49. New 20(10→5)*abeo*-Diterpenoids from *Pygmaeopremna herbacea*

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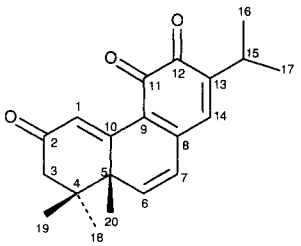
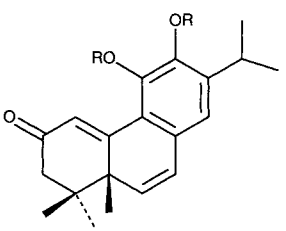
Two new 20(10→5)*abeo*-abietane diterpenoids, pygmaeocins B (**1**) and C (**2**), have been isolated from the roots of *Pygmaeopremna herbacea* (Verbenaceae). Their structures were elucidated on the basis of spectroscopic data, and chemically, both compounds were correlated with each other. The absolute configurations were assigned tentatively on the basis of biogenetic pathway.

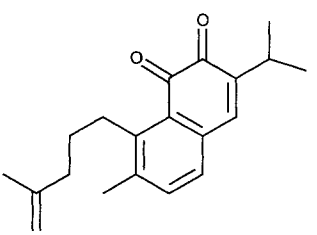
Pygmaeopremna herbacea (ROXB.) MOLDENKE (Verbenaceae) is a small shrub growing in the Yunnan and the Hainan provinces of China and in the north of India. It is used in Yunnan as a folk medicine against inflammation and malaria. From the roots of *P. herbacea*, several diterpenoids [1] and a coumarin [2] have been already isolated. In this paper, we report on two new diterpenoids, the pygmaeocins B (**1**) and C (**2**), both isolated from the roots. Both are 20(10→5)*abeo*-abietane diterpenoids [3][1]). Several 20(10→5)*abeo*-4,5-seco-abietane diterpenoids such as aethiopinone (**3**) have been isolated from some *Salvia* spp. [4]. However, so far no example of 20(10→5)*abeo*-abietane skeleton with an intact C(4)–C(5) bond is known. Pygmaeocin B (**1**; $C_{20}H_{22}O_3$) is a purple solid. The proposed structure is supported by the ^{1}H- and ^{13}C-NMR data and NOE correlations.



1



2 R = H
4 R = Ac



3

The ^{1}H-NMR of **1** displays signals for three Me groups attached to quaternary C-atoms and for one i-Pr group. The only CH_2 group indicated by ^{13}C-NMR *(Table)* is confirmed by a typical geminal H,H coupling constant (J = 17 Hz) for the signals at 2.15 and 2.62 ppm. Furthermore, four olefinic protons appear at 6.26, 6.73, 6.77, and 7.17 ppm. The signals at 6.26 and 6.77 ppm (J = 10) are attributed to the *cis*-related H–C(6) and H–C(7) [5]. The ^{13}C-NMR of **1** (*Table*) shows an *ortho*-quinone system at 179.3 and 179.4 ppm [6] and a keto

[1]) Abietane numbering is used for convenience. Systematic names are given in the *Exper. Part.*

C=O at 198.0 ppm, as also confirmed by IR data. Of the four peaks at 1650–1667 cm^{-1} with almost the same intensity, three are attributed to the *ortho*-quinone system [7] and the fourth to a conjugated ketone.

The connectivities in **1** are supported by NOE results. Thus, the proton at 6.73 ppm (H–C(14)) correlates with the proton at 6.26 ppm (H–C(7)), so that the double bond should be between C(6) and C(7). H–C(6) (6.77 ppm) has NOE correlations with Me(18), Me(19), and Me(20). Therefore, Me(20) should be attached to C(5) instead of C(10). H–C(1) does not correlate with any other proton.

A five-bond H,H coupling (^5J = 1 Hz) between H–C(1) and H–C(6) confirms the structure of **1**, because appreciable long-range couplings are usually found between protons at the ends of a chain of *zig-zag* configuration, being generally conjugated and part of a cyclic molecule (J = 0.4–2.0 Hz) [8].

The four-bond H,H couplings between H–C(1) and H_α–C(3) (^4J = 0.8 Hz) and between H_β–C(3) and H–C(18) (^4J = 0.6 Hz) also support structure **1** (for '*W*' conformations, J = 1–2 Hz [9]).

