ABSOLUTE STEREOSTRUCTURES OF PAEONISOTHUJONE, A NOVEL SKELETAL MONOTERPENE KETONE, AND DEOXYPAEONISUFFRONE, AND ISOPAEONISUFFRAL, TWO NEW MONOTERPENES, FROM MOUTAN CORTEX

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Three new labile monoterpenes named paeonisothujone, deoxypaeonisuffrone, and isopaeonisuffral were isolated from Chinese Moutan Cortex, the root cortex of *Paeonia suffruticosa* ANDREWS. The absolute stereostructures of paeonisothujone, deoxypaeonisuffrone, and isopaeonisuffral were elucidated on the basis of chemical and physicochemical evidence which included the application of the modified Mosher's method. Paeonisothujone is the first natural example of ortho-menthane type monoterpene having a cyclopropane ring.

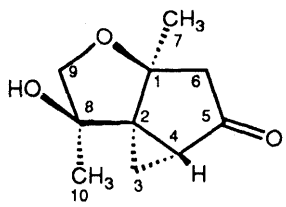
KEYWORDS Moutan Cortex; *Paeonia suffruticosa*; Paeoniaceae; paeonisothujone; deoxypaeonisuffrone; isopaeonisuffral

In search of bioactive constituents of Moutan Cortex,[1] the dried root cortex of *Paeonia suffruticosa* ANDREWS (Paeoniaceae, Botanpi in Japanese), we have reported the isolation of two new monoterpenes named paeonisuffrone and paeonisuffral from Chinese Moutan Cortex, which is now in common use in Chinese medicinal preparations in Japan, and elucidated their absolute stereostructures.[2] In a continuing study, we isolated three new labile monoterpenes designated as paeonisothujone(**1**), deoxypaeonisuffrone(**5**), and isopaeonisuffral(**7**) from the same Chinese Moutan Cortex. This paper deals with the evidence for the absolute stereostructures of paeonisothujone(**1**), deoxypaeonisuffrone(**5**) and isopaeonisuffral(**7**).[3]

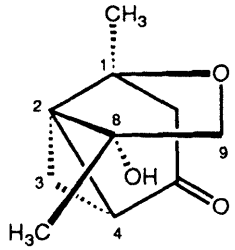
The acetone extract(prepared below 25°C)[4] of the cortex was subjected to ordinary and reversed phase silica gel column chromatography and HPLC to furnish **1**(0.0008%, from the crude drug), **5**(0.0001%), and **7**(0.0001%) together with paeoniflorigenone(**8**, 0.0012%),[5] paeonisuffrone(0.0011%),[2] paeonisuffral(**9**, 0.0004%),[2] and 3-*O*-methylpaeonisuffral (0.0001%).[2]

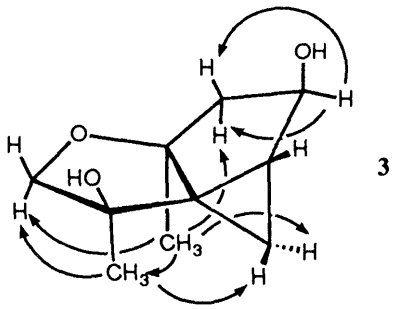
Paeonisothujone(**1**), a white powder, $[\alpha]_D$ -15.1°(MeOH), $C_{10}H_{14}O_3$, IR(KBr, cm^{-1}): 3430, 1721, 1383, 1030, showed the quasimolecular ion peak at m/z 183$(M+H)^+$ in its positive FAB-MS spectrum. The ^1H NMR(CD_3OD) spectrum of **1** indicated the presence of a cyclopropane ring [δ 1.37(dd, J= 3.7, 5.5, 3-*endo*-H), 1.54(ddd, J= 1.3, 5.5, 9.6, 3-*exo*-H), 1.98(br dd, J= 3.7, 9.6, 4-H)] adjacent to ketone, one methylene [δ 2.32(br d, J= 19.2, 6α-H), 2.45(dd, J= 1.3, 19.2, 6β-H), one oxymethylene[δ 3.92, 4.01(ABq, J= 9.9, 9-H_2)] and two tert. methyl[δ 1.31(s, 7-H_3), 1.40(s, 10-H_3)] functions. Furthermore, the ^{13}C NMR study of **1**(Table I) exhibited the signals due to a ketone carbonyl, two quart. carbons bearing oxygen function, and quart. carbon; it also showed the characteristic ^{13}C-^1H coupling constants of cyclopropane ring[164.1(C-3), 171.8(C-4)Hz]. The ortho-menthane skeleton with a cyclopropane ring of **1** was confirmed from the HMBC experiment. Namely, long-range correlations were observed between carbons and protons of **1** (1-C & 7-H_3; 2-C & 7-H_3, 10-H_3; 8-C & 10-H_3).

