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TWO NOVEL DITERPENOIDS FROM *PYGMAEOPREMNA HERBACEA*

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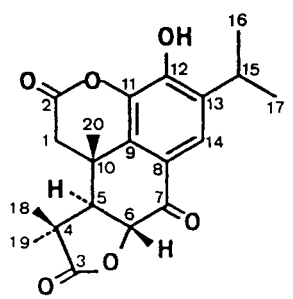
ABSTRACT.—Two diterpenoids with a new carbon skeleton, pygmaeocin A [**1**] and 5,6-didehydropygmaeocin A [**2**] together with a known diterpenoid, sugiol [**3**], were isolated from the roots of *Pygmaeopremna herbacea*. Their structures were deduced on the basis of spectroscopic evidence. The relative configuration of **1** was determined by an nOe experiment and **2** was correlated with **1** by catalytic hydrogenation of **2**. A biosynthetic pathway of the new carbon skeleton from abietane was proposed.

Pygmaeopremna herbacea (Roxb.) Moldenke (Verbenaceae) is a folk medicine against inflammation and malaria in the Yunnan province of China. From the roots of *P. herbacea* a diterpenoid with a new carbon skeleton (1) and a new coumarin (2) have been isolated previously. Two further novel constituents, pygmaeocin A [**1**] and 5,6-didehydropygmaeocin A [**2**] together with a known compound, sugiol [**3**], are reported in this paper.

Pygmaeocin A [**1**], $C_{20}H_{22}O_6$, a colorless crystalline solid, shows in its ir spectrum the presence of a hydroxyl group (3364) and a benzene ring (1604, 1580, 1490 cm^{-1}). The ^{1}H-nmr spectrum of **1** indicated three methyl groups, four methine groups, one aromatic proton, and one isopropyl group. The ^{13}C-nmr spectrum of **1** (Table 1) showed three carbonyl signals at δ 188.4 (s), 180.3 (s), and 168.0 (s). However, there were only two carbonyl bands (1778 and 1690 cm^{-1}) in the ir spectrum (KBr) of **1**. The band at 1778 cm^{-1} is rather broad so we assume that two carbonyls absorb at the same position. The three carbonyl bands in the ir spectrum of **2** confirmed this conclusion.



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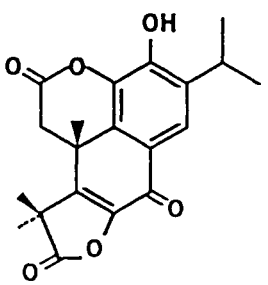

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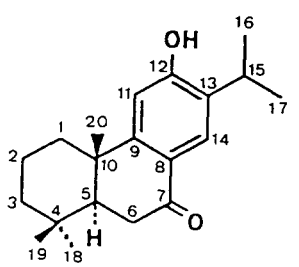

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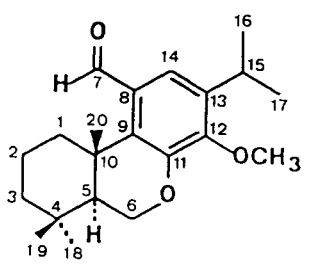

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TABLE 1. ^{13}C-nmr Spectral Data of Compounds **1**, **2**, and **3**.[a]

Carbon	Compound		
	1	**2**	**3**
C-1	41.7 t	38.2 t	39.1 t
C-2	168.0 s[b]	166.3 s	16.7 t
C-3	180.3 s	180.0 s	33.5 t
C-4	44.7 s	47.9 s	30.9 s
C-5	57.4 d	145.3 s	47.4 d
C-6	76.6 d	141.5 s	33.9 t
C-7	188.4 s	173.0 s	195.6 s
C-8	113.4 s	120.8 s	131.5 s
C-9	138.2 s[c]	137.6 s[d]	156.0 s
C-10	37.3 s	36.7 s	39.1 s
C-11	138.9 s[c]	138.0 s[d]	107.6 d
C-12	158.1 s	149.0 s	159.4 s
C-13	132.4 s	131.9 s	121.8 s
C-14	122.5 d	121.0 d	124.0 d
C-15	27.1 d	27.6 d	24.7 d
C-16[e]	22.4 q	22.2 q	20.3 q
C-17[e]	22.6 q	22.4 q	20.4 q
C-18	20.2 q	23.3 q	30.2 q
C-19	25.2 q	25.1 q	19.0 q
C-20	19.5 q	27.9 q	20.9 q

[a]Spectra were run in pyridine-d_5 at 50 MHz with TMS as internal standard. Multiplicities were assigned by DEPT sequence.

