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PAEONILACTONE-A, -B, AND -C, NEW MONOTERPENOIDS FROM PAEONY ROOT

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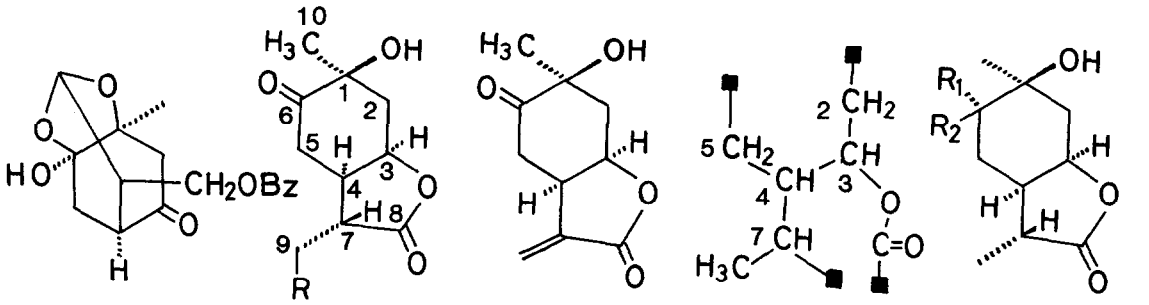
Abstract: Three new monoterpenoids named paeonilactone-A, -B, and -C were isolated from Paeony root (roots of *Paeonia albiflora* PALLAS *var. trichocarpa* BUNGE) and their structures were determined to be 2, 3, and 4, respectively, by means of chemical and spectroscopic studies.

Paeony root (roots of *Paeonia albiflora* PALLAS *var. trichocarpa* BUNGE) is one of the most important crude drugs used in several Chinese medicinal prescriptions. Previously we reported the isolation of paeoniflorigenone (1),[1] a new monoterpenoid having a blocking effect on the neuromuscular junction in phrenic nerve diaphragm preparations of mice,[2] from this crude drug. Further investigation on the pharmacologically active principles of Paeony root led to the isolation of three minor components, named paeonilactone-A (2), -B (3), and -C (4).

The ether-soluble fraction of the water extract from Paeony root was fractionated by a combination of silica gel column chromatography, Sephadex LH-20 chromatography, and preparative thin-layer chromatography (Merck Kieselgel G-60) to give small amounts of 2, 3, and 4, along with 1.

Paeonilactone-A (2), $C_{10}H_{14}O_4$, colorless needles (from ether-MeOH), mp 128-129°C, $[\alpha]_D^{25}$ -18.8° (MeOH), showed the molecular ion peak at m/z 198 in its mass spectrum. The IR spectrum of 2 exhibited a hydroxyl absorption (3500 cm^{-1}) and two carbonyl absorptions (1750 and 1710 cm^{-1}). As shown in Table 1, the ^1H- and ^{13}C-NMR spectra indicated the presence of a ketone, a γ-lactone, a tertiary and a secondary methyl, a tertiary hydroxyl, two methylene, and three methine groups in 2 and detailed spin decoupling experiments suggested the presence of the partial structure 5.

Reduction of 2 with $NaBH_4$ afforded epimeric diols, 6, mp 109-110°C (from ether), $C_{10}H_{16}O_4$, MS: m/z 182 ($M^+ - H_2O$), and 7, amorphous, $C_{10}H_{16}O_4$, MS: m/z 200



1 2: R = H 3 5 6: R_1 = OH, R_2 = H
4: R = C_6H_5COO 7: R_1 = H, R_2 = OH

Fig. 1

Table 1. ^{1}H-NMR (200 MHz) and ^{13}C-NMR spectral data of paeonilactone-A (2), -B (3), and -C (4) (ppm in $CDCl_3$)[a]

