Non-Amine Based Analogues of Lavendustin A as Protein-Tyrosine Kinase Inhibitors

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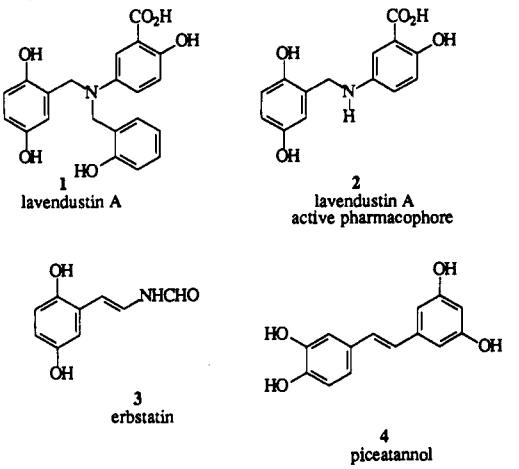
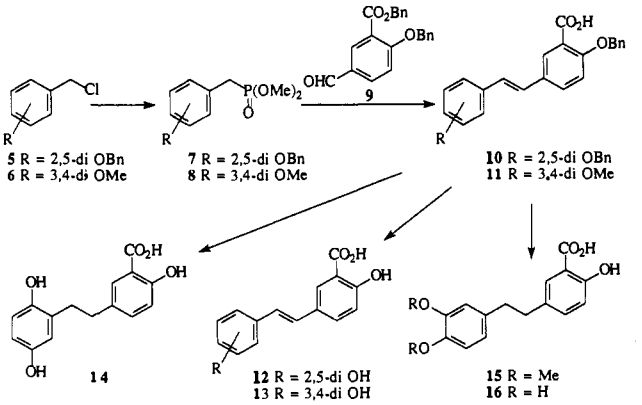
Received April 23, 1993®

The fermentation product lavendustin A (1) is a protein-tyrosine kinase (PTK) inhibitor whose active pharmacophore has previously been shown to reside in the more simplified salicyl-containing benzylamine 2. Amine 2 bears some structural resemblance to two other natural product PTK inhibitors, erbstatin (3) and piceatannol (4). Non-amine containing analogues of 2 were therefore synthesized which incorporated additional aspects of either erbstatin or piceatannol. Examination of these inhibitors in immunoprecipitated $p56^{lck}$, epidermal growth factor receptor (EGFR), and c-*erb* B-2/HER 2/neu PTK preparations showed that compound 12 (IC_{50} = 60 nM) was one of the most potent $p56^{lck}$ inhibitors reported to date. These results demonstrate that nitrogen is not an essential component of the lavendustin A pharmacophore 2 and that 1,2-diarylethanes and -ethenes bearing a salicyl moiety appear to be valuable structural motifs for the construction of extremely potent PTK inhibitors.

Protein-tyrosine kinases (PTKs) mediate important signaling events associated with cellular activation, differentiation, and mitogenesis. The possibility that highly potent and selective PTK inhibitors could serve as pharmacological probes of the biochemical roles played by tyrosine phosphorylation as well as their potential use as antiproliferative therapeutics has made the development of PTK inhibitors an important area of research.[1] We have found one useful approach toward deriving new PTK inhibitors of the lymphocyte-specific PTK, $p56^{lck}$ to be the rational modification of preexisting inhibitors.[2] A survey of known PTK inhibitors in many cases reveals common structural features.[1] One example is lavendustin A (1) a natural product inhibitor of the epidermal growth factor receptor (EGFR) PTK, whose active pharmacophore has been shown to consist of the more simplified benzylamine 2.[3] Structure 2 is of note in that it contains the 2,5-dihydroxyphenyl ring of erbstatin (3)[4] as well as the diaryl pattern of piceatannol (4),[5] both of which are natural product PTK inhibitors. Another distinctive feature of the lavendustin A pharmacophore 2 is the salicyl moiety. A study was therefore undertaken to derive new PTK inhibitors by combining the salicyl ring of 2 with other features displayed by erbstatin and piceatannol. Two sets of compounds resulted: One set (compounds 12 and 13) was based on the stilbene-like nucleus of piceatannol having, in addition to a salicyl ring, a second aryl ring containing either the 2,5-dihydroxy pattern found in erbstatin and lavendustin A (compound 12) or the 3,4-dihydroxy pattern of piceatannol (compound 13). The second set (compounds 14 and 16) was identical to the first, except that the ethylene bridge had been hydrogenated. These latter analogues more closely represent the lavendustin A pharmacophore 2 in which the nitrogen has been replaced by a carbon. Herein we report the synthesis and PTK inhibitor potency of these compounds in nonreceptor PTK $p56^{lck}$ as well as receptor PTK EGFR and c-*erb* B-2 (also known as HER 2/neu) preparations.



