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THE ISOLATION AND STRUCTURAL ELUCIDATION OF THREE NEW NEOLIGNANS, PUBERULINS A, B, AND C, AS PLATELET ACTIVATING FACTOR RECEPTOR ANTAGONISTS FROM *PIPER PUBERULUM*

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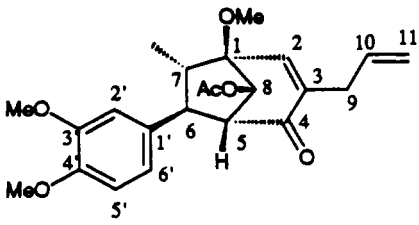
ABSTRACT.—Three new neolignans, puberulins A [**1**], B [**2**], and C [**3**], were isolated from *Piper puberulum*. Their structures and relative stereochemistries were determined from spectral data and the X-ray crystallographic analysis of **1**. Compounds **1** and **3** inhibit specific platelet activating factor receptor binding with IC_{50} values of 7.3 and 5.7 μM, respectively.

The stems of *Piper puberulum* (Benth.) Maxim (Piperaceae), a plant indigenous to the southern People's Republic of China, together with other plants of the genus *Piper*, are known as "Hai Feng Teng" in Chinese folk medicine and have been used for the treatment of asthma and arthritic conditions. Many lignans have been isolated from the leaves of *P. futokadsura*, which are also used like "Hai Feng Teng" (1–4). One of the components, piperone, was reported to show insect antifeeding activity (2). A number of neolignans have also been isolated from *P. futokadsura* (5). Some of these were reported to inhibit the binding of platelet activating factor (PAF) to a receptor site preparation isolated from rabbit platelet membrane (5). In order to investigate the active principles of *P. puberulum*, we screened extracts of its stems in a PAF bioassay. Bioactivity-directed fractionation of the active Et_2O extract has led to the isolation and characterization of three new neolignans, puberulins A, B, and C, together with other biogenetically related neolignans including isoguianin (6,7). We report herein the structural elucidation and the PAF receptor binding inhibitory activity of puberulins A, B, and C.

RESULTS AND DISCUSSION

Puberulin A [**1**], obtained as colorless cubic crystals (mp 130–131°), has the molecular formula $C_{23}H_{28}O_6$ as revealed by its hrms (*m/z* 400.1877). Singlets at δ 3.35 (3H) and 3.84 (6H) in its ^{1}H-nmr spectrum (Table 1) pointed, respectively, to the presence of one aliphatic OMe and two aromatic OMe groups while a further singlet at δ 2.14 (3H) indicated the presence of one OAc group. Expansion of the formula to $C_{18}H_{16}O.(OMe)_3.OAc$ suggested that **1** was a neolignan. One of the C_6-C_3 moieties was assigned the partial structure **4a**, since the ms indicated cleavage of the molecular ion



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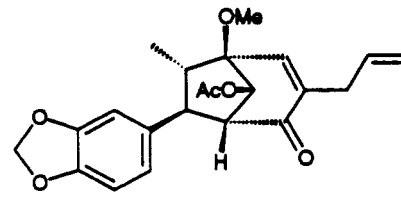

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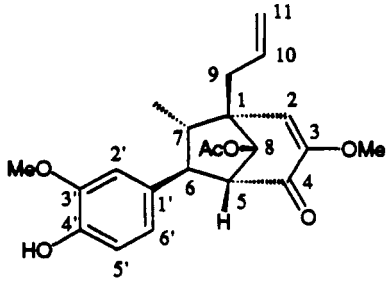

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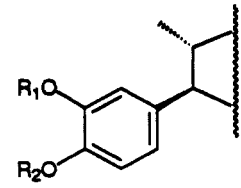
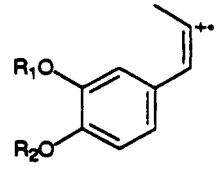


4a $R_1=R_2=Me$
4b $R_1-R_2=CH_2$
4c $R_1=Me$, $R_2=H$



5a $R_1=R_2=Me$, *m/z* 178
5b R_1-$R_2=CH_2$, *m/z* 162
5c $R_1=Me$, $R_2=H$, *m/z* 164

TABLE 1. ^{1}H-Nmr Chemical Shifts of Compounds **1–3**.

