H, R^2 = Me, R^3 = H, R^4 = CO_2H$
(22) $R^1 = H, R^2 = Me, R^3 = COCF_3, R^4 = CO_2H$
(23) $R^1 = Me, R^2 = R^4 = H, R^3 = COCF_3$



(24)



(25)
(26) R = CF_3



(27) $R^1 = Me, R^2 = Br, R^3 = CF_3$
(28) $R^1 = Me, R^2 = Br, R^3 = OEt$
(29) $R^1 = R^2 = H, R^3 = CF_3$

Incubation of the acids with hydrogen peroxide and horseradish peroxidase gave results similar to those obtained with laccase. The phenolic acids were smoothly and consistently decarboxylated (kinetic data in the Table) while the methylated acids (7)—(9) were not; hydrogen peroxide by itself had no effect on any acid.

Values of maximum velocity V, and Michaelis constant K_M for the enzyme catalysed decarboxylation of isoquinoline-1-carboxylic acids

	$10^4 K_M$/M		$10^7 V$/mol min^{-1}	
Acid	Laccase	Peroxidase	Laccase	Peroxidase
(3)	1.2	2.1	0.5	2.1
(5)	0.4	1.1	0.2	0.6
(6)	12.0	20.0	8.3	18.5
(10)	3.7	0.9	0.2	0.04
(21)	2.5	11.7	3.7	12.0

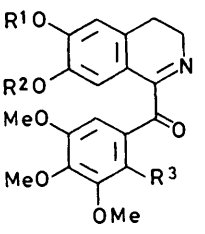
It seems established, therefore, that phenolic tetrahydroisoquinoline-1-carboxylic acids are oxidatively decarboxylated by enzymes which occur widely in plant cells, with the results obtained in the present study being broadly parallel to those found with anodic oxidation.

The isolation [11] in low yield of the quinonoid compound (24) from anodic oxidation of *N*-acetylated acid (11) raises the possibility that such compounds might, by intra- or inter-molecular nucleophilic addition to the enone system, give rise to a variety of isoquinoline alkaloids. It was therefore decided to study the oxidation of *N*-acyl acids of type (20), in which the polymethoxylated benzyl residue might be sufficiently nucleophilic for Michael addition to the quinone, and being symmetrical would give rise only to product (25). The acid (21) resisted *N*-acylation with acetyl chloride, ethyl chloroformate, acetic–formic anhydride,[16] or αα-dicyanoethyl acetate,[17] but formed a trifluoroacetamide (22), which was used in all subsequent oxidations.

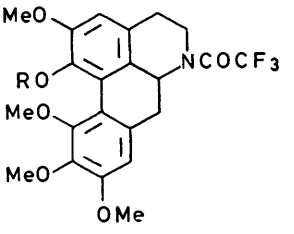
In view of the low yield of aporphine products anticipated from the oxidative coupling, an independent synthesis of (26) was attempted to facilitate its identification. Irradiation of bromostilbenes in butanol–butoxide mixtures is reported [18] to give a good yield of 6a,7-didehydroaporphines.* Accordingly the enamide (27) was prepared by standard procedures; although of sharp m.p. and chromatographically homogeneous, its spectral properties suggest that it exists as an equal mixture of geometrical isomers, or of amide conformers.† When compound (27) was dissolved in basic media, it was immediately converted to the keto-imine (30), which did not cyclise to an oxoaporphine [19] on irradiation. Starting material only was recovered from irradiation of the *N*-ethoxycarbonyl compound (28), and from the cathodic reduction of (27), although compounds analogous to the latter have been reported [20] to undergo ready electrochemical transformation to didehydroaporphines. It may be that steric crowding in the benzyl portion of the stilbenes inhibited cyclisation, and attempts to prepare (26) were discontinued.

The ring closure of 7-hydroxy-1-benzyltetrahydroisoquinoline to 1-hydroxyaporphine has recently been effected by oxidation with lead tetra-acetate [21] or vanadium oxytrifluoride; [22] their use on the tetrahydro-compound (23) gave the novel aporphine (33) in moderate yield. However, when the corresponding benzylidene compound (29) was treated with these reactants, no didehydroaporphine was obtained, the sole product being the keto-imine (31). The aporphine (33) could be converted with some difficulty into the pentamethoxyaporphine (34), but this, unexpectedly, was not oxidised by iodine [23] to a didehydroaporphine.

The isoquinaldic acid (22) was subjected to oxidation by laccase–peroxidase–active manganese dioxide–potassium ferricyanide in a two-phase system, dichlorodicyanobenzoquinone, and anodically in a variety of solvents. Because of the previous failure to prepare the target compound (26) all oxidations of the acid (22) were followed by methylation and reduction procedures to convert aporphine products into the synthesised (34).



(30) $R^1 = R^2 = Me, R^3 = Br$
(31) $R^1 = Me, R^2 = R^3 = H$
(32) $R^1 = R^3 = H, R^2 = Me$



(33) R = H
(34) R = Me

Although these operations may not have been quantitative, no trace of a pentamethoxyaporphine was detected, the sole product being the keto-imine (32). Since this keto compound is formed readily by aerial oxidation of the dihydroisoquinoline (18), it is not possible to say whether it was produced by oxidation of the acid, or of a decarboxylated intermediate. It seems clear, however, that nucleophilic attack of the benzyl residue on a possible quinone methide intermediate does not take place, and that the predominant reaction, after, or concurrent with, decarboxylation is the removal of the *N*-blocking group, accompanied by oxidation of the benzylic methylene group. Attempts to synthesise benzylic isoquinoline-1-carboxylic acids in which the methylene protons were replaced by methyl groups were unsuccessful.