Scheme I



Synthesis

Lavendustin A (1), benzylamine 2, and the imine 17 were prepared as previously reported.[3] Synthesis of the remaining analogues is shown in Scheme I. Horner-

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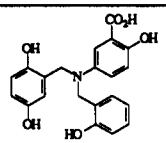
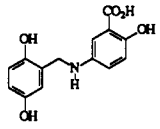
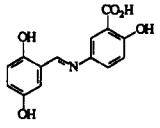
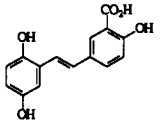
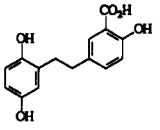
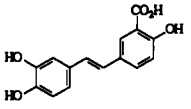
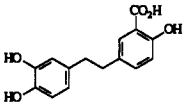
® Abstract published in *Advance ACS Abstracts*, September 1, 1993.

0022-2623/93/1836-3010$04.00/0

Table I. Inhibition of Immunoprecipitated PTK Autophosphorylation (IC_{50} μM)

no.		p56[lck]	EGFR	c-*erb* B-2
1		8	7	9
2		10	4	0.9
17		85	9	70
12		0.06	2	0.6
14		1	20	4
13		a	5	0.2
16		1	12	0.6

[a] Could not be determined.

Emmons condensation of the appropriate 2,5-dibenzyloxy (**7**)- or 3,4-dimethoxy (**8**)-substituted dimethyl benzylphosphonates (obtained from the corresponding benzyl chlorides **5** and **6** by reaction with trimethyl phosphite) with the benzyl-protected 5-formyl salicylate **9** gave the respective ethenyl compounds **10** and **11**. Of note is the concomitant monodebenzylation, providing the free carboxylic acids. Boron tribromide-mediated dealkylation of **10** and **11** provided the final 2,5- and 3,4-dihydroxy-substituted stilbenes **12** and **13**, respectively. Hydrogenation of **10** directly gave the saturated 2,5-dihydroxylated product **14**. The analogous 3,4-dihydroxylated compound **16** was obtained in a less direct manner by initial H_2/Pd·C mediated hydrogenation of stilbene **11** to yield the saturated 3,4-dimethoxy compound **15**, which was then demethylated using boron tribromide to give **16**.

Results and Discussion

Protein-tyrosine phosphorylation and dephosphorylation are important events in controlling a variety of biological processes. Enzymes which condense a phosphate group to the 4′-hydroxyl of tyrosyl residues (protein-tyrosine kinases, PTKs) can be broadly classified as being either receptor or nonreceptor, depending on whether they do or do not contain an extracellular ligand-binding domain. PTKs play key roles in initiation and regulation of various signaling cascades associated with cellular activation, differentiation, and proliferation. Significant progress has been made toward understanding the basic physiological functions of both receptor and nonreceptor PTKs.[6]

The p56[lck] PTK is a member of the src family of nonreceptor PTKs which associate with a number of cell-surface receptors and participate in activation and mitogenic signal transduction through these surrogate receptors. Several members of the src family play important roles in the immune system, and recent results from different laboratories emphasize the critical role that expression of these PTKs has on the development of T and B lymphocytes, making these enzymes potential targets for the therapy of lymphomas and certain autoimmune diseases.[7]

