Bull. Inst. Chem. Res., Kyoto Univ., Vol. 67, No. 3, 1989

Terpenoids. LV The Structure and Absolute Configuration of Macrocalyxoformin E

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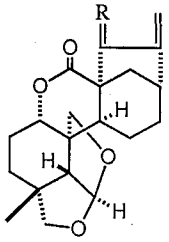
Received July 14, 1989

On the basis of chemical evidence and spectroscopic investigations, the structure and absolute configuration of macrocalyxoformin E, which had been isolated from *Rabdosia macrocalyx* (Dunn.) Hara form (Labiatae) were established as *ent*-6,20: 7,20α-diepoxy-6α, 15α-dihydroxy-7-oxo-6,7-seco-16-kaurene (**5**).

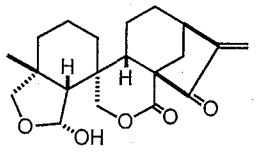
KEY WORDS: *Robdosia macrocalyx* (Dunn.) Hara form/ Diterpenoid/ Macrocalyxoformin E/ 6,7-Secokaurene/ A Ring Conformation/ Absolute configuration/ Hydride Shift/

From the leaves of *Rabdosia macrocalyx* (Dunn.) Hara *form* (Labiatae), five new diterpenoids, macrocalyxoformin A-E (**1-5**), have been isolated and their structures have been previously reported[2]. Among these, the structure of macrocalyxoformin E (**5**) has been speculated without sufficient chemical or spectral evidence, leaving ambiguity in the configuration of several functional groups. We reexamined the structure of macrocalyxoformin E and clarified the whole structure including the A ring conformation and the absolute configuration. Here, we describe the structure elucidation of macrocalyxoformin E.

In the previous report[2], it was speculated that macrocalyxoformin E (**5**) has an *ent*-6,7-seco-16-kaurene skeleton and, has an internal acetal group at C-20 form-



1 : R = O
2 : R = α-OH, β-H



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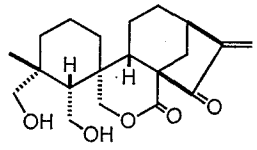

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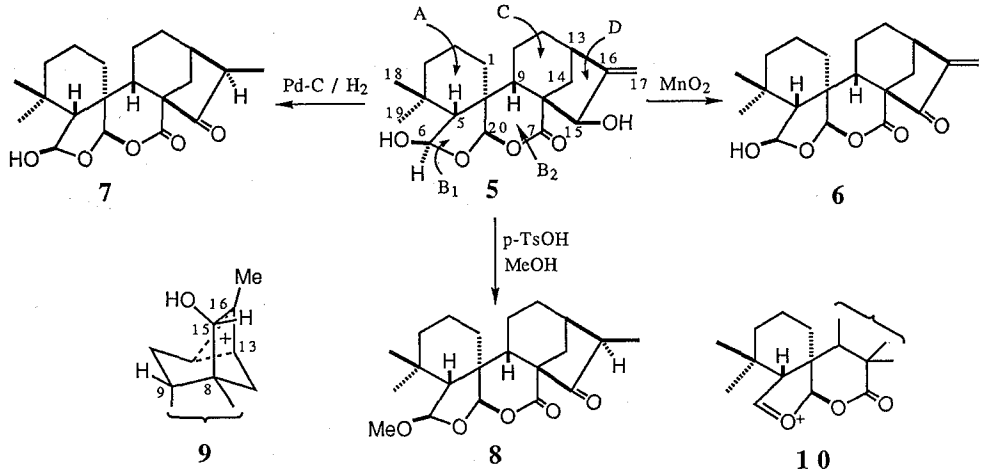
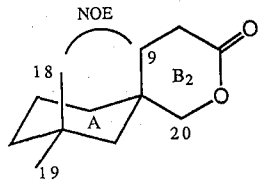
ing a lactone ring, a hemiacetal group at C-6, and an allyl alcohol on the D ring. However, there is no decisive evidence for the stereochemistry of the functional groups at C-6, C-15, and C-20 and for the absolute configuration of **5**. At first, we examined several chemical reactions to get some chemical evidence for the structures of macrocalyxoformin E. The oxidation of **5** with manganese oxide afforded α, β-unsaturated ketone **6**. This reaction proved the existence of an allylic alcohol system on the D ring. If the hydroxyl group of the allylic alcohol system is located on the endo (β)-side of the C/D ring system, the allylic alcohol system will be converted to the methyl ketone **7** by the Garryfoline-Cuauchichicine rearrangement[3] under acidic conditions. The reaction of **5** with *p*-toluenesulfonic acid in methanol caused the above rearragement, involving the stereoselective hydride shift from the exo-H at C-15 to the exo-H at C-16 through the nonclassical carbonium ion **9**, to give methyl ketone **8** with concomitant acetalization at C-6. Because of this rearrangement, the stereochemistry of the hydroxy group at C-15 was proved to be endo (β-configuration). Methyl acetalization under acidic conditions should proceed *via* the oxonium ion **10** which is attacked by the nuclepohile (MeOH) from the less hindered (β)-side to give the acetal **8**. Since the coupling constants of C-6-H in compounds **5** and **8** showed the same value (ca. 6 Hz) in ^{1}H-NMR spectra, the above speculation leads to a β-configuration for C-6-OH of **5**. Attempted catalytic hydrogenations of **5** afforded the keton **7** unexpectedly through the same type of rearrangement.[4]