EXPERIMENTAL

M.p.s are corrected. I.r. spectra were recorded with a Perkin-Elmer 137G spectrometer and ^1H n.m.r. on a JEOL JNM C-60HL spectrometer with tetramethylsilane as the internal standard in the solvent indicated. High and low resolution mass spectra were determined by the Boots Co. Ltd., Nottingham. Anodic oxidations were controlled by a Wenking potentiostat 70 TSI against a saturated calomel reference electrode. T.l.c. was performed on silica gel, Polygram SIL G/UV_{254}, and p.l.c. on silica gel GF, Anachem Uniplate. All solvents for chromatographic and photolytic work were redistilled.

Preparations of Substrates and Standards for Enzymatic Oxidations.—Compounds (3),[24] (7),[25] (15),[26] (16),[26] (17),[27] and (19) [28] have been previously described.

Preparation of 7-Benzyloxy-Reissert Compounds (13) *and* (14).—To the Reissert compound [11] (12) (1.7 g) in dry DMF (8 cm^3) was added sodium hydride (0.2 g) over 15 min, followed by iodomethane (1 g) in DMF (2 ml). After 4 h the mixture was poured onto ice and extracted with chloroform which was washed with dilute hydrochloric acid, sodium hydroxide solution, and dried ($MgSO_4$). Evaporation of the solvent gave *7-benzyloxy-2-benzoyl-1-cyano-6-methoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline* (13) (1.4 g,

* Although the term 'dehydroaporphine' is commonly used for aporphines with an additional double bond, according to IUPAC recommendations the correct term is didehydroaporphine, and this is the usage adopted in this paper.

† We thank Professor G. W. Kirby for this useful suggestion.

80%) as needles (from methanol), m.p. 179.5—181° (Found: C, 75.5; H, 5.7; N, 6.9. $C_{26}H_{24}N_2O_3$ requires C, 75.7; H, 5.8; N, 6.8%); $\nu_{max.}$ (KBr) 2 220 (C≡N) and 1 665 cm^{-1} (C=O); τ($CDCl_3$) 2.02—3.08 (12 H, m, aromatic), 4.85 (2 H, s, PhCH_2O), 6.06 (3 H, s, CH_3O), 6.25 and 6.88 (4 H, 2t, CH_2CH_2, and 7.53 (3 H, s, CH_3). The *1-benzyl analogue* (14) was prepared similarly from (12) and benzyl chloride in 67% yield, m.p. (from methanol) 178.5—180.5° (Found: C, 79.2; H, 5.9; N, 5.7. $C_{32}H_{28}N_2O_3$ requires C, 78.7; H, 5.7; N, 5.7%); $\nu_{max.}$ (Nujol) 2 220 (C–N) and 1 640 cm^{-1} (C=O); τ($CDCl_3$) 3.03—3.87 (17 H, m, aromatic), 4.97 (2 H, s, PhCH_2O), 5.66 (2 H, d, PhCH_2), and 6.15 (3 H, s, CH_3O).

Preparation of Dimethoxy Acids (8) *and* (9).—*2-Benzoyl-1-cyano-6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline* was prepared from the corresponding 1-hydrogen-Reissert compound [29] by the above procedure, m.p. 205—206° (from ethyl acetate–chloroform) (Found: C, 71.6; H, 5.9; N, 8.2. $C_{20}H_{20}N_2O_3$ requires C, 71.4; H, 5.9; N, 8.3%); $\nu_{max.}$ (KBr) 2 240 (C≡N) and 1 653 cm^{-1} (C=O); τ($CDCl_3$) 2.5 (5 H, s, phenyl) 2.95 and 3.37 (2 H, 2 s, isoquinoline), 6.07 and 6.11 (6 H, 2 s, 2 × OCH_3), 6.4 and 7.15 (4 H, 2 t, CH_2-CH_2), and 7.89 (3 H, s, 1-CH_3). This compound (5 g, 0.015 mol) was heated in phosphoric acid (85%, 40 cm^3) at 120° under nitrogen for 20 min. The resulting solution was cooled and added to ice–water (50 cm^3); the precipitate of benzoic acid was filtered off and the pH of the filtrate adjusted to 7.0 with ammonia solution and left overnight to give the *acid* (8) (2.2 g, 59%), m.p. 298—300° (from aqueous acetic acid) (Found: C, 61.9; H, 6.8; N, 5.2. $C_{13}H_{15}$-NO_4 requires C, 62.1; H, 6.8; N, 5.6%); $\nu_{max.}$ (KBr) 2 880—2 430 (amine salt) and 1 640 cm^{-1} (C=O). In an analogous manner, the appropriate 1-benzyl-Reissert compound [29] was hydrolysed in 74% yield to the *acid* (9), m.p. 281—286° (decomp.) which formed a *hydrochloride*, m.p. 238—40° (decomp.) (ethanol–concentrated HCl), $\nu_{max.}$ (KBr) 2 785—2 700 (salt bands) and 1 725 cm^{-1} (CO_2H); τ(D_2O) 2.76—3.21 (7 H, m, aromatic), 6.04 and 6.07 (6 H, 2 s, 2 CH_3O), 6.23 (2 H, s, PhCH_2), and 6.26 and 6.83 (4 H, 2 t, CH_2CH_2).