The c-*erb* B-2 protein is a transmembrane receptor PTK closely related to the EGFR PTK. Activation of c-*erb* B-2 initiates multiple cellular events culminating in DNA synthesis, cellular proliferation, and potentially neoplastic transformation. The over-expression of c-*erb* B-2 correlates with a poor prognosis in breast, ovarian, gastric, and non-small cell lung carcinoma. Since the aberrant expression or function of both receptor PTKs (EGFR and c-*erb* B-2) and nonreceptor PTKs (p56[lck]) can lead to neoplastic transformation, development of specific PTK inhibitors not only may aid in further studies of PTK signal transduction pathways, but also may offer novel therapeutic approaches.[1]

The p56[lck], EGFR, and c-*erb* B-2 PTKs have been extensively studied, and multiple reports have appeared on the development of inhibitors of these enzymes. The design of new inhibitors is impeded by the lack of a detailed understanding of the three-dimensional structure of a PTK catalytic domain and by a less than clear understanding of the mode of binding and interaction of known PTK inhibitors with their target enzymes. In light of these limitations, we are attempting to design new PTK inhibitors by rational modification of structures which have previously demonstrated potent PTK inhibition.[2,8] Prior reports detailed our preparation of conformationally constrained analogues whose design was based on hypothetical preferential modes of binding of a known parent PTK inhibitor.[2c,d] In the present work, our approach is different in that we make no assumptions as to the mode of interaction of the parent compound, lavendustin A (**1**), with the PTK catalytic site. Rather, we have taken note that lavendustin A bears a salicyl ring which, except for some synthetic sulfonylbenzoyl nitrostyrenes,[9] is unique among PTK inhibitors. It was also observed that certain structural elements of lavendustin A are found in other PTK inhibitors. For example, the 2,4-dihydroxyphenyl moiety of erbstatin **3** and the diaryl motif of piceatannol **4**. Therefore, using the lavendustin A active pharmacophore, salicyl benzylamine **2** as a starting point, we have designed new analogues that incorporate aspects of these other inhibitors. The resulting compounds, **12–14** and **16**, can be considered as non-amine containing analogues of lavendustin A in which **12** and **14** bear the 2,5-dihydroxy pattern of erbstatin, and **13** and **16** have the 3,4-dihydroxy pattern of piceatannol. Finally, the set of target compounds can be further differentiated into etheno analogues (**12** and **13**), which may be viewed as a subclass of the stilbene-like PTK inhibitors,[5,10] and the saturated ethano compounds (**14** and **16**), which are more directly des-amino analogues of the lavendustin A pharmacophore **2**.

A number of conclusions can be drawn from this study. Of primary note is the extremely high potency of compound **12** against immunoprecipitated p56[lck] (IC_{50} = 60 nM), being one of the most potent inhibitors reported to date in the

indicated test system. A nearly 1400-fold increase in potency against p56^{lck} resulted when the imine nitrogen of compound 17 (IC_{50} = 85 μM) was replaced by a carbon to give compound 12. Substitution of the analogous nitrogen of the saturated lavendustin A pharmacophore 2 (IC_{50} = 10 μM) with a methylene (giving compound 14; IC_{50} = 1 μM) resulted in only a 10-fold increase in potency. It can also be seen that moderate selectivity toward p56^{lck} is displayed by 12, which is 40-fold less potent against EGFR (IC_{50} = 2 μM) and 10-fold less potent against c-*erb* B-2 (IC_{50} = 0.6 μM).

