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ERICACEOUS CONSTITUENTS: SEVENTEEN TRITERPENOIDS ISOLATED FROM THE BUDS OF RHODODENDRON MACROCEPALUM

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From the bursting buds of Rhododendron macrocepalum, four triterpenoid mono-ols (**1**, **4-6**) and nine diols (**7-10**, **13-17**) including four new compounds were isolated and characterized together with **2**, **3** and **11**, **12**. The structures of two 2β,3β-diols (motidiol and germanidiol) hitherto reported are revised to 2α,3α-diols.

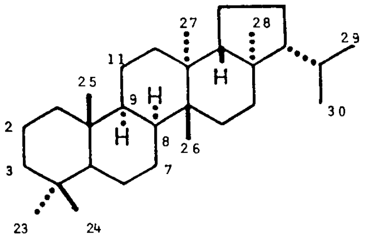
KEYWORDS——triterpenoid; olean-18-ene-2α,3α-diol; fern-7-ene-2α,3α-diol; olean-12-ene-2α,3α-diol; neohop-12-ene-2α,3α-diol; olean-12-ene-2α,3β-diol; lup-20(29)-ene-2α,3β-diol; adian-5-ene-2α,3α-diol; ^{1}H-NMR spectrum; Rhododendron macrocepalum

A Japanese wild azalea, Rhododendron macrocepalum Maxim. (R. linearifolium Sieb. et Zucc. var. macrocepalum Makino, Japanese name "Mochi-tsutsuji", Ericaceae) is one of the most common species in the central part of Honshû and Shikoku. The calyx of the bursting buds is very rich in mucilaginous hair. The triterpenoid components of the buds were first reported by Kariyone et al. (motiol, motic acid and ursolic acid)[1] and then by Hirata et al. (motiol, neomotiol, germanidiol, motidiol, adianenediol, fernenediol, epigermanidiol) with their structural elucidation.[2,3] This paper concerns reinvestigation of the same materials resulting in the isolation and characterization of seventeen compounds including six 3β-ols (**1-6**), two 3β-ol acids (**11**, **12**), five 2α,3α-diols (**7-10**, **17**) and four 2α,3β-diols (**13-16**) belonging to migrated hopane, oleanane, ursane and lupane groups as shown in Table I.[4]

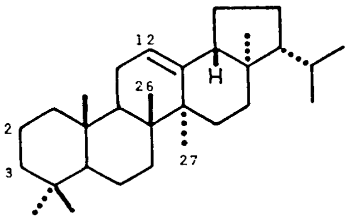
Table I. Triterpenoids Isolated from the Buds of Rhododendron macrocepalum

Compounds	Common name	Alcohols		lit.		Derived peracetates				lit.		Ref.
		mp°C	$[\alpha]_D$	mp°C	$[\alpha]_D$		Rt_R a)	mp°C	$[\alpha]_D$	mp°C	$[\alpha]_D$	
friedelan-3β-ol(**1**)	epifriedelanol	281-283	+23.5	265	+27	**1a**	4.97	>290	+40.5	299	+28	6)
olean-12-en-3β-ol(**2**)	β-amyrin	identified by GC-MS and ^{1}H-NMR spectrum										
ursan-12-en-3β-ol(**3**)	α-amyrin											
fern-7-en-3β-ol(**4**)	motiol	221-223	-28.7	218	-44	**4a**	5.32	262-263	-6.6	247	-3	2)
neohop-12-en-3β-ol(**5**)	neomotiol	227-228	+35.5	228	-24	**5a**	5.29	212-214	+35.8	234	-24	2,5)
fern-9(11)-en-3β-ol(**6**)	fernenol	193-194	-24.0	193	-19.4	**6a**	4.69	218-220	-9.4	223	-8.9	7)
olean-18-ene-2α,3α-diol(**7**)	germanidiol	275-277	+15.4	275	+36.8	**7a**	4.72	260-261	-6.0	264	+36.8	2,11)
olean-12-ene-2α,3α-diol(**8=A**)		286-288	+74.3	---	---	**Aa**	4.83	>290	+40.0	---	---	
fern-7-ene-2α,3α-diol(**9**)	motidiol	234-236	-23.7	233	-22.3	**9a**	7.09	259-260	-22.9	252	-12.2	2)
neohop-12-ene-2α,3α-diol(**10**)		244-245	+30.8	---	---	**10a**	7.06	233-235	+4.6	---	---	
3β-hydroxyolean-12-en-28-oic acid(**11**)	oleanolic acid	identified by GC-MS and ^{1}H-NMR spectrum										
3β-hydroxyursan-12-en-28-oic acid(**12**)	ursolic acid											
olean-12-ene-2α,3β-diol(**13**)		210-211	+79.4	---	---	**13a**	6.19	183-184	+29.9	---	---	
fern-9(11)-ene-2α,3β-diol(**14**)	fernenediol	202-204	-36.9	204	-34	**14a**	7.65	242-244	-52.6	240	-65	3)
lup-20(29)-ene-2α,3β-diol(**15**)		232-233	+18.6	---	---	**15a**	6.51	128-130	+5.4	---	---	
olean-18-ene-2α,3β-diol(**16**)	epigermanidiol	220-222	-2.0	221	-1.4	**16a**	6.25	171-172	-17.5	171	-13	3)
adian-5-ene-2α,3α-diol(**17**)	adianenediol	234-236	+2.3	231	+3	**17a**	7.32	222-223	-21.5	211	-30	2)