The values of ^4J and ^5J in the two types of systems discussed above 'fall off rapidly with departures from planarity' [10]. The structure of **1** agrees well with this rule, because H–C(1) is coplanar with both H–C(6) and H_α–C(3), and H_β–C(3) is coplanar with one of the H–C(18) in a '*W*' conformation.

Pygmaeocin C (**2**; $C_{20}H_{24}O_3$) is a yellow foam. All spectral data of **2** suggest that it is the dihydro derivative of pygmaeocin B (**1**).

The IR of **2** shows the presence of OH groups. The ^{1}H-NMR spectrum is quite similar to that of **1**. Of interest is the difference of the chemical shifts of H–C(6): in **1**, H–C(6) is deshielded by C(11)=O by conjugation; in the case of **2**, a reverse effect is exerted on H–C(6) by HO–C(12)($\Delta\delta$ = 0.85 ppm). In the ^{13}C-NMR *(Table)* of **2**, no signals corresponding to an *ortho*-quinone system can be detected. The signal of C(11) is at rather low field (163.6 ppm) because of the electron-withdrawing effect of C(2)=O.

Table. *^{13}C-NMR Data of Pygmaeocin B* (**1**), *Pygmaeocin C* (**2**), *and Pygmaeocin C Diacetate* (**4**)[a])

C-atom	**1**	**2**	**4**
C(1)	124.4(*d*)	123.9(*d*)	124.4(*d*)
C(2)	198.0(*s*)	201.2(*s*)	198.5(*s*)
C(3)	48.0(*t*)	48.2(*t*)	48.5(*t*)
C(4)	39.5(*s*)	38.8(*s*)	38.6(*s*)
C(5)	46.4(*s*)	45.5(*s*)	45.1(*s*)
C(6)	148.0(*d*)	130.3(*d*)	134.0(*d*)
C(7)	135.9(*d*)	125.6(*d*)	125.8(*d*)
C(8)	122.7(*s*)	114.9(*s*)	122.4(*s*)
C(9)	150.9(*s*)	142.5(*s*)	144.3(*s*)
C(10)	153.1(*s*)	144.7(*s*)	157.4(*s*)
C(11)	179.3(*s*)[b])	163.6(*s*)	140.2(*s*)[c])
C(12)	179.4(*s*)[b])	138.7(*s*)	141.2(*s*)[c])
C(13)	143.3(*s*)	127.1(*s*)	132.7(*s*)
C(14)	128.4(*d*)	116.7(*d*)	122.1(*d*)
C(15)	27.6(*d*)	27.3(*d*)	27.7(*d*)
C(16)[d])	21.2(*q*)	21.2(*q*)	20.2(*q*)
C(17)[d])	21.3(*q*)	22.2(*q*)[e])	20.3(*q*)
C(18)	27.2(*q*)	26.1(*q*)	26.1(*q*)
C(19)	23.2(*q*)[f])	22.5(*q*)[e])	20.9(*q*)
C(20)	23.9(*q*)[f])	24.3(*q*)	24.1(*q*)
CH_3CO_2C(11)[g])			22.5(*q*)
CH_3CO_2C(12)[g])			22.7(*q*)
CH_3CO_2C(11)[h])			167.7(*s*)
CH_3CO_2C(12)[h])			168.3(*s*)

[a]) Spectra were run in $CDCl_3$ at 50 MHz, TMS as internal standard. Multiplicities were assigned by DEPT sequence.

[b])–[h]) Assignments may be interchanged.

Treatment of pygmaeocin C (**2**) with Ac_2O/pyridine yielded the diacetate **4**, confirming the presence of two phenolic OH groups. Pygmaeocins B (**1**) and C (**2**) were easily correlated with each other. Catalytic hydrogenation of **1** over Pt/C selectively reduced the *ortho*-quinone system to a catechol system. On the other hand, Ag_2CO_3 oxidation of **2** quantitatively afforded **1**.