Preparation of 6-Hydroxy Acids (5), (6), *and* (21).—(a) A solution of glyoxylic acid monohydrate (100 cm^3 of a 1% solution) was added to 3-hydroxy-4-methoxyphenylethylamine hydrochloride (2 g, 0.01 mol) dissolved in 0.05M-sulphuric acid (100 cm^3). The mixture was heated on a steam-bath for 4 h, the solution adjusted to pH 7 with ammonia, reduced to half-volume and kept at 4° overnight. The crystalline precipitate was dissolved in boiling methanol–hydrochloric acid (100 : 1), cooled, and ether added to give *6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid hydrochloride* (1.2 g, 47%), m.p. 209—212° (Found: C, 47.8; H, 5.7; N, 4.9. $C_{11}H_{14}$-$ClNO_4 \cdot H_2O$ requires C, 47.6; H, 5.8; N, 5.0%): $\nu_{max.}$ (KBr) 3 370 (OH), 2 790—2 530 (salt bands), and 1 738 cm^{-1} (CO_2H); τ(D_2O) 2.68 and 3.09 (2 H, 2 s, aromatic), 6.02 (3 H, s, CH_3O), 6.46 (1 H, s, 1-H), and 6.29 and 6.94 (4 H, 2 t, CH_2CH_2).

(b) To phenylpyruvic acid (1.6 g, 0.01 mol) dissolved in the minimum volume of 1M-sodium hydroxide solution was added a solution of 3-hydroxy-4-methoxyphenylethylamine hydrochloride (2 g, 0.01 mol) in water (30 cm^3), the pH of the mixture was adjusted to 6.5—7.0 with dilute hydrochloric acid, and the reaction left under nitrogen for 24 h. The resulting precipitate recrystallised from methanol–concentrated hydrochloric acid–ether gave the *hydrochloride of acid* (6) (1.7 g, 55%), m.p. 245—249° (decomp.) (Found: C, 61.4; H, 5.8; N, 3.8. $C_{18}H_{19}NO_4 \cdot HCl$ requires C, 61.8; H, 5.7; N, 4.0%); $\nu_{max.}$ (KBr) 3 480 (OH), 3 170 (NH_3^+), 2 730—2 400 (salt bands), and 1 710 cm^{-1} (CO_2H); τ(D_2O) 2.93—3.56 (7 H, m, aromatic) and 6.10 (3 H, s, CH_3O).

In an analogous manner from 3,4,5-trimethoxyphenylpyruvic acid (7.6 g) and the phenylethylamine hydrochloride (6.1 g) was prepared the *hydrochloride of acid* (21) (6.7 g, 56%), m.p. 252—254° (decomp.) (Found: C, 56.9; H, 6.1; N, 2.7. $C_{21}H_{25}NO_7 \cdot HCl$ requires C, 57.3; H, 5.9; N, 3.2%); $\nu_{max.}$ (KBr) 3 320 (OH), 3 160 (NH_2^+), 2 665—2 430 (salt bands), and 1 710 cm^{-1} (CO_2H); τ(D_2O) 2.48, 3.10, and 3.39 (4 H, 3 s, aromatic), 5.93 (2 H, s, ArCH_2), 6.02 and 6.15 (12 H, 2 s, 4CH_3O), and 6.40 and 7.03 (4 H, 2 t, CH_2CH_2).

Preparation of Dihydroisoquinoline (18). Heating the salt obtained from 3,4,5-trimethoxyphenylacetic acid (4.3 g, 0.019 mol) and 3-benzyloxy-4-methoxyphenylethylamine (5.0 g, 0.019 mol) at 180° for 1 h gave the corresponding *phenylacetamide* (7.1 g, 79%), m.p. 124—126.5° (from ethyl acetate) (Found: C, 69.4; H, 6.7; N, 3.0. $C_{27}H_{31}NO_6$ requires C, 69.7; H, 6.7; N, 3.0%); $\nu_{max.}$ (KBr) 3 320 (NH) and 1 635 cm^{-1} (C=O); τ($CDCl_3$) 2.68—3.69 (10 H, m, aromatic), 4.57br (1 H, s, NH), 4.95 (2 H, s, PhCH_2O), 6.17 and 6.23 (12 H, 2 s, 4CH_3O), 6.63 (2 H, s, ArCH_2), and 6.68 and 7.40 (4 H, 2 t, CH_2CH_2). Cyclisation of the amide with phosphorus oxychloride in toluene afforded the *hydrochloride* of *1-(3,4,5-trimethoxybenzyl)-6-benzyloxy-7-methoxy-3,4-dihydroisoquinoline*, m.p. 184—186° (ethanol–ether) (Found: C, 66.4; H, 6.0; N, 2.8. $C_{27}H_{29}NO_5$,HCl requires C, 67.0; H, 6.2; N, 2.9%); $\nu_{max.}$ (KBr) 2 690 (salt band) and 1 655 cm^{-1} (C=N); τ($CDCl_3$) 2.58—3.26 (9 H, m, aromatic), 4.83 (2 H, s, PhCH_2O), 5.42 (2 H, s, ArCH_2), 6.13, 6.22, and 6.28 (12 H, 3 s, 4CH_3O), and 7.10 (2 H, t, CH_2CH_2N). Debenzylation of the compound with ethanolic hydrochloric acid gave the *hydrochloride of the dihydroisoquinoline* (18), m.p. 233—236° (from ethanol–ether) (Found: C, 60.9; H, 6.1; N, 3.2. $C_{20}H_{23}NO_5 \cdot HCl$ requires C, 61.0; H, 6.1; N, 3.5%); $\nu_{max.}$ (KBr) 3 450 cm^{-1} (OH), 2 750 (salt band), and 1 650 cm^{-1} (C=N); τ(D_2O) 2.80, 3.18, and 3.43 (4 H, 3 s, aromatic), 6.25 (12 H, s, 4CH_3O), and 7.15 (2 H, t, CH_2CH_2N).