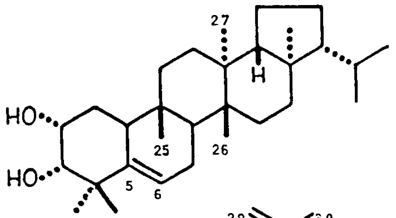
a) GC were run with a Hitachi 163 on Chromosorb G HP coated with SE-30 (1.4%) at 260°C in a flow of nitrogen. Cholestane was used as reference and its retention time was set at 3.5 min.



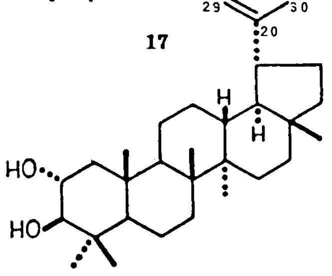
4 Δ^{7} 3β-OH
6 Δ$^{9(11)}$ 3β-OH
9 Δ^{7} 2α,3α-di-OH
14 Δ$^{9(11)}$ 2α,3β-di-OH



5 3β-OH
10 2α,3α-di-OH



17



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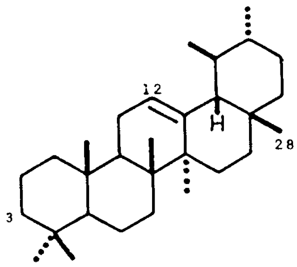
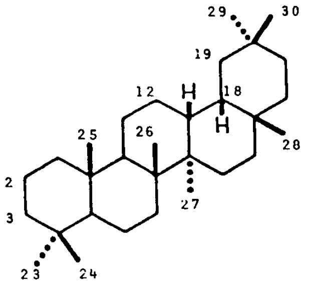
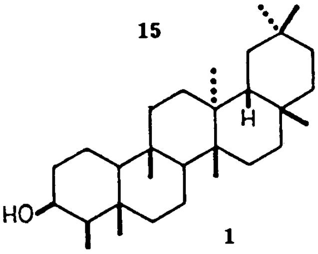


3 3β-OH
12 3β-OH, 28=COOH



2 Δ^{12} 3β-OH
7 Δ^{18} 2α,3α-di-OH
8 Δ^{12} 2α,3α-di-OH
11 Δ^{12} 3β-OH, 28=COOH
13 Δ^{12} 2α,3β-di-OH
16 Δ^{18} 2α,3β-di-OH



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Hexane extraction of the fresh bursting buds (1.69 kg) collected at Higashiyama, Kyoto city, on May gave an extract (20.3 g) with water (420 g). The extract was separated by silica gel chromatography into 18 fractions using a hexane-benzene-ether solvent system. The main part of Frac. 8 (benzene, 0.37 g) was friedelan-3β-ol (epifriedelanol, **1**),5) which as well as the derived acetate were identified by mp and IR and ^{1}H-NMR spectra with samples obtained from friedelin. Frac. 9 (benzene-ether 8:2, 2.07 g) was a mixture (2:1) of β-amyrin (**2**) and α-amyrin (**3**), which was determined by GC-MS and the ^{1}H-NMR spectrum applied to a mixture of samples. Frac. 10 (hexane-benzene 7:3, 2.44 g) was separated into three components by recrystallization and 20% $AgNO_3$-silicai gel chromatography after acetylation. Compounds **4** and **5** were identified with the authentic samples of motiol and neomotiol,2) respectively, by mp and IR and ^{1}H-NMR spectra of the alcohols and their corresponding acetates.6) Compound **6** was the most soluble one of the three, and identified with an authentic sample of fernenol7) by method similar to that used for **4** or **5**. All the three alcohols and their acetates gave ^{1}H-NMR spectra (Table II) reasonably comparable with those of the corresponding hydrocarbons.8)

