# Characterization of the Light- and Base-Mediated Instability of Calbistrin A

Wendy S. Horn, Kathleen K. Bierilo, Gerald F. Bills, Anne W. Dombrowski, Gregory L. Helms, E. Tracy Jones, David L. Linemeyer, David F. Sesin, Robert E. Schwartz, and Kenneth E. Wilson

*J. Nat. Prod.*, **1993**, 56 (10), 1779-1785• DOI:
10.1021/np50100a018 • Publication Date (Web): 01 July 2004

*Journal of Natural Products*
*Vol. 56, No. 10, pp. 1779-1785, October 1993*



# CHARACTERIZATION OF THE LIGHT- AND BASE-MEDIATED INSTABILITY OF CALBISTRIN A

WENDY S. HORN,* KATHLEEN K. BIERILO, GERALD F. BILLS, ANNE W. DOMBROWSKI, GREGORY L. HELMS, E. TRACY JONES, DAVID L. LINEMEYER, DAVID F. SESIN, ROBERT E. SCHWARTZ, and KENNETH E. WILSON

*Merck Research Laboratories, R80Y-340, PO Box 2000, Rahway, New Jersey 07065*

ABSTRACT.—The isolation and characterization of calbistrin A [**1**] were complicated by the compound's instability. The instability was caused by light, which led to a complex degradation of **1**. The nature of the light instability was elucidated by forming the dimethyl acetal derivative **2**, which isomerized upon exposure to light, leading to an equilibrium mixture of **2** and **3**. Alkaline conditions also caused decomposition of **1**, leading to the formation of **4**.

Three independent research teams appear to have isolated calbistrin A in approximately the same time frame (1–3). The antifungal agent L-702,285 [**1**] was isolated here from a solid fermentation of *Aspergillus versicolor* (ATCC 74035, MF2664) (4,5) and given the trivial name versilumen (1). Versilumen is now referred to as calbistrin A for the sake of clarity. The isolation and characterization of calbistrin A were complicated by the compound's instability. Initial isolation efforts were characterized by a low overall recovery of calbistrin A. It was discovered that the instability was caused by light, which led to a complex degradation of **1**. The isolation, structure elucidation, and biological characterization were performed in near darkness in order to maintain calbistrin A's integrity. The nature of the light instability was elucidated using the dimethyl acetal **2**, which isomerized upon exposure to light leading cleanly to an equilibrium mixture of **2** and **3**. Independent of the light-catalyzed instability, alkaline conditions also caused decomposition of **1**, leading to the formation of **4** (Figure 1).

Success in isolating as well as biologically and chemically characterizing calbistrin A depended upon minimizing the decomposition of the compound during manipulations. The influences of a number of experimental parameters were investigated in order to understand the origins of the instability. These parameters included temperature, light, pH, solvent, compound concentration, and oxygen. The results of these studies indicated that light and pH were the two major factors leading to compound degradation and that temperature, compound concentration, and the presence of oxygen did not contribute to the instability. Solvent effects were found to be dependent on the presence of light.

The key role played by light was evident when solutions of calbistrin A remained stable at room temperature when stored in the dark. This observation was independent of the solvent used, the concentration of compound and whether the solution was exposed to air. In contrast, the same solutions decomposed when exposed to ambient laboratory light. Radical scavengers such as BHA (0.2%), Tinuvin 144 and 622 LD, and Chimassorb 944 FL (200 μg/ml) did not ameliorate the effects of light. Reversed-phase hplc analysis indicated different light-catalyzed decomposition profiles depending upon whether a protic or an aprotic solvent was used (Figure 2). For example, exposure of a MeOH solution of calbistrin A to light resulted in the partial disappearance of the hplc peak due to calbistrin A and the concomitant appearance of two later-eluting peaks with the same uv chromophore as calbistrin A. Nmr data showed that each new peak was a mixture of at least two compounds (data not shown). In contrast, when aprotic solutions of calbistrin A were exposed to light the decomposition of calbistrin A was accompanied by the loss of the uv chromophores at 239 and 340 nm, with no significant new peak being detected by hplc. Although light clearly induced the decomposition of calbistrin