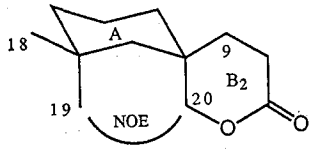


Next, we conducted the nuclear Overhauser enhancement (NOE) experiment on **5** to determine the configuration of C-20. Two kinds of ring A conformation (A and B) have been shown to exist in the *Rabdosia* diterpenoids of the spirolactone-type.[5] Each conformer is distinguishable from the other by the NOE between 18-H_3 and 9-H or between 19-H_3 and 20-H. The chemical shifts of the protons required for the NOE inspection is easily determined from the ^{1}H-^{1}H correlation spectroscopy (COSY) NMR spectrum (Fig. 1). The results of NOE experiment on **5** are

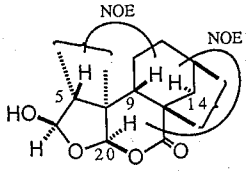
shown in Table 1. The NOEs (12% and 2%) between 18-H_3 and 9-H (run 1 and 4) indicate that the A ring of **5** exists in the A conformation. The NOEs between C-5-H and C-9-H, and between C-20-H and C-14-H are expected in the structure C, while the NOEs between C-5-H, C-9-H and C-20-H are also expected in the



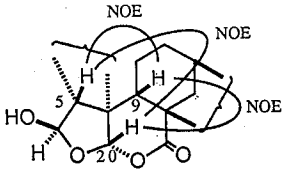
A



B



C



D

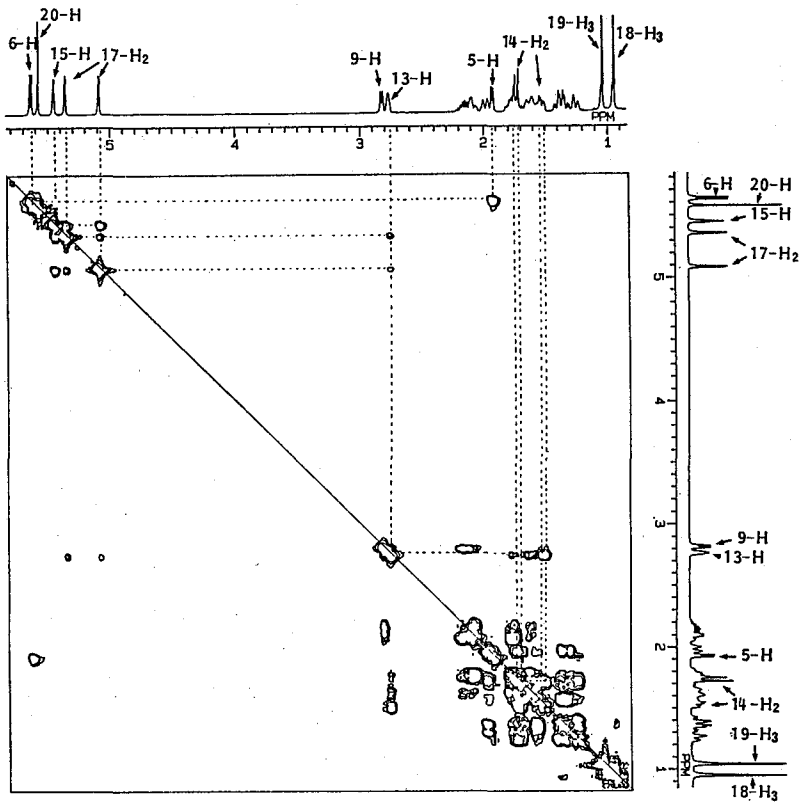


Fig. 1. ^1H-^1H COSY Spectrum of Macrocalyxoformin E(5) in $CDCl_3$-C_5D_5N(2: 1).