Enzymatic Oxidations.—Polyporus versicolor was cultured, and laccase production induced with 2,5-dimethylaniline as described in the literature.[30] The laccase activity was determined using catechol [31] as substrate. Commercially available horseradish peroxidase was assayed against guaiacol.[32]

Laccase Oxidation of Acid (3).—To the acid (0.5 g) in a mixture of phosphate buffer (0.1M; pH 6.0; 200 cm^3) and ethanol (50 cm^3) was added laccase solution (activity 2.8 units cm^{-3}; 100 cm_3) and the mixture was incubated at 35°. Further laccase solution (60 cm^3) was added after 24 and 48 h. The mixture was made alkaline with ammonia, and continuously extracted for 12 h with chloroform to give dihydroisoquinoline (16) (0.07 g), identified by t.l.c. (acetonitrile–15% water) and picrate formation.

Reactions for Spectrophotometric Assay.—(*a*) *Laccase.* The reaction mixture consisted of the isoquinoline-1-carboxylic acid (0.05—1.0 cm^3 of a *ca.* 0.000 5M solution), laccase solution (0.1 cm^3), and phosphate buffer (0.1M, pH 6.0) to a final volume of 3.0 cm^3. The increase in absorbance at the relevant wavelength was measured against a blank containing the acid and buffer, but no enzyme.

(b) *Horseradish peroxidase.* Reaction mixtures contained the acid (0.05—1.0 cm^3 of a *ca.* 0.000 5M solution),

hydrogen peroxide solution (0.000 87M, 1.0 cm^3), peroxidaes solution (0.1 g dm^{-3} in pH 6.0 phosphate buffer; 1.0 cm^3), and phosphate buffer (0.1M; pH 6.0) to a final volume of 3.0 cm^3. The increase in absorbance was measured at the appropriate wavelength against an acid–peroxide–buffer blank.

Compd.	(15)	(16)	(17)	(18)	(19)
$\lambda_{max.}$/nm	389	375	389	392	355
ε	12 870	7 883	10 947	17 212	6 550

Preparation of Trifluoroacetamide (22).—The hydrochloride salt of amino-acid (21) (1.5 g, 3.4 mmol) suspended in dry chloroform (25 cm^3) containing triethylamine (2 cm^3) was cooled and stirred during the dropwise addition of just sufficient trifluoroacetic anhydride (*ca.* 2 cm^3) to dissolve the suspended material. Stirring was continued for a further 3 h, after which the organic phase was washed (2M-HCl, then water), dried ($MgSO_4$), and evaporated. Trituration of the resultant gum with ether–petroleum (b.p. 40—60°) gave a solid which recrystallised from aqueous ethanol to afford the amide (22) (1.2 g, 71%), m.p. 191—192.5° (Found: C, 55.2; H, 5.0; N, 2.9%; M^+, 499.147 9. $C_{23}H_{24}F_3NO_8$ requires C, 55.3; H, 4.8; N, 2.8%; *M*, 499.145 5); $\nu_{max.}$ (KBr) 3 370 (OH), 1 715 (CO_2H), and 1 685 cm^{-1} ($NCOCF_3$); τ($CDCl_3$) 2.86, 3.35, and 4.12 (4 H, 3 s, aromatic) 6.05 (2 H, s, ArCH_2), 6.21 and 6.40 (12 H, 2 s 4CH_3O), and 7.18 (2 H, t, CH_2CH_2N).