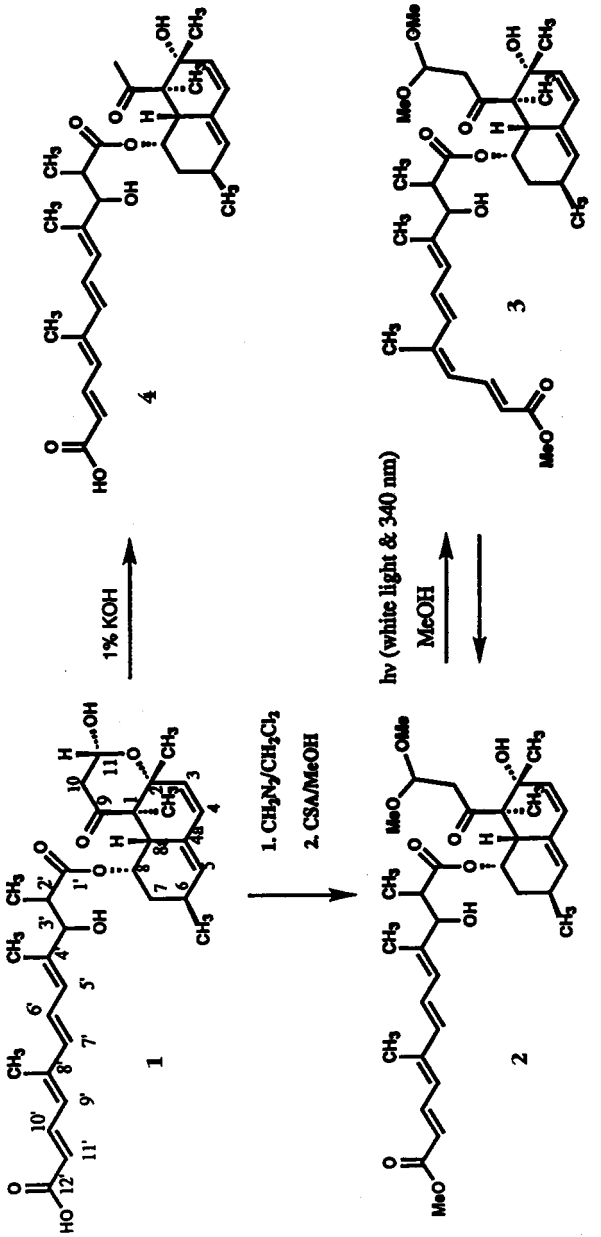


FIGURE 1. The conversion of calbistrin A [1] to its dimethyl acetal methyl ester **2**, and light-induced conversion of **2** to **3**. When calbistrin A is treated with 1% methanolic KOH, a 50% conversion to the base decomposition product **4** occurs.

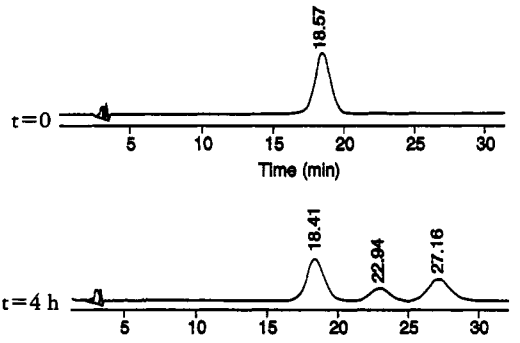


FIGURE 2. Hplc chromatograms showing the light decomposition of calbistrin A over time. The hplc trace at t=0 shows calbistrin A before it is exposed to light, while the trace at t=4 shows the effect of exposure to white light for 4 h when stored in a protic solvent (MeOH). Calbistrin A degrades completely in aprotic solvents, without concomitant appearance of other chromatographically similar compounds [Phenomenex ODS C20, MeCN-10 mM potassium phosphate buffer pH 7 (30:70), 1 ml/min, monitored at 340 nm].

A, the different decomposition profiles seen in protic versus aprotic solvent suggested that, in the presence of light, other factors were involved that contributed additional degradation pathways.