Proton	Compound		
	1	**2**	**3**
H-2	6.83, s	6.73, s	5.66, s
H-5	2.98, s	2.97, s	3.04, s
H-6	2.41, d, J=8.5 Hz	2.41, d, J=8.5 Hz	2.57, d, J=8.0 Hz
H-7	2.73, dt, J=8.5, 7.0 Hz	2.70, dt, J=8.5, 7.0 Hz	2.45, dt, J=8.0, 6.5 Hz
H-8	5.29, s	5.31, s	5.17, s
H-9	3.01, d, J=6.5 Hz	3.04, d, J=6.5 Hz	2.38, d, J=12.5 Hz
H-10	5.82, ddd, J=6.5, 1.0, 13.5 Hz	5.85, ddd, J=6.5, 1.0, 13.5 Hz	5.85, dd, J=12.5, 5.5 Hz
H-11	5.10, dd, J=1.0, 13.5 Hz	5.15, dd, J=1.0, 13.5 Hz	5.10, d, J=5.5 Hz
H-2′	6.78, s	6.73, s	6.76, d, J=1.0 Hz
H-5′	6.80, d, J=9.0 Hz	6.89, d, J=12.0 Hz	6.81, d, J=8.0 Hz
H-6′	6.84, d, J=9.0 Hz	6.89, d, J=12.0 Hz	6.81, dd, J=8.0, 1.0 Hz
Me-7	0.98, d, J=7.0 Hz	1.00, d, J=7.0 Hz	1.01, d, J=6.5 Hz
MeO-1	3.35, s	3.37, s	
MeO-3			3.68, s
MeO-3′	3.84, s		3.88, s
MeO-4′	3.84, s		
OH-4′			5.55, s
AcO-8	2.14, s	2.20, s	2.14, s
OCH_2O		5.95, s	

into a fragment of *m/z* 178 (26.1%), which must be represented by **5a**. That **1** contained a 1,3,4-trisubstituted aromatic system was revealed by a doublet at δ 6.89 (1H, J=2.0 Hz), a double doublet at δ 6.77 (1H, J=2.0 and 8.0 Hz), and a doublet at δ 6.55 (1H, J=8.0 Hz) in C_6D_6. The structure of the other C_6-C_3 moiety was deduced from the remaining ^{1}H-nmr signals. The ^{1}H-^{1}H COSY nmr spectrum showed the presence of a spin-system of an allyl moiety (-CH_2-CH=CH_2). Peaks in the ir (1685 cm^{-1}) and uv (231 nm) spectra characterized an α,β-unsaturated ketone, with a single hydrogen located most probably at the β-carbon because the corresponding singlet (δ 6.83) in the ^{1}H-nmr spectrum occurs at relatively low field. The α-carbon must be substituted by the allyl group as the signal for the allylic methylene protons occurs at δ 3.01 (>δ 2.60), indicating that the allyl moiety must be linked to an sp^2 carbon (8). The ^{1}H-nmr (Table 1), ^{13}C-nmr (Table 2), DEPT, and HETCOR nmr spectra revealed that one *tert*-C-OMe, one-CHOAc, and one -CH were present in this C_6-C_3 moiety. Significant features of the ^{1}H-nmr spectrum were singlets at δ 2.98 and 5.29 for H-5 and H-8, respectively, and a doublet at δ 2.41 for H-6, reflecting the fact that the H–C-8–C-5–H and H–C-5–C-6–H dihedral angles must lie close to 90°; for such an arrangement, the Karplus equation (9) gives J_{vic}=0 Hz. A Dreiding molecular model attested to the feasibility of this assignment [the torsion angles derived from an X-ray crystallographic analysis (see below) are 75(3)° and −105(3)°, respectively]. Consequently, the acetoxy group at C-8 must be oriented towards the five-membered ring, cis to the benzylic proton. The trans relationship between the vicinal aryl and methyl substituents was suggested by the chemical shift of the C-7 methyl protons (δ 0.98) and by the large coupling constant ($J_{6,7}$=8.5 Hz). In 2-aryl-3-methyl-2,3-dihydrobenzofuran analogues, the Me-group

TABLE 2. ^{13}C-Nmr Data for Compounds **1–3**.

