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Studies on Fungal Products. VII.[1] The Structures of Meleagrin and 9-*O*-*p*-Bromobenzoylmeleagrin

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The structure of meleagrin (**1**), isolated from *Penicillium meleagrinum*, was determined by X-ray crystallographic analysis of 9-*O*-*p*-bromobenzoylmeleagrin (**7**) monohydrate. The crystal structure of **7** monohydrate was determined by the heavy atom method; the final *R* value without hydrogen atoms was 0.094. The structure of meleagrin was established as the 9-*O*-demethyl compound of oxaline (**4**), isolated from *Penicillium oxalicum*. The absolute configurations at N_1, C_2, and C_3 are *S*, *S*, and *R*, respectively.

Keywords——meleagrin; *p*-bromobenzoylmeleagrin; oxaline; *Penicillium meleagrinum*; X-ray analysis

In the course of screening *Penicillium* species for antimicrobial metabolites, meleagrin (**1**), $C_{23}H_{23}N_5O_4$, was isolated as a main component from the culture filtrate of *Penicillium meleagrinum* BIORGE IFO 8143.[1] It was clarified that meleagrin showed structural similarity with the tremorgenic mycotoxins formed from two amino acids and one isoprenyl moiety, but its structure could not be established. In this paper, we determined the structure of meleagrin as **1**.

On acetylation, **1** afforded a monoacetate (**2**), $C_{25}H_{25}N_5O_5$, which showed a proton nuclear magnetic resonance (^{1}H-NMR) signal at δ 2.27 (3H, s) assigned to the methyl protons of an acetoxyl group. The ^{1}H-NMR signal of one of the olefinic protons at δ 5.50 (s) in **1** shifted to δ 5.97 (s) after acetylation. This suggested the presence of an enol group in **1**. The signal of methoxy protons appeared at δ 3.73 (s) in **1**. Thus two of the four oxygen functions in **1** have been assigned to one hydroxyl and one methoxyl. The remaining two oxygens must be the carbonyls of amide groups in view of the signals at δ 159.9 and 166.7 in the carbon 13 nuclear magnetic resonance (^{13}C-NMR) spectrum. On methylation with diazomethane, **1** gave a monomethyl derivative (**3**), $C_{24}H_{25}N_5O_4$, which showed a ^{1}H-NMR signal at δ 2.45 (s) assigned to methyl protons of an *N*-methyl group. No other significant changes were observed at **3** compared with **1**. The vinyl proton signals at δ 5.06 (1H, d, J=10.6 Hz), 5.10 (1H, d, J=17.2 Hz) and 6.12 (1H, dd, J=17.2 and 10.6 Hz) disappeared on hydrogenation of **1** to the dihydro compound (**5**), in which ethyl group signals appeared at δ 0.88 (3H, t, J=9 Hz) and 1.28 (2H, q-like). At the same time, two methyl signals at δ 1.24 and 1.35 in **1** shifted to higher field (δ 1.12). The presence of the vinyl group was confirmed by a decoupling experiment with irradiation at δ 6.12. The doublets at δ 5.06 and 5.10 changed to singlets. In addition to the above evidence, the base peak at *m/z* 364 in the mass spectrum of **1** attributable to the loss of a C_5H_9 radical suggested the presence of a 1,1-dimethylallyl group in the molecule of **1**. On hydrogenation with zinc and acetic acid, **1** afforded another dihydro compound (**6**), which yielded histidine on subsequent acid hydrolysis. The above result suggested the presence of a dehydrohistidine moiety in **1**, and therefore the remaining nitrogen atoms were considered to

TABLE I. Crystal Data for **7** Monohydrate

Monoclinic	$C_{30}H_{26}BrN_5O_5 \cdot H_2O$
Space group $C2$	$F_W = 634.49$
$a = 37.825\ (20)$ Å	$F(000) = 1404$
$b = 7.833\ (4)$ Å	$Z = 4$
$c = 11.086\ (6)$ Å	$\mu(CuK\alpha) = 21.7\,cm^{-1}$
$\beta = 104.72\ (5)^\circ$	$D_c = 1.326\,g \cdot cm^{-3}$
$V = 3177\ (5)$ Å^3	$D_m = 1.330\,g \cdot cm^{-1}$