Of note is the apparent discrepancy in the EGFR inhibitory potency we observed for lavendustin A (1) (IC_{50} = 7 μM) relative to the inhibitory potency originally reported by Onoda et al. (IC_{50} = 0.01 μM).[3] This difference may be due to several factors. We utilized a cell line (DHER 14) different from Onoda (A431) and measured inhibition of autophosphorylation in contrast to Onoda's use of a small peptide substrate. As pointed out previously,[11] inhibition of autophosphorylation requires a higher concentration of inhibitor because of the effectively elevated local concentration of "substrate", which is the enzyme itself. Another difference which may contribute even more to the discrepancy is the assay conditions. A previously reported[12] kinetic analysis of the inhibition of immunoprecipitated EGFR PTK by lavendustin A indicated a complex interaction of enzyme, inhibitor, and substrates which suggested that lavendustin A is a "slow tight binder". The implication of this relative to observed IC_{50} values is that apparent inhibitory potency is dependent on the order and time course of addition to the enzyme of substrate (ATP) and inhibitor. Preincubation with lavendustin A, as performed by Onoda, is required for optimal inhibitory potency. In contrast, our experimental protocol[13] utilized simultaneous addition of ATP and inhibitor, and this may account for the reduced IC_{50} value relative to Onoda.

Previous work has shown the importance of the hydroxy substitution pattern relative to both potency and interkinase specificity.[2,3,14] While the 2,5-dihydroxylated compound 12 behaved in an expected manner in p56^{lck}, the corresponding 3,4-substituted analogue 13 exhibited an extremely unusual inhibition "curve". Inhibition went abruptly from very low to very high levels at low micromolar concentrations, providing an "all or nothing" effect. For this reason, an IC_{50} value could not be assigned to this compound.

Perhaps the most fundamental structure–activity relationships which can be drawn from this study are that the nitrogen is not an essential component of the lavendustin A pharmacophore 2 and that salicyl-containing 1,2-diarylethanes and -ethenes are valuable structural motifs for the construction of extremely potent PTK inhibitors. The high potency of the saturated, ethano-based compounds 14 and 16 is surprising in light of much previous work demonstrating the importance of the "styryl nucleus" or its equivalent for PTK inhibition. In a related study,[10a] 1,2-diarylethanes were found to be inactive against p56^{lck}. The fact that these latter analogues did not contain the salicyl moiety may indicate the importance of this group as a key component of the active pharmacophore. This is further supported by the extremely high potency observed with other salicyl-containing PTK inhibitors.[9] Further work is in progress to apply these findings toward the design of novel PTK inhibitors.

Experimental Section

Biochemical Assay. In vitro p56^{lck} PTK[2d] and EGFR PTK[13] assays were conducted as previously described. Assays against c-*erb* B-2/HER2/neu were conducted in a similar manner[2d] with slight modification. Immunoprecipitation of c-*erb* B-2 from SK-BR-3 breast cancer cells (American Type Culture Collection) was carried out with HER2/neu antibody no. 2 (Oncogene Science) using 1–2 × 10^6 cells per assay using a solid-phase immunoisolation technique. Briefly, 96-well U-bottom plastic plates (Falcon Labware, Oxnard, CA) were coated with goat anti-mouse immunoglobulin (0.1 mg/mL) at 37 °C for 2 h. After washing with PBS, 50-μL aliquots of mAb solution in PBS (20 μg/mL) were applied and incubated at 4 °C for 6 h. Unoccupied binding sites on the plastic were blocked by 0.2% gelatin and 1% glycine in PBS at 37 °C for 1 h. Lysates were applied and incubated on ice over night. After washings with lysis buffer, the contents of the wells were subjected to in vitro kinase assays. Contents of wells were incubated with 50 μL of 25 mM HEPES (pH 7.2), containing 3 mM $MnCl_2$, 20 mM $MgCl_2$, 0.1% NP-40, and 1 μCi of [γ-^{32}P]ATP. After incubation for 20 min at 25 °C, protein was eluted with SDS-PAGE sample buffer, and the phosphoprotein products were resolved by 7.5% reducing SDS-PAGE gel. The resulting gels were scanned by a Hoefer Scientific Instruments Scanning Densitometer, and inhibition was calculated from the relative band densities.