Preparation of Bromostilbenes (27) *and* (28).—Bromine (5.6 g) was added dropwise over 30 min to a stirred solution of 3,4,5-trimethoxyphenylacetic acid (8 g) in acetic acid (20 cm^3) cooled to 10—15°. The mixture was stirred for a further 2 h, poured into water, and the precipitate recrystallised from chloroform to give *2-bromo-3,4,5-trimethoxyphenylacetic acid* (9.8 g, 91%), m.p. 150—152° (Found: C, 42.9; H, 4.2; Br, 26.9. $C_{11}H_{13}BrO_5$ requires C, 43.3; H, 4.3; Br, 26.2%); $\nu_{max.}$ (KBr) 1 710 cm^{-1} (CO_2H); τ($CDCl_3$) −0.4 (1 H, s, exchangeable, CO_2H), 3.41 (1 H, s, aromatic), 6.17 and 6.20 (9 H, 2s, 3CH_3O), and 6.25 (2 H, s, ArCH_2). The bromo-acid (12.1 g, 0.04 mol) was dissolved in ether (500 cm^3) and 3,4-dimethoxyphenethylamine was added dropwise until precipitation of salt was complete. The salt was collected and heated at 180° for 1 h to produce a tan oil, which was dissolved in dichloromethane (150 cm^3), washed with water, and dried ($MgSO_4$). Recrystallisation from toluene–petroleum (b.p. 100—120°) gave crystals of *N-[2-(3,4-dimethoxyphenethyl)]-2-bromo-3,4,5-trimethoxyphenyl)acetamide* (16.7 g, 90%), m.p. 110.5—112° (Found: C, 53.6; H, 5.6; N, 3.4; Br, 17.0. $C_{21}H_{26}BrNO_6$ requires C, 53.8; H, 5.6; N, 3.0; Br, 17.0%); $\nu_{max.}$ (KBr) 3 260 (NH) and 1 645 cm^{-1} (C=O); τ($CDCl_3$) 3.20—3.52 (4 H, m, aromatic), 4.44br (1 H, s, N*H*), 6.14—6.20 (15 H, 4 s, 5 CH_3O), 6.38 (2 H, s, ArCH_2), and 6.58—7.31 (4 H, 2 t, CH_2CH_2). To a cooled solution of the bromoacetamide (0.95 g, 2 mmol) in dry chloroform was added phosphorus pentachloride (1.3 g). The mixture was left overnight, the solvent removed, and the residual gum triturated with ether to give *1-(2-bromo-3,4,5-trimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline hydrochloride* (0.60 g, 61%), m.p. 207—209°. The *free base* had m.p. 107—109° [from petroleum (b.p. 60—80°)] (Found: C, 56.1; H, 5.2; N, 3.1; Br, 17.4. $C_{21}H_{24}BrNO_5$ requires C, 56.0; H, 5.3; N, 3.1; Br, 17.8%); $\nu_{max.}$ (KBr) 1 625 cm^{-1} (C=N); τ($CDCl_3$) 3.05—3.32 (3 H, 3 s, aromatic), 5.85 (2 H, s, ArCH_2), 6.10—6.26 (17 H, m, 5 CH_3O and CH_2CH_2N), and 7.36 (2 H, t, CH_2CH_2N). To a solution of the dihydroisoquinoline hydrochloride (7.2 g, 0.015 mol) in dry chloroform (70 cm^3), cooled in an ice-bath, was added under nitrogen triethylamine (3 g), followed by trifluoroacetic anhydride (24 g, 0.114 mol) over 15 min. The mixture was stirred for 4 h at room temperature, washed with water and 2M-hydrochloric acid, and dried ($MgSO_4$). Removal of the solvent and recrystallisation of the residue from ethanol yielded *1-(2-bromo-3,4,5-trimethoxybenzylidene)-6,7-dimethoxy-2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinoline* (27) (3.8 g, 47%), m.p. 154—155° (Found: C, 50.2; H, 4.3; N, 2.4; Br, 14.4%; M^+, 545.068 8, 547.065 7. $C_{23}H_{23}BrF_3NO_6$ requires C, 50.5; H, 4.2; N, 2.6; Br, 14.6%; *M*, 545.066 2, 547.064 3); $\nu_{max.}$ (KBr) 1 695 ($COCF_3$) and 1 645 cm^{-1} (C=C); τ($CDCl_3$) 2.78—3.39 (4 H, m, aromatic and vinylic), 6.04, 6.10, and 6.20 (15 H, 5 s, 5 CH_3O), and 6.98 (2 H, t, CH_2CH_2N). ^{19}F N.m.r. showed two singlets at 70.19 and 70.87 p.p.m. upfield of $CFCl_3$. To a stirred solution of the dihydroisoquinoline hydrochloride (2.0 g, 4 mmol) in chloroform (30 cm^3) and 10% sodium carbonate solution (30 cm^3) was added ethyl chloroformate (1.81 g, 0.017 mol) in chloroform (8 cm^3) over 20 min. After 3 h, the mixture was worked up as above to afford *stilbene* (28) (0.75 g, 35%), m.p. 97—99° (from ethanol) (Found: C, 55.2; H, 5.4; N, 2.7; Br, 15.3. $C_{24}H_{28}BrNO_7$ requires C, 55.2; H, 5.4; N, 2.7; Br, 15.3%); $\nu_{max.}$ (KBr) 1 690 cm^{-1} (C=O); τ($CDCl_3$) 2.68, 3.02, 3.08, and 3.35 (4 H, 4 s, aromatic and vinylic), 6.03—6.38 (19 H, m, 5 CH_3O, CH_2CH_3, and CH_2-CH_2N), 7.10 (2 H, t, CH_2CH_2N), and 9.17 (3 H, t, CH_2CH_3).

Keto-imine (30).—(a) When the preparation of stilbene (27) by trifluoroacetylation of the dihydroisoquinoline was carried out using pyridine as solvent, the reaction mixture contained two products, separable by preparative t.l.c. (silica gel–chloroform). The material of higher R_F was (27), that of lower R_F was identified as *1-(2-bromo-3,4,5-trimethoxybenzoyl)-6,7-dimethoxy-3,4-dihydroisoquinoline* (30), m.p. 137.5—139° (from ether) (Found: C, 52.8; H, 4.6; N, 2.7%; M^+, 464. $C_{21}H_{22}BrNO_6$ requires C, 54.3; H, 4.7; N, 3.0%; *M*, 464); *m/e* 384.145 7 (100%) and 272.975 5 (calc. for $C_{21}H_{22}NO_6$ 384.144 8 and for $C_{10}H_{10}$-BrO_4 272.982 2); $\nu_{max.}$ (KBr) 1 660 cm^{-1} (C=O); τ($CDCl_3$) 2.80, 2.97, and 3.28 (3 H, 3 s, aromatic), 6.07—6.29 (17 H, m, 5 CH_3O and CH_2CH_2N), and 7.29 (2 H, t, CH_2CH_2N).