[b]See Polonsky *et al.* (21).

[c,d,e]Assignments may be interchangeable.

The signal at δ 180.3 in the ^{13}C-nmr spectrum of **1** together with the ir absorption at 1778 cm^{-1} indicated a γ-lactone unit (2,3). The signal at δ 188.4 in the ^{13}C-nmr spectrum of **1** was attributed to a keto carbonyl group. In view of its ir absorption at a lower frequency (1690 cm^{-1}), it was concluded to be attached to the benzene ring, which was confirmed by additional ir bands of the benzene ring (4). The signal at δ 168.0 in the ^{13}C-nmr spectrum of **1** is characteristic of a δ-lactone unit. Its ir absorption at a higher frequency (1778 cm^{-1}) indicated that its acyloxy group was attached to the benzene ring, which was in turn supported by the strong (as strong as a carbonyl absorption) ir absorption at 1604 cm^{-1} of the benzene ring.

The aromatic displacement pattern of **1** was quite similar to that of **4**, a diterpenoid isolated from *Coleus barbatus* Bentham (5). In the 2D nOe spectrum of **1**, correlations between the methyls of the isopropyl group and the sole aromatic proton (δ 8.11) were observed.

So far, the main structural features of **1** have been deduced. The stereochemistry at C-5, C-6, and C-10 was established as follows. In the ^{1}H-COSY spectrum of **1**, δ 5.76 (d, 1H, J = 13 Hz) correlated with δ 2.92 (d, 1H, J = 13 Hz), and δ 3.44 (d, 1H, J = 15 Hz) correlated with δ 3.01 (d, 1H, J = 15 Hz). According to their chemical shifts and coupling constants, δ 5.76 and 2.92 were attributed to H-6 and H-5 respectively, and δ 3.44 and 3.01 to geminal H-1. In the 1D nOe experiment (Figure 1), when the methyl group at δ 1.43 was irradiated nOe intensities were observed for two other methyl groups at δ 1.61 and 1.49 and H-6, and the signal at δ 1.43 was assigned to Me-18. When the methyl group at δ 1.61 was irradiated nOe intensities were observed for H-6, Me-18, and an H-1 at δ 3.44. Therefore, Me-18, Me-20, and H-6 were all assigned β. Signals at δ 3.44 and 3.01 were assigned to H-1β and H-1α, respectively. When Me-19 was irradiated nOe intensity was observed for H-5, and when H-5

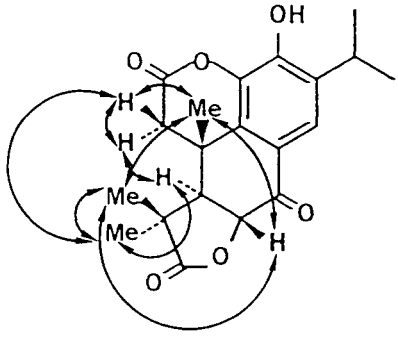


FIGURE 1. Observed nOe enhancements of pygmaeocin A [**1**].

was irradiated nOe intensity was observed for H-1α, so H-5 was assigned α. However, in the 2D nOe spectrum of **1** (Figure 2) a weak correlation between H-5 and H-6 was observed. This strange spot resulted from the big scalar coupling correlation which was not completely suppressed in the 2D nOe spectrum. In contrast, in the 1D nOe spectrum when H-5 was irradiated no nOe intensity was observed for H-6. In addition, be-

