Preussomerins and Deoxypreussomerins: Novel Inhibitors of Ras Farnesyl-Protein Transferase

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Farnesylation of Ras (p21) by farnesyl-protein transferase (FPTase) precedes association of Ras with the plasma membrane which is essential for Ras-mediated cell-transforming activity. Our continuing search for FPTase inhibitors led us to isolate a number of novel preussomerins and deoxypreussomerins from the extracts of a dung-inhabiting coelomycetous fungus from Chaco Province, Argentina. Isolation, structure elucidation, chemistry, and structure–activity relationships of the natural products and derivatives have been described.

Ras farnesyl-protein transferase (FPTase) is a heterodimeric enzyme that catalyses the transfer of the farnesyl group from farnesyl pyrophosphate (FPP) onto cysteine 186 at the C-terminus of the Ras peptide.[1] This is a mandatory process before further post-translational modification and association of the Ras peptide to the plasma membrane which is required for triggering *ras* oncogene toward tumor formation.[1,2] There is now evidence that inhibitors of FPTase will have an antitumorgenic effect.[3] This is considered to be a first step toward the development of an effective agent for treatment of cancers, particularly those with mutated *ras* gene such as colon and pancreatic carcinomas.[2]

Biological screening of natural products is a most effective method for discovering novel inhibitors of relevant target enzymes. Last year, we reported the discovery of chaetomellic acids[4] as highly specific low nanomolar FPP mimic inhibitors of FPTase, and most recently we uncovered fusidienol,[5] from *Fusidium griseum* as a potent and specific inhibitor of FPTase. Zaragozic acids,[6] which are a class of bicyclotricarboxylic acids originally discovered as highly potent inhibitors of squalene synthase, are also efficient inhibitors of FPTase. Microbial products which have been recently reported by other groups as inhibitors of FPTase are pepticinnamins[7] (a series of peptides), gliotoxin,[8] 10′-desmethoxystreptonigrin,[9] and several analogs of manumycin.[10] Continued efforts toward the discovery of novel inhibitors of FPTase in our laboratories have culminated in the discovery of other interesting compounds. In this paper we wish to report preussomerins (**1**–**4**) and deoxypreussomerins (**5**, **6**) as novel inhibitors of FPTase (Chart 1).

The preussomerins[11] are a class of fungal metabolites which were recently isolated as antifungal agents from the coprophilous fungus[11a,b] *Preussia isomera* and the endophytic fungus[11c] *Harmonema dematioides*. These compounds are comprised of two unsaturated decalin units connected *via* three oxygens bridges through two spiroketal carbons located in each of the upper and lower decalin units. The deoxypreussomerins are a class of compounds which lack one of the bridge oxygens and thus one of the spiroketal carbons. All of the examples of this class of compounds have been reported[12] during or after 1993, while this study was in progress; with the exception

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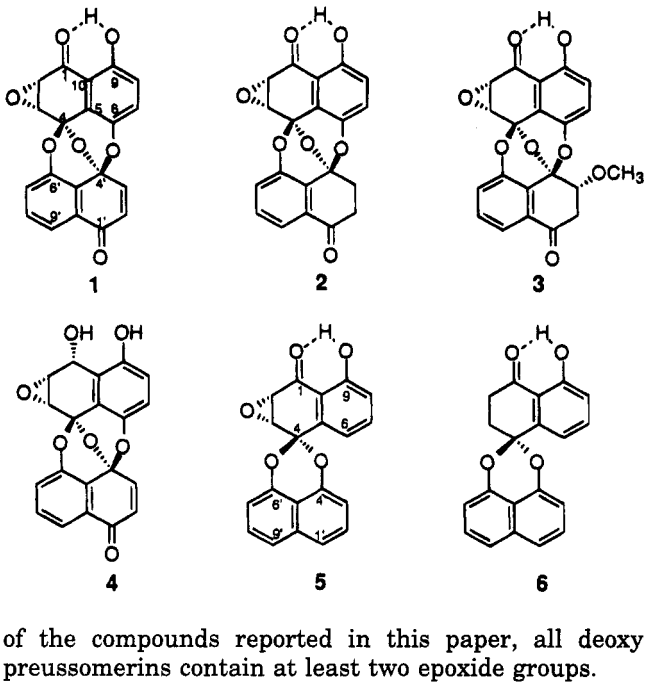
Chart 1



of the compounds reported in this paper, all deoxy preussomerins contain at least two epoxide groups.

Isolation

Methyl ethyl ketone extract of the fermentation broth of an unidentified coelomycetes (MF 5916) was chromatographed over a Sephadex LH-20 column in methanol. Crystallization of the FPTase active fractions from boiling acetone gave deoxypreussomerin A (**5**). Subsequent crystallization of the mother liquor from hot methanol followed by silica gel chromatography of the crystalline material gave preussomerin G (**1**). The minor components—preussomerin D (**4**), preussomerin H (**2**), preussomerin I (**3**), and deoxypreussomerin B (**6**)—were isolated from the filtrate by chromatography on Whatman C-18 followed by silica gel column chromatography or preparative TLC.

Structure Elucidation

Preussomerin G (1). Electron-impact mass-spectral analysis of preussomerin G (**1**) gave a molecular ion at m/z 362 which was analyzed for the molecular formula $C_{20}H_{10}O_7$ by exact mass measurement and was supported by the ^{13}C NMR spectrum. The infrared spectrum of **1** showed absorption bands for conjugated carbonyls. Examination of the ^1H NMR spectrum (Table 1) revealed two sets of *ortho*-coupled protons and a 1,2,3-trisubstituted aromatic ring with at least one oxygen substitution as well as a highly chelated phenolic OH group (δ 10.21 ppm) which slowly exchanged with deuterium upon shaking with D_2O. The ^{13}C NMR spectrum (Table 2) of **1** exhibited 20 carbon signals which were assigned with the help of an HMQC experiment. Two of the quaternary carbon signals appeared at δ 89.95 and 93.82 ppm, characteristic of the spiroketal carbons of the preussomerins.[11] The other carbons were identified as two conjugated carbonyls appearing at δ 195.35 and 183.68 ppm, seven olefinic/aromatic methines, one of which appeared at δ 143.30 ppm and was assigned to a carbon β to the carbonyl group. Two carbon signals at δ 52.07 and 53.60 ppm correspond to the epoxide-bearing methines. The remaining seven carbons are quaternary and were assigned as shown in Table 2. As preussomerin G was our first example of this class of compounds, we decided to get an independent structural proof by X-ray crystallography. Suitable crystals were obtained by slow crystallization from methanol and X-ray analysis[13] confirmed the proposed structure **1**.

The X-ray structure (Figure 1) clearly demonstrated that the aromatic rings are perpendicular to each other and shows that only the bottom face of the lower half of the molecule is accessible to nucleophilic reagents (*vide infra*). The mass spectrum of preussomerin G gave two major characteristic fragment ions at m/z 189 and 174. The fragmentation pattern of **1** is depicted in Scheme 1.