(b) To a solution of trifluoroacetamide (27) (1.0 g) in dry benzene (950 cm^3) was added a solution of potassium t-butoxide (0.8 g) in t-butyl alcohol (50 cm^3); the mixture turned yellow immediately, and no amide was detectable by t.l.c. The organic phase was washed with water and evaporated to yield a gum which on trituration with ether gave (30) (0.8 g), identical with the previously prepared material.

Preparation of Stilbene (29).—To a solution of 1-(3,4,5-trimethoxybenzyl)-6-methoxy-7-benzyloxy-3,4-dihydroisoquinoline (35) hydrochloride [21] (2.8 g, 5.8 mmol) in dry chloroform (30 cm^3), stirred and cooled to 10°, was added triethylamine (3 cm^3) and trifluoroacetic anhydride (5 cm^3). The usual work-up yielded *N-trifluoroacetyl-1-(3,4,5-trimethoxybenzylidene)-6-methoxy-7-benzyloxy-1,2,3,4-tetrahydroisoquinoline* (1.4 g, 45%), m.p. 178—179° (ethanol) (Found: C, 63.5; H, 5.3; N, 2.5. $C_{29}H_{28}F_3NO_6$ requires C, 64.0; H, 5.2; N, 2.6%); $\nu_{max.}$ (KBr) 1 700 cm^{-1} ($COCF_3$), τ($CDCl_3$) 2.55—3.45 (10 H, m, aromatic and vinylic), 4.81 (2 H, s, PhCH_2O), 6.15 (12 H, s, 4CH_3O), and 6.58 and 6.98 (4 H, 2 t, CH_2CH_2). To a solution of this trifluoroacetamide (2.0 g) in ethyl acetate (250 cm^3) was added 5% palladised charcoal (1.5 g). Hydrogenation of this mixture gave a

glass which on trituration with ethanol and recrystallisation from ethyl acetate–petroleum (b.p. 60—80°) yielded *stilbene* (29), (1.3 g, 78%), m.p. 161—163° (Found: C, 57.9; H, 4.9; N, 2.9. $C_{22}H_{22}F_3NO_6$ requires C, 58.2; H, 4.9; N, 3.1%); $\nu_{max.}$ (KBr) 3 390 (OH), 1 705 ($COCF_3$), and 1 635 cm^{-1} (C=C); τ($CDCl_3$) 2.70—3.50 (5 H, m, aromatic and vinylic), 4.23 (1 H, s, exchanges with D_2O, OH), 6.15 (12 H, s, 4 CH_3O), and 6.65 and 7.00 (4 H, 2 t, CH_2CH_2).

Keto-imine (31).—(a) To a stirred solution of (29) (0.18 g, 0.39 mmol) in glacial acetic acid (3 cm^3) was added lead tetra-acetate (0.21 g, 0.47 mmol). Stirring was continued for 30 min, water (10 cm^3) was added and the solution carefully basified with solid sodium hydrogencarbonate. Extraction with chloroform (3 × 25 cm^3), drying ($MgSO_4$), and removal of solvent afforded an oil (0.16 g), $\nu_{max.}$ (thin film) 1 750 ($OCOCH_3$), 1 675, 1 645, and 1 630 cm^{-1} (dienone). The oil was dissolved in dichloromethane (15 cm^3) to which was added trifluoroacetic acid (0.75 cm^3) followed by stirring at room temperature for 2 h. The organic solvent was washed with sodium hydrogencarbonate and water, and evaporated to give solid (80 mg) which, after preparative t.l.c. (silica gel, ethyl acetate–petroleum 1 : 1) gave *keto-imine* (31) (55 mg), m.p. 183—186° (ethyl acetate–petroleum) (Found: C, 63.9; H, 5.9; N, 3.6%; M^+, 371. $C_{20}H_{21}NO_6$ requires C, 64.7; H, 5.7; N, 3.8%; *M*, 371); $\nu_{max.}$ (KBr) 2 742, 2 660, 2 617 (salt bands), 1 665 (C=O), and 1 623 cm^{-1} (C=N); τ($CDCl_3$) 2.63, 3.00, 3.20 (4 H, 3 s, aromatic), 4.15br (1 H, s, exchanges with D_2O, OH), 6.03, 6.10 (12 H, 2 s, 4 CH_3O), and 6.07 and 7.22 (4 H, 2 t, CH_2-CH_2). Compound (31) gave a positive 2,4-dinitrophenylhydrazine test.

(b) To a solution of (29) (0.46 g) in dichloromethane (16 cm^3) at −10° was added a 20 : 1 w/w mixture of trifluoroacetic acid and trifluoroacetic anhydride (4 cm^3). The solution was stirred during the addition of vanadium oxytrifluoride (0.31 g) dissolved in the minimum volume of a 1 : 1 solution of ethyl acetate and trifluoroacetic acid–trifluoroacetic anhydride (20 : 1 w/w). After 10 min the mixture was poured into water and the organic phase was washed and evaporated to give (31), identical with the previous product.