One of the factors that may have complicated the interpretation of the above-mentioned hplc profiles was the presence of the β-keto-aldehyde moiety, which could exist in solution as an equilibrium of two cyclic diastereomeric hemiacetals and several open forms. To eliminate this possibility, **1** was converted to the dimethylacetal methyl ester **2** (Figure 1) by treating the methyl ester of calbistrin A briefly with MeOH/camphorsulfonic acid. In contrast to the natural product **1**, the light-induced transformation of **2** was very clean. Exposure of MeOH solutions of **2** to ambient laboratory light over 14 h led to a 23:77 mixture of **2** and the geometric isomer **3** with excellent overall mass balance as judged by the quantitative recovery of absorbance of the tetraene uv

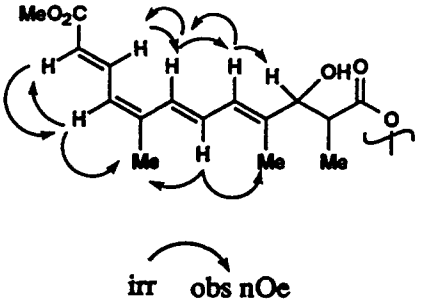


FIGURE 3. Observed nOe's in **3** suggested that the light catalyzed isomerization of the C-8′–C-9′ double bond in **2** caused the formation of **3** (Varian Unity 500 MHz nmr, $CD_2Cl_2$).

chromophore. The final 23:77 mixture reflected equilibrium conditions, since MeOH solution of **3** was transformed by light to the same 23:77 mixture. The clean, reversible light-induced interconversion of **2** and **3** was also observed in solutions of $CH_2Cl_2$. This is in direct contrast with $CH_2Cl_2$ solutions of **1**, in which decomposition was accompanied by the complete loss of the tetraene chromophore at 340 nm.

Mass spectral data indicated that **3** was isomeric with **2**, and uv data of **3** [ λ max (MeOH) 240 nm, 341 nm] showed that the diene and tetraene chromophores remained intact. The structure of **3** was determined based on $^1$H-nmr data. The chemical shifts of the protons about the bicyclic ring and the attached β-keto-acetal appendage of **3** were essentially identical to those of the corresponding protons of **2**. The only marked difference in proton chemical shifts between **2** and **3** was observed in the unsaturated fatty acyl residue at C-8. Specifically, the H-7′ was observed at 6.35 ppm in **2** but was downfield-shifted to 6.96 ppm in **3**. For all other protons of the unsaturated fatty acyl side chain, the corresponding chemical shifts were very similar ($<\pm 0.15$ ppm).

The data suggested that **3** arose from the isomerization of the trisubstituted double bond C-8′, C-9′ of **2**. This conclusion was verified by the observed nOe effects shown in Figure 3. This isomerization suggests that the compound referred to as calbistrin B (2, 6, 7) may be an artifact of conducting the isolation of **1** in ambient light.

Alkaline pH was another factor that caused decomposition of **1**. Calbistrin A was buffered at pH 3, 5, 7, 9, and 11 as a 50% aqueous MeOH solution. It was unstable when buffered at pH 9 and pH 11, partially decomposing (ca. 40%) within 5 h at pH 9 and pH 11, without any further decomposition observed. Calbistrin A was exposed to harsher conditions in order to obtain as much of the decomposition product as possible for structure elucidation. Exposure to 1% KOH in MeOH for 24 h resulted in a 50% conversion to the new degradation product, which was subsequently purified by preparative hplc (Figure 4). The structure of this base conversion product was deter-

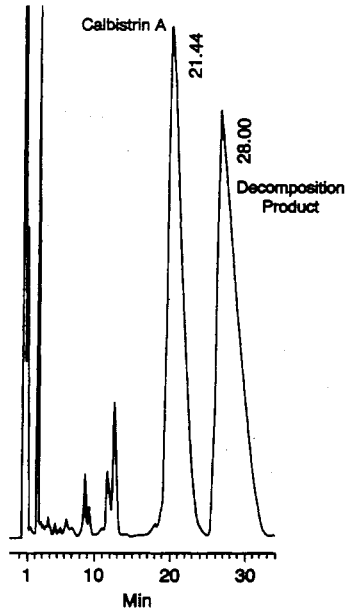


FIGURE 4. Calbistrin A formed one major base decomposition product in 1% methanolic KOH. (Phenomenex ODS C30 column, MeCN/10 mM potassium phosphate buffer pH 7, 4 ml/min, monitored at 340 nm).

mined by nmr and mass spectral studies to be **4**, the methyl ketone apparently arising from a retro-aldol condensation.