Preussomerin H (2). Mass spectral analysis of preussomerin H gave a molecular ion at m/z 364 and the molecular formula $C_{20}H_{12}O_7$ which indicated that preussomerin H has two additional protons compared to preussomerin G. The ^1H NMR spectrum (Table 1) of **2** was devoid of the two doublets (δ 6.60 and 7.24 ppm) assigned to *cis*-olefinic protons H-2′ and H-3′ of preussomerin G and instead displayed four self-coupled aliphatic protons in the upfield region of the spectrum, which consequently were assigned to H-2′ and H-3′ protons. The other parts of the spectra were virtually identical, and thus structure **2** was assigned to preussomerin H. The mass spectral fragmentation of the dihydro compound **2** was different from that of **1**. Unlike the situation in preussomerin G, it seems that the formation of the first intermediate is not allowed in preussomerin H due to the lack of extended conjugation and thus the corresponding fragment ions presented in Scheme 1 were not formed. Instead, the mass spectrum of preussomerin H produced a fragment ion (m/z 159) corresponding to the lower decalin unit which has one oxygen atom less than the corresponding fragment ion derived from preussomerin G.

Preussomerin I (3). Mass spectral analysis of preussomerin I gave a molecular formula of $C_{21}H_{14}O_8$. Comparison of the ^1H NMR spectrum of **3** with that of preussomerin G indicated absence of the C-2′, C-3′ double bond, the presence of a methoxy group at δ 3.55 ppm, and an apparent triplet of a oxygenated methine proton at δ 4.35 ppm as well as two self-coupled aliphatic proton signals which in turn were coupled to the methine proton. On the basis of these observations a methanol adduct structure **3** was assigned to preussomerin I. This structural assignment was fully corroborated by the mass spectral fragmentation pattern which was much like that of preussomerin H and gave an ion (m/z 189: $C_{11}H_9O_3$) corresponding to the lower part of the molecule. The α stereochemistry was assigned to the new stereogenic center at C-3′ based on the following arguments. The coupling constant of 2.7 Hz between H-2′ and H-3′ in the ^1H NMR spectrum of **3** suggested that H-3′ must occupy a *pseudo*equatorial position and thus the methoxy group must be placed in a *pseudo* axial position of the pseudo-chair ring conformation. This observation was strongly supported by examination of the Drieding model of both preussomerin G and preussomerin I. The β oxygen (O-3) at C-4′ blocks the β face of the preussomerins (see ORTEP drawing, Figure 1), and thus any nucleophilic attack must come from the bottom face of the molecule, in particular at C-3′. Preussomerin I has been isolated in relatively small amounts compared to preussomerin G. This raised the obvious question as to whether

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Table 1. ^{1}H NMR Spectral Data of Preussomerins 1-3 and Deoxypreussomerins 5 and 6 in $CDCl_3$ Solutions

position	1 δH	2 δH	3[a] δH	5 δH	6 δH
2	3.85, d, 4.0	3.84, d, 3.9	3.85, d, 4.2	3.68, d, 4.0	2.84, t, 6.3
3	4.24, d, 4.0	4.23, d, 3.9	4.31, d, 4.2	4.09, d, 3.5	2.50, t, 6.9
6				7.43, dd, 8.0, 1.5	7.45, dd, 7.8, 1.0
7	7.05, dd, 9.4, 0.4	7.05, d, 9.3	7.04, d, 9.0	7.64, brt, 6.6	7.63, t, 7.8
8	6.95, d, 9.2	6.95, d, 9.6	6.95, d, 9.3	7.13, dd, 8.5, 1.5	7.10, dd, 8.4, 0.9
9-OH	10.21, d, 0.4	10.14, s	10.14, s	11.36, s	12.45, s
1′				7.59, dd, 9.0, 1.0	7.54, dd, 8.4, 0.9
2′	6.60, d, 10.0	2.90, m, 3.28, ddd, 19, 14, 6	3.10, dd, 18, 2.7, 3.37, dd, 18, 3.0	7.45, t, 8.0	7.45, t, 7.2
3′	7.24, d, 10.0	2.52, dt, 13.5, 5.4, 2.81, m	4.35, t, 2.7	6.92, dd, 7.5, 1.0	6.97, dd, 7.5, 0.9
7′	7.04, dd, 8.4, 1.2	7.06, d, 8.0	7.06, dd, 7.2, 0.9	7.18, dd, 7.0, 0.5	6.97, dd, 7.5, 0.9
8′	7.41, dd, 8.4, 8.0	7.40, t, 8.0	7.40, t, 8.0	7.53, t, 7.5	7.45, t, 7.2
9′	7.63, dd, 7.8, 1.2	7.65, d, 7.8	7.66, dd, 7.8, 0.9	7.56, brd, 8.0	7.54, dd, 8.4, 0.9

[a] Coupling are in Hz (3-OCH_3 3.55, s).

Table 2. ^{13}C NMR Spectral Data of Preussomerins 1 and Deoxypreussomerins 5 in $CDCl_3$ Solutions

position	1 δC	5 δC	5 (HMBC)
1	195.35	196.53	H-2, H-3, 9-OH
2	53.60	53.25	
3	52.07	53.25	H-2
4	93.82	95.96	H-3, H-6
5	109.74	136.86	H-3, H-7
6	143.31	119.08	H-7, H-8
7	126.74	137.69	
8	121.23	120.07	H-6, 9-OH
9	156.00	161.86	H-7, H-8, 9-OH
10	109.74	112.25	H-2, H-6, H-8, 9-OH
1′	183.68	121.35	H-3, H-9
2′	133.46	127.65	
3′	140.65	109.34	
4′	89.85	146.66	H-2′, H-3′
5′	120.50	112.78	H-3′, H-7′, H-9′
6′	148.63	146.91	H-7′, H-8′
7′	120.67	110.18	H-9′
8′	131.11	127.79	H-9′
9′	120.81	121.45	H-7′
10′	130.22	134.17	H-2′, H-8′, H-9′

preussomerin I is an isolation artifact. In order to answer this question preussomerin G was reacted with an excess of methanol in CD_3CN or $CDCl_3$, and the progress of the reaction was monitored by NMR over a 10-day period. No preussomerin I was formed. A similar observation was made by Gloer and his co-workers in their study of methoxypreussomerin.[11b] The identity of preussomerin D (**4**) was confirmed by comparison (HPLC, NMR) with an authentic sample.[11c]

Deoxypreussomerin A (5) and Deoxypreussomerin B (6). High resolution mass spectral analysis of deoxypreussomerin A (**5**) gave a molecular ion at *m/z* 332 and the molecular formula $C_{20}H_{12}O_5$ which is consistent with a formula derived from ^{13}C NMR spectrum (assigned by HMQC, Table 2). The ^{1}H NMR spectrum of **5** displayed two oxymethine and two *ortho*-coupled aromatic signals similar to those in the upper half of preussomerins G–I. Three other signals (two protons each) were reminiscent of a 1,2,3-trisubstituted aromatic ring in general and 1,8-dioxynaphthalene in particular. These assignments were verified by an HMBC experiment, and most of the correlations are presented in Table 2. The ^{13}C NMR spectrum of deoxypreussomerin A exhibited only one spiroketal carbon at δ 95.96 ppm which was connected to 1,8-dioxynaphthalene. Besides indicating losses of units of oxygen and CO, the mass spectrum of **5** gave two characteristic fragment ions at *m/z* 173 and 160 (see Scheme 2) derived from splitting the molecule in to upper and lower halves, with either a loss of hydride or addition of two hydrogens, respectively.