Carbon	Compound		
	1	**2**	**3**
C-1	54.1 s	88.4 s	53.8 s
C-2	148.8 d	146.4 d	119.7 s
C-3	133.8 s	135.1 s	151.6 s
C-4	197.6 s	197.4 s	193.0 s
C-5	60.7 d	60.7 d	61.4 d
C-6	51.3 d	51.5 d	53.6 d
C-7	46.3 d	46.6 d	48.1 d
C-8	75.2 d	75.0 d	79.3 d
C-9	32.5 t	32.4 t	36.6 t
C-10	134.4 d	134.3 t	134.0 d
C-11	117.6 t	117.6 t	118.2 t
C-1′	140.0 s	140.0 s	133.3 s
C-2′	111.5 d	108.2 d	111.2 d
C-3′	149.0 s	148.8 s	146.4 s
C-4′	149.0 s	148.8 s	144.5 s
C-5′	111.2 d	108.3 d	114.4 d
C-6′	120.2 d	121.5 d	120.5 d
Me-7	13.2 q	13.0 q	13.8 q
OMe-1	54.1 q	54.0 q	
OMe-3			55.9 q
OMe-3′	55.9 q		
OMe-4′	55.9 q		
OCH_2O		101.0 t	
OAc-8	21.2 q	21.1 q	21.1 q
	169.1 s	169.1 s	169.1 s

that is cis to the vicinal aryl falls into the magnetically shielded region above the aromatic system and leads to a signal at about δ 0.7 and to a smaller coupling (8,10,11).

From the foregoing evidence, the structure of **1** was deduced as 3-allyl-8-acetoxy-1-methoxy-7-methyl-4-oxo-6-(3′,4′-dimethoxyphenyl)-bicyclo[3,2,1]oct-2-ene. An X-ray crystallographic analysis unequivocally established the complete structure and relative stereochemistry; attempts to determine the absolute stereochemistry by use of anomalous scattering effects were inconclusive. Carbon and oxygen atom fractional coordinates are listed in Table 3. A view of the solid-state conformation, with the atom numbering scheme, is provided in Figure 1. Bond lengths agree in general with expected values (12). Bond strain is, however, reflected in the elongated C-5–C-6 distance of 1.577(5) Å. An approximate C_2 symmetry axis passing through C-6 and the mid-point of the C-1–C-8 bond relates the cyclopentane ring torsion angles and thus it has a half-chair form. Torsion angles characterizing the cyclohexenone ring are related by an approximate mirror plane of symmetry passing through C-3 and C-8 and, with two small adjacent values [C-1–C-2–C-3–C-4 = −0.8(6)°, C-2–C-3–C-4–C-5 = 5.9(6)°], the ring has a half-boat (envelope, 1,2-diplanar) form. Atoms C-6, O-7′, and O-9′ deviate by small amounts [Δ = 0.022, 0.002, 0.029 Å, respectively] from the least-squares plane through C-1′–C-6′ [Δ max = 0.006 Å]. Moreover, in common with the usually preferred approximately coplanar arrangement of aryl methoxy groups (13–20) in the solid state,

TABLE 3. Non-hydrogen Atom Fractional Coordinates and Equivalent Isotropic Thermal Parameters for Puberulin A [**1**], with Estimated Standard Deviations in Parentheses.