TABLE II. Final Atomic Parameters ($\times 10^4$, for Br $\times 10^5$) and Equivalent Thermal Parameters, with Estimated Standard Deviations in Parentheses

	x	y	z	$B_{eq.}$ (Å^2)
BR	6987 (7)	0 (0)	105419 (21)	7.71 (.05)
N1	−1558 (3)	5978 (19)	4131 (12)	3.8 (0.2)
C2	−1412 (4)	7664 (24)	4656 (14)	3.6 (0.3)
C3	−1070 (4)	7940 (28)	4111 (16)	4.3 (0.3)
C3a	−1208 (5)	7030 (29)	2854 (15)	4.6 (0.3)
C4	−1058 (6)	7016 (34)	1814 (18)	6.4 (0.4)
C5	−1222 (6)	5929 (38)	849 (19)	7.8 (0.5)
C6	−1517 (6)	4920 (47)	879 (18)	8.6 (0.5)
C7	−1669 (5)	4827 (36)	1938 (16)	6.4 (0.4)
C7a	−1497 (5)	6001 (30)	2912 (17)	5.6 (0.4)
C8	−767 (5)	6778 (25)	4855 (17)	4.6 (0.3)
C9	−763 (4)	6178 (23)	5982 (16)	3.9 (0.3)
C10	−1030 (4)	6659 (23)	6671 (15)	3.5 (0.3)
N11	−1324 (3)	7583 (19)	6037 (11)	3.6 (0.2)
C12	−1585 (4)	8516 (22)	6527 (14)	3.2 (0.2)
C13	−1822 (4)	9430 (24)	5425 (13)	3.8 (0.3)
N14	−1681 (3)	8999 (18)	4419 (12)	3.5 (0.2)
C15	−1589 (5)	8574 (26)	7743 (15)	4.4 (0.3)
C16	−1835 (5)	9480 (26)	8295 (15)	4.9 (0.3)
N17	−2151 (4)	10374 (25)	7750 (12)	5.2 (0.3)
C18	−2301 (6)	10893 (33)	8592 (17)	6.3 (0.4)
N19	−2100 (5)	10319 (31)	9741 (14)	7.2 (0.4)
C20	−1803 (5)	9447 (32)	9564 (16)	5.9 (0.4)
C21	−923 (5)	9887 (31)	4166 (16)	5.0 (0.3)
C22	−902 (5)	10612 (24)	5476 (19)	5.5 (0.4)
C23	−1048 (6)	11974 (29)	5729 (23)	6.8 (0.5)
C24	−1174 (6)	10912 (31)	3082 (19)	6.3 (0.4)
C25	−508 (5)	9909 (40)	4043 (21)	7.4 (0.5)
C26	−1952 (6)	4268 (33)	4806 (23)	7.6 (0.5)
C27	−194 (5)	5682 (25)	7398 (17)	4.7 (0.3)
C28	31 (4)	4278 (24)	8095 (14)	3.5 (0.3)
C29	311 (4)	4773 (34)	9118 (15)	5.2 (0.3)
C30	509 (5)	3496 (31)	9830 (16)	5.2 (0.4)
C31	425 (5)	1776 (28)	9502 (16)	5.0 (0.4)
C32	153 (4)	1276 (27)	8496 (15)	4.3 (0.3)
C33	−37 (5)	2610 (25)	7786 (15)	4.1 (0.3)
O1	−1921 (3)	5782 (17)	4121 (11)	4.7 (0.2)
O2	−499 (3)	5039 (19)	6596 (9)	4.2 (0.2)
O3	−967 (3)	6336 (18)	7813 (10)	4.9 (0.2)
O4	−2072 (3)	10332 (18)	5393 (9)	4.3 (0.2)
O5	−129 (4)	7190 (19)	7458 (14)	6.6 (0.3)
Ow	−2203 (6)	10296 (34)	2180 (11)	13.1 (0.5)

be those of another amino acid moiety in the molecule. The ^{1}H-NMR signals of aromatic protons in **1** appeared at δ 6.96 (br d, J=7.5 Hz), 7.07 (br t, J=7.5 Hz), 7.30 (br t, J=7.5 Hz) and 7.58 (br d, J=7.5 Hz). This result suggests that the tryptophan moiety of **1** has no substituent on the aromatic ring. Hence the methoxyl group must be on the nitrogen (N_1).