Synthesis. Petroleum ether was of the boiling range 35–60 °C, and removal of solvents was performed by rotary evaporation under reduced pressure. Silica gel filtration was carried out using TLC grade silica gel (5–25 μ; Aldrich). Melting points were determined on a Mel Temp II melting point apparatus and are uncorrected. Elemental analyses were obtained from Atlantic Microlab Inc., Norcross, GA, and are within 0.4% of theoretical values unless otherwise indicated. Fast atom bombardment mass spectra (FABMS) were acquired with a VG Analytical 7070E mass spectrometer under the control of a VG 2035 data system. ^{1}H NMR data were obtained on a Bruker AC250 (250 MHz) instrument and are reported in ppm relative to TMS and referenced to the solvent in which they were run.

3,4-Dimethoxybenzyl Chloride (6). To 3,4-dimethoxybenzyl alcohol (7.25 mL, 50 mmol) in anhydrous $CHCl_3$ (75 mL) at 0 °C was added Et_3N (7.7 mL, 55 mmol) followed by $SOCl_2$ (5.45 mL, 75 mmol). The resulting yellow solution was stirred at 0 °C (10 min) and then at room temperature (1.5 h). The reaction mixture was washed successively with H_2O (100 mL), 1 M HCl (100 mL), and saturated $NaHCO_3$ (100 mL) and then dried ($MgSO_4$), and the solvent was removed to give crude 6 as an oil (12.4 g). Trituration with petroleum ether (100 mL) containing Et_2O (5 mL) at −78 °C afforded benzyl chloride 6 as a colorless solid (8.42 g, 90%): mp 50–52 °C; ^{1}H NMR ($CDCl_3$) δ 6.92 (dd, 1 H, J = 7.96, 2.04 Hz, H_6), 6.90 (s, 1 H, H_2), 6.81 (d, 1 H, J = 7.96 Hz, H_5), 4.55 (s, 2 H, CH_2Cl), 3.89 (s, 3 H, OCH_3), 3.87 (s, 3 H, OCH_3). Anal. ($C_9H_{11}O_2Cl$) C, H.

Dimethyl [(2,5-Bis(dibenzyloxy)phenyl)methyl]-phosphonate (7). To benzyl chloride 5 (2.83 g, 8.35 mmol)[15] was added $P(OMe)_3$ (4.0 mL, 33.4 mmol). The mixture was warmed to 110 °C and stirred overnight, and then excess $P(OMe)_3$ was removed by distillation. The resulting yellow oil was chromatographed [EtOAc–petroleum ether (2:1) followed by EtOAc] to afford pure 7 as a colorless solid (3.05 g, 89%): mp 63–65 °C; ^{1}H NMR ($CDCl_3$) δ 7.38 (m, 10 H), 6.98 (t, 1 H, J = 2.66 Hz, H_6), 6.82 (m, 2 H, H_3 & H_4), 5.03 (s, 2 H, OCH_2), 5.00 (s, 2 H, OCH_2), 3.63 (s, 3 H, $POCH_3$), 3.59 (s, 3 H, $POCH_3$), 3.26 (d, 2 H, J = 21.8 Hz, PCH_2). Anal. ($C_{23}H_{25}O_5P$) C, H.

Dimethyl [(3,4-Dimethoxyphenyl)methyl]phosphonate (8). Phosphonate 8 was prepared in a manner similar to that described for 7. Chromatography of the crude product [gradient elution with EtOAc–hexanes (2:1), then EtOAc, and then EtOAc–MeOH (3:1)] afforded 8 as a clear yellow oil (1.73 g, 99%): ^{1}H NMR ($CDCl_3$) δ 6.81 (m, 3 H), 3.86 (s, 3 H, OCH_3), 3.84 (s, 3 H, OCH_3), 3.67 (s, 3 H, P-OCH_3), 3.63 (s, 3 H, P-OCH_3), 3.09 (d, 2 H, J = 21.2 Hz, P-CH_2).