Atom	*x*	*y*	*z*	B_{eq} (Å^2)
C-1	0.0405 (2)	0.1827 (2)	0.1456 (3)	3.28 (6)
C-2	0.0651 (3)	0.0933 (2)	0.0916 (4)	3.63 (7)
C-3	0.1114 (3)	0.0784 (2)	−0.0292 (4)	3.87 (8)
C-4	0.1393 (3)	0.1542 (3)	−0.1164 (4)	4.07 (8)
C-5	0.1195 (3)	0.2418 (2)	−0.0491 (4)	3.44 (7)
C-6	0.1814 (2)	0.2529 (2)	0.0872 (4)	3.39 (6)
C-7	0.1209 (2)	0.2290 (2)	0.2162 (3)	3.24 (6)
C-8	0.0238 (2)	0.2380 (2)	0.0118 (4)	3.37 (7)
C-9	0.1373 (4)	−0.0102 (3)	−0.0823 (5)	5.4 (1)
C-10[a]	0.1571 (6)	−0.0715 (5)	0.0328 (10)	5.0 (2)
C-11	0.1170 (5)	−0.1393 (3)	0.0715 (7)	8.3 (2)
C-12	0.1672 (3)	0.1763 (3)	0.3328 (4)	4.47 (8)
O-13	−0.0303 (2)	0.1784 (2)	0.2474 (3)	4.22 (5)
C-14	−0.1135 (3)	0.1473 (4)	0.1925 (5)	6.7 (1)
O-15	0.1763 (2)	0.1480 (2)	−0.2310 (3)	6.35 (7)
O-16	−0.0064 (2)	0.3222 (2)	0.0571 (3)	4.12 (5)
C-17	−0.0588 (3)	0.3665 (3)	−0.0331 (4)	4.31 (8)
O-18	−0.0792 (3)	0.3391 (2)	−0.1491 (4)	7.87 (9)
C-19	−0.0851 (4)	0.4524 (3)	0.0267 (6)	6.8 (1)
C-1′	0.2268 (2)	0.3409 (2)	0.1033 (4)	3.31 (6)
C-2′	0.3021 (2)	0.3444 (2)	0.1936 (4)	3.53 (7)
C-3′	0.3488 (3)	0.4205 (3)	0.2153 (4)	3.76 (7)
C-4′	0.3224 (3)	0.4960 (2)	0.1433 (4)	3.92 (7)
C-5′	0.2482 (3)	0.4926 (2)	0.0537 (4)	3.91 (7)
C-6′	0.2012 (3)	0.4153 (3)	0.0334 (4)	3.91 (8)
O-7′	0.4225 (2)	0.4291 (2)	0.3020 (3)	5.19 (6)
C-8′	0.4476 (3)	0.3549 (3)	0.3837 (5)	5.6 (1)
O-9′	0.3733 (2)	0.5689 (2)	0.1665 (3)	5.10 (6)
C-10′	0.3549 (4)	0.6427 (3)	0.0799 (5)	5.8 (1)
C-10′′′[a]	0.0921 (6)	−0.0830 (5)	−0.0099 (11)	5.5 (2)

[a]Occupancy factor = 0.50.

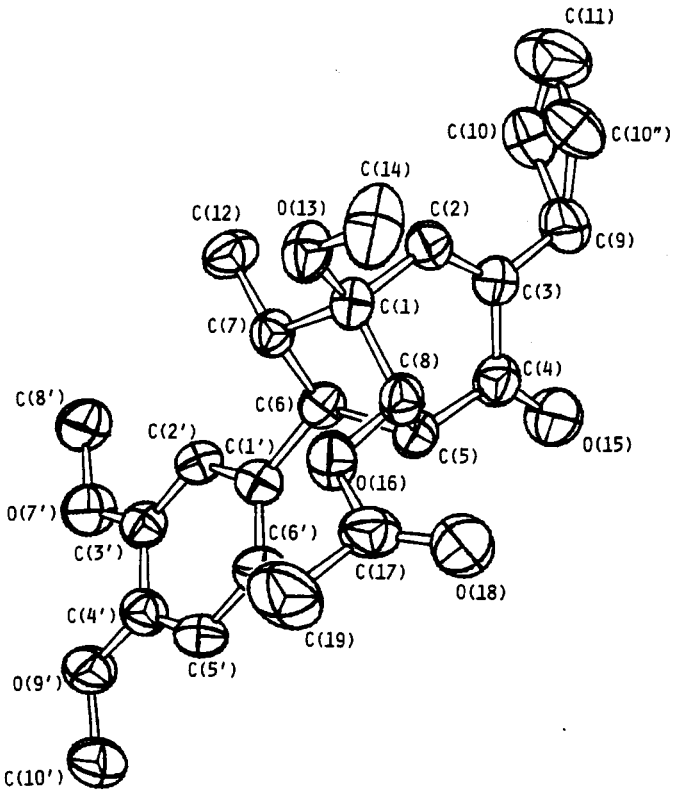


FIGURE 1. ORTEP diagram (50% probability ellipsoids) showing the crystallographic atom numbering scheme and solid-state conformation of puberulin A [**1**]; one of the allyl carbon atoms is disordered over two positions [C-10 and C-10"]. Hydrogen atoms have been omitted for clarity.