Meleagrin (**1**) is closely similar in all of the spectroscopic data, except for the absence of one (*O*)-methyl, to oxaline (**4**), which was recently isolated from *Penicillium oxalicum*.[3,4] From a comparison of the ^{1}H-NMR spectra of **1** and **4**, meleagrin was suggested to be represented by the chemical formula **1**, which is 9-*O*-demethyloxaline. We tried to obtain oxaline (**4**) by methylation of **1**, but only N_{14}-methylmeleagrin (**3**) was obtained. In order to determine the exact structure of **1** including the absolute stereochemistry, an X-ray structure analysis of 9-*O*-*p*-bromobenzoylmeleagrin (**7**) monohydrate was undertaken.

Crystals of **7** monohydrate were grown in chloroform–ethanol solution as pale yellow prisms. The size of the X-ray specimen was about 0.3 × 0.07 × 0.15 mm. The lattice constants and intensity data were collected on a Philips PW 1100 diffractometer using Cu$K\alpha$ radiation monochromated by a graphite plate. The crystal data are summarized in Table I. A total of 2455 reflections were measured in a 2θ range of 6—130° as above the 2σ (I) level; 296 of these were Friedel reflections. Intensities were corrected for Lorenz and polarization factors, but no absorption correction was applied.

The crystal structure was solved by the heavy atom method, and the refinement was carried out by the block-diagonal least-squares method using HBLS IV.[5] To determine the absolute configuration, the crystal structure was first refined with anisotropic thermal parameters to an R value of 9.5% excluding hydrogen atoms, and then the anomalous dispersion corrections were applied to the bromine atoms. A comparison between the observed and calculated amplitude ratios of $|F(hkl)|/|F(\bar{h}\bar{k}\bar{l})|$ for 296 Friedel pairs indicated that 88 out of 96 pairs for which the observed and calculated ratios differed by more than 5% from unity showed the same absolute configuration. The final atomic parameters are listed in Table II.[6]

The absolute structure of 9-*O*-*p*-bromobenzoylmeleagrin was determined to be as shown

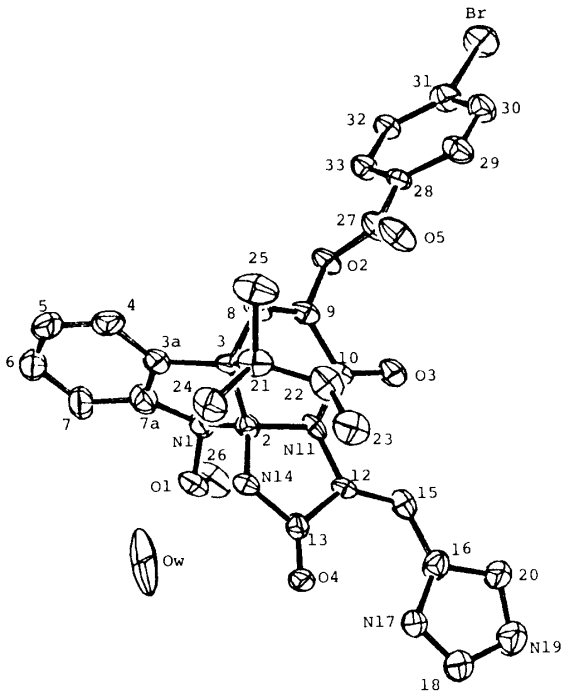
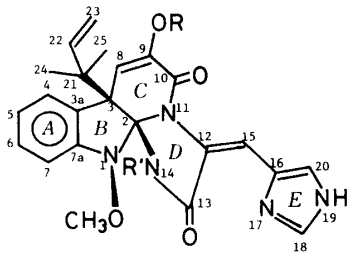


Fig. 1. The Molecular Structure of 9-*O*-*p*-Bromobenzoylmeleagrin (**7**) monohydrate



1: R=R′=H
2: R=CH_3CO, R′=H
3: R=H, R′=CH_3
4: R=CH_3, R′=H
5: R=R′=H, 22,23-dihydro
6: R=R′=H, 12,15-dihydro
7: R=*p*-BrC_6H_4CO, R′=H