## EXPERIMENTAL

FERMENTATION.—*A. versicolor* (ATCC 74035) was of unknown origin and was identified by its morphological features which conformed well with those of Domsch *et al.* (8) and Raper and Fennell (9). Frozen vegetative mycelia of *A. versicolor* were used to inoculate flasks of seed medium, which consisted of yeast extract 0.4%, malt extract 1%, and dextrose 0.4%. The seed medium was prepared with distilled $H_2O$, adjusted to pH 7, dispensed into 250-ml Erlenmeyer flasks (54 ml each), and sterilized at 121°, 1.05 kg/cm$^2$ for 20 min. Culture development began by inoculating 1 ml from an FVM into 54 ml of seed medium and incubating for 4 days at 25°, 220 rpm. Sterile ceramic balls and cylinders were added to the flask, and the flask was shaken at 150 rpm for 2 h to obtain a more homogeneous seed for inoculation. Prior to inoculation the liquid phase of the production medium was dispensed at 425 ml per liter Erlenmeyer flask and sterilized at 121°, 1.05 kg/cm$^2$ for 15 min. The production medium consisted of dextrose 15.0%, urea 0.4%, NZ amine type A 0.4%, $K_2HPO_4$ 0.05%, $MgSO_4 \cdot 7H_2O$ 0.025%, KCl 0.025%, $ZnSO_4 \cdot 7H_2O$ 0.9%, and $CaCO_3$ 1.65% in distilled $H_2O$. Eighteen ml of the seed culture described above was transferred aseptically into the liquid phase of the production medium. This inoculated medium was added to a 4-liter roller jar containing 1250 ml of vermiculite which had been sterilized for 60 min at 121°, 1.05 kg/cm$^2$ prior to inoculation. The inoculated roller jars were mixed vigorously to coat the vermiculite. The roller jar production vessels were incubated on a roller machine at 25° for 13 days.

GENERAL METHODS.—All isolation, structure elucidation, and chemical manipulations were carried out in a darkened environment except where otherwise indicated. Uv spectra were recorded using a Beckman DU-70 spectrophotometer. Ir spectra were recorded as neat sample on a ZnSe multiple internal reflectance crystal using a Perkin-Elmer 1750 FT-ir spectrophotometer. Mass spectral data were acquired on Finnigan-MAT212 (ei mode at 90 eV) and TSQ70 spectrometers. Exact mass measurements were taken using hreims. $^1$H- and $^{13}$C-nmr spectra were acquired in $CD_3CN$-$CD_2Cl_2$ (15:85) or $CD_2Cl_2$ using a Varian Unity 500 MHz spectrometer or in $CD_2Cl_2$ using a Varian XL 300 MHz spectrometer. Hplc analyses were done using a Spectraphysics 8700 pump, Spectraphysics 8780 autoinjector, an LKB 2151 UV detector equipped with a 10 mm pathlength cell, monitoring at 340 nm, and a Spectraphysics 4200 integrator. Hplc conditions included a Phenomenex ODS C20 (4.6 mm i.d.×25 cm) column maintained at 40°, an eluting solvent of MeCN-10 mM potassium phosphate buffer, pH 7 (30:70), a flow rate of 1 ml/min and the column effluent monitored at 340 nm. Under these conditions **1** and **4** eluted with retention times of 20.0 min and 24.1 min, respectively. Hplc analyses to support the preparation and studies of **2** and **3** used a Rainin Dynamax C8 column (4.6 mm i.d.×25 cm) at ambient temperature and an eluting solvent of MeCN-20 mM phosphoric acid buffer pH 2.45 (80:20). Under these conditions the retention times of **2** and **3** were 6.5 min and 7.1 min, respectively.

ISOLATION AND CHARACTERIZATION OF CALBISTRIN A [**1**].—Fourteen roller jars containing a solid fermentation were extracted sequentially using a roller machine for mixing, first for 6 h with 10 liters of EtOAc and again overnight, with 8 liters of EtOAc. The extract was then vacuum-filtered through Whatman #1 filter paper. A 13-liter portion of the EtOAc extract was concentrated to dryness and reconstituted in 500 ml of MeOH-$H_2O$ (90:10). This 90% MeOH solution was partitioned with an equal volume of hexane-isopropyl acetate (6:4). The aqueous MeOH lower layer was concentrated, in vacuo, yielding 6 g of solids. These solids were reconstituted in $CH_2Cl_2$ containing 1% HOAc and applied to a silica column (1.5 liters of Si gel 60) packed in $CH_2Cl_2$/1% HOAc. The column was eluted using a $CH_2Cl_2$-to-EtOAc step gradient which contained 1% HOAc. Calbistrin A eluted in the EtOAc-$CH_2Cl_2$-1% HOAc (1:1:0.01) eluate. Fractions were combined based on hplc and concentrated to yield 770 mg of an amorphous yellow solid. This solid (770 mg) was dissolved in MeOH (200 mg/ml), and 100-mg samples were chromatographed sequentially on a preparative hplc column [Phenomenex ODS 30, 225 mm i.d.×25 cm, 10 ml/min, MeCN-1 mM potassium phosphate buffer pH 7 (30:70)]. Fractions eluting between 24 and 30 min were combined and partitioned with an equal volume of $CH_2Cl_2$ containing 1% HOAc.