C-8′ (Δ=0.098 Å) and C-10′ (Δ=0.221 Å) lie close to the phenyl ring plane [torsion angles: C-2′–C-3′–O-7′–C-8′ = −5.2(6)°; C-5′–C-4′–O-9′–C-10′ = −7.8(6)°].

Puberulin B [**2**], molecular formula $C_{22}H_{24}O_6$ from its hrms (*m/z* 384.1541), was obtained as a colorless amorphous solid. ^{1}H- and ^{13}C-nmr spectral data were in agreement with this formulation and the fact that **2** was also a neolignan. As with **1**, the ir (1685 cm^{-1}) and uv (233 nm) spectra reflected the presence of an α,β-unsaturated ketone. The ^{1}H-nmr spectrum of **2** closely resembled that of **1** except that it contained a distinctive signal at δ 5.95 (2H, s) for the methylenedioxy protons and only one aliphatic methoxy signal at δ 3.37 (3H, s). A mass spectral fragment at *m/z* 162 was indicative of the partial structure **4b**. These data led to the characterization of **2** as 3-allyl-8-acetoxy-1-methoxyl-7-methyl-4-oxo-6-piperonyl-bicyclo[3,2,1]oct-2-ene.

Puberulin C [**3**], obtained as needles (mp 205°), had a molecular formula of $C_{22}H_{26}O_6$, as determined from its hrms (*m/z* 386.1689). Its ^{1}H- and ^{13}C-nmr spectral data supported this formulation and indicated that **3** was also a neolignan. Furthermore, the ir (1690 cm^{-1}) and uv (231 nm) spectra revealed that **3** also contained an α,β-unsaturated ketone. The presence of an aromatic hydroxy group was evidenced by an ir absorption at 3495 cm^{-1} and a D_2O-exchangeable signal at δ 5.55 (1H, s) in the ^{1}H-nmr spectrum. The ^{1}H-nmr spectrum of **3** was similar to that of **1** except for the signals of the OMe and allyl groups. This spectrum revealed the presence of an aromatic OMe group (δ 3.88, 3H, s) and an enolic OMe group (δ 3.68, 3H, s). The NOESY spectrum

showed cross-peaks between the signals at δ 3.88 (Ar-OMe) and δ 6.76 (H-2′) as well as between the signals at δ 3.68 (OMe-3) and δ 5.66 (H-2), thereby indicating that the Ar-OMe group was located at C-3′ while the enolic OMe was situated at C-3. The position of the signal for the allylic methylene protons [δ 2.38 (< δ 2.60)] reflects the fact that the allyl moiety must be linked to an sp^3 carbon (8). A mass spectral fragment at *m/z* 164 indicated the partial structure **4c**. These data led to the identification of **3** as 1-allyl-8-acetoxy-3-methoxy-7-methyl-4-oxo-6-(3′-methoxy-4′-hydroxyphenyl)-bicyclo[3,2,1]oct-2-ene.

Puberulins A [**1**], B [**2**], and C [**3**] were tested for in vitro inhibition of PAF binding to rabbit platelets. The data are listed in Table 4. Puberulin C [**3**] is the most potent neolignan in this group. It inhibits the specific binding of ^{3}H-PAF to its receptor site on isolated rabbit platelet plasma membranes with an IC_{50} value of 5.7 μM. The IC_{50} value of compound **1** was 7.3 μM.

TABLE 4. Inhibition of PAF Binding to Rabbit Platelets by Puberulins A [**1**], B [**2**], and C [**3**].

Compound	% Inhibition at 10 μM	IC_{50} (μM)	Ki
1	63	7.3	4.7±0.9 μM
2	43	≥10	—
3	66	5.7	3.7±0.6 μM

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Kofler micro-melting point apparatus and are uncorrected. Ir spectra were recorded as KBr pellets on a Perkin-Elmer 983 spectrophotometer. Uv spectra were measured on a Shimadzu *UV-2101PC* spectrophotometer in absolute MeOH. Ms were determined on a JMSO-D 300S mass spectrometer. ^{1}H- and ^{13}C-nmr spectra were measured on Bruker AC-300 and Varian XL-400 spectrometers with TMS as an internal standard in $CDCl_3$. Si gel H (10–40 μm Qing Dao) was used for cc under 0.5–2 kg/cm^2. Analytical tlc was performed on Si gel plates with cyclohexane-Et_2O (3:1). Neolignans were detected by spraying with a 10% H_2SO_4 solution containing 1% $CeSO_4$, followed by heating.

