Studies on the diterpenoid constituents from Rabdosia angustifolia

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Abstract Two new ent-kaurene diterpenoids, angustifolin B and natural hydrogen-bonded 1:1 complex, angustifolin C, as well as four known diterpenoids, angustifolin, isodonal, sodoponin, trichorabdal B, were isolated from the leaves of Rabdosia angustifolia.

Keywords Rabdosia angustifolia, angustifolin B, angustifolin C, diterpene

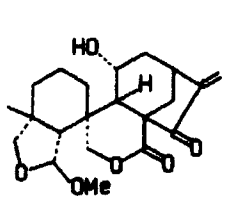
Introduction

Rabdosia angustifolia (Dunn) Hara is distributed mainly over Yunnan, China. It is used by Chinese people against pyrexia, abdominal distension and inflammation. From its dried leaves, angustifolin (**1**), isodonal (**2**), β-sitosterol, were isolated previously.[1] As a continuation for biological active constituents of the same plant, the other six constituents, sodoponin (**3**),[2] trichorabdal B (**4**),[3] cirsiliol,[4] daucosterol as well as two minor ent-kaurene diterpenoid constituents: angustifolin B (**5**) and a hydrogen-bonded 1:1 complex angustifolin C (**6**) were afforded. This paper reports mainly the structure elucidation of two new compounds (**5, 6**) by detailed spectroscopic evidence and X-ray analysis.

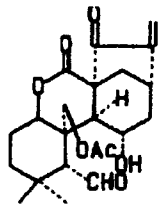
Results and discussion

An alcohol extract of dried Rabdosia angustifolia leaves was fractionated by column chromatography on silica gel. Further purifications of **1**—**6**, six diterpenoids, were achieved by recrystallization, column chromatography (silica gel, sephadex-LH20, Rp-8) and preparative HPLC.

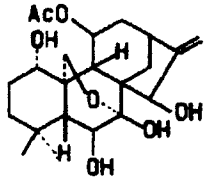
[0]Received July 29, 1996; revised August 18, 1997.



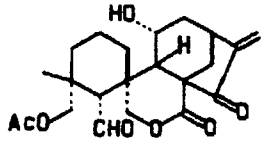
angustifolin(1)



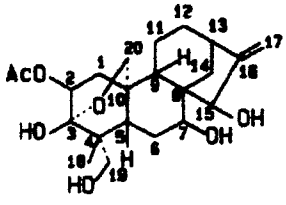
isodonal(2)



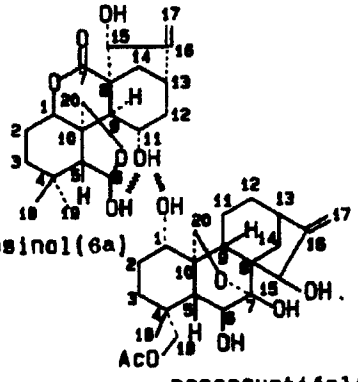
sodoponin(3)



trichorabdal B(4)



angustifolin B(5)



epinodosinol(6a)

neoangustifolin(6b)

angustifolin C

Angustifolin B (**5**), $C_{22}H_{32}O_7$, ([M^+] at m/z 408), colourless crystal, was shown to have four hydroxys [δ_H: 8.35(d, J=5.2 Hz, 1H), 8.02(s, 1H), 7.18(s, 1H), 5.93(d, J=3.9 Hz, 1H), which disappeared on addition of D_2O]; an exo-methylene group [ν_{max}(KBr): 1658 cm^{-1}; δ_H: 5.50, 5.19(ABs, each 1H); δ_C: 162.3(s), 107.1(t)]; an acetal carbon [δ_C: 97.9(s)]; an acetoxy group [δ_C: 170.9(s), 20.7(q); δ_H: 2.00(s, 3H)]; three oxygenated methines [δ_H: 3.78(br.s, 1H), 4.48(t, J=3.2 Hz, 1H), 5.52(s, 1H); δ_C: 64.9(d), 73.8(d), 75.4(d)]; two oxygenated methylenes [δ_H: 4.85, 4.55(ABd, J=11.2 Hz, each 1H), 4.29, 4.08(ABd, J=9.8 Hz, each 1H); δ_C: 66.4(t), 67.3(t)]; as well as additional presence of three methines, five methylenes, three quaternary carbons and one tertiary methyl (see Table 1).