Chart 1

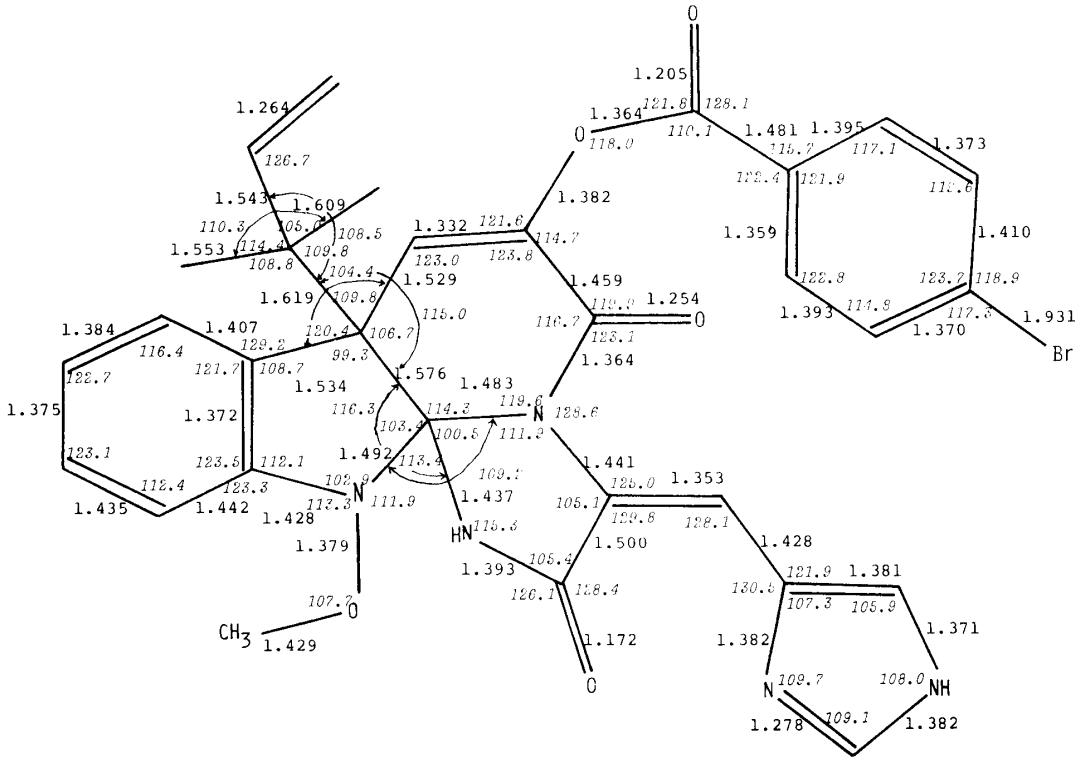


Fig. 2. The Bond Lengths (Å) and Angles ( )

The mean standard deviations of the bonds connecting C, N and O are 0.026 Å and 1.6 .

in Fig. 1 and in **7**. The present X-ray analysis of **7** monohydrate established the structure of meleagrin, including the absolute configuration, as **1** in accordance with the result of chemical and spectroscopic investigations. The absolute configurations at N_1, C_2, and C_3 are *S*, *S*, and *R*, respectively. The stereochemistry at N_1 of the present compound (**7**) is the same as that of oxaline,[3] but that of **1** in solution has not yet been determined.

The bond lengths and angles for **7** monohydrate are given in Fig. 2. These values are not significantly different from the expected ones. The five-membered rings *B* and *D* both adopt envelope forms, but ring *D* is much more planar than ring *B*, while the six-membered ring *C* shows a half-chair. The two aromatic rings and the imidazole ring are almost planar, as expected. The non-bonding distances of 2.827 and 2.933 Å found between N_{14} and O_w, and N_{19} and O_w may represent hydrogen bonds of the NH–O type. The molecules are packed together mainly in the *c* direction through the hydrogen bonding between the molecules of **7** and water in the crystals.

Experimental

Melting points are uncorrected. Infrared (IR) and ultraviolet (UV) spectra were taken with a Hitachi 215 grating spectrophotometer and a Hitachi 124 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were measured on a JEOL JNM-FX100 spectrometer or a Nicolet 500 MHz NMR spectrometer using an Oxford superconducting magnet with tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were obtained on a JEOL JMD-D 300 spectrometer. Optical rotation and circular dichroism (CD) spectra were measured with a JASCO DIP 181 spectrometer and a JASCO J-40 spectrophotometer, respectively.