Deoxypreussomerin B (**6**) was similarly characterized by ^{1}H NMR and high resolution mass spectral data. The mass spectral data suggested that deoxypreussomerin B had one less oxygen atom and one degree less of unsaturation compared to **5** which could be explained by the loss of the epoxide ring. This supposition was im-

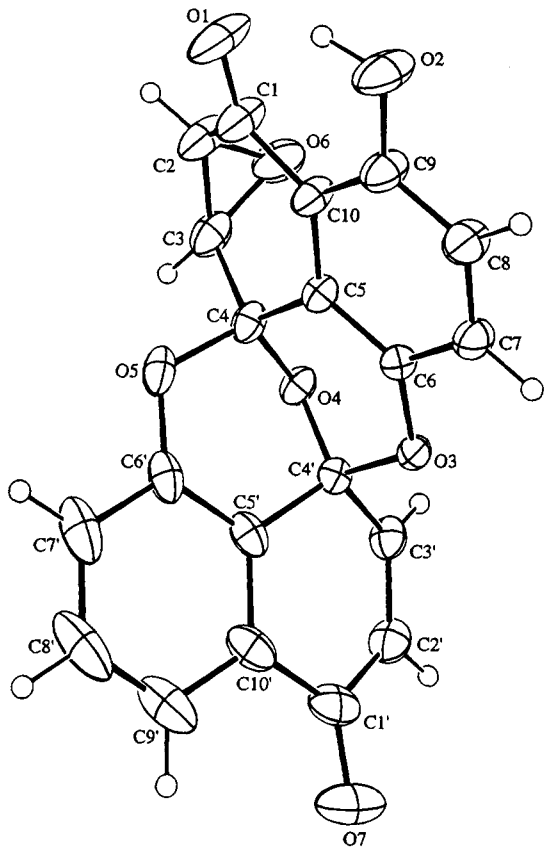
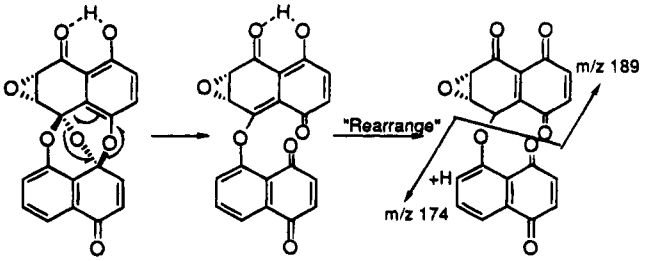
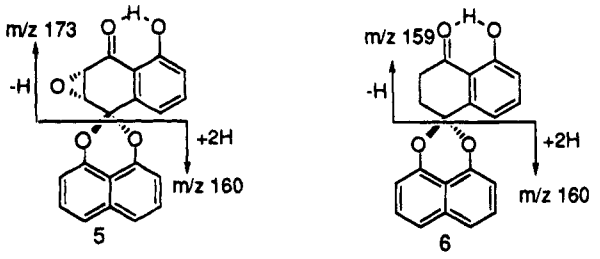


Figure 1. ORTEP view of preussomerin G (**1**) showing the crystallographic labeling scheme. Atoms are drawn with 30% probability thermal ellipsoids except for the hydrogen atoms which are an arbitrary size.

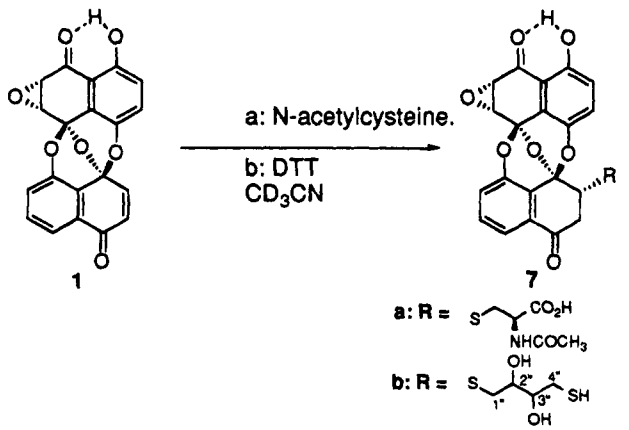
Scheme 1. Mass Spectral Fragmentations of Preussomerin G



Scheme 2. Mass Spectral Fragmentations of 5 and 6



Scheme 3. Michael Adducts of Preussomerin G



mediately apparent from examination of the ^{1}H NMR spectrum (Table 1) which did not have the characteristic oxymethine epoxide doublets and showed the presence of two self-coupled magnetically equivalent CH_2 groups. Deoxypreussomerin B gave two characteristic fragment ions at m/z 159 and 160 by a mechanism similar to that of deoxypreussomerin A (Scheme 2).

The absolute stereochemistry of the preussomerins has been determined by Gloer and co-workers[11a,b] by chemical correlations of a degradation product of preussomerin A to (−)-regiolone. Since all preussomerins including preussomerins G–H and deoxypreussomerin A have large negative optical rotations they all must have the same absolute stereochemistry.

Michael Reactions of Preussomerin G. Strong nucleophiles add to preussomerin G (**1**) in a highly stereospecific Michael fashion at the C-3′ position to give the C-3′ adduct quantitatively. Thus, reaction of **1** with 1 molar equiv of *N*-acetylcysteine gave α adduct **7a** (see Scheme 3). Strong steric hindrance by the β (O-3) oxygen makes the top face of preussomerin G inaccessible to nucleophiles, and thus Michael addition takes place only from the more accessible α face. The stereochemistry at C-3′ was assigned based on the coupling constants (4.8 and 2.0 Hz) of quasiequatorial H-3′ with H-2′ (quasiequatorial, quasiaxial). A larger coupling would be expected between a quasiaxial H-3′ and quasiaxial H-2′. A similar reaction with dithiothreitol gave a clean H-3α′ adduct (^{1}H NMR) **7b** which was not characterized and was used as a C-2" and C-3" diastereomeric mixture. Formation of **7b** was extremely fast and the reaction was complete (monitored by ^{1}H NMR for disappearance of H-2′ and H-3′) within 10 min. On the other hand, reaction of *N*-acetylcysteine with preussomerin G was very slow. Less than 20% of the product (**7a**) was formed after 30 min, and the reaction took more than 3 days for completion.