Acetylation of **1** with Ac_2O-pyridine-4-dimethylaminopyridine(DMAP) at room temperature afforded an aromatic compound **2**, $C_{14}H_{18}O_5$, IR(KBr): 1744, 1464, 1375, 1211, ^1H NMR($CDCl_3$, δ): 1.61(s, 10-H_3), 2.07(s, 9-OAc), 2.29(s, 5-OAc), 2.56(s, 7-H_3), 4.27, 4.50(ABq, J= 11.6, 9-H_2), 6.88(dd, J= 2.3, 9.2, 4-H), 6.90(d, J= 2.3, 6-H), 7.41(d, J= 9.2, 3-H), EI-MS: 266(M^+), which may be formed *via* enolization of 5-keto group followed by cleavage of cyclopropane and 1, 9-oxide rings in **1**, suggesting the ortho-menthane skeleton with a cyclopropane ring of **1**. By treatment of **1** with $NaBH_4$ in EtOH, two reduction products (**3** and **4**) were obtained in a 1:1 ratio : **3**, $[\alpha]_D$ +6.1°(EtOH), $C_{10}H_{16}O_3$, IR(KBr, cm^{-1}): 3330, 1458, 1379, 1116, ^1H NMR(CD_3OD, δ): 0.39(dd, J= 4.3, 6.0, 3-*endo*-H), 0.93(dd, J= 6.0, 8.9, 3-*exo*-H), 1.16(s, 7-H_3), 1.32(s, 10-H_3), 1.55(br dd, J=4.3, 8.9, 4-H), 1.73(dd, J= 5.9, 15.7, 6α-H), 2.08(ddd, J= 1.0, 1.6, 15.7, 6β-H), 3.99(s, 9-H_2), 4.12(dd, J= 1.0, 5.9, 5-H), positive FAB-MS: m/z 185$(M+H)^+$, and **4**, $[\alpha]_D$ -20.0°(EtOH), $C_{10}H_{16}O_3$, IR(KBr, cm^{-1}): 3330, 1458, 1379, 1016, ^1H NMR(CD_3OD, δ): 0.87(2H, m, 3-H_2), 1.18(s, 7-H_3), 1.32(s, 10-H_3), 1.33(dd, J=6.9, 14.9, 6α-H), 1.71(ddd, J= 5.6, 5.6, 7.9, 4-H), 2.34(ddd, J= 8.2, 14.9, 6β-H), 3.84, 3.91(2H, ABq, J= 9.9, 9-H_2), 4.62(ddd, J= 5.6, 6.9, 8.2, 5-H), EI-MS: m/z 184(M^+). As shown in the Figure, the NOE correlations were observed in the following pairs of protons[7-H_3 & 3-*endo*-H; 7-H_3 & 6α-H; 7-H_3 & 9α-H; 7-H_3 & 10-H_3; 10-H_3 & 9α-H; 10-H_3 & 3-*exo*-H] in the difference NOE experiments



paeonisothujone (1)