PLANT MATERIAL.—The stems of *P. puberulum* were collected in September 1988, in Fujian Province, People's Republic of China. A voucher specimen is deposited at the School of Pharmacy, Shanghai Medical *University, Shanghai, People's Republic of China.*

EXTRACTION AND ISOLATION.—The stems of *Piper puberulum* (2.0 kg) were ground and soaked in 20 liters of 95% EtOH for 48 h at room temperature. The extract was filtered and concentrated *in vacuo* to yield 45 g of dark green semi-solid with a pepper-like aroma. This material was dissolved in 80% MeOH solution, then extracted with petroleum ether four times. After evaporation of the defatted 80% MeOH solution, the residue was dissolved in Et_2O, after which the solution was filtered and concentrated to yield 8 g of residue. The residue was chromatographed on Si gel under low pressure, employing cyclohexane-Et_2O (3:1 to 1:1) as eluent, to give five fractions. The third fraction was further purified by chromatography with cyclohexane-Et_2O (3:1) as eluent to afford 70 mg (0.0035% yield) of puberulin A [**1**] and 90 mg (0.0045% yield) of puberulin B [**2**]. Further purification of the fourth fraction by chromatography using cyclohexane-Et_2O (3:2) as eluent yielded 40 mg (0.002% yield) of puberulin C [**3**].

Puberulin A [**1**].—Colorless cubic crystals (Et_2O), mp 130–131° (Et_2O); $[\alpha]_D$ −28.1° (c=5.1, $CHCl_3$); uv λ max (log ε) 280 (3.39), 231 (4.05) nm; ir ν max 3010, 2940, 2840, 1750, 1685, 1595, 1540, 1460, 1440, 1380, 1365, 1235, 1045, 990, 930, 860, 810 cm^{-1}; ^{1}H nmr (C_6D_6) δ 6.89 (1H, d, *J*=2.0 Hz, H-2′), 6.77 (1H, dd, *J*=2.0 and 8.0 Hz, H-6′), 6.55 (1H, d, *J*=8.0 Hz, H-5′), 6.55 (1H, s, H-2), 5.74 (1H, m, H-10), 5.40 (1H, s, H-8), 5.03 (2H, m, H-11), 3.42 (3H, s, ArO-Me), 3.39 (3H, s, ArO-Me), 3.18 (1H, s, H-5), 3.10 (3H, s, C-Me), 3.04 (1H, m, H-7), 2.90 (2H, m, H-9), 2.55 (1H, d, *J*=8.5 Hz, H-6), 1.64 (3H, s, AcO-8), 1.05 (3H, d, *J*=7.0 Hz, Me-7); ^{1}H-nmr data ($CDCl_3$), see Table 1; ^{13}C-nmr data, see Table 2; eims *m/z* 400 (100), 341 (13.1), 340 (46.1), 195 (34.8), 194 (15.9), 193 (25.2), 180 (28.5), 178 (26.1), 177 (13.7), 165 (62.9), 164 (24.1), 163 (17.9), 151 (18.4), 137 (13.2), 105 (11.5), 91 (13.8).

Puberulin B [**2**].—Colorless amorphous solid, $[\alpha]_D$ −33.9° (c=3.2, $CHCl_3$); uv λ max (log ε) 288 (3.52), 233 (3.92) nm; ir ν max 3100, 2940, 2880, 2840, 1750, 1685, 1648, 1508, 1490, 1450, 1375, 1255,

1225, 1040, 995, 930, 860, 815 cm^{-1}; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; eims *m/z* 384 (100), 325 (21.4), 324 (68.2), 309 (13.3), 221 (15.0), 193 (13.0), 180 (60.3), 179 (43.6), 178 (18.2), 177 (32.2), 175 (12.9), 165 (56.1), 164 (43.5), 163 (19.3), 162 (38.7), 161 (20.9), 151 (12.9), 149 (47.2), 147 (16.4), 137 (25.6), 131 (14.2), 105 (26.0), 103 (20.9), 91 (29.2).