Hydrogenation of preussomerin G (1). Palladium-catalyzed hydrogenation (Scheme 4) of preussomerin G

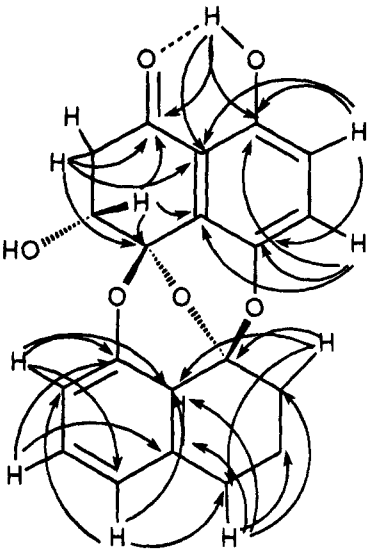
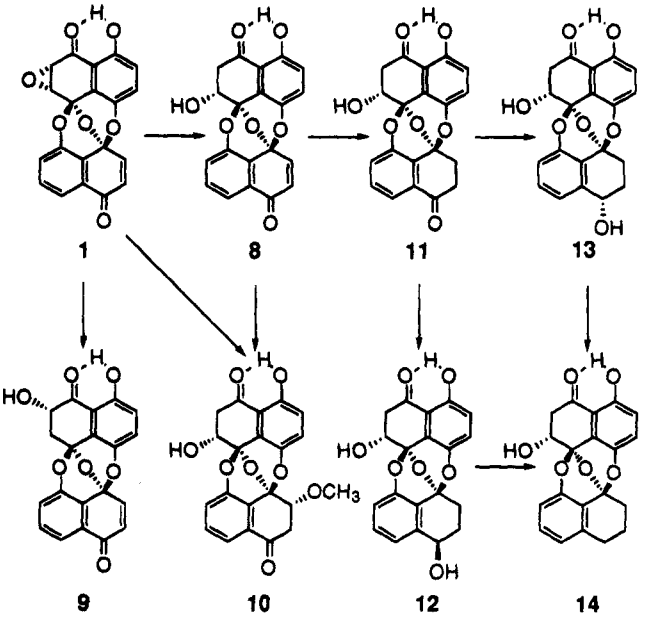


Figure 2. HMBC Correlations of 14.

Scheme 4. Hydrogenation of Preussomerin G



in ethyl acetate overnight gave exclusively compound **14**. The structure of **14** was elucidated with the help of HMQC and HMBC experiments. The HMBC correlations are presented in Figure 2. This rather unexpected outcome prompted us to investigate the course of this reaction (Scheme 4) in detail. The time course of a similar hydrogenation reaction of preussomerin G in ethyl acetate was carefully monitored by TLC and was terminated after 3 h (more than 2 h were needed for complete consumption of preussomerin G). The reaction gave a mixture of products in a ratio (determined by ^{1}H NMR) of 1:3.5:2.2, which were isolated and characterized to be compounds **8**, **11**, **12/13**, respectively. The final product **14** had not yet been formed.

Many interesting observations were made in this reaction, e.g.: (I) reduction of the C-2, C-3 epoxide was regiospecific and was faster than reduction of conjugated olefin C-2′, C-3′. Furthermore, it appears to be the first step, as the mixture does not contain any epoxide-containing compounds as determined by ^{1}H NMR; (II) hydrogenolysis of the C-1′ benzo ketone was also regiospecific; and (III) none of the three bridged benzylic oxygens were hydrogenolyzed. To ascertain if the re-

Table 3. Ras Farnesyl-Protein Transferase (Bovine Brain) Activity of Preussomerins and Analogs[a]

compd	IC_{50} (μM)	compd	IC_{50} (μM)
1	1.2	**8**	27.5
2	12	**9**	1.4
3	17	**10**	25.3
4	1.2	**11**	IA
5	10	**12**	IA
6	12	**13**	IA
7a	21.7	**14**	IA
7b	28.5		

[a] IC_{50} is the concentration of compound which inhibited the reaction of Ras-CVLS and FPP catalyzed by bovine brain FPTase by 50%. IA = inactive up to ~70 μM.

giospecificity of the hydrogenolysis of benzo ketone C-1′ was due to hydrogen bonding of the C-1 benzo ketone, the hydrogenation reaction was performed in a mixture of ethyl acetate–methanol (poor methanol solubility at room temperature) for 3 h; this gave compounds **8**, **9**, **10**, **12/13**, and **14** in a ratio of 3.6, 0.1, 0.2, 4.0, and 0.5. Since the C-1 benzo ketone was intact in all of the products formed, we, therefore, conclude that intramolecular hydrogen bonding is not responsible for the regiospecificity of the hydrogenolysis of the C-1′ benzo ketone.

Two important differences were observed between the protic and aprotic solvent reactions besides the differences in the ratios of the products formed. (I) The regiospecificity of epoxide reduction was slightly lower in the protic solvent (a small amount of the other regioisomer, **9**, was formed). (II) Compound **11** was the major product in the aprotic (ethyl acetate) solvent reaction, whereas it was not present in the reaction (after 3 h) conducted in the protic solvent (methanol). This is undoubtedly due to the rapid hydrogenolysis of compound **11** to **12/13** in the latter reaction as explained in the experiments below. The reaction in methanol was extremely fast. Preussomerin G was completely used up in about 5 min, and compound **8** (observed by TLC) and compound **9** were the sole products formed. After 10 min compounds **8**, **9**, **11**, and **12/13** in a ratio of 7:3:6:6 were obtained. The ratio of the products formed was determined by integration of H-2 of **9** and H-3 of the others in the ^1H NMR spectrum. It was surprising to see that the C-1 ketone and benzylic bridge oxygens were untouched under these hydrogenation conditions.

Ras Farnesyl-Protein Transferase Structure–Activity Relationship. Farnesyl-protein transferase inhibitory activity was measured against partially purified FPTase enzyme prepared[13] from bovine brain and Ras-CVLS acceptor peptide using an assay protocol which has been described previously.[4d,13] The IC_{50} data of all the compounds is presented in Table 3. Preussomerin G (**1**) and preussomerin D (**4**) exhibited an IC_{50} of 1.2 μM, and compound **9** showed an IC_{50} of 1.4 μM. These are the most active compounds in this series. The other compounds in Table 3 either have modest activity or are inactive. On the basis of the activity of this small set, these compounds could be grouped into three classes. The first set (those most active) of compounds (**1**, **4**, and **9**) contain a conjugated ketone in the lower half of the molecule and either an epoxide or at least a 1,2-dioxygen in the upper half of the preussomerin molecule. The second set (modestly active) of compounds (**2**, **3**, **5-8**, **10**) have only one of these two structural features. The third set (inactive) of compounds (**11-14**) has neither. The most active compounds (**1**, **4**, and **9**) in the series have conjugated ketones. This characterisic seems to be critical for activity and may serve as a Michael acceptor for nucleophilic Ras-CVLS. The reduced compounds (**2** and **11**) and Michael adducts (**3** and **7**) were either significantly less active or were inactive. The FPTase assay contains 5 mM DTT and prescribes 30 min of incubation time, which should be sufficient for DTT to react with the conjugated ketone in question as evidenced by the formation of the DTT adduct of preussomerin G in CD_3CN. However, this may not be happening in the actual assay milieu as the adduct (**7b**) showed ~20 times less activity compared to preussomerin G. Other explanations for the high activity of compounds **1**, **4**, and **9** may be that they are either true inhibitors or are acting as nonspecific Michael substrates for Ras-CVLS and depleting it from the substrate pool. In the latter case the rate of Ras-CVLS reaction must be faster than the rate of DTT reaction at pH 7.4 (assay pH). This would seem improbable since the DTT reaction is 1 order of magnitude faster than the *N*-acetylcysteine reaction. This may not be the sole reason for higher activity of compounds **1**, **4**, and **9** because compound **8**, which has the intact conjugated ketone, is significantly less active. On the basis of these observations one could conclude that the 1,2-dioxygen structural feature of preussomerin G is important for FPTase activity and that the conjugated ketone in the lower half of the molecule has an additive effect with regard to inhibitory activity. The exact mechanism of inhibition is still unclear. Preussomerin G (**1**) gave an IC_{50} of 20 μM when evaluated against bovine geranyl-geranyl transferase I (GGTase I), thus showing a 18-fold selectivity for FPTase over GGTase.