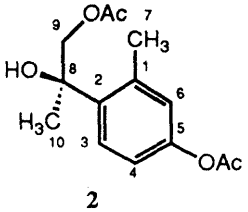




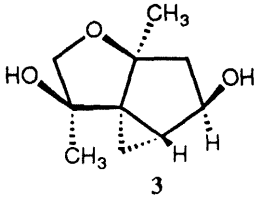
3

Ac$_2$O / pyridine / DMAP

NaBH$_4$ / EtOH



2



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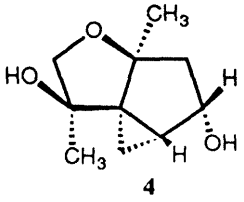

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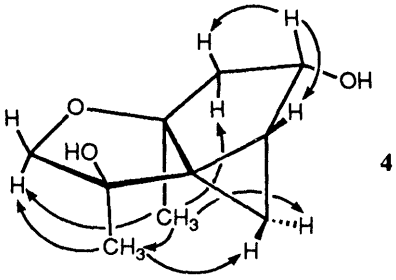
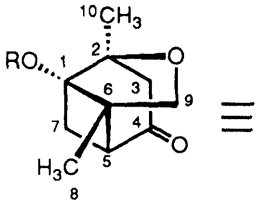
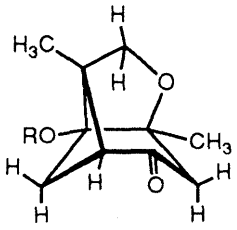


4

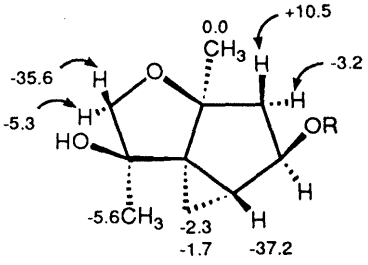
difference NOE experiments of 3 and 4





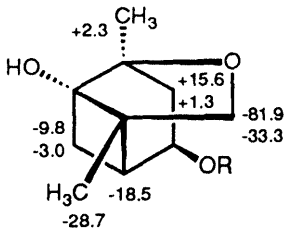
deoxypaeonisuffrone (5) : R = H

5a : R = Ac



3a : R = (-)-(*S*)-MTPA

3b : R = (+)-(*R*)-MTPA

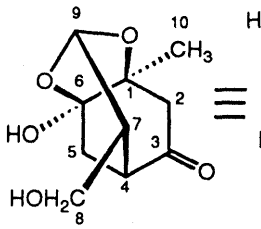


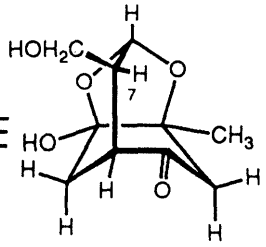
6 : R = H

6a : R = (-)-(*S*)-MTPA

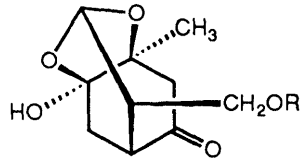
6b : R = (+)-(*R*)-MTPA

Δδ values in Hz(= δS-δR, measured in 270 MHz)





isopaeonisuffral (7)



paeoniflorigenone(8): R = Bz

paeonisuffral(9, keto form): R = H

Table I. ^{13}C NMR Data for 1, 3, 4, 5, 5a, 6, and 7

	1[a]	3[a]	3[b]	4[a]	4[b]	5[a]	5[b]	5a[b]	6[a]	6[b]	7[b]
1	87.0	93.0	91.8	91.9	90.7	81.7	77.2	85.4	81.9	81.3	79.3
2	54.5	50.2	48.8	49.3	49.8	87.2	85.1	85.6	90.3	88.3	48.8
3	14.6	9.3	9.2	8.2	8.1	50.2	48.4	49.1	45.6	45.2	214.0
4	34.5	31.4	30.3	29.2	28.2	213.3	210.9	209.3	70.8	70.3	47.6
5	213.8	75.0	74.1	72.5	72.1	54.1	52.5	54.8	44.0	42.7	47.1
6	50.5	49.5	46.7	47.7	47.5	58.7	57.2	56.8	54.3	53.6	32.4
7	20.7	20.9	19.5	21.2	20.7	31.4	30.6	31.5	30.5	30.2	47.6
8	75.7	76.2	75.7	76.1	75.8	15.6	15.2	15.6	16.1	15.5	61.5
9	80.8	80.9	80.5	80.4	79.9	74.0	73.0	72.9	72.6	71.7	101.4
10	26.5	25.9	25.6	26.3	25.6	19.7	19.3	20.1	20.8	20.4	21.9