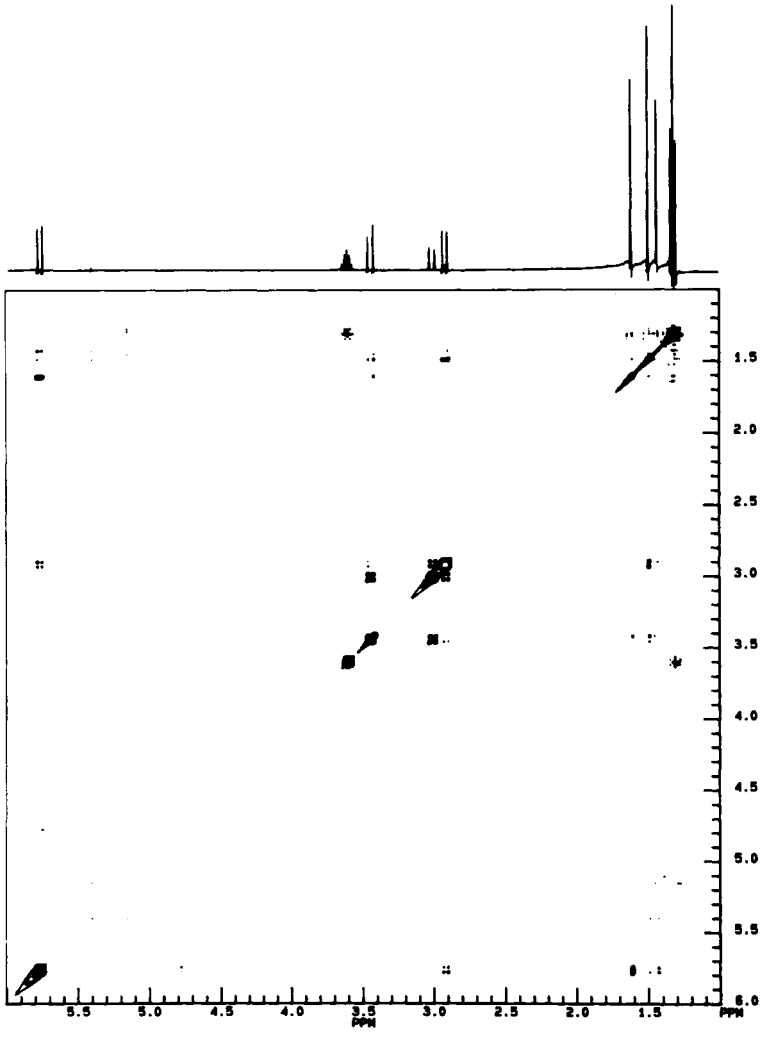


FIGURE 2. 2D nOe spectrum of pygmaeocin A [**1**].

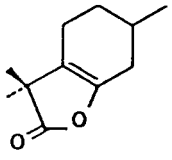
cause Me-19 was nearer to H-1β than to H-1α when Me-19 was irradiated nOe intensity was observed for H-1β instead of H-1α (only trace nOe).

All the spectral data suggest the 5,6-didehydropygmaeocin A [**2**], $C_{20}H_{20}O_6$, a slightly yellow crystalline solid, has the same carbon skeleton as **1**. The only difference between them is an additional double bond in the molecule of **2**. H-5α and H-6β of **1** are not present in the ^{1}H-nmr spectrum of **2**, and C-5 and C-6 shift to the sp^2-carbon region in the ^{13}C-nmr spectrum of **2** (Table 1). The ir spectrum of **2** exhibited a typical C,C double bond peak at 1643 cm^{-1}. Therefore the double bond should be between C-5 and C-6. In contrast to **1**, the ir peaks of the γ-conjugated and δ-lactone carbonyls in **2** were separate from each other. The γ-lactone carbonyl absorbed at a higher frequency (1794 cm^{-1}) than the corresponding carbonyl of **1** because a double bond was introduced to it. On the other hand, the keto carbonyl of **2** absorbed at a lower frequency (1668 cm^{-1}) because of the extended conjugation system. The three quaternary methyl groups were assigned unambiguously using nOe experiments. All the three methyl groups exhibited nOe correlation with a proton at δ 3.40, so δ 3.40 and 3.22 were assigned as H-1β and H-1α, respectively. One methyl group (δ 1.49) exhibited nOe correlation with both H-1α and H-1β and was assigned as Me-19. Another methyl group (δ 1.58), which showed nOe correlation with Me-19, was assigned as Me-18. When Me-20 (δ 1.53) was irradiated, nOe was observed only for H-1β.

High resolution mass spectra revealed that the peak $[M-42]^+$ both of pygmaeocin A [**1**] and 5,6-didehydropygmaeocin A [**2**] derived from the loss of ketene. It is very interesting to note that the peak $[M-42]^+$ (*m/z* 314) of **2** is the base peak while the relative intensity of the corresponding peak (*m/z* 316) of **1** is only 18%. The resultant ion of **2** from the loss of ketene is more stable than that of **1** because the conjugated system is extended to the additional double bond in the case of **2**.