Puberulin C [**3**].—Colorless needles, mp 205–205.5° (Et_2O) $[\alpha]_D$ =5.8° (c=1.2, $CHCl_3$); uv λ max (log ε) 259 (3.70), 231 (3.84) nm; ir ν max 3495, 3100, 2940, 2840, 1735, 1695, 1620, 1520, 1460, 1445, 1365, 1270, 1235, 1200, 1033, 985, 925, 870, 840, 830 cm^{-1}; eims *m/z* 386.1689 (30.6), 307 (26.5), 285 (11.8), 181 (22.3), 165 (10.5), 164 (10.6), 154 (100), 153 (30.6), 139 (12.9), 137 (30.6), 136 (60.0), 135 (67.0), 107 (22.3), 91 (15.3).

X-RAY CRYSTAL STRUCTURE ANALYSIS OF PUBERULIN A [**1**].[1]—*Crystal data.*—$C_{23}H_{28}O_6$, mol wt=400.48, orthorhombic, a=14.903(2), b=15.398(2), c=9.298(2) Å, V=2134(1) $Å^3$, Z=4, D_c=1.247 g cm^{-3}, μ(Cu-Kα radiation, λ=1.5418 Å)=7.0 cm^{-1}; crystal dimensions 0.07×0.10×0.70 mm. Space group $P2_12_12_1$(D_2^4) uniquely from the Laue symmetry and systematic absences: *h*00 when *h*≠2*n*, 0*k*0 when *k*≠2*n*, 00*l* when *l*≠2*n*.

Preliminary unit-cell parameters and space group information were derived from oscillation and Weissenberg photographs. One octant of intensity data was recorded on an Enraf-Nonius CAD-4 diffractometer [Cu-Kα radiation, graphite monochromator; ω-2θ scans, θ max 75°, scanwidth (0.80+0.14tanθ)°]. The intensities of four reference reflections, remeasured every 2 h during data collection, showed no significant variation (<1.0%) throughout. Refined unit-cell parameters were derived by least-squares treatment of the diffractometer setting angles for 25 reflections (36°<θ<40°) widely separated in reciprocal space. From a total of 2491 independent measurements, those 1499 reflections with $I>3.0\sigma(I)$ were retained for the structure analysis following correction for the usual Lorentz and polarization effects.

The crystal structure was solved by direct methods (MULTAN11/82). Approximate coordinates for the non-hydrogen atoms were derived in part from an *E*-map and from a series of weighted F_o Fourier syntheses phased successively by an increasing number of atoms. One of the carbon atoms of the allyl group [C(10)] was found to be disordered over two positions. Following several rounds of full-matrix least-squares adjustment of positional and thermal parameters of these atoms (at first isotropic, then anisotropic), evaluation of a difference Fourier synthesis revealed significant positive regions at positions calculated for most of the hydrogen atoms. Positional and isotropic thermal parameters of the hydrogen atoms (other than those associated with the disordered allyl group where they were incorporated at their calculated positions) were then included as variables during the next series of least-squares calculations. An extinction correction (g) was also refined during the later iterations. The parameter refinement converged at $R=\Sigma||F_o|-|F_c||/\Sigma|F_o|=0.039$ $\{R_w=[\Sigma w(|F_o|-|F_c|)^2/\Sigma w|F_o|^2]^{1/2}=0.053$, $g=1.7(3)\times10^{-6}$, $GOF=[\Sigma w\Delta^2/(N_{observations}-N_{parameters})]^{1/2}=1.16\}$. No unusual features were present in a final difference Fourier synthesis.

Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf-Nonius Structure Determination Package (SDP). In the least-squares iterations, $\Sigma w\Delta^2\{w=1/\sigma^2(|F_o|), \Delta=(|F_o|-|F_c|)\}$ was minimized. For all structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from the literature (21).

INHIBITION OF PAF RECEPTOR BINDING.—The inhibition of ^{3}H-PAF binding to isolated rabbit platelet plasma membranes was carried out according to a literature method (22).

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[1]Hydrogen coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre and can be obtained upon request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

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