Concentration of the $CH_2Cl_2$ layer yielded 303 mg of calbistrin A [**1**]: $^1$H nmr [500 MHz, $CD_3CN$-$CD_2Cl_2$ (15:85)] δ 8.89 (br s, COO*H*), 7.62 (dd, *J*=15.0, 12.0 Hz, 1H), 6.72 (dd, *J*=15.2, 11.2 Hz, 1H), 6.35 (d, *J*=15.4 Hz, 1H), 6.26 (d, *J*=11.8 Hz, 1H), 6.10 (d, *J*=11.0 Hz, 1H), 5.97 (d, *J*=10 Hz, 1H), 5.96 (br s, 1H), 5.86 (d, *J*=15 Hz, 1H), 5.71 (br s, 1H), 5.60 (d, *J*=9.8 Hz, 1H), 5.15 (dt, *J*=7.7, 3.7 Hz, 1H), 4.54 (d, *J*=7.3 Hz, O*H*), 4.03 (d, *J*=8.4 Hz, 1H), 3.16 (br s, 1H), 2.85 (br q, obs, *J*=2.9 Hz, 1H), 2.71 (dd, *J*=8.4 Hz, 14, 1H), 2.48 (dq, *J*=7.3 Hz, 9.0, 1H), 2.45 (br s, 1H), 2.36 (dd, *J*=14.1 Hz, 4.0, 1H), 2.09 (ddt, *J*=14.0, 4.5 Hz, 1.0, 1H), 2.01 (br s, 3H), 1.72 (br s, 3H), 1.32 (dd, *J*=1.2, 10.2, 14 Hz, 1H), 1.25 (s, 3H), 1.18 (s, 3H), 1.00 (d, *J*=7.2 Hz, 3H), 0.88 (d, *J*=7.2 Hz, 3H); $^{13}$C nmr [125 MHz, $CD_3CN$-$CD_2Cl_2$ (15:85)] δ 209.91, 175.42, 168.02, 145.24, 141.57, 141.30, 137.18, 136.22, 131.49,

131.10, 129.26, 129.17, 128.62, 128.47, 120.89, 92.06, 80.16, 78.01, 70.22, 54.17, 47.80, 44.93, 40.43, 35.77, 27.58, 23.34, 21.08, 18.06, 14.75, 13.23, 11.91; λ max (MeOH) 239 nm (ε=17,820) 340 nm (MeOH, ε=42,822); hreims *m/z* $[M-H_2O]^+$ 522.2623 (calcd 522.2618).