Table 1. NOE data for macrocalyxoformin E (**5**)[a]

run	Monitor proton (δ, ppm)	Observed proton[b] (Intensity increase)		
1	18-H_3	5-H,	9-H	
	(0.97)	(8.6%)	(11.7%)	
2	19-H_3	5-H,	6-H,	18-H_3
	(1.03)	(7.8%)	(13.7%)	
3	5-H	9-H,	18-H_3,	19-H_3
	(1.86)	(12.5%)	(1.2%)	(1.4%)
4	9-H	5-H,	18-H_3	
	(2.75)	(14.3%)	(1.8%)	
5	20-H	14α-H		
	(5.50)	(6.7%)		
6	14α-H	20-H,	13-H	
	(1.71)	(9.8%)		

a, Spectra at 400 MHz NMR; solution in $CDCl_3$-C_5D_5N (2:1).
b, The existence of the observed protons without any % of intensity increase was indicated by the subtractive spectra from the NOE experiment.

structure D. The NOE experiment in Table 1 (run 3–6) coincides with the former structure C. Thus, the relative configuration of all functional groups in the structure **5** was established.

Finally, in order to determine the absolute configuration of macrocalyxoformin E, the circular dichroism (CD) spectrum of its rearranged product **9** was measured. On the basis of a negative Cotton effect at 306 nm on the CD curve[6], the absolute configuration of macrocalyxoformin E was established as that shown as **5**. Consequently, the structure of macrocalyxoformin E should be represented as *ent*-6, 20:7,20α-diepoxy-6α,15α-dihydroxy-7-oxo-6,7-seco-16-kaurene (**5**).

EXPERIMENTAL SECTION

The melting points were determined on a Yanagimoto melting pionts apparatus and are uncorrected. Infrared (IR) spectra were recorded with a JASCO IR-810 spectrophotometer, and ultraviolet (UV) spectra were taken with a JASCO UVI-DEC-610C spectrophotometer. Optical rotations were measured with a JASCO DIP-181 digital polarimeter, and CD spectra were measured with a JASCO J-600 spectropolarimeter. Mass spectra (MS) and the fast atom bombardment mass spectra (FAB-MS) were determined on a JEOL JMS-DX300 mass spectrometer. ^{1}H-NMR spectra and ^{13}C-NMR spectra were measured with a JEOL GX-400, Varian VXR-200, and JEOL FX-100, and recorded in δ values with tetramethylsilane as an internal reference. Kieselgel 60 (0.05–0.2 mm, Merk) was used for column chromatography and precoated silica gel plates F_{254} (0.25 and 0.5 mm in thickness) were used for thin layer chromatography (TLC).

Isolation—Dried leaves (5Kg) of *R. macrocalyx* (Dunn.) Hara form collected in

the southern district of Anhui (China) in August, 1985, were extracted with ethanol (40 l) under reflux for 3h. The plant material was further extratced 2 times in the same manner. The combined ethanol extract was concentrated under reduced pressure to 20 l. The extract was treated with activated charcoal (350 g), and evaporated under reduced pressure to give a sirupy residue which was dissolved in boiling ethylacetate (8 l). The ethylacetate solution was shaken with 5% aqueous Na_2CO_3 (2 l) to remove any acidic substances. After being washed with water and dried with anhydrous Na_2SO_4, the ethylacetate extract was concentrated under reduced pressure to sirupy residue (200 g), which was chromatographed on a silica gel (1.2 Kg) column with chloroform-acetone. The eluates (1.5 g) from choroform-acetone (9:1) were rechromatographed on silica gel (100 g) with dichloromethane-acetone as eluants. The eluates (500 mg) from dichloromethane-acetone (9:1) was recrystsllized from methanol to give macroclyxoformin E (**5**) (100 mg).