Above mentioned evidence suggested that **5** contains neither a δ-lactone nor a five membered ring hemiacetal, which are usually found in the B-secokaurene-type diterpenoids, but does bear a tertiary hydroxy group. Thus, it was assumed that **5** may have a kaurene-type 7-hemiketal structure or kaurene-type 3-hemiketal structure.

Table 1 ^1H, ^{13}C NMR data of angustifolin B (**5**) in C_5D_5N

No.	H	C	No.	H	C
1		32.6(t)	11		15.1(t)
2	3.78(1H, br, s)	64.9(d)	12		27.4(t)
3		97.9(s)	13	2.69(1H, dd, 4.6, 8.7)	37.3(d)
4		41.2(s)	14		27.4(t)
5	2.52(1H, d, 5.2)	54.4(d)	15	5.25(1H, s)	75.4(d)
6	1.97(overlap)	28.6(t)	16		162.3(s)
7	4.48(1H, t, 3.2)	73.8(d)	17	5.50, 5.19(2H, ABs)	107.1(t)
8		52.0(s)	18	1.37(3H, s)	27.2(q)
9	3.31(1H, dd, 5.6, 11.6)	36.7(d)	19	4.85,4.55(2H, ABd, 11.2)	67.3(t)
10		37.8(s)	20	4.29,4.08(2H, ABd, 9.8)	66.4(t)
			OAc	2.00(3H, s)	170.9(s)
					20.7(q)

Assignments are based on ^1H-^1H, ^{13}C-^1H COSY and NOESY measurements.

The second assumed frame was confirmed reasonably as follows: a) δ_C: 36.7(d), which was assigned as 9-C according to ^1H NMR spectrum [δ_H: 3.31(dd, J=5.6, 11.6 Hz, 1H, 9β-H)], and observation of NOE's between 5β-H [δ_H: 2.52(d, J=5.2 Hz, 1H)] and 9β-H, is abnormally upfield shift because of the synergism of γ-gauche effect from both 7β-OH and 15β-OH. This evidence suggested that carbon-7 is bearing a hydroxy group. b) The triplet signal [δ_H: 4.48(t, J=3.2 Hz, 1H)], which is caused by the coupling with 6-H_2, was attributable to 7α-H. c) The lowfield shifts of C-4 [δ_C 41.2(s)] is similar to coetsoidin A.[5] d) The signal appearing as a doublet at 4.29(d, J=9.8 Hz, 1H) was firmly supported by the W-coupling of 20-Ha with 5β-H.[6]

Alternatively, on the second assumed frame, the other hydroxy group was settled at 19-C, because the chemical shift, the coupling pattern and the coupling constants of 19-H_2 [δ_H: 4.85, 4.55(ABd, J=11.2 Hz, 2H)], and the observation of NOESY between 18-CH_3 and 5β-H, explained satisfactorily the hydroxy at 19-C. The acetoxy group was supposed to be presented at 2-C with β-configuration, considering the data δ_C 64.9(d), δ_H 3.78(br.s, 1H); the coupling signal of 2α-H and 1α-H from the ^1H-^1H COSY spectrum and the upfield shift of 1-C [32.6(t)] due to spatial effect of 2β-OAc.

Accordingly, the foregoing facts established that angustifolin B (**5**) was ent-3α,7α,15-α,19-tetrahydroxy-2α-acetoxy-3β,20-epoxy-kaur-16-ene.

Angustifolin C (**6**), colourless crystal, $[\alpha]_D^{19}$=−46.5°(c 0.16, MeOH), which was shown to be homogeneous on TLC and HPLC analysis, was shown to have forty-one carbon atoms in the ^{13}C NMR spectrum.