Experimental Section

General Procedure. All the reagents and deuterated solvents were obtained from Aldrich Chemical Co. and were used without any purification. The medium components used in this study were obtained from the following sources: yeast extract (Fidco a division of Nestle Co., Inc.), tomato paste (Beatrice/Hunt-Wesson, Inc.), oat flour (Quaker Oat Co.), and [2-(*N*-morpholino)ethanesulfonic acid] monohydrate (MES) (Sigma). All other materials were reagent grade. E. Merck (Darmstadt) and/or Analtech silica gel plates (0.25 mm) were used for TLC and developed either with 3% ceric sulfate in 3 N H_2SO_4 spray and/or iodine vapors. Stationary phases used for column chromatography were E. Merck silica gel (70–230 or 40–63 mesh). Melting points were uncorrected.

Spectral Procedures. The IR absorption spectra were obtained with a multiple internal reflectance cell (MIR, ZnSe) on neat 10–20 μg samples. Mass spectra were recorded on a JEOL SX-102A (electron impact, EI, 70 eV and fast atom bombardment, FAB) instrument. Exact mass measurements were performed at high resolution (HR-EI) using perfluorokerosene (PFK) as internal standard. Trimethylsilyl derivatives were prepared with a 1:1 mixture of BSTFA–pyridine at room temperature. The FAB spectrum was run in a matrix of dithiothreitol/dithioerythritol (20/80).

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions, and the chemical shifts are given relative to the solvent peaks at δ 5.32 and 53.8 ppm, respectively.

^1H–^1H COSY were recorded using the standard pulse sequence. The 2K–2K data set was accumulated in 512 increments with 16–32 transients, respectively, for each value of t_1 for full phase cycling. HMQC and HMBC experiments were performed using the pulse sequence of Bax *et al.*[14] The 1K × 4K data set was recorded for HMQC experiment on a Unity 400 MHz spectrometer employing Bird nulling of 0.300

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s, number of increments = 512, eight transients per increment, 0.9 s relaxation delay per transient, and J_{CH} = 140 Hz. The HMBC experiment was recorded using a similar experiment with 32 transients per increment, 2.5 s of relaxation delay, and $^{n}J_{CH}$ optimized for 7 Hz.

Producing Fungus and Its Fermentation. The producing fungus was isolated from the dung of pig (*Sus scrofa*) collected in Bajo Verde, province of Chaco, Argentina. In agar culture the fungus forms moderately fast-growing dematiaceous mycelium and sporulates by scattered minute, black ostiolate pycnida with doliform, phialidic conidiogenous cells. Conidia are hyaline, asepate ellipsoidal, 2–3 × 1–2 μm. The pycnidia are reminiscent of the coelomycetous form-genus *Phoma*, but it is possible that the pycnidia are the spermatial state of an ascomycete. *Phoma*-like pycnidial or spermatial states have been reported in some members of the Sporormiaceae which includes the coprophilous genera *Preussia* and *Sporormiella*.[16] Thus, the combination of *Phoma*-like pycnidia, dematiaceous hyphae, coprophilous habit, and production of preussomerins leads one to speculate that this fungus is a member of the Sporormiaceae. The isolate is maintained in the Merck Microbial Resources Culture Collection as MF5916.

A vegetative culture was prepared by inoculating a 54-mL portion of seed medium[16] in a 250-mL unbaffled Erlenmeyer flask with 2-mL of mycelia in 10% glycerol (MF5916) that had been stored at −80 °C. This seed culture was incubated for 3 days on a rotary shaker at 25 °C with 50% relative humidity. This and subsequent cultures were shaken at 220 rpm with a 5 cm throw in a room with continuous fluorescent light. Two-mL portions of the resulting culture were used to inoculate 50-mL portions of liquid production medium (consisting of mannitol, 75 g/L; oat flour, 15 g/L; yeast extract, 5 g/L; L-glutamic acid, 4 g/L; and MES, 16.2 g/L; pH was adjusted to 6.0 with NaOH before autoclaving) in 250 mL unbaffled Erlenmeyer flasks. These production flasks were incubated at 25 °C on a rotary shaker with 50% relative humidity for up to 21 days. The products appeared in the fermentation as early as 7 days with maximal accumulation observed between 14 and 21 days. The post sterile pH of the liquid production medium was 6.1 with a harvest pH at all time points of 6.5.

Isolation of Preussomerins and Deoxypreussomerins. Fermentation whole broth of MF5916 (1.3 L) was extracted with methyl ethyl ketone (2.0 L) by shaking for 60 min. The methyl ethyl ketone extract was concentrated under reduced pressure to a small volume, and residual water was finally removed by freeze-drying. The residue was dissolved in methanol–methylene chloride (3:1, 50 mL) and was chromatographed over a Sephadex LH-20 (1.0 L) column. Elution of the column yielded the active components (1.1 g) after 1.1–1.4 column volumes of methanol. This fraction was dissolved in boiling acetone (20 mL) and cooled to give granules which were collected by filtration to afford deoxypreussomerin A (**5**) (100 mg). The filtrate thus obtained was concentrated to dryness, dissolved in hot methanol (25 mL), and allowed to cool at room temperature for 2 h. Filtration gave 500 mg of fraction A, composed of a 80:20 mixture of preussomerin G (**1**) and deoxypreussomerin A (**5**), and filtrate (fraction B). Fraction A was chromatographed on a small silica gel column and was washed with hexane. Deoxypreussomerin A (15 mg) was eluted with 15% ethyl acetate in hexane and elution with 25% ethyl acetate gave preussomerin G (160 mg) as a yellow powder which was crystallized from methylene chloride–methanol to give yellow shining prisms.

A portion of fraction B was chromatographed over a Whatman C-18 (22 × 250 mm) HPLC column, eluted initially with 40% acetonitrile–water containing 0.2% trifluoroacetic acid for a period of 20 min at a flow rate of 10 mL per minute followed by a gradient to 70% acetonitrile over a period of 40 min. This column gave three active fractions: 30–41 (95.8 mg, fraction C), 42–49 (45 mg, preussomerin G), and 50–70 (38 mg, fraction D) minutes. Fraction C was rechromatographed on the same column and eluted for 20 min with 40% acetonitrile followed by a 40 min gradient to 50% acetonitrile. Fractions eluting between 22 and 24 min yielded 1.8 mg of preussomerin D (**4**) and those at 28–35 minutes afforded fraction E (10.7 mg). Preparative TLC of fraction E using hexane–ethyl acetate (10:3) as developing solvent and elution of bands with acetone furnished preussomerins H (4.1 mg, **2**) and I (5.0 mg, **3**).