Macrocalyxoformin E (**5**); colorless needless, mp 242-244°C, $[\alpha]_D^{20}$-164.5° (c=0.23, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3490, 3400, 1740, 1665, 1055, 1000. ^{1}H-NMR (GX-400, $CDCl_3$: C_5D_5N=1:1) δ: 0.95, 1.04 (each 3H, s, 18-H_3 and 19-H_3), 2.76 (1H, br.s, 13-H), 2.81 (1H, d, J=6.8, 9-H), 5.08 (1H, d, J=2.4, 17-Ha), 5.35 (1H, br.s, 15-H), 5.45 (1H, br.s, 17-Hb), 5.57 (1H, s, 20-H), 5.63 (1H, d, J=5.9, 6-H). ^{13}C-NMR (FX-100, C_5D_5N) δ: 19.3 (t), 20.9(t), 26.8(t), 27.3 (q, C-19), 30.1 (s), 30.9 (q, C-18), 33.0(t), 33.8(t), 34.0(t), 35.7(d), 39.9(d), 48.2(s), 51.9(s), 60.7(d), 75.6(d, C-15), 101.7 (d, C-20), 105.9(t, C-17), 107.2(d, C-6), 156.0(s, C-16), 173.6 (s, C-7). FAB-MS m/z: 371 (M^++Na). Anal. calcd for $C_{20}H_{28}O_5$: C, 68.96; H, 8.04. Found: C, 68.72; H, 8.18.

Oxidation of macrocalyxoformin E (**5**)———To a solution of macrocalyxoformin E (**5**) (10 mg) in acetone (3 ml) was added active MnO_2 (50 mg). The mixture was stirred for 4h at room temperature, then filtered and washed with acetone. After evaporation of the filtrate under reduced pressure, the residue was chromatographed on a preparative TLC plate with $CHCl_3$-Me_2CO (8:2) to afford **6** (5 mg). Recrystallization with methanol gave **6** as colorless prisms, mp 215–217°C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3570, 1765, 1720, 1645, 1300. UV λ_{max}^{MeOH} mμ: 235 (ε=7,200). ^{1}H-NMR (VXR-200, C_5D_5N)δ: 0.85, 1.08 (each 3H, s, 18-H_3 and 19-H_3), 2.55 (1H, d, J=6, 5-H), 3.06 (1H, br.s, 13-H), 5.30 (1H, s, 17-Ha), 5.62 (1H, s, 20-H), 5.68 (1H, d, J=6, 6-H), 6.05 (1H, s, 17-Hb). High MS Calcd for $C_{20}H_{26}O_5$(M^+): 346.1779. Found 346.1763.

Acidic Treatment of 5———To a solution of macrocalyxoformin E (**5**) (10 mg) in methanol (3 ml) was added *p*-toluenesulfonic acid (8 mg). The mixture was refluxed for 15h under nitrogen atmosphere and the mxiture was poured into ice-water, and extracted with CH_2Cl_2. After the usual treatment, the extract was concentrated *in vacuo* to give a residue which was chromatographed on a preparative TLC plate with $CHCl_3$-Me_2CO (9:1) to give **8** (8 mg). The product was recrystallized with MeOH, mp 196–198°C. CD (c=0.2, MeOH) $[\theta]^{25}$ (nm): −1, 450 (306) (negative maximum). IR $\nu_{max}^{CHCl_3}$ cm^{-1}:1770, 1726, 1250, 1100. ^{1}H-NMR (FX-100, $CDCl_3$) δ: 0.94 (6H, s, 18-H_3 and 19-H_3), 1.14 (3H, d, J=7, 17-H_3), 3.43 (3H, s, -OMe), 4.90 (1H, d, J=6, 6-H), 5.40 (1H, s, 20-H). High MS Calcd for $C_{21}H_{30}O_5$

(M^+): 362.2093. Found 326.2125.

Rearragement Reaction of 5———To a solution of macrocalyxoformin E (**5**) (10 mg) in methanol (3 ml) was added a catalytic amount of 10% Pd-carbon and the mixture was stirred under the atmospheric pressure of hydrogen at room temperature overnight. The catalyst was filtered off, and the filtrate was evaporated *in vacuo* to give a crystalline product **7** (9 mg), which was recrystallized from MeOH to give colorless prisms, mp 238–240°C, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3575, 1770, 1725, 1245, 1127, 982. ^{1}H-NMR (VXR-200, C_5D_5N) δ: 0.82, 1.10 (each 3H, s, 18-H_3 and 19-H_3), 1.08 (3H, d, J=7, 17-H_3), 5.67 (1H, s, 20-H), 5.72 (1H, d, J=6, 6-H). High MS Calcd for $C_{20}H_{28}O_5$ (M^+): 348.1925. Found 348.1919.

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