a) were measured in CD_3OD, b) were in $CDCl_3$.

for **3** and **4**. Based on these findings, the relative stereostructure of **1** was elucidated; also, detailed ^{1}H NMR examination of **3** and **4** including NOE observation between the following proton pairs in **3**[5-H & 6α-H; 5-H & 6β-H] and **4** [5-H & 6β-H; 5-H & 4-H] led us to conclude the structures of **3** and **4**.

The absolute stereostructure of **1** was determined by the application of the modified Mosher's method.[6] Treatment of **3** with (-)-(*S*)- and (+)-(*R*)-α-methoxy-α-trifluoromethylphenylacetic acid(MTPA) and dicyclohexylcarbodiimide(DCC) in CH_2Cl_2 in the presence of DMAP furnished the (-)-(*S*)-MTPA ester(**3a**) and (+)-(*R*)-MTPA ester(**3b**), respectively. The signals due to protons on the C-3, 4, and 9 of **3a** appeared at higher field than those of **3b**, while the 6β-H signal[7] of **3a** was observed at lower field than that of **3b**, so that the absolute configuration at the C-5 has been presumed to be *R* orientation. In addition, the absolute stereostructure of **1** was concluded by the application of the front octant rule of CD spectrum for α-cyclopropyl-ketone derivative.[8] Namely, the CD spectrum of **1** showed a positive Cotton effect ($[\theta]_{283}$ +16600). Based on this evidence, a novel skeletal monoterpene structure of paeonisothujone(**1**) was elucidated as shown.

Deoxypaeonisuffrone(**5**), white powder, $[\alpha]_D$ -30.0°(MeOH), showed the absorption bands due to hydroxyl(3424 cm^{-1}) and ketone(1725 cm^{-1}) functions in its IR spectrum. The EI-MS of **5** showed the molecular ion peak at m/z 182$(M)^+$, and the high-resolution MS measurement of **5** revealed the molecular formula to be $C_{10}H_{14}O_3$. The ^1H NMR(CD_3OD, δ) and ^{13}C NMR data of **5** showed signals ascribable to two tert.-methyls [1.27(s, 8-H_3), 1.33(s, 10-H_3)], one methine [2.53(dd, J= 1.0, 6.6, 5-H)], two methylenes [2.19(d, J=8.3, 7α-H), 2.30(dd, J= 1.0, 17.5, 3β-H), 2.40(ddd, J= 1.3, 6.6, 8.3, 7β-H), 2.90(dd, J= 1.3, 17.5, 3α-H)], and one oxymethylene[3.57(2H, s, 9-H_2)] together with one carbonyl, two quart. carbons bearing oxygene function and one quart. carbon.