Fraction D was chromatographed over a small silica gel flash column and eluted with 20% ethyl acetate–hexane to give a fraction which was rechromatographed on a preparative silica gel plate developed in hexane–ethyl acetate (10:3); elution of the bands with acetone gave 1 mg of deoxypreussomerin B (**6**) and additional amounts of deoxypreussomerin A (**5**).

Preussomerin G (1): mp 222–25 °C dec; $[\alpha]_D$ −688° (*c* 0.66; 25 °C, CH_2Cl_2); UV λ_{max} ($CHCl_3$) 356 (ε 3079), 271 (6630), 241 (9020) nm; IR (ZnSe) 2924, 1672, 1664, 1633, 1590, 1370, 1349, 1317, 1293, 1272, 1214, 1158, 1144, 1115, 1089, 1023, 974, 945, 930, 902, 887, 834, 800, 785, 758, 739, 706 cm^{-1}; HREIMS *m/z* 362.0400 (M^+, 100, calcd for $C_{20}H_{10}O_7$ 362.0426), 333.0416 (42, calcd for $C_{19}H_9O_6$ 333.0399, M − CHO), 289.0163 (17, calcd for $C_{17}H_5O_5$ 289.0137), 189.0169 (6, calcd for $C_{10}H_5O_4$ 189.0188), 174.0243 (15, calcd for $C_{10}H_6O_3$ 174.0317); ^1H NMR (CD_3CN) δ 3.93 (1H, d, *J* = 4.2 Hz, H-3), 4.38 (1H, d, *J* = 4.2 Hz, H-2), 6.61 (1H, d, *J* = 10.2 Hz, H-2′), 6.98 (1H, d, *J* = 9.3 Hz, H-8), 7.10 (2H, brd, *J* = 9.3 Hz, H-7 and H-7′), 7.36 (1H, d, *J* = 10.2 Hz, H-2′), 7.47 (1H, dd, *J* = 8.4, 7.8 Hz, H-8′), 7.59 (1H, dd, *J* = 7.8, 0.8 Hz, H-9′), 10.05 (1H, brs, OH-9); see Tables 1 and 2 for other ^1H NMR and ^{13}C NMR data.

Preussomerin H (2): $[\alpha]_D$ −371° (*c* 0.07; 25 °C, CH_2Cl_2); UV: λ_{max} ($CHCl_3$) 365 (ε 3467), 312 (3102), 252 (12 518) nm; IR (ZnSe) 2925, 1693, 1661, 1625, 1592, 1471, 1365, 1315, 1284, 1211, 1175, 1156, 1117, 1086, 1044, 1023, 1000, 972, 953, 930, 886, 832, 808, 781, 737, 705 cm^{-1}. HREIMS (*m/z*) 364.0582 (M^+, 100, calcd for $C_{20}H_{12}O_7$ 364.0583), 318.0446 (15, calcd for $C_{19}H_{10}O_5$ 318.0528), 159.0439 (10, calcd for $C_{10}H_7O_2$ 159.0446), 131.0487 (8, calcd for C_9H_7O 131.0497). For ^1H NMR see Table 1.

Preussomerin I (3): UV λ_{max} ($CHCl_3$) 370 (ε 2767), 325 (2174), 255 (9169) nm; IR (ZnSe) 2924, 1696, 1664, 1625, 1593, 1471, 1341, 1315, 1278, 1261, 1211, 1156, 1106, 1024, 986, 950, 929, 887, 832, 789, 738, 705 cm^{-1}; HREIMS *m/z* 394.0681 (M^+, 100, calcd for $C_{21}H_{14}O_8$ 394.0689), 379.0393 (6, calcd for $C_{20}H_{11}O_8$ 379.0454), 364.0609 (23, calcd for $C_{20}H_{12}O_7$ 364.0583), 279.0053 (11, calcd for $C_{19}H_3O_3$ 279.0082), 189.0485 (23, calcd for $C_{11}H_9O_3$ 189.0552). ^1H NMR data are listed in Table 1.

Deoxypreussomerin A (5): mp 235–36 °C (dec, shrinks at 200 °C); $[\alpha]_D$ −300° (*c* 0.21; 25 °C, CH_2Cl_2); UV λ_{max} ($CHCl_3$) 328 (ε 7933), 314 (7766), 300 (8433), 267 (9000), 239 (11 833) nm; IR (ZnSe) 2936, 1650, 1610, 1455, 1410, 1380, 1331, 1301, 1268, 1238, 1177, 1156, 1114, 1065, 1036, 1016, 969, 917, 874, 818, 806, 739, 720 cm^{-1}; HREIMS *m/z* 332.0683 (M^+, 100, calcd for $C_{20}H_{12}O_5$ 332.0684), 316.0737 (9, calcd for $C_{20}H_{12}O_4$ 316.0736), 303.0659 (11, calcd for $C_{19}H_{11}O_4$ 303.0659), 287.0700 (21, calcd for $C_{19}H_{11}O_3$ 287.0708), 173.0240 (9, calcd for $C_{10}H_5O_3$ 173.0240), 160.0497 (5, calcd for $C_{10}H_8O_2$ 160.0524). ^1H NMR data are listed in Table 1.

Deoxypreussomerin B (6): UV λ_{max} ($CHCl_3$) 328 (ε 4140), 314 (4554), 301 (4777), 255 (4458), 239 (6719) nm; IR (ZnSe) 2924, 1645, 1608, 1585, 1456, 1412, 1380, 1331, 1271, 1238, 1214, 1181, 1108, 1076, 1040, 1016, 994, 924, 888, 822, 806, 756 cm^{-1}; HREIMS *m/z* 318.0892 (M^+, 43, calcd for $C_{20}H_{14}O_4$ 318.0892), 301.0851 (14, calcd for $C_{20}H_{13}O_3$ 301.0865), 160.0497 (17, calcd for $C_{10}H_8O_2$ 160.0524), 159.0443 (73, calcd for $C_{10}H_7O_2$ 159.0446), 131.0493 (97, calcd for C_9H_7O 131.0497), 114.0184 (100, calcd for C_8H_2O 114.0106). For ^1H NMR refer to Table 1.