Benzyl 5-Formyl-2-(benzyloxy)benzoate (9). To 5-formylsalicylic acid (5.00 g, 30 mmol) in dry DMF (45 mL) was added anhydrous K_2CO_3 (16.6 g, 120 mmol) under argon. Benzyl bromide (9.0 mL, 75.2 mmol) was added, and the resulting mixture

was warmed to 110 °C and stirred (16 h). After cooling to 0 °C, the solution was poured into EtOAc (100 mL), the salts were filtered, the EtOAc was washed with 1 N KOH (50 mL) and then H_2O (50 mL) and dried ($MgSO_4$), and solvent was removed to yield **9** as a tan solid (9.85 g, 95%): mp 80–85 °C; ^1H NMR ($CDCl_3$) δ 9.89 (s, 1 H, CHO), 8.34 (d, 1 H, J = 2.18 Hz, H_6), 7.97 (dd, 1 H, J = 8.67, 2.22 Hz, H_4), 7.35 (m, 10 H), 7.14 (d, 1 H, J = 8.70 Hz, H_3), 5.36 (s, 2 H, CO_2CH_2), 5.26 (s, 2 H, OCH_2). Anal. ($C_{22}H_{18}O_4$) C, H.

***trans*-1-(3′-Carboxy-4′-(benzyloxy)phenyl)-2-(2″,5″-bis(benzyloxy)phenyl)ethene (10).** To phosphonate **7** (412 mg, 1.0 mmol) in dry DMF (4.0 mL) at 0 °C under argon was added potassium *tert*-butoxide (168 mg, 1.5 mmol). The resulting yellow solution was stirred (20 min), and then aldehyde **9** (346 mg, 1.0 mmol) in DMF (1.0 mL) was added. The mixture was warmed to room temperature and stirred (4.5 h), then 10 N NaOH (1.0 mL) was added, and stirring was continued (1 h). The reaction mixture was diluted with H_2O (25 mL), acidified (6 N HCl) then extracted with CH_2Cl_2 (3 × 30 mL), and dried ($MgSO_4$). Evaporation of solvent gave crude **10** as a yellow syrup (750 mg). Silica gel chromatography [hexanes–EtOAc (5:1)] afforded **10** as a light yellow tinted powder (431 mg, 79%). Recrystallization (CH_2Cl_2–hexanes) provided an analytical sample of **10** as a colorless powder: mp 139–140 °C; ^1H NMR ($CDCl_3$) δ 8.31 (d, 1 H, J = 2.36 Hz, $H_{2'}$); 7.63 (dd, 1 H, J = 8.70, 2.40 Hz, $H_{6'}$), 7.37 (m, 11 H), 7.19 (d, 1 H, J = 2.58 Hz, $H_{6''}$), 7.08 (d, 1 H, J = 8.64 Hz, $H_{5'}$), 7.06 (d, 1 H, J = 16.52 Hz, C=CH), 6.84 (m, 2H, $H_{3''}$ and $H_{4''}$), 5.29 (s, 2 H, OCH_2), 5.09 (s, 2 H, OCH_2), 5.05 (s, 2 H, OCH_2). Anal. ($C_{36}H_{30}O_5$) C, H.

***trans*-1-(3′-Carboxy-4′-(benzyloxy)phenyl)-2-(3″,4″-dimethoxyphenyl)ethene (11).** Preparation of compound **11** was carried out on a 4 mmol scale following a procedure similar to that reported for the synthesis of compound **10**. Chromatographic purification [hexanes–EtOAc (3:1)] afforded **11** as a yellow solid (744 mg, 47%). Recrystallization (CH_2Cl_2–hexanes) gave analytically pure **11** as colorless crystals: mp 138–139 °C; ^1H NMR ($CDCl_3$) δ 10.8 (br s, 1 H, CO_2H), 8.34 (d, 1 H, J = 2.37 Hz, $H_{2'}$), 7.63 (dd, 1 H, J = 8.66, 2.40 Hz, $H_{6'}$), 7.42 (m, 5 H), 7.10 (d, 1 H, J = 8.84 Hz, $H_{5'}$), 7.04 (m, 3 H, $H_{6''}$ and $H_{2''}$ and H_1), 6.90 (d, 1 H, J = 17.2 Hz, H_2), 6.85 (d, 1 H, J = 9.01 Hz, $H_{5''}$), 5.31 (s, 2 H, CH_2O), 3.92 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3). Anal. ($C_{24}H_{22}O_5 \cdot {}^1/_2CH_2Cl_2$) C, H.