^{1}H \ Compd.	2	3	4
2α - H	2.61, dd (14, 6)	2.51, dd (14, 6)	2.65, dd (14, 6)
2β - H	1.89, dd (14, 11)	1.98, dd (14, 9)	1.97, dd (14, 10)
3 - H	4.93, ddd (11, 6, 6)	5.01, m	5.00, ddd (10, 8, 6)
4 - H	2.77, br.ddd (12, 8, 6)	3.69, m	3.19, m
5α - H	2.94, dd (15, 8)	2.97, dd (16, 8)	2.95, dd (16, 8)
5β - H	2.64, dd (15, 2)	2.78, dd (16, 4)	2.81, dd (16, 4)
7 - H	2.38, dq (12, 7)	—	2.85, m
9 - H	1.26, d 3H (7)	5.70, d (3)	4.60, dd (12, 5)
		6.36, d (3)	4.81, dd (12, 4)
10 - CH_3	1.47, s	1.40, s	1.47, s
OH	3.84, s	3.47, s	3.73, s

^{13}C \ Compd	2	3	4
C-1	73.6 s	74.0 s	73.9 s
C-2	42.4 t[b]	41.4 t[b]	42.0 t[b]
C-3	73.6 d	73.2 d	73.4 d
C-4	37.9 d	39.1 d	38.7 d
C-5	35.6 t[b]	37.0 t[b]	35.8 t[b]
C-6	210.6 s	210.0 s	210.3 s
C-7	44.2 d	136.7 s	43.4 d
C-8	170.0 s	169.1 s	173.6 s
C-9	13.3 q	123.1 t	60.9 t
C-10	25.0 q	25.0 q	27.8 q
CO			166.1 s
Phenyl			128.6 d 129.0 d 129.7 d 133.6 s

a) Values in parentheses are coupling constants (Hz).
b) Assignments may be interchanged in each compound.

(M^+), which showed the ^{1}H-NMR signals due to the newly produced carbinol methine protons at δ 3.74 (dd, J=10, 3.5 Hz) and at δ 3.58 (m, $W_{1/2}$=11 Hz), respectively. Both of these protons were found by decoupling experiments to be coupling with the C-5 methylene protons, but not with the C-2 protons. Therefore, the structure of