Acetylcysteine Adduct of Preussomerin G (7a). Preussomerin G (4.4 mg, 0.012 mmol) in CD_3CN (0.8 mL) was reacted with *N*-acetylcysteine (44 mg, 0.27 mmol) in a NMR tube, and the reaction was monitored over several days. Preussomerin G (90%) was converted to product (**7a**) after 72 h and almost completely after 15 days. When the reaction was performed using preussomerin G (1.81 mg, 0.005 mmol) in CD_3CN (0.8 mL) and *N*-acetylcysteine (0.005 mmol), the rate of Michael addition was at least three to four times faster. The adduct **7a** was purified from the first reaction by reversed phase HPLC using a Zorbax RX C-8 column (22 × 250 mm) and eluting at a flow rate of 10 mL/min with a gradient of

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40–60% of CH_3CN–H_2O (both containing 0.1% TFA) for 12 min followed by isocratic elution with 60% acetonitrile. The fractions eluted between 15 and 19 min were lyophilized to give a fluffy powder of adduct **7a** (3 mg): ^{1}H NMR (CD_3CN) δ 1.89 (3H, s, $COCH_3$), 2.98 (1H, dd, J = 18.4, 2.4 Hz, H-2′α), 3.17 (1H, dd, J = 13.6, 7.2 Hz, *Cys*: H-3), 3.31 (1H, dd, J =13.6, 4.8 Hz, *Cys*: H-3), 3.79 (1H, dd, J = 18.4, 5.2 Hz, H-2′ β), 3.95 (1H, d, J = 4.0 Hz, H-3), 4.17 (1H, dd, J = 4.8, 2.0 Hz, H-3′β), 4.51 (1H, d, J = 4.4 Hz, H-2), 4.64 (1H, brddd, J = 7.5, 7.5, 4.8 Hz, *Cys*: H-2), 6.81 (1H, brd, J = 9.0 Hz, *Cys*: NH), 7.0 (1H, d, J = 9.2 Hz, H-8), 7.16 (1H, dd, J = 8.0, 0.8 Hz, H-7′), 7.17 (1H, d, H = 9.2 Hz, H-7), 7.48 (1H, dd, J = 8.4, 7.8 Hz, H-8′), 7.59 (1H, dd, J = 8.0, 1.2 Hz, H-9′), 10.00 (1H, brs, OH-9); ESIMS m/z 526 (M + H).

Dithiothreitol Adduct of Preussomerin G (7b). To a solution of preussomerin G (3.62 mg, 0.01 mmol) in CD_3CN (1 mL) was added dithiothreitol (1.54 mg, 0.01 mmol), the solution was transferred to an NMR tube, and the progress of the reaction was monitored by ^{1}H NMR. Adduct **7b** was formed almost instantaneously. Acetonitrile was removed after 30 min, under reduced pressure, to give an amorphous powder of adduct **7b**.

Hydrogenation of Preussomerin G. To a solution of preussomerin G (10 mg) in ethyl acetate (3 mL) was added 10% Pd/C (10 mg), and the suspension was maintained under slightly positive pressure of hydrogen dispensed from a balloon. The hydrogenation was continued for a period of 20 h by which time all of the reactant was consumed and a final product (**9**) was formed which had an R_f value (SiO_2: hexane–ethyl acetate, 1/1) very close to that of preussomerin G but was fluorescent under 356 nm UV light. The catalyst was removed by filtration through a bed of Celite, and ethyl acetate was removed under reduced pressure to give a chromatographically homogeneous powder of **14**: ^{1}H NMR ($CDCl_3$) 2.07 (2H, m, H-2′ and H-3′), 2.46 (2H, m, H-2′ and H-3′), 2.80 (1H, m, H-1′), 2.99 (1H, dd, J = 18.4, 2.4 Hz, H-2), 3.05 (1H, m, H-1′), 3.43 (1H, dd, J = 18.4, 3.6 Hz, H-2), 4.62 (1H, dd, J = 3.2, 2.8 Hz, H-3), 6.63 (1H, dd, J = 8.0, 0.8 Hz, H-7′), 6.80 (1H, dd, J = 7.6, 1.2 Hz, H-9′), 6.90 (1H, d, J = 9.2 Hz, H-8), 7.02 (1H, d, J = 9.2 Hz, H-7), 7.15 (1H, dd, J = 8.0, 8.0 Hz, H-8′), 11.57 (1H, brs, OH-9); ^{13}C NMR ($CDCl_3$) 17.13 (C-2′), 26.33 (C-1′), 31.90 (C-3′), 41.13 (C-2), 70.32 (C-3), 93.21 (C-4), 95.15 (C-4′), 112.83 (C-10), 113.00 (C-7′), 117.61 (C-5), 118.60 (C-5′), 120.45 (C-8), 121.77 (C-9′), 126.01 (C-7), 130.11 (C-8′), 138.14 (C-10′), 143.56 (C-6), 149.02 (C-6′), 156.56 (C-9), 200.30 (C-1). HREIMS m/z 352.0938 (M^+, 73, calcd for $C_{20}H_{16}O_6$ 352.0947), 323.0903 (45, calcd for $C_{19}H_{15}O_5$ 323.0920), 190.0292 (29, calcd for $C_{10}H_6O_4$ 190.0266), 176.0482 (43, calcd for $C_{10}H_8O_3$ 176.0473), 174.0302 (38, calcd for $C_{10}H_6O_3$ 174.0317), 162.0374 (100, calcd for $C_9H_6O_3$ 162.0317), 160.0482 (38, calcd for $C_{10}H_8O_2$ 160.0524), 134.0369 (30, calcd for $C_8H_6O_2$ 134.0368).

Partial Hydrogenation of Preussomerin G (1). Hydrogenation reaction of preussomerin G (10 mg) in ethyl acetate (2 mL) using 10% Pd/C (10 mg) was repeated as described above. The reaction was stopped after 3 h, and the mixture was purified by preparative TLC (hexane–ethyl acetate (7:3)) to give compounds (listed in the decreasing R_f order) **11** (3.5 mg), **8**, (1.0 mg), and **12/13** (1:2, 2.2 mg) as amorphous solids.