Preparation of Aporphine (33).—To a solution of the dihydroisoquinoline hydrochloride (35) (3.9 g, 8 mmol) in 1 : 1 glacial acetic acid–water (40 cm^3) was added zinc metal (20 g) and the mixture was heated under reflux for 3 h, poured into water, and extracted with chloroform. The organic phase was washed, dried (K_2CO_3), and evaporated to give the corresponding tetrahydroisoquinoline (3.5 g, 97%) as an oil. To a stirred solution of this oil in dry chloroform (30 cm^3) and triethylamine (2 cm^3) was added dropwise with cooling trifluoroacetic anhydride (7.4 g.) After 4 h stirring the mixture was worked up as before to give N-*trifluoroacetyl*-1-(3,4,5-*trimethoxybenzyl*)-6-*methoxy*-7-*benzyloxy*-1,2,3,4-*tetrahydroisoquinoline* (2.0 g, 47%), m.p. 155—156° (from ethanol) (Found: C, 64.1; H, 5.5; N, 2.2. $C_{29}H_{30}F_3NO_6$ requires C, 63.9; H, 5.5; N, 2.6%); $\nu_{max.}$ (KBr) 1 685 cm^{-1} (C=O); τ($CDCl_3$) 2.60 (5 H, s, $PhCH_2O$), 3.35, 3.56, and 3.76 (4 H, 3 s, aromatic), 5.00 (2 H, s, $PhCH_2$-O), 6.10, 6.14, and 6.23 (13 H, 3 s, 4 CH_3O and 1-H), 6.68 and 7.35 (4 H, 2 t, CH_2CH_2), and 6.96 (2 H, d, $PhCH_2$). Debenzylation of this compound by hydrogenation using 5% palladised charcoal catalyst afforded the *phenolic tetrahydroisoquinoline* (23), m.p. 126—127° [ethyl acetate–petroleum (b.p. 60—80°)] (Found: C, 57.3; H, 5.4; N, 2.8. $C_{22}H_{24}F_3NO_6$ requires C, 58.0; H, 5.3; N, 3.1%); $\nu_{max.}$ (KBr) 3 400 (OH) and 1 693 cm^{-1} (C=O); τ($CDCl_3$) 2.93—3.26 (4 H, 3 s, aromatic), 4.25 (1 H, exchangeable, OH), 6.03, 6.14, and 6.16 (12 H, 3 s, 4 CH_3O), and 7.14 (2 H, t, CH_2CH_2N). Lead tetra-acetate oxidation of compound (23) (1.0 g) was carried out as described above. A brown oil (1.15 g) was obtained, $\nu_{max.}$ 1 745 ($OCOCH_3$), 1 690 ($NCOCF_3$), 1 678, 1 655, and 1 630 cm^{-1} (dienone). This oil was stirred for 2 h in dichloromethane (85 cm^3) and trifluoroacetic acid (4.2 cm^3), followed by washing with sodium hydrogencarbonate solution and water. The organic phase was dried and evaporated to give the 1-*hydroxyaporphine* (33), (0.35 g, 35%), m.p. 238—239.5° (from ethanol) (Found: C, 58.0; H, 4.8; N, 3.0%; M^+, 453.141 1. $C_{22}H_{22}F_3NO_6$ requires C, 58.3; H, 4.9; N, 3.1%; *M*, 453.140 0); $\nu_{max.}$ (KBr) 3 200 (OH) and 1 680 cm^{-1} ($NCOCF_3$); τ($CDCl_3$) 1.12 (1 H, s, OH), 3.28 and 3.34 (2 H, 2 s, aromatic), 6.10, 6.13, and 6.23 (12 H, s, 4 CH_3O), and 7.16 (2 H, t, CH_2-CH_2N); $\lambda_{max.}$ (EtOH) 222 (ε 32 576), 280 (11 320), and 303 nm (6 792). Vanadium oxytrifluoride oxidation of (23) carried out as described earlier gave a 10% yield of the aporphine (33), m.p. 235—238°.

Preparation of Aporphine (34).—Sodium hydride (0.48 g) was added to a solution of the hydroxyaporphine (33) (0.30 g, 0.66 mmol) in anhydrous DMF (15 cm^3), followed by iodomethane (1 g, 7 mmol). After 1 h the mixture was poured into water, extracted with chloroform, and the organic phase washed, dried, and evaporated to yield the *pentamethoxyaporphine* (34) (0.2 g, 65%), m.p. 179—180° (from ethanol) (Found: C, 59.5; H, 5.3; N, 2.8. $C_{23}H_{24}F_3NO_6$ requires C, 59.1; H, 5.1; N, 3.0%); $\nu_{max.}$ (KBr) 1 685 cm^{-1} ($NCOCF_3$); τ($CDCl_3$) 3.37 and 3.43 (2 H, 2 s, aromatic), 6.15, 6.24, and 6.40 (15 H, 3 s, 5 CH_3O), and 7.29 (2 H, t, CH_2CH_2N); $\lambda_{max.}$ (ethanol) 226 and 284 nm.