DIMETHYL ACETAL **2**.—All operations were carried out in a darkened environment. A $CH_2Cl_2$ solution of **1** was placed on ice, and 200 μl of freshly distilled $CH_2N_2$ was added. The mixture was left standing for 5 min and then concentrated to dryness under vacuum. Calbistrin A methyl ester (6 mg, 0.108 mmol) was treated with 4 ml of 0.1% MeOH camphorsulfonic acid at room temperature for 4 h. The reaction was diluted with 10 ml of $H_2O$ and extracted with EtOAc. The extract was washed twice with 20 mM sodium phosphate buffer (pH 6.3) and once with $H_2O$. After drying over anhydrous $Na_2SO_2$, the extract was evaporated to dryness. The residue was chromatographed on 1 ml Si gel in hexane-EtOAc (1:1), affording **2** as a colorless oil (3.3 mg, 51% yield): $^1$H nmr (300 MHz, $CD_2Cl_2$) δ 7.68 (dd, *J*=15.0, 11.9 Hz, 1H), 6.68 (dd, *J*=15.1, 11.0 Hz, 1H), 6.35 (d, *J*=15.2 Hz, 1H), 6.24 (d, *J*=11.9, Hz, 1H), 6.147 (dd, *J*=11.0 Hz, 1.3 Hz, 1H), 5.94 (d, *J*=10.3 Hz, 1H), 5.90 (d, *J*=15.7 Hz, 1H), 5.64 (br s, 1H), 5.39 (d, *J*=9.7 Hz, 1H), 5.38 (m, obs, 1H), 4.86 (dd, *J*=7.8, 3.6 Hz, 1H), 4.13 (br d, *J*=9.2 Hz, 1H), 3.72 (s, 3H), 3.35 (s, 3H), 3.32 (s, 3H), 3.18 (dd, *J*=16.2, 7.9 Hz, 1H), 3.12 (m, 1H), 2.56 (dd, *J*=9.1, 7.2 Hz, 1H), 2.50 (dd, *J*=16.1, 3.5 Hz, 1H), 2.40 (br m, 1H), 2.04 (d, *J*=1.2 Hz, 3H), 2.04 (m, obs, 1H), 1.78 (d, *J*=1.3 Hz, 3H), 1.32 (s, 3H), ca. 1.28 (m, 1H), 1.17 (s, 3H), 1.02 (d, *J*=7.1 Hz, 3H), 0.97 (d, *J*=7.2 Hz, 3H); ir (film) 3476 (br, m), 1713 (s), 1698 (s), 1614 (m), 1584 (m) $cm^{-1}$; uv (MeOH) 346 (ε=43,000), 239 (ε=19,900) nm; hreims *m/z* $[M]^+$ 600 (weak), $[M-H_2O]^+$ 582, $[M-MeOH]^+$ 568.3036 (568.3036 calcd for $C_{33}H_{44}O_8$.

PHOTOLYSIS PRODUCT **3**.—A MeOH solution of **2** (1.5 mg, 0.0025 mmol) was exposed to ambient laboratory light and temperature. Isomerization of **2** was monitored by hplc as described above. Isomerization proceeded over 14 h to an equilibrium mixture of **2** and **3** of approximately 23:77. The following operations were carried out in a darkened environment. The MeOH solution of **2** and **3** after 7 h exposure to light was evaporated to dryness, reconstituted in 0.2 ml of MeCN-$H_2O$ (6:4), and chromatographed on a Rainin Dynamax C8 column (25 cm×4.6 mm) in MeCN-20 mM sodium phosphate buffer, pH 2.45 (70:30) at 1 ml/min. Fractions containing **3** were combined, diluted with 2 volumes of $H_2O$, extracted with EtOAc, and dried over anhydrous $Na_2SO_4$, affording 0.82 mg of **3** as a colorless oil. From the uv spectrum of **3** it was determined that $\epsilon_{341}$=28,700, based on the assumption that $\epsilon_{240}$ for **3** was the same as that for **2** since the diene chromophores of **2** and **3** are identical. The hplc relative response of **3** versus **2** at 340 nm was then determined to be 28,700/43,000. $^1$H nmr (300 MHz, $CD_2Cl_2$) δ 7.81 (dd, *J*=15.2 Hz, 12.1 Hz, 1H), 6.96 (d, *J*=15.0 Hz, 1H), 6.66 (dd, *J*=15.1, 11.1 Hz, 1H), 6.21 (d, *J*=10.8 Hz, 1H), 6.12 (d, *J*=11.4 Hz, 1H), 5.95 (d, *J*=9.6 Hz, 1H), 5.83 (d, *J*=15.2 Hz, 1H), 5.65 (br s, 1H), 5.39 (d, *J*=9.4 Hz, 1H), 5.39 (m, obs, 1H), 4.87 (dd, *J*=7.8, 3.5 Hz, 1H), 4.15 (dd, *J*=9.0, 4.2 Hz, 1H), 3.72 (s, 3H), 3.35 (s, 3H), 3.32 (s, 3H), 3.18 (dd, *J*=16.1, 7.8 Hz, 1H), 3.13 (m, 1H), 2.57 (dd, *J*=8.9, 7.0 Hz, 1H), 2.51 (dd, *J*=16.3, 3.7 Hz, 1H), 2.40 (br m, 1H), 2.03 (m, obs, 1H), 2.00 (s, 3H), 1.79 (d, *J*=1.2 Hz, 3H), 1.33 (s, 3H), 1.28 (m, obs, 1H), 1.17 (s, 3H), 1.03 (d, *J*=7.2 Hz, 3H), 0.98 (d, *J*=7.2 Hz, 3H); uv (MeOH) 341 (ε=28,700), 240 (ε=19,900) nm; ir (film) 3464 (m), 1715 (s), 1698 (s), 1610 (m) $cm^{-1}$; hreims *m/z* $[M]^+$ 600 (weak), $[M-MeOH]^+$ 568.3041 (568.3036 calcd for $C_{33}H_{44}O_8$).