A similar hydrogenation of preussomerin G (10 mg) in a mixture of ethyl acetate–methanol (1:1, 2 mL) after 3 h gave a mixture which upon purification by preparative TLC gave (in order of R_f) **14** (0.5 mg), **9** (0.1 mg), **8** (3.6 mg), **10** (0.2 mg), and **12/13** (1:4, 4.0 mg) as a powders. The mixture of **12/13** from both reactions was combined and was purified on a preparative reversed phase Zorbax C-8 RX HPLC column (22 × 250 mm). Elution with a 20%–30% gradient (30 min) of acetonitrile–water containing 0.1% trifluoroacetic acid at a flow rate of 10 mL per minute gave HPLC pure **12** and **13** as amorphous powders after lyophilization. **8:** ^{1}H NMR ($CDCl_3$) 2.83 (1H, dd, J = 17.2, 3.6 Hz, H-2), 3.12 (1H, dd, J = 17.6, 2.8 Hz, H-2), 4.55 (1H, dd, J = 3.2, 3.2 Hz, H-3), 6.85 (1H, d, J = 8.4 Hz, H-2′), 7.03 (1H, d, J = 8.0 Hz, H-3′), 7.04 (1H, dd, J = 7.6, 0.4 Hz, H-7′), 7.09 (1H, d, J = 9.2 Hz, H-8), 7.26 (1H, d, J = 9.2 Hz, H-7), 7.50 (1H, dd, J = 7.6, 8.4 Hz, H-8′), 7.53 (1H, s, OH), 7.83 (1H, dd, J = 8.4, 0.8 Hz, H-9′), 12.25 (1H, s, OH); EIMS m/z 364 (M^+, 60), 190 (50), 174 (100). **9:** ^{1}H NMR ($CDCl_3$) 3.05 (1H, dd, J = 18.4, 2.8 Hz, H-3), 3.45 (1H, dd, J = 18.8, 2.8 Hz, H-3), 4.72 (1H, brdd, J = 3.2, 3.2 Hz, H-2), 6.59 (1H, d, J = 10 Hz, H-2′), 6.95 (1H, d, J = 9.2 Hz, H-8), 6.99 (1H, s, OH), 7.04 (1H, d, J = 8.8 Hz, H-7), 7.06 (1H, dd, J = 8.0, 0.8 Hz, H-7′), 7.21 (1H, d, J = 9.6 Hz, H-3), 7.42 (1H, dd, J = 7.6, 8.4 Hz, H-8′), 7.64 (1H, dd, J = 7.2, 0.8 Hz, H-9′), 11.60 (1H, s, OH); EIMS m/z 364 (M^+, 90), 335 (70), 207 (20), 190 (20), 174 (60). **10:** ^{1}H NMR ($CDCl_3$) 3.04 (1H, dd, J = 18.8, 2.8 Hz, H-2), 3.13 (1H, dd, J = 18.4, 2.8 Hz, H-2′), 3.39 (1H, dd, J = 18.4, 3.2 Hz, H-2′), 3.46 (1H, dd, J = 18.4, 3.2 Hz, H-2), 3.49 (3H, s, OCH_3), 4.32 (1H, dd, J = 2.8, 2.8 Hz, H-3′), 4.78 (1H, dd, J = 3.2, 3.2 Hz, H-3), 6.95 (1H, d, J = 9.2 Hz, H-8), 7.04 (1H, d, J = 9.2 Hz, H-7), 7.09 (1H, dd, J = 8.0, 0.8 Hz, H-7′), 7.41 (1H, dd, J = 8.0, 8.0 Hz, H-8′), 7.67 (1H, dd, J = 7.6, 1.2 Hz, H-9′), 11.59 (1H, s, OH); EIMS m/z 396 (M^+, 70), 366 (20), 190 (20), 174 (20), 170 (20), 149 (100). **11:** ^{1}H NMR ($CDCl_3$) 2.49 (1H, ddd, J = 13.6, 13.6, 5.6 Hz, H-3′), 2.78 (1H, ddd, J = 13.2, 5.6, 1.6 Hz, H-3′), 2.90 (1H, ddd, J = 18.8, 5.6, 1.6 Hz, H-2′), 3.04 (1H, dd, J = 18.8, 2..4 Hz, H-2), 3.30 (1H, ddd, J = 18.8, 13.6, 5.6 Hz, H-2′), 3.45 (1H, dd, J = 18.4, 3.6 Hz, H-2), 4.69 (1H, dd, J = 2.8, 2.8 Hz, H-3), 6.94 (1H, d, J = 8.8 Hz, H-8), 7.04 (1H, d, J = 9.2 Hz, H-7), 7.08 (1H, dd, J = 8.4, 1.2 Hz, H-7′), 7.41 (1H, dd, J = 8.0, 8.0 Hz, H-8′), 7.65 (1H, dd, J = 7.6, 0.8 Hz, H-9′), 11.59 (1H, s, OH); EIMS m/z 366 (M^+, 100), 348 (10), 337 (30), 190 (10), 170 (40), 149 (25). **12:** ^{1}H NMR ($CDCl_3$) 2.12 (1H, m, H-2′), 2.46 (1H, m, H-3′), 2.50 (1H, m, H-3′), 2.60 (1H, m, H-2′), 3.01 (1H, dd, J = 18.3, 2.4 Hz, H-2), 3.43 (1H, dd, J = 18.6, 3.6 Hz, H-2), 4.63 (1H, dd, J = 2.7, 2.7 Hz, H-3), 4.88 (1H, dd, J = 6.9, 6.9 Hz, H-1′), 6.77 (1H, dd, J = 8.4, 1.2 Hz, H-7′), 6.92 (1H, d, J = 9.3 Hz, H-8), 7.04 (1H, d, J = 9.3 Hz, H-7), 7.16 (1H, brd, J = 7.8 Hz, H-9′), 7.30 (1H, dd, J = 7.8, 7.8 Hz, H-8′), 11.58 (1H, brs, OH-10); EIMS m/z 368 (M^+, 60), 350 (70), 318 (65), 170 (40), 160 (45), 149 (85). **13:** ^{1}H NMR ($CDCl_3$) 2.03 (1H, m, H-2′), 2.29 (1H, ddd, J = 13.4, 10.8, 6.6 Hz, H-3′), 2.46 (1H, ddd, J = 13.5, 6.0, 4.5 Hz, H-3′), 2.76 (1H, dddd, J = 14.1, 10.8, 5.7, 5.7 Hz, H-2′), 3.00 (1H, dd, J = 18.3, 2.7 Hz, H-2), 3.43 (1H, dd, J = 18.3, 3.3 Hz, H-2), 4.64 (1H, dd, J = 3.3, 2.7 Hz, H-3), 5.02 (1H, dd, J = 5.1, 5.1 Hz, H-1′), 6.77 (1H, brd, J = 7.5 Hz, H-7′), 6.90 (1H, d, J = 9.3 Hz, H-8), 7.00 (1H, d, J = 9.0 Hz, H-7), 7.13 (1H, brd, J = 7.5 Hz, H-9′), 7.30 (1H, dd, J = 7.8, 7.8 Hz, H-8′), 11.57 (1H, brs, OH-10); EIMS m/z 368 (M^+, 65), 350 (70), 318 (65), 170 (40), 160 (45), 149 (85).

X-ray Crystallography of Preussomerin G (1). Preussomerin G (**1**) $C_{20}H_{10}O_7$, M_r = 362.299, monoclinic, $P2_1$, a = 8.374(1) Å, b = 8.0696(7) Å, c = 11.814(1) Å, b = 99.66(1)°, V = 787.0(3) Å^3, Z = 2, D_x = 1.529 g cm^{-3}, monochromatized radiation λ(Cu Kα) = 1.541 838 Å, μ = 0.95 mm^{-1}, F(000) = 372, T = 294 K. Data were collected on a Rigaku AFC5 diffractometer to a θ limit of 71° with 1378 observed (with $I \geq 3\sigma(I)$ as the criterion for being observed) reflections out of 1697 measured. The structure was solved by direct methods (SHELXS-86)[17a] and refined using full-matrix least-squares on F using 244 parameters.[17b] All non-hydrogen atoms were refined with anisotropic thermal displacements. The final agreement statistics are as follows: R = 0.037, wR = 0.037, S = 2.23 with $(\Delta/\sigma)_{max}$ = 0.03. The least-squares weights were defined using $1/\sigma^2(F)$. The maximum peak height in a final difference Fourier map is 0.16(4) e Å^{-3}, and this peak is without chemical significance. The atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.

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Supplementary Material Available: Copies of ^{1}HNMR spectra of **1–5**, **7a**, and **8–14** (13 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(17) (a) Sheldrick, G. M. *Acta Crystallogr.* **1990**, *A46*, 467. (b) Structure Determination Package Version 3, Enraf-Nonius, Delft, 1985.