In the MS of pygmaeocin B (**1**), instead of the molecular-ion peak, a $[M+2]^{+\cdot}$ peak was observed. This kind of 'signal shifts' are characteristic for quinones (more pronounced in *ortho*-quinones), as they can be partially reduced by residual moisture in the inlet system and the ion source [11]. The *ortho*-quinone system of **1** being completely reduced, its MS was the same as that of pygmaeocin C (**2**). On the other hand, pygmaeocin C diacetate (**4**) exhibited the expected molecular-ion peak. By a *retro-Diels-Alder* reaction of the molecular ion, loss of 2-methylpropene results in the dihydroxyketene ion (*m/z* 256). Subsequent cleavage of the C=O group leads to the base peak at *m/z* 228.

The absolute configurations of the new diterpenoids were not determined directly. However, the CD spectra (*Fig.*) of **1** and **2** indicated the same configuration. Because of the co-occurrence of sugiol (**5**) [1b], **1**, and **2** in the same plant, it is plausible to assume that they are biogenetically interrelated. A migration of Me(20) from C(10) to C(5) in 5,6-didehydrosugiol could lead ultimately to **1** and **2** (see the *Scheme*). This migration has been realized chemically, first by *Eugster et al.* [12] and later by *Tahara et al.* [13] [14]; the latter established that the β-configuration of the Me group was retained after migration [13]. These facts suggest β-configuration for Me(20) in pygmaeocins B (**1**) and C (**2**). To our knowledge, this is the first reported isolation of 20(10→5)*abeo*-abietane diterpenoids with intact C(4)–C(5) bond from natural source.

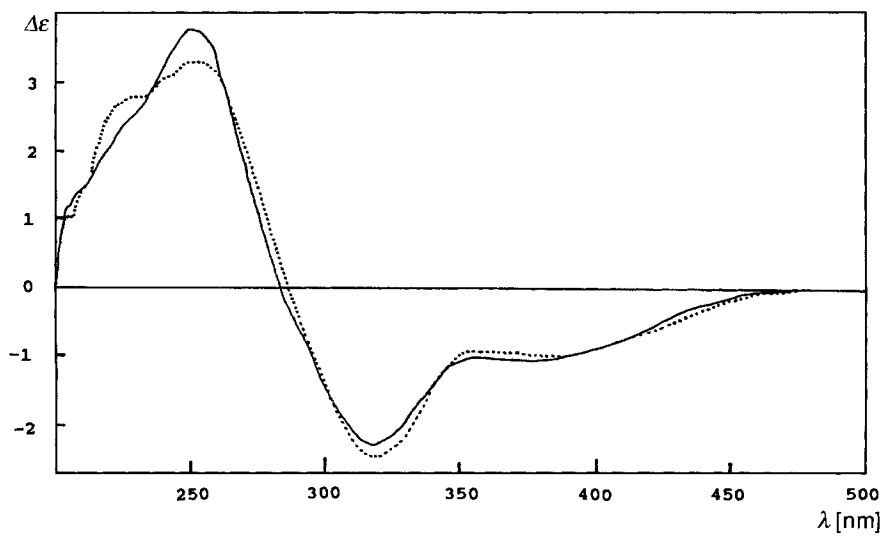
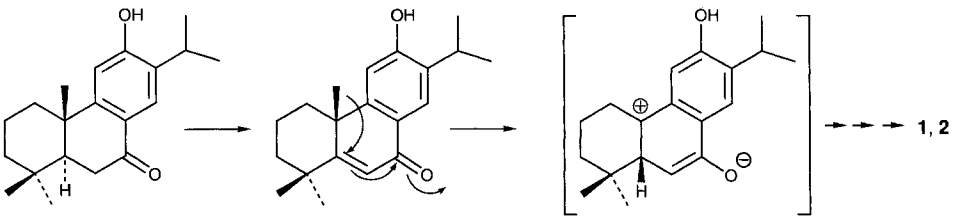


Figure. *CD Spectra of pygmaeocins B* (**1**; —) *and C* (**2**; ····)

Scheme. *Biosynthetic Pathway Leading to Pygmaeocins B* (**1**) *and C* (**2**)



Sugiol (**5**)

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Experimental Part

General. 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. M.p.: *Mettler FP5/FP52* melting-point apparatus, uncorrected. UV spectra: in MeOH, *Perkin-Elmer 555* spectrophotometer. CD spectra: in MeOH, *JACO J500A* instrument. IR spectra: *Perkin-Elmer 297* instrument. ^{1}H-NMR spectra: *Varian XL-200* (200 MHz) or *Bruker AM 400* instrument (400 MHz) with TMS as internal standard. ^{13}C-NMR spectra: *Varian XL-200* instrument (50 MHz) with TMS as internal standard. MS (70 ev): *Varian MAT-112S* spectrometer. HR-MS: *Varian-MAT 711* spectrometer.