***trans*-1-(3′-Carboxy-4′-hydroxyphenyl)-2-(2″,5″-dihydroxyphenyl)ethene (12).** To **10** (1.34 g, 2.47 mmol) in anhydrous CH_2Cl_2 (50 mL) at −78 °C under argon was added BBr_3 (12.3 mL, 1 M in CH_2Cl_2) dropwise via syringe. The solution was warmed to room temperature and stirred 4 h. After recooling to −78 °C, H_2O (10 mL) and EtOAc (20 mL) were added, and the solution was again warmed to room temperature. Extractive workup (H_2O/EtOAc), drying ($MgSO_4$), and removal of the solvent gave crude **12** (1.44 g). Recrystallization from hexanes–acetone (4:1) provided an orange solid which was triturated with CH_2Cl_2 and filtered to afford **12** as a yellow powder (428 mg, 64%): mp 221–223 °C dec; ^1H NMR (DMSO-d_6) δ 11.28 (br s, 1 H, CO_2H), 9.01 (s, 1 H, OH), 8.72 (br s, 1 H, OH), 7.91 (d, 1 H, J = 2.27 Hz, $H_{2'}$), 7.73 (dd, 1 H, J = 8.66, 2.27 Hz, $H_{6'}$), 7.23 (d, 1 H, J = 16.54 Hz, C=CH), 7.03 (d, 1 H, J = 16.58 Hz, C=CH), 6.96 (d, 1 H, J = 8.60 Hz, $H_{5'}$), 6.92 (d, 1 H, J = 2.86 Hz, $H_{6''}$), 6.66 (d, 1 H, J = 8.61 Hz, $H_{3''}$), 6.51 (dd, 1 H, J = 8.61, 2.86 Hz, $H_{4''}$), 3.40 (br s, 1 H, OH); high-resolution negative ion FABMS m/z calcd for $C_{15}H_{11}O_5$ 271.0606, found 271.0590. Anal. ($C_{12}H_{14}O_5 \cdot {}^1/_4H_2O$) C, H.

***trans*-1-(3′-Carboxy-4′-hydroxyphenyl)-2-(3″,4″-dihydroxyphenyl)ethene (13).** Treatment of compound **11** in a manner similar to that described for the conversion of **10** to **12** yielded compound **13** as a green powder (25% yield): mp 226–228 °C; ^1H NMR (DMSO-d_6) δ 11.2 (br s, 1 H, CO_2H), 9.08 (br s, 1 H, OH), 8.90 (br s, 1 H, OH), 7.88 (d, 1 H, J = 1.97 Hz, $H_{2'}$), 7.74 (dd, 1 H, J = 8.64, 2.01 Hz, $H_{6'}$), 6.93 (m, 4 H, $H_{5'}$ and $H_{2''}$ and H_1 and H_2), 6.84 (dd, 1 H, J = 8.16, 1.70 Hz, $H_{6''}$), 6.70 (d, 1 H, J = 8.13 Hz, $H_{5''}$); FABMS m/z 271 (M − H). Anal. ($C_{15}H_{12}O_5 \cdot {}^1/_4H_2O$) C, H.