To correlate **1** and **2** with each other, **2** was hydrogenated by H_2/Pd-C. The product was identical with **1** (physical data including cd spectrum). Therefore, it can be concluded that the stereochemistry at C-10 in **2** was the same as that in **1**. The catalytic hydrogenation of the double bond in **2** gave the trans instead of the expected cis isomer. Two main mechanisms for getting trans derivatives during catalytic hydrogenation can be considered: either by an in situ rearrangement of an intermediate cis form or by cis-1,4 addition to an enol system (6). Examples of in situ rearrangement can be found in the catalytic hydrogenations of 1,2-dimethylcyclohexene (7–9), 1,2,3,4,5,6,7,8-octahydronaphthalene ($\Delta^{9,10}$-octalin) (10), and 7,11-dioxolanost-8-en-3β-yl acetate (11). Examples of 1,4 addition can be found in the catalytic hydrogenation of some hydrophenanthrones (12). One explanation for the formation of the trans product **1** from **2** is a 1,4 addition of hydrogen to the α,β-unsaturated ketone system. The resulting enol system is in an equilibrium with the trans product. The catalytic hydrogenation of lactones **5** and **6** supports our proposal. The C,C double bond in **5** could not be catalytically reduced (13) while the corresponding double bond in **6** has been catalytically reduced in high yield due to facilitation of 1,4 addition (14).

Compound **3**, $C_{20}H_{28}O_2$, colorless needles, was identified on the basis of spectral data as the known abietane diterpenoid sugiol [**3**] (15–17).



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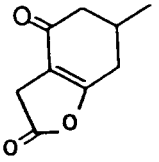

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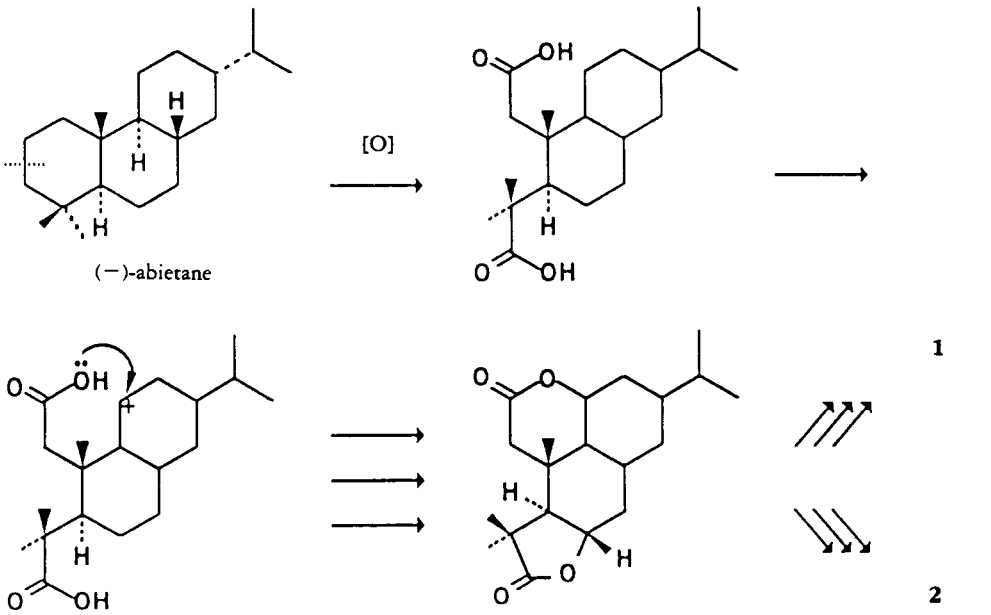
To our knowledge, pygmaeocin A [**1**] and 5,6-didehydropygmaeocin A [**2**] are diterpenoids with a new carbon skeleton. We propose to name this carbon skeleton pygmaeocane A. The configurations given in the structures are relative. Because of the cooccurrence of sugiol [**3**], pygmaeocin A [**1**], and 5,6-didehydropygmaeocin A [**2**] in the same plant we assume they are interrelated biogenetically. It is reasonable to postulate that pygmaeocin A and 5,6-didehydropygmaeocin A are derived from an abietane diterpene as shown in Scheme 1. The C-10 methyl group of abietane diterpenoids has a β configuration (18–20) as in sugiol [**3**]. If the biogenetic pathway for pygmaeocane A given in Scheme 1 is correct, the absolute configurations of pygmaeocin A and 5,6-didehydropygmaeocin A are as shown in **1** and **2**, respectively. The numbering system of pygmaeocane A was set up according to the biogenetic pathways in Scheme 1.