Isolation and Separation. Roots of *Pygmaeopremna herbacea* (18 kg) were extracted with Et_2O at r.t. 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°), benzene, and Et_2O in a *Soxhlet* apparatus. The benzene extract (250 g) was dissolved in MeOH (600 ml), and H_2O(300 ml) was added. Upon standing, the soln. was filtered. The filtrate was extracted with benzene (5 × 800 ml) and evaporated. The sticky residue was chromatographed (silica gel, CCl_4/AcOEt/MeOH from 10:1:0 to 10:5:1). One of the fractions obtained was first chromatographed over silica gel (CH_2Cl_2/MeOH 100:1) and then over *Merck* precoated anal. TLC silica-gel plates (CH_2Cl_2/MeOH 20:1) to give *pygmaeocin B* (**1**; purple solid, 57 mg) and *pygmaeocin C* (**2**; yellow foam, 34 mg).

Pygmaeocin B (= *8,8a-Dihydro-2-isopropyl-8,8,8a-trimethylphenanthren-3,4,6(7*H*)-trione*; **1**). M.p. 108.5–110° (CH_2Cl_2). UV (MeOH): *ca.* 227 (3.24), 260 (3.29), 323 (3.17), 512 (2.13); min.: 241 (3.23), 285 (2.98), 449 (1.99). CD: see *the Figure*. IR ($CHCl_3$): 3007, 2972, 1667–1650 (*ca.* 4 peaks), 1608, 1519, 1415, 1391, 1351, 1283. ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.94 (br. *s*, Me(18)); 1.15 (*d*, *J* = 7, Me(16) or Me(17)); 1.16 (*d*, *J* = 7, Me(17) or Me(16)); 1.20 (*s*, Me(19)); 1.27 (*s*, Me(20)); 2.15 (*dd*, *J* = 17, 0.8, H_α–C(3)); 2.62 (*dd*, *J* = 17, 0.6, H_β–C(3)); 3.01 (*dsept.*, *J* = 7, 1, H–C(15)); 6.26 (*d*, *J* = 10, H–C(7)); 6.73 (*d*, *J* = 1, H–C(14)); 6.77 (*dd*, *J* = 10, 1, H–C(6)); 7.17 (br. *s*, H–C(1)). ^{1}H,^{1}H-decoupling NMR ($CDCl_3$, 400 MHz; correlated signals): 0.94–2.62; 1.15–3.01; 1.16–3.01; 2.15–2.62; 2.15–7.17; 3.01–6.73; 6.26–6.77; 6.77–7.17. ^{1}H-NOE ($CDCl_3$, 400 MHz; correlated signals): 0.94–1.20; 0.94–2.15; 0.94–6.77; 1.20–1.27; 1.20–2.15; 1.20–2.62; 1.20–6.77; 1.27–2.62; 1.27–6.77; 3.01–6.73; 6.26–6.73; 6.26–6.77. ^{13}C-NMR see the *Table*. EI-MS: 312 (19, $[M + 2]^+$), 256 (65, $C_{16}H_{16}O_3$), 228 (100, $C_{15}H_{16}O_2$), 213 (26), 183 (19), 41 (22). HR-MS: 312.1722 ($C_{20}H_{24}O_3$, calc. 312.1725).