Closer examination of the NMR spectra disclosed that they were derived from the presence of two diterpene substrates in the same abundance. A single-crystal X-ray analysis (Fig. 1) of angustifolin C (**6**) revealed that it consisted of epinodosinol (**6a**) and a new diterpene designated as neoangustifolin (**6b**, Fig. 2), 19-acetoxy-1α,6β,7β,15β-tetrahydroxy-7α,20-epoxy-ent-kaur-16-ene.

Although there is some overlap of resonance in the NMR spectra of angustifolin C, all of the ^1H and ^{13}C NMR data could be unambiguously assigned (Table 2) by a combination of DEPT, ROESY, HETCOR and FLOCK, *etc.*, NMR experiments.

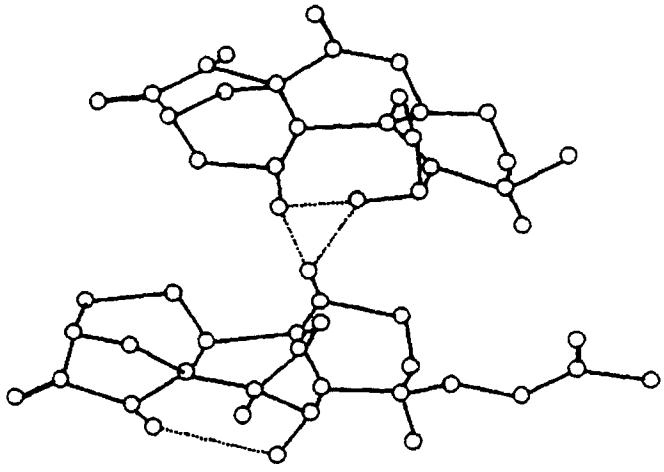


Fig. 1 X-Ray structure of **6** showing the binding site and inter- and intramolecular hydrogen bonds with dotted line.

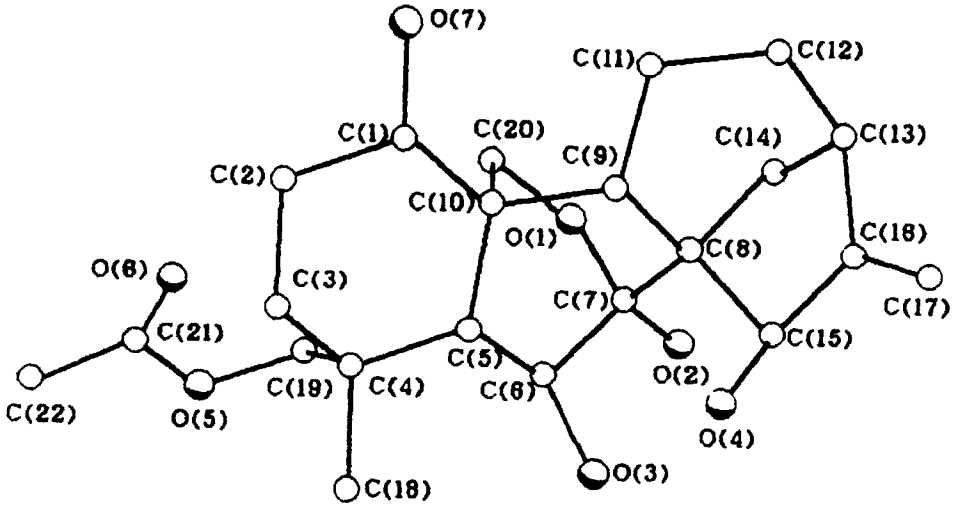


Fig. 2 ORTEP drawing of the crystal structure of neoangustifolin (**6b**).

X-ray crystallography of angustifolin C (**6**): colorless crystals, $C_{42}H_{60}O_{13}$, MW= 772.93, monoclinic, space group C_2, with a=2.1893(5) nm, b=0.6496(1) nm, c=2.8561(6) nm, β=73.65(2)°, V=3.89821 nm^3, Z=4. Collection and processing were carried out on a R3m/E four-circle diffractometer, graphite monochromated Cu K_α X-radiation in the range $0°<\theta<57°$, W/2Q scan.