BASE DECOMPOSITION PRODUCT **4**.—The base decompositon product was purified by acidification, subsequent partitioning into EtOAc, and finally preparative hplc (Phenomenex ODS C30 column, MeCN/10 mM potassium phosphate buffer pH 7, 4 ml/min, monitored at 340 nm). $^1$H nmr (500 MHz, $CD_2Cl_2$) δ 7.75 (d, *J*=12 Hz, 1H), 6.71 (dd, *J*=15.1, 11.3 Hz, 1H), 6.37 (d, *J*=15.1 Hz, 1H), 6.27 (d, *J*=12.0, 1H), 6.17 (br d, *J*=11.3 Hz, 1H), 5.98 (d, *J*=9.6, 1H), 5.90 (d, *J*=15.1, 1H), 5.65 (br s, 1H), 5.42 (br t, *J*=3, 1H), 5.37 (d, *J*=9.6 Hz, 1H), 4.15 (d, *J*=8.7, 1H), 2.83 (br dt, *J*=3.1, 2.6 Hz, 1H), 2.59 (dq, *J*=8.7, 7.2 Hz, 1H), 2.40 (br m, 1H), 2.20 (s, 3H), 2.07 (m, 1H), 2.06 (s, 3H), 1.80 (d, *J*=0.9 Hz, 3H), 1.32 (s, 3H), 1.27 (m, 1H), 1.16 (s, 3H), 1.02 (d, *J*=7.1, Hz, 3H), 1.01 (d, *J*=7.2 Hz, 3H); λ max (MeOH) 239 nm (ε=13,897) 342 nm (ε=27,494). For peak matching the methyl ester of **4** was prepared by reacting the sample in MeOH with trimethyl silyl $CH_2N_2$. Hreims **4** methyl ester, *m/z* 526.2930 (526.2931 calcd for $C_{31}H_{42}O_7$). By difference the empirical formula of the underivatized acid is $C_{30}H_{40}O_7$.

## LITERATURE CITED

1. W. Horn, G. Bills, A. Dombrowski, G. Helms, E.T. Jones, M. Kurtz, D. Linemeyer, J. Onishi, W. Rozdilsky, and R. Schwartz, *Abstracts of Papers*, 33rd Annual Meeting of the Am. Soc. Pharmacognosy: Williamsburg, Virgina, 1992; Abstract O-25.
2. G.M. Brill, R.H. Chen, R.R. Rasmussen, D.N. Whittern, and J.B. McAlpine, *J. Antibiot.*, **46**, 39 (1993).
3. H. Agematu, Y. Watanabe, H. Chiba, R. Kaneto, N. Shibamoto, T. Yoshioka, T. Kumamoto, H. Nishida, and R. Okamoto, Patent no. EP514884-A1.

4. G.L. Helms, W.S. Horn, E.T. Jones, and D.L. Linemeyer, United States Patent no. 5,182,298.
5. D.L. Linemeyer, W.S. Horn, A.W. Dombrowski, E.T. Turner Jones, L. Koupal, K.F. Bartizal, and W. Rozdilsky, Patent no. EP0505135A2.
6. M. Jackson, J.P. Karwowski, P.E. Humphrey, W.L. Kohl, G.J. Barlow, and S.K. Tanaka, in: "Abstracts." ICAAC 32nd annual conference, October 11–14, 1992, Abstract #499.
7. G.M. Brill, R.H. Chen, J.B. McAlpine, R.R. Rasmussen, and D.N. Whittern, in: "Abstracts." ICAAC, 32nd annual conference, October 11–14, 1992, Abstract #500.
8. K.H. Domsch, W. Gams, and T. Anderson, in: "Compendium of Soil Fungi." Ed. by K.H. Domsch, Academic Press, NY, 1980, Vol. 1, pp. 94–98.
9. K.B. Raper and D.I. Fennell, "The Genus Aspergillus," Robert E. Krieger Publishing Company, Malabar, Florida, 1965.

*Received 22 March 1993*