Meleagrin (1)——Pale yellow leaves from $CHCl_3$ or CH_2Cl_2. mp 250 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 3180 (NH). 1700 (C=O). UV λ_{max}^{EtOH} nm (log ε): 232 (4.44), 285 sh (3.92), 349 (4.44). MS *m/z*: 433.1754 (M^+, 433.1749 required for $C_{23}H_{23}N_5O_4$), 366, 365, 364 ($M^+ - C_5H_9$, base peak). *Anal.* Calcd for $C_{23}H_{23}N_5O_4$: C, 63.73; H, 5.35. Found: C, 63.09; H, 5.35. CD (c=0.017, MeOH) $[\theta]^{20}$ (nm): -1.39×10^5 (224) (negative maximum), $+5.62\times10^4$

(247) (positive maximum), $+5.09\times10^4$ (257) (negative maximum), $+6.14\times10^4$ (275) (positive maximum), -3.94×10^4 (342) (negative maximum). ^{1}H-NMR ($CDCl_3$) δ: 1.24 and 1.35 (3H, each, s, 24- and 25-CH_3's), 3.73 (3H, s, 1-OCH_3), 5.06 (1H, d, J=10.6 Hz, 23-H), 5.10 (1H, d, J=17.2 Hz, 23-H), 5.50 (1H, s, 8-H), 6.12 (1H, dd, J=17.2 and 10.6 Hz, 22-H), 6.30 (1H, br, NH or OH), 6.96 (1H, br d, J=7.5 Hz), 7.07 (1H, br t, J=7.5 Hz), 7.25 (1H, s, 20-H), 7.30 (1H, br t, J=7.5 Hz), 7.58 (1H, br d, J=7.5 Hz), 7.61 (1H, s, 18-H), 8.27 (1H, s, 15-H), 12.72 (1H, br NH). ^{1}H-NMR (in acetone-d_6) δ: 1.32 and 1.38 (3H each, s, 24- and 25-CH_3's), 3.74 (3H, s, 1-OCH_3), 5.05 (1H, d, J=10 Hz, 23-H), 5.10 (1H, d, J=16 Hz, 23-H), 5.50 (1H, s, 8-H), 6.18 (1H, br, 22-H), 6.97 (1H, d, J=8 Hz), 7.07 (1H, t, J=8 Hz), 7.26 (1H, t, J=8 Hz), 7.46 (1H, s, 20-H), 7.65 (1H, d, J=8 Hz), 7.95 (1H, s, 18-H), 8.50 (1H, s, 15-H).

9-*O*-Acetylmeleagrin (2)——**1** (50 mg) was acetylated with acetic anhydride (1 ml) and pyridine (0.5 ml) at room temperature for 48 h to give the monoacetate (**2**) (40 mg) as a pale yellow crystalline powder, mp 247 °C from benzene–petroleum ether. IR ν_{max}^{KBr} cm^{-1}: 3400, 3300 (NH), 1770 (COO), 1708, 1690 (C=O), 1197, 1112. UV λ_{max}^{EtOH} nm (log ε): 204 (4.68), 223 sh (4.45), 295 sh (4.17), 347 (4.50). MS m/z: 475.1871 (M^+, 475.1876 required for $C_{25}H_{25}N_5O_5$), 444, 433, 407 ($M^+-C_5H_8$, base peak), 406. ^{1}H-NMR ($CDCl_3$) δ: 1.31 and 1.35 (3H, each, s, 24- and 25-CH_3's), 2.27 (3H, s, 9-OAc), 3.77 (3H, s, 1-OCH_3), 5.04 (1H, d, J=10 Hz, 23-H), 5.10 (1H, d, J=17 Hz, 23-H), 5.97 (1H, s, 8-H), 6.09 (1H, dd, J=17 and 10 Hz, 22-H), 6.6—7.6 (4H, m, aromatic-H's), 7.34 (1H, s, 20-H), 7.50 (1H, s, 18-H), 8.28 (1H, s, 15-H).