SCHEME 1. Hypothetical biosynthetic pathways of pygmaeocin A [**1**] and 5,6-didehydropygmaeocin A [**2**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were measured on a Reichert melting point apparatus and were uncorrected. Ir spectra were obtained on a Nicolet 200XV PFT instrument in KBr. Uv spectra were measured in MeOH on a Perkin-Elmer 555 spectrophotometer. Cd spectra were measured in MeOH on a JACO J500A instrument. ^{1}H-nmr spectra were recorded at 200 MHz on a Varian XL-200 instrument, at 300 MHz on a Bruker WM 300 instrument, or at 400 MHz on a Bruker AM 400 instrument with TMS as internal standard. ^{13}C-nmr spectra were recorded at 50 MHz on a Varian XL-200 instrument with TMS as internal standard. Mass spectra were obtained at 70 eV on a JMS-OIU spectrometer. High resolution mass spectra were obtained on a Varian MAT 711 spectrometer. Plant material was collected in Shuangjiang county, Yunnan province, China. A voucher specimen is located at Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, Sichuan, China.

ISOLATION AND SEPARATION.—Roots of *P. herbacea* (18 kg) were extracted with Et_2O at room temperature three times. The crude extract was concentrated (682 g), mixed with the same amount of Kieselgur and extracted in turn with petroleum ether (60–90°), C_6H_6, and Et_2O in a Soxhlet apparatus. The petroleum ether extract (130 g) was chromatographed over 1500 g Si gel packed in petroleum ether, 500-ml fractions being collected as follows: 1–20 (petroleum ether), 21–70 (petroleum ether/Et_2O, gradient elution), and 71–95 (petroleum ether/Et_2O/MeOH, gradient elution). Fractions 79–83, which exhibited the same tlc plots, were combined and chromatographed over Si gel packed in petroleum ether, being eluted with petroleum ether/$CHCl_3$ (gradient elution). One of the fractions obtained was once again chromato-

graphed over Si gel packed in C_6H_6, being eluted with C_6H_6-Me_2CO (100:1). Pygmaeocin A [**1**] was then obtained and recrystallized (60 mg) from MeOH.

Fractions 20–28, which exhibited the same tlc spots, were combined and chromatographed over a fortyfold excess of Si gel packed in petroleum ether, being eluted with petroleum ether-C_6H_6 (2:1). Sugiol [**3**] was then obtained and recrystallized as colorless needles (42 mg) from $CHCl_3$-MeOH (3:1).

The C_6H_6 extract (250 g) was dissolved in MeOH (600 ml), and H_2O (300 ml) was added. Upon standing the solution was filtered. The filtrate was extracted with C_6H_6 (5 × 800 ml) and concentrated under reduced pressure to a sticky solid, which was chromatographed over Si gel using CCl_4-EtOAc-MeOH (10:1:0, 10:2:0, 10:5:0, and 10:5:1) as eluent. One of the fractions obtained was further chromatographed over Si gel several times using C_6H_6-EtOAc (10:1) as eluent. 5,6-Didehydropygmaeocin A [**2**] was then obtained and recrystallized as a slightly yellow crystalline solid (45 mg) from MeOH.

PYGMAEOCIN A [**1**].—Colorless crystalline solid: mp 281–283°; elementary analysis $C_{20}H_{22}O_6$ (calcd C 67.02, H 6.19; found C 67.13, H 6.09); uv λ max (MeOH) 225 (ε 1.60×10^7), 287 (ε 1.15×10^7), 3.50 (ε 3.25×10^6) nm; cd ($c = 4.41 \times 10^{-7}$, MeOH) $[\theta]_{278}$ $+1.46 \times 10^7$, $[\theta]_{245}$ $+4.65 \times 10^7$, $[\theta]_{218}$ -3.30×10^7; ir ν max (KBr) 3364, 2973, 2938, 2874, 1778 (br), 1690, 1604 1580, 1490, 1468, 1443, 1397, 1350, 1309, 1237, 1162, 1095, 1035, 1011 cm^{-1}; ^1H nmr (200 MHz, pyridine-d_5, TMS) δ 8.11 (s, H-14), 6.44 (br s, disappeared in D_2O, 12-OH), 5.76 (d, H-6β, $J = 13$ Hz), 3.59 (br septet, H-15, $J = 7$ Hz), 3.44 (d, H-1β, $J = 15$ Hz), 3.01 (d, H-1α, $J = 15$ Hz), 2.92 (d, H-5α, $J = 13$ Hz), 1.61 (s, Me-20), 1.49 (s, Me-19), 1.43 (s, Me-18), 1.33 (d, Me-16 or Me-17, $J = 7$ Hz), 1.31 (d, Me-17 or Me-16, $J = 7$ Hz); ^1H-COSY (200 MHz, pyridine-d_5, TMS) see discussion; ^1H 1D nOe see Figure 1; ^1H 2D nOe spectrum see Figure 2; ^{13}C nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 358 ($C_{20}H_{22}O_6$) (100), 343 (37), 316 ($C_{18}H_{20}O_5$) (18), 272 (16), 259 (20), 245 (61), 218 (28), 203 (45), 96 (25), 83 (16), 70 (21), 55 (11).