Preparation of Keto-imine (32).—A solution of acid (21) (0.4 g, 1 mmol) in 0.1M-sodium hydrogencarbonate solution in 3 : 2 methanol–water was oxidised at 280 mV (carbon felt anode, calomel standard electrode) for 2.5 h. The graphite felt anode was washed with methanol (3 × 100 cm^3), and washings plus electrolyte were evaporated to near dryness. Addition of water, followed by extraction with chloroform, afforded the *keto-compound* (32) (0.22 g, 60%), m.p. 135—136.5° (from ether) (Found: C, 63.3; H, 5.6; N, 3.5. $C_{20}H_{21}NO_6 \cdot \frac{1}{2}H_2O$ requires C, 63.2; H, 5.8; N, 3.7%); $\nu_{max.}$ (KBr) 1 670 cm^{-1} (C=O); τ($CDCl_3$) 2.78—3.27 (4 H, 3 s, aromatic), 6.11, 6.14, and 6.21 (12 H, 3 s, 4 CH_3O), and 7.26 (2 H, t, CH_2CH_2N).

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REFERENCES

[1] J. M. Bobbitt, T. Y. Cheng, and I. G. C. Coutts, presented in part at the 6th Natural Products Symposium, University of West Indies, Jamaica, 1976.

[2] G. Hahn and F. Rumpf, *Ber.*, 1938, **71**, 2141.

[3] E. Leete and J. D. Braunstein, *Tetrahedron Letters*, 1969, 451.

[4] G. J. Kapadia, G. S. Rao, E. Leete, M. B. E. Fayez, Y. N. Vaishnav, and H. M. Fales, *Lloydia*, 1969, **32**, 525.

[5] G. J. Kapadia, G. S. Rao, M. B. E. Fayez, Y. N. Vaishnav, and H. M. Fales, *J. Amer. Chem. Soc.*, 1970, **92**, 6943.

[6] M. Slaytor and I. J. McFarlane, *Phytochemistry*, 1972, **11**, 229.

[7] M. L. Wilson and C. J. Coscia, *J. Amer. Chem. Soc.*, 1975, **97**, 431.

[8] A. R. Battersby, R. C. F. Jones, and R. Kazlauskas, *Tetrahedron Letters*, 1975, 1873.

[9] B. S. Bhakuni, A. N. Singh, S. Tewari, and R. S. Kapil, *J.C.S. Perkin I*, 1977, 1662.

[10] W. H. Whalley and T. R. Govindachari, *Org. Reactions*, 1951, **6**, 151.
[11] J. M. Bobbitt and T. Y. Cheng, *J. Org. Chem.*, 1976, **41**, 443.
[12] J. M. Bobbitt, C. L. Kulkarni, and P. Wiriyachitra, *Heterocycles*, 1976, **4**, 1645.
[13] (*a*) V. Ullrich and W. Duppel, in ' The Enzymes,' ed. P. D. Boyer, Academic Press, New York, 1975, 3rd edn., vol. XII, p. 296; (*b*) B. G. Malmström, L. E. Andréasson, and B. Reinhammer, *ibid.*, p. 575.
[14] S. M. Bocks, R. C. Cambie, and T. Takahashi, *Tetrahedron*, 1963, **19**, 1109; G. Benfield, S. M. Bocks, K. Bromley, and B. R. Brown, *Phytochemistry*, 1964, **3**, 79.
[15] R. Malkin and B. G. Malmström, *Adv. Enzymol.*, 1970, **33**, 177.
[16] J. C. Sheehan and D.-D. H. Yang, *J. Amer. Chem. Soc.*, 1958, **80**, 1154.
[17] T.-L. Ho, *Synth. Comm.*, 1974, **4**, 351.
[18] M. P. Cava, P. Stern, and K. Wakisaka, *Tetrahedron*, 1973, **29**, 2245; M. P. Cava and S. S. Libsch, *J. Org. Chem.*, 1974, **39**, 577.
[19] T. Kametani, R. Nitadori, H. Terasawa, K. Takahashi, and M. Ihara, *Heterocycles*, 1975, **3**, 821.
[20] R. Gottlieb and J. L. Neumeyer, *J. Amer. Chem. Soc.*, 1976, **98**, 7108.
[21] M. Hara, O. Hoshino, and B. Umezawa, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 262.
[22] S. M. Kupchan, O. P. Dhingra, and C.-K. Kim, *J. Org. Chem.*, 1976, **41**, 4049.
[23] M. P. Cava, A. Venkateswarlu, M. Srinivasane, and D. L. Edie, *Tetrahedron*, 1972, **28**, 4299.
[24] S. Agurell, *Lloydia*, 1969, **32**, 40.
[25] F. M. Hershenson, *J. Org. Chem.*, 1975, **40**, 740.
[26] H. Bruderer and A. Brossi, *Helv. Chim. Acta*, 1965, **48**, 1945.
[27] A. Brossi, A. I. Rachlin, and S. Teitel, *J. Heterocyclic Chem.*, 1967, **4**, 417.
[28] K. Kratzl and G. Billek, *Monatsh.*, 1952, **83**, 1409.
[29] M. Shamma and C. D. Jones, *J. Org. Chem.*, 1970, **35**, 3119.
[30] G. Fahreus and B. Reinhammer, *Acta Chem. Scand.*, 1967, **21**, 2367.
[31] N. A. Pickard and D. W. S. Westlake, *Canad. J. Microbiol.*, 1969, **15**, 869.
[32] T. M. Devlin in ' Methods in Enzymology,' eds. S. P. Colowick and N. O. Kaplan, Academic Press, New York, 1955, vol. 2, p. 770.