Acetylation of **5** with Ac_2O-pyridine-DMAP afforded the monoacetate(**5a**), $[\alpha]_D$ -23.6°(EtOH), $C_{12}H_{16}O_4$, IR(KBr, cm^{-1}): 1732, 1717(sh), 1240, 1021, ^1H NMR($CDCl_3$, δ): 1.33(s, 8-H_3), 1.36(s, 10-H_3), 2.14(s, OAc), 2.49(dd, J=1.0, 17.5, 3β-H), 2.64(ddd, 1.3, 6.9, 11.8, 7β-H), 2.74(d, J= 11.8, 7α-H), 2.75(dd, J=1.0, 6.9, 5-H), 2.89(dd, J= 1.3, 17.5, 3α-H), 3.66, 3.71, (ABq, J=9.9Hz, 9-H_2), positive FAB-MS : m/z 225$(M+H)^+$. The connectivities of the quart. carbons(C-1, 2, 6) were clarified by HMBC experiment on **5a**. Namely, long-range correlations were observed between the following carbons and protons (1-C: 7-H_2, 8-H_3, 10-H_3; 2-C; 3-H_2, 10-H_3; 6-C: 5-H, 9-H_2, 8-H_3). Furthermore, the NOE correlations were observed in the pairs of protons in **5**[9-H_2 & 3β-H, 9-H_2 & 8-H_3; 3-H_2 & 10-H_3; 7β-H&8-H_3]. Finally, comparison of the ^1H NMR and ^{13}C NMR(Table) data for **5** and **5a** with those for paeonisuffrone and its derivatives[2] led us to formulate the stereostructure of **5**.

The absolute configuration of **5** was determined by the application of the modified Mosher's method.[5] Thus, reduction of **5** with $NaBH_4$ provided **6**, $[\alpha]_D$ -16.4°(MeOH), $C_{12}H_{16}O_3$, IR(KBr, cm^{-1}): 3222, 1267, 1138, 1013, ^1H NMR(CD_3OD, δ): 1.16(s, 8-H_3), 1.23(s, 10-H_3), 1.52(d, J=9.9Hz, 7α-H), 1.78(dd, J= 1.0, 15.5, 3β-H), 1.96(dd, J=6.6, 9.9, 7β-H), 2.15(ddd, 1.0, 4.3, 6.6, 5-H), 2.26(dd, J=7.6, 15.5, 3α-H), 3.40, 4.54(both d, J=8.1Hz, 9-H_2), 4.07(dd, J= 4.3, 7.6, 4-H), EI-MS: m/z 184(M^+), which was derivated to the (-)-(*S*)-MTPA ester(**6a**) and (+)-(*R*)-MTPA ester(**6b**). The signals due to protons on C-3 and C-10 in the (+)-(*R*)-MTPA ester(**6b**) appeared at higher fields than those of the (-)-(*S*)-MTPA ester(**6a**), while the signals due to protons attached to C-5, 7 and 9 of **6b** were observed at lower fields compared to those of **6a**. Consequently, the absolute configuration at C-4 has been elucidated to be *R*, and the absolute structure of deoxypaeonisuffrone(**5**) was determined as shown.

Isopaeonisuffral(**7**), a white powder, $[\alpha]_D$ -27.3°(MeOH), $C_{10}H_{14}O_5$, IR(KBr, cm^{-1}): 3423, 1721, 1123, ^1H NMR(CD_3OD, δ): 1.22(s, 10-H_3), 1.74(br dd, J= 7.6, 7.6, 7-H), 2.08(dd, J= 2.3, 13.9, 5α-H), 2.30(dd, J= 3.3, 13.9, 5β-H), 2.53(d, J= 16.8, 2β-H), 2.64(m, 4-H), 2.84(d, J= 16.9, 2α-H), 3.53, 3.72(both dd, J= 7.6, 11.0, 8-H), 5.33(br s, 9-H), showed the quasimolecular ion peak at m/z 213$(M-H)^-$ in the negative FAB-MS spectrum. The ^1H NMR and ^{13}C NMR(Table) of **7** were fairly similar to those of paeoniflorigenone(**8**)[5] and the keto form of paeonisuffral(**9**).[2] The observation of NOE correlations between following protons of **7** (8-H_2 & 4-H, 8-H_2 & 5β-H) indicated the relative stereostructure of **7**. Finally, the CD spectrum of **7** showed a negative Cotton effect($[\theta]_{292}$ -3900), which is correspondent to the CD spectra for paeoniflorigenone(**8**, $[\theta]_{295}$ -6900) and paeonisuffral(**9**, $[\theta]_{294}$ -1100), so that the absolute stereostructure of isopaeonisuffral (**7**) was elucidated as shown.

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