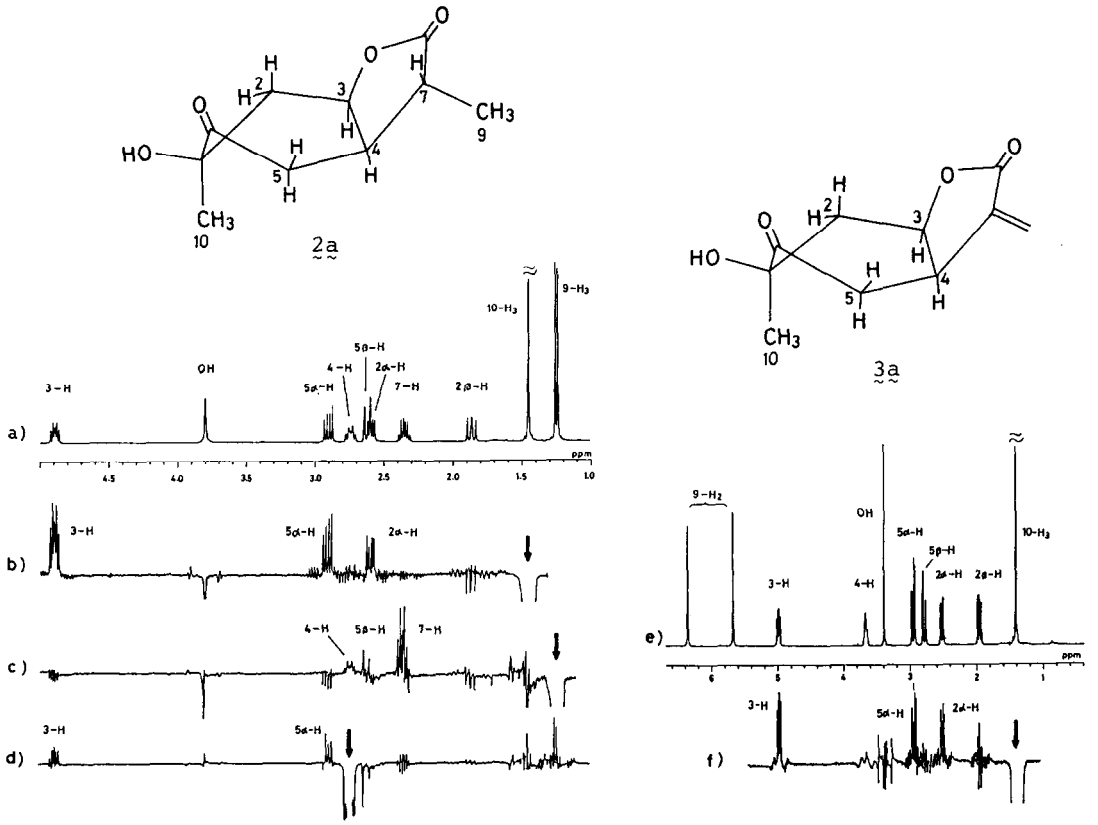


Fig. 2 a) Normal ^{1}H-NMR spectrum (400 MHz) of paeonilactone-A (2). b - d) NOE difference spectra of 2 on irradiation at δ1.47, δ1.26, and δ2.77, respectively. e) Normal ^{1}H-NMR spectrum (400 MHz) of paeonilactone-B (3). f) NOE difference spectrum of 3 on irradiation at δ1.40.

paeonilactone-A can be formulated as the formula 2. The relative stereochemistry was elucidated on the basis of the coupling constants of each proton and NOE experiments of 2. Irradiation at the tert-CH_3 (δ 1.47) and the sec-CH_3 group (δ 1.26) caused the increase of signal intensity of the 2α-, 3-, and 5α-protons and the 4-, 5β-, and 7-protons, respectively. Irradiation at the 4-proton (δ 2.77) gave rise to the increase of signal intensity of the 3- and 5α-protons (Fig.2, b - d). These observations led us to conclude the stereostructure of paeonilactone-A to be 2a.

Paeonilactone-B (3), $C_{10}H_{12}O_4$, colorless needles (from ethyl acetate), mp 88-89°C, $[\alpha]_D^{25}$ +23.2° (MeOH), MS: m/z 196 (M^+), exhibited the IR absorptions at 3450 (OH), 1745 (CO), 1700 (CO), and 1650 (C=C) cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra indicated the presence of an exo methylene group conjugated with a lactone, together with a ketone, a tert-hydroxyl, a tert-CH_3, two methylene, and two methine groups (Table 1). From these spectral data this compound is considered

to be an analog of 2 and its structure was assigned to 3. The stereochemistry was determined as 3a on the basis of the coupling constants of each proton and the NOE experiment, in which the increase of signal intensity of the 2α-, 3-, and 5α-protons was observed on irradiation at the tert-CH_3 signal (Fig. 2, f).

Paeonilactone-C (4), $C_{17}H_{18}O_6$, colorless needles (from aq. MeOH), mp 132-133°C, $[\alpha]_D^{25}$ -31.6° (MeOH) showed UV absorptions at 229 (log ε: 4.80), 267_{sh}, 272 (log ε: 3.71) and 279_{sh} nm and IR absorptions at 3300 (OH), 1770, 1730, 1720 (CO), 1605 and 1595 (phenyl) cm^{-1}. The EI mass spectrum of 4 failed to give the molecular ion peak, but showed significant peaks at m/z 196 (M^+ - C_6H_5COOH), 178, 122 (base peak, $C_6H_5COOH^+$), 105 ($C_6H_5CO^+$), and 77 ($C_6H_5^+$). The ^1H- and ^{13}C-NMR spectra of 4 are similar to those of 2 except for the appearance of signals due to a benzoylmethylene grouping instead of the secondary methyl group in 2 (Table 1). Thus the structure of paeonilactone-C was assigned to the formula 4.

The absolute configuration of paeonilactone-A, -B, and -C was determined as 2, 3, and 4, respectively, based on the fact that all of these compounds showed the negative Cotton effect due to the optically active ketone chromophore in the CD spectra in MeOH (CD maximum: 2, $[\theta]_{292}$ -2450; 3, $[\theta]_{293}$ -3110; 4, $[\theta]_{295}$ -3340). Octant projection of the structures 2a and 3a reasonably supports these assignments, which are parallel with the absolute configuration of paeoniflorigenone (1).

Paeonilactone-C (4) was found to suppress both directly and indirectly stimulated muscle twitchings of frog sciatic nerve-sartorius muscle preparations at 100 μg/ml.[3] Biological activities of the other two compounds are now under investigation.

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