1-(3′-Carboxy-4′-hydroxyphenyl)-2-(2″,5″-dihydroxyphenyl)ethane (14). A mixture of **10** (200 mg, 0.37 mmol) and 10% Pd-C (40 mg) in EtOAc (3.7 mL) and EtOH (3.7 mL) was exposed to H_2 (1 atm) for 6.5 h. Filtration through Celite and evaporation of the solvent gave crude **14** (104 mg) which was crystallized from hexanes–acetone (3:1) to afford **14** as a dark orange powder (92 mg, 91%): mp 188–190 °C; ^1H NMR (DMSO-d_6) δ 11.30 (br s, 1 H, CO_2H), 8.54 (s, 1 H, OH), 8.49 (br s, 1 H, OH), 7.59 (d, 1 H, J = 2.24 Hz, $H_{2'}$), 7.34 (dd, 1 H, J = 8.46, 2.25 Hz, $H_{6'}$), 6.85 (d, 1 H, J = 8.43 Hz, $H_{5'}$), 6.57 (d, 1 H, J = 8.42 Hz, $H_{3''}$), 6.44 (d, 1 H, J = 2.81 Hz, $H_{6''}$), 6.38 (dd, 1 H, J = 8.43, 2.93 Hz, $H_{4''}$), 3.33 (br s, 1 H, OH), 2.69 (m, 4 H, CH_2-CH_2); FABMS m/z 273 (M − H). Anal. ($C_{15}H_{14}O_5$), C, H.

1-(3′-Carboxy-4′-hydroxyphenyl)-2-(3″,4″-dimethoxyphenyl)ethane (15). A solution of **11** (200 mg, 0.51 mmol) in absolute EtOH (5 mL) was hydrogenated in a Parr apparatus over 10% Pd-C (40 mg) under 50 psi H_2 (3.5 h). Filtration through Celite and removal of solvent gave crude **15** (154 mg, 100%). Crystallization from $CHCl_3$–hexanes afforded pure **15** as a colorless powder: mp 165–168 °C (softened at 150 °C); ^1H NMR ($CDCl_3$) δ 10.31 (br s, 1 H, CO_2H), 7.66 (d, 1 H, J = 2.21 Hz, $H_{2'}$), 7.28 (dd, 1 H, J = 8.57, 2.31 Hz, $H_{6'}$), 6.90 (d, 1 H, J = 8.54 Hz, $H_{5'}$), 6.77 (d, 1 H, J = 8.07 Hz, $H_{6''}$), 6.65 (m, 2 H, $H_{2''}$ and $H_{5''}$), 3.84 (s, 3 H, OCH_3), 3.82 (s, 3 H, OCH_3), 2.84 (s, 4 H, CH_2-CH_2). Anal. ($C_{17}H_{18}O_5$), C, H.

1-(3′-Carboxy-4′-hydroxyphenyl)-2-(3″,4″-dihydroxyphenyl)ethane (16). Treatment of compound **15** in a manner similar to that described for the conversion of **10** to **12** yielded **16** as a tan powder (60%). Purification by reverse-phase HPLC (15–50% MeCN in H_2O; linear gradient over 30 min) provided analytically pure **16** as a colorless fluffy solid: mp 186–188 °C (turns brown at 178 °C); ^1H NMR (DMSO-d_6) δ 11.08 (br s, 1 H, CO_2H), 8.64 (br s, 1 H, OH), 8.60 (br s, 1 H, OH), 7.58 (d, 1 H, J = 2.12 Hz, $H_{2'}$), 7.32 (dd, 1 H, J = 8.46, 2.19 Hz, $H_{6'}$), 6.83 (d, 1 H, J = 8.45 Hz, $H_{5'}$), 6.59 (d, 1 H, J = 7.96 Hz, $H_{5''}$), 6.56 (d, 1 H, J = 1.81 Hz, $H_{2''}$), 6.42 (dd, 1 H, J = 8.06, 1.88 Hz, $H_{6''}$), 2.68 (m, 4 H, CH_2-CH_2); FABMS m/z 273 (M-H). Anal. ($C_{15}H_{14}O_5 \cdot {}^1/_4H_2O$), C, H.

Acknowledgment. Appreciation is expressed to Dr. James Kelley and Ms. Pamela Russ of the LMC for providing mass spectral analysis.

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