5,6-DIDEHYDROPYGMAEOCIN A [**2**].—Slightly yellow crystalline solid: mp 211–213°; $C_{20}H_{20}O_6$; uv λ max (MeOH) 254 (ε 1.61×10^7), 331 (ε 6.40×10^6), 418 (ε 2.85×10^6) nm; cd ($c = 3.09 \times 10^{-7}$, MeOH) $[\theta]_{356}$ $+3.10 \times 10^6$, $[\theta]_{314}$ -2.94×10^6, $[\theta]_{277}$ $+7.87 \times 10^5$, $[\theta]_{274}$ $+3.05 \times 10^5$, $[\theta]_{253}$ $+1.12 \times 10^7$, $[\theta]_{237}$ 0, $[\theta]_{226}$ $+3.13 \times 10^6$; ir ν max (KBr) 3525, 2969, 2937, 2872, 1794, 1700, 1668, 1643, 1608, 1576, 1493, 1468, 1450, 1330, 1247, 1229, 1117, 1054, 1014 cm^{-1}; ^1H nmr (400 MHz, DMSO-d_6, TMS) δ 10.52 (br s, 12-OH), 7.70 (s, H-14), 3.40 (d, H-1β, $J = 16$ Hz), 3.30 (septet, H-15, $J = 7$ Hz), 3.22 (d, H-1α, $J = 16$ Hz), 1.58 (s, Me-19), 1.53 (s, Me-20), 1.49 (s, Me-18), 1.21 (d, Me-16 or Me-17, $J = 7$ Hz), 1.19 (d, Me-17 or Me-16, $J = 7$ Hz). ^1H 1D nOe experiment (400 MHz, DMSO-d_6, TMS): when δ 1.58 was irradiated nOe intensities were observed for δ 3.40 and 1.49; when δ 1.53 was irradiated nOe intensity was observed for δ 3.40; when δ 1.49 was irradiated nOe intensities were observed for δ 3.40, 3.22, and 1.58. ^{13}C nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 356 ($C_{20}H_{20}O_6$) (77), 341 (55), 314 ($C_{18}H_{18}H_5$) (100), 313 (51), 299 (11), 286 (53), 285 (28), 271 (30), 258 (24), 259 (24), 243 (33), 190 (18), 128 (10), 129 (10), 115 (14), 91 (13), 77 (13), 70 (12), 43 (19), 41 (21).

CATALYTIC HYDROGENATION OF 5,6-DIDEHYDROPYGMAEOCIN A.—A mixture of 5% Pd-C (14.3 mg) in EtOH (5 ml) was saturated with H_2 at atmospheric pressure overnight. 5,6-Didehydropygmaeocin A (35.7 mg, 0.10 mmol) in EtOH (5 ml) was added under a sealed atmosphere, and H_2 was introduced. After 1 equivalent of H_2 was absorbed (about 1 h), H_2 was stopped and the mixture was stirred for another 1 h. Then the mixture was filtered, and the filtrate was concentrated under reduced pressure to a solid which was chromatographed over 10 g Si gel using CH_2Cl_2 as eluent. The reduction product (10.8 mg, 30%) and the starting material (11.4 mg, 32%) were obtained. The reduction product was identical with pygmaeocin A [**1**] based on the physical data recorded above including a cd spectrum.

SUGIOL [**3**].—Colorless needles: mp 294–295°; elementary analysis $C_{20}H_{28}O_2$ (calcd C 80.00, H 9.33, found C 79.78, H 9.40); $[\alpha]^{25}D$ +29.3° ($c = 3.25$, pyridine).

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