Table 2 ^1H, ^{13}C NMR data of angustifolin C (**6**) in C_5D_5N

Carbon	^1H		^{13}C	
	6a	**6b**	**6a**	**6b**
1	4.79(t,6.5)	3.77(dd,5.0,11.0)	76.4	73.5
2	1.84(m)	1.82(m)	30.2	24.3
3	1.18(m)	1.83(m,α);1.17(m,β)	37.2	33.7
4			31.7	37.6
5	3.11(br.s)	1.84(br,s)	54.3	58.9
6	5.66(br.s)	4.36(d,7.0)	102.2	73.8
7			175.2	97.3
8			53.3	52.7
9	3.53(d,9.5)	2.63(m)	46.3	43.7
10			51.0	41.5
11	4.38(ddd,11.5,9.5,8.5)	1.93(m),2.29(m)	63.0	19.1
12	1.87(m),2.90(m)	1.58(m),2.23(m)	45.6	33.1
13	2.71(m)	2.60(m)	37.1	37.1
14	1.67(m)	2.04(m)	34.5	26.9
15	5.21(br.s)	5.13(br s)	77.7	75.3
16			158.1	162.3
17	5.44(br.s),5.17(br.s)	5.42(br.s),5.17(br.s)	108.5	106.9
18	0.93(s)	1.31(s)	33.1	27.0
19	0.94(s)	4.85,4.46(ABd,11.0)	23.2	66.4
20	4.18,4.13(ABd,11.0)	4.82,4.31(ABd,11.0)	73.2	64.2
-OAc		1.91(s)		170.8
				20.7

Experimental

Mps were uncorrected. ^1H and ^{13}C NMR were recorded at 400 and 100.16 MHz, respectively, using TMS as internal standard. IR was taken for KBr disc. Optical rotations were recorded on SEPA-300 with 2 cm cell. FABMS was measured with VG Auto spec-3000 mass spectrometer.

Plant material: The air-dried leaves of Rabdosia angustifolia were collected in Lijiang, Yunnan, China, in Sept. 1986, and were identified by Prof. H. W. Li of Kunming Institute of Botany where a voucher specimen has been deposited.

Extraction and isolation: Dried and powdered leaves (4.0 kg) were extracted with 95% alcohol. After evaporation of the alcohol, the residue was extracted with petroleum, ether, AcOEt, *n*-BuOH, respectively. The extract of AcOEt fraction, which was evaporated to give a residue 70 g, gave by column chromatography (silica gel, sephadex-LH20) and HPLC (partisil-ODS), to afford, **1**(240 mg), **2**(536 mg), **3**(425 mg), **4**(46 mg), **5**(18 mg), **6**(64 mg), respectively.

Angustifolin B (**5**), colourless crystal from acetone, mp 213—215°C, $[\alpha]_D^{19}=-46.5°$ (*c* 0.12, MeOH). ν_{max}(KBr): 3464, 3380—3340, 3268, 1735, 1658 cm^{-1}. *m/z*: 408 [M^+], 390[$M-H_2O$], 362, 348, 330, 312, 299, 269, 255, 199, 105, 95, 69, 55, 43(base peak). Found: C, 64.15; H, 7.76; requires: C, 64.69; H, 7.90. ^1H, ^{13}C NMR data see Table 1.

Angustifolin C (**6**), colourless crystal from acetone, mp 225—228°C, $[\alpha]_D^{19}=-46.5°$ (*c* 0.16, MeOH). λ_{max}(MeOH) 203 nm (ϵ 16050). ν_{max}(KBr) 3474, 3315, 1738, 1724,

1660, 1235 cm^{-1}. *m/z*: 408 [M^+_{6b}], 390 [$M^+_{6b}-H_2O$], 364[M^+_{6a}], 346[$M^+_{6a}-H_2O$], 330, 318, 302, 282, 256, 223, 199(base peak), 181, 168, 163, 149, 135, 107, 95, 81, 69, 57, 43. Found: C, 65.72; H, 7.91; requires: C, 65.27, H, 7.82. ^1H, ^{13}C NMR data see Table 2.

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