N(14)-Methylmeleagrin (3)——**1** (100 mg) was methylated in ether with CH_2N_2 overnight at room temperature. The reaction mixture was chromatographed on silica gel with 1% MeOH–$CHCl_3$ to give the monomethyl derivative (**3**) (65 mg) as pale yellow needles, mp 212 °C (dec.) from MeOH. IR ν_{max}^{KBr} cm^{-1}: 3400, 3150 (NH, OH), 1715, 1680 (C=O). UV λ_{max}^{MeOH} nm (log ε): 229 (4.02), 285 sh (3.63), 347 (4.06). MS m/z: 447.1902 (M^+, 447.1905 required for $C_{24}H_{25}N_5O_4$). CD (c=0.0088, MeOH) $[\theta]^{20}$ (nm): -1.47×10^5 (226) (negative maximum), $+4.88\times10^4$ (247) (positive maximum), $+4.37\times10^4$ (256) (negative maximum), $+5.99\times10^4$ (280) (positive maximum), -4.78×10^4 (345) (negative maximum). ^{1}H-NMR ($CDCl_3$) δ: 1.21 and 1.32 (3H each, s, 24- and 25-CH_3's), 2.45 (3H, s, NCH_3), 3.75 (3H, s, 1-OCH_3), 5.02 (1H, d, J=17.2 Hz, 23-H), 5.03 (1H, d, J=10.6 Hz, 23-H), 5.44 (1H, s, 8-H), 6.11 (1H, dd, J=17.2 and 10.6 Hz, 22-H), 6.8—7.2 (3H, m, aromatic-H's), 7.37 (1H, s, 20-H), 7.53 (1H, d, J=7.7 Hz), 7.70 (1H, s, 18-H), 8.31 (1H, s, 15-H), 13.05 (1H, br, NH). The reaction of **1** with Ag_2O and MeI gave a monomethyl derivative, which was identified as **3**.

22,23-Dihydromeleagrin (5)——Catalytic reduction of **1** (80 mg) with 5%-Pd/C (20 mg) in AcOEt (20 ml) afforded **5** (55 mg) as pale yellow needles, mp 210 °C (dec.) from chloroform. IR ν_{max}^{KBr} cm^{-1}: 3400, 3150 (NH, OH), 1700, 1670 (C=O). UV λ_{max}^{EtOH} nm (log ε): 232 (4.06), 348 (4.08). MS m/z: 435.1936 (M^+, 435.1906 required for $C_{23}H_{25}N_5O_4$), 366, 365 (base peak). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=9 Hz, 23-CH_3), 1.12 (6H, s, 24- and 25-CH_3's), 1.28 (2H, q-like, 22-CH_2), 3.74 (3H, s, 1-OCH_3), 5.50 (1H, s, 8-H), 6.20 (1H, br, NH or OH), 6.9—7.6 (4H, m, aromatic-H's), 7.30 (1H, s, 20-H), 7.61 (1H, s, 18-H), 8.31 (1H, s, 15-H), 12.65 (1H, br, NH).

12,15-Dihydromeleagrin (6)——**1** (5 mg) was treated with zinc powder (40 mg) in acetic acid (0.5 ml) at 70 °C for 3 h to give a dihydromeleagrin (**6**) (3 mg). MS m/z: 435.1920 (M^+, 435.1907 required for $C_{23}H_{25}N_5O_4$), 405.1822 (M^+-OCH_2, 405.1801 requires for $C_{22}H_{23}N_5O_3$).

9-*O*-*p*-Bromobenzoylmeleagrin (7)——**1** (80 mg) was treated with *p*-bromobenzoyl chloride (200 mg) and pyridine (2 ml) at room temperature for 24 h. The reaction mixture was chromatographed on silica gel with benzene–acetone (10 : 1) to afford the *p*-bromobenzoate (**7**) (75 mg) as pale yellow plates, mp 190 °C (dec.) from $CHCl_3$–MeOH or $CHCl_3$–EtOH. IR ν_{max}^{KBr} cm^{-1}: 3200—2800 (NH), 1730 (COO), 1700, 1670 (C=O). MS m/z: 615 and 617 (M^+). *Anal.* Calcd for $C_{30}H_{26}BrN_5O_5\cdot H_2O$: C, 56.79; H, 4.53; N, 11.04. Found: C, 56.32; H, 4.53; N, 10.86. ^{1}H-NMR ($CDCl_3$) δ: 1.36 and 1.41 (3H each, s, 24- and 25-CH_3's), 3.84 (3H, s, 1-OCH_3), 5.11 (1H, d, J=10.8 Hz, 23-H), 5.18 (1H, d, J=17.2 Hz, 23-H), 6.13 (1H, s, 8-H), 6.18 (1H, dd, J=17.2 and 10.8 Hz, 22-H), 6.90—7.37 (4H, m, aromatic-H's), 7.19 (1H, s, 20-H), 7.62 (2H, d, J=8.5 Hz), 7.63 (1H, s, 18-H), 8.00 (2H, d, J=8.5 Hz), 8.34 (1H, s, 15-H).

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6) A list of F_o and F_c values is available from one of the authors (K. K.) upon request.