Pygmaeocin C (= *1,10a-Dihydro-5,6-dihydroxy-7-isopropyl-1,1,10a-trimethylphenanthren-3(2*H*)-one*; **2**). UV (MeOH): *ca.* 224 (3.49), 246 (3.52), 258 (3.52), 339 (3.34); min.: 243 (3.48), 251 (3.51), 300 (2.89). CD: see the *Figure*. IR ($CHCl_3$): 3500, 3140 (br.), 3010, 2967, 2940, 2880, 1630 (br.), 1581, 1440, 1417, 1391, 1348, 1310, 1318, 1134, 1041, 911, 877. ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.96 (*s*, Me(18)),; 1.19 (*s*, Me(19)); 1.23 (*d*, *J* = 7, Me(16) or Me(17)); 1.24 (*s*, Me(20)); 1.27 (*d*, *J* = 7, Me(17) or Me(16)); 2.16 (*d*, *J* = 18, H_α–C(3)); 2.67 (*d*, *J* = 18, H_β–C(3)); 3.26 (*sept.*, *J* = 7, H–C(15)); 5.92 (br. *d*, *J* = 10, H–C(6)); 6.37 (*d*, *J* = 10, H–C(7)); 6.56 (br. *s*, H–C(14)); 7.00 (br. *s*, H–C(1)); *ca.* 7.80 (OH). ^{13}C-NMR: see the *Table*. EI-MS and HR-MS: identical to those of **1**.

Pygmaeocin C Diacetate (= *6,7,8,8a-Tetrahydro-2-isopropyl-8,8,8a-trimethyl-6-oxophenanthrene-3,4-diyl Diacetate*; **4**). To a soln. of **2** (25.4 mg, 0.081 mmol) in Ac_2O (5 ml), pyridine (10 drops) was added, and the mixture was stirred at r.t. overnight. The soln. was poured into ice/H_2O (10 ml) and extracted with CH_2Cl_2 (2 × 10 ml), the extract dried ($MgSO_4$) and evaporated, and the yellow oil obtained was chromatographed (*Merck* precoated anal silica-gel TLC plates, CH_2Cl_2/MeOH 20:1): **4** (yellow solid; 26.0 mg, 81%). M.p. 142.5–143.5°. $[\alpha]_D^{22}$ = –425.5 (*c* = 0.235, MeOH). UV (MeOH): *ca.* 213 (2.81), 232 (2.88), 257 (sh, 2.86), 263 (2.89), 304 (2.44); min.: 221 (2.80), 250 (2.68), 260 (sh, 2.87), 277 (2.19). IR ($CHCl_3$): 3004, 2975, 2938, 2879, 1775 (br.), 1661, 1592, 1434, 1373, 1330, 1286, 1184, 1130, 1044, 889. ^{1}H-NMR ($CDCl_3$, 200 MHz): 0.98 (*s*, Me(18)); 1.17 (*s*, Me(19)); 1.20 (*d*, *J* = 7, Me(16) or Me(17)); 1.23 (*s*, Me(20)); 1.25 (*d*, *J* = 7, Me(17) or Me(16)); 2.13 (*dd*, *J* = 17, 1, H_α–C(3)); 2.24 (*s*, AcO–C(11) or AcO–C(12)); 2.34 (*s*, AcO–C(12) or AcO–C(11)); 2.66 (*dd*, *J* = 17, 0.8, H_β–C(3)); 2.98 (*sept.*, *J* = 7, H–C(15)); 6.10 (*dd*, *J* = 10, 1, H–C(6)); 6.43 (*d*, *J* = 10, H–C(7)); 6.57 (br. *s*, H–C(1)). ^{13}C-NMR: see the *Table*. EI-MS: 396 (5, M^+), 354 (6), 312 (19), 298 (36), 294 (30), 256 (100), 228 (15), 55 (17), 44 (90), 43 (89).

Hydrogenation of **1**. In the presence of 5% Pt/C (6.5 mg), **1** (12.2 mg) in 2 ml of EtOH was hydrogenated with H_2 for 1 min under stirring. The mixture was filtered and the filtrate evaporated. The yellow oil was chromatographed (*Merck* precoated anal. silica-gel TLC plates, CH_2Cl_2/MeOH 20:1): **2** (7.4 mg, 60%). Identification by spectroscopic data. When the hydrogenation was run overnight, the result was the same as described above.

Oxidation of **2**. To a soln. of **2** (3.9 mg) in CH_2Cl_2 (1 ml), Ag_2CO_3 (21.1 mg) was added and the mixture shaken at r.t. for 5 min. Then, it was filtered and the filtrate evaporated: pure **1** (3.9 mg).

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