Next, two diols (**7**, **8**) obtained from Frac. 14 (benzene-ether 1:1, 2.48 g) and another two (**9**, **10**) from Frac. 15 (benzene-ether 1:1, 1.99 g) appeared to be the same type of 2,3-diols because they demonstrated similar patterns of vicinal protons signals attached to the C-2 and C-3 of the diols and also of the derived diacetates. To clarify the structures of these diols, olean-12-ene-2α,3α-diol (**A**) and olean-12-ene-2β,3β-diol (**B**) were synthesized from β-amyrin via olean-2,12-diene according to the method for the oleanolic acid derivatives.9,10) The coupling patterns of two protons attached to C-2 and C-3 demonstrated their structures as shown in Table II [C-2β (axial) and C-3β (equatorial) for **A**, and C-2α (equatorial) and C-3α (axial) for **B**], while the methyl signals of C-24 and 25 were observed at remarkably lower fields in **B** by the effect of the C-2β (axial) hydroxyl group. All the four natural diols gave patterns similar to those of **A** to exhibit the 2α,3α-diol structure. Incidentally, compounds **7** and **9** were proved to be identical with germanidiol and motidiol by comparing the mp, $[\alpha]_D$, and their IR, ^{1}H-NMR and MS spectra with those of the authentic samples of diols and derived diacetates.2,11) Thus the structures of germanidiol and motidiol must be revised to olean-18-ene-2α,3α-diol (**7**) and fern-7-ene-2α,3α-diol (**9**), respectively. Although **8**

Table II. ^{1}H-Chemical Shifts (δ) in $CDCl_3$ Solution (JEOL FX 100)

	Methyl Signals of C- 23	24	25	26	27	28	29	30	Proton attached to C- 2	3	Olefinic proton	Acetyl methyl
4	0.966	0.856	0.746	0.990	0.892	0.732	0.828d,0.898d (6.8)		----	3.24dd (5.2,10.0)	[7] 5.37ddd (3.0,3.2,3.6)	---
4a	0.848	0.926	0.770	0.990	0.894	0.736	0.828d,0.896d (6.6)		---- ----	4.51dd (6.5,8.7)	[7] 5.36ddd (3.4,3.4,4.0)	2.046
5	0.990	0.786	0.882	0.732	1.110	0.754	0.846d,0.932d (6.6)		----	3.23dd (5.8,9.9)	[12] 5.05ddd (2.2,2.2,4.4)	---
5a	0.866	0.866	0.910	0.738	1.118	0.758	0.846d,0.934d (6.6)		----	4.50dd (6.6,9.1)	[12] 5.05ddd (2.3,2.3,4.5)	2.042
6	0.964	0.870	1.068	0.732	0.816	0.760	0.828d,0.890d (6.4)		----	3.21dd (6.1,9.1)	[11] 5.30ddd (2.2,2.9,4.2)	---
6a	0.848	0.942	1.086	0.730	0.806	0.760	0.830d,0.890d (6.4)		----	4.48dd (6.6,8.6)	[11] 5.30ddd (2.3,2.7,4.1)	2.044
7	1.004	0.848	0.938	1.066	0.742	1.014	0.938	0.938	3.99ddd (2.9,4.0,12.0)	3.41d (2.9)	[19] 4.85d (1.5)	---
7a	0.876	0.978	1.000	1.086	0.786	1.020	0.944	0.944	5.24ddd (2.8,4.2,12.0)	4.95d (2.8)	[19] 4.85d (1.4)	1.962 2.112
A=8	1.028	0.870	0.986	0.956	1.140	0.828	0.870	0.870	4.00ddd (2.5,4.6,11.0)	3.43d (2.5)	[12] 5.19dd (3.0,3.0)	---
Aa=8a	0.878	1.000	1.066	0.976	1.184	0.838	0.878	0.878	5.25ddd (2.8,4.6,11.6)	4.97d (2.8)	[12] 5.19dd (3.0,3.0)	1.960 2.108
B	1.020	1.020	1.260	0.988	1.128	0.834	0.870	0.870	4.09ddd (3.2,3.7,3.7)	3.22d (3.7)	[12] 5.20dd (3.5,3.5)	---
Ba	0.912	1.066	1.218	1.000	1.128	0.830	0.872	0.872	5.32ddd (3.9,3.9,4.0)	4.63d (3.9)	[12] 5.18dd (3.0,3.0)	2.030 2.044
9	0.992	0.898	0.804	0.992	0.928	0.732	0.830d,0.892d (6.5)		4.01ddd (2.5,6.5,11.0)	3.46d (2.5)	[7] 5.38ddd (2.9,2.9,3.2)	---
9a	0.874	1.066	0.874	1.000	0.934	0.744	0.832d,0.902d (6.7)		5.23ddd (2.6,6.6,10.9)	5.01d (2.6)	[7] 5.39bd (3.0)	1.972 2.098
10	1.024	0.870	0.936	0.726	1.116	0.752	0.844d,0.934d (6.1)		4.00ddd (2.6,5.2,11.6)	3.43d (2.6)	[12] 5.06ddd (2.4,2.4,4.1)	---
10a	0.876	0.996	0.996	0.742	1.152	0.764	0.846d,0.934d (6.0)		5.24ddd (2.6,4.8,12.0)	4.97d (2.6)	[12] 5.04ddd (2.0,2.0,4.2)	1.966 2.130
13	1.042	0.836	1.018	0.968	1.134	0.836	0.876	0.876	3.70ddd (4.2,9.1,9.1)	3.01d (9.1)	[12] 5.19dd (3.4,3.4)	---
13a	0.916	0.916	1.084	0.966	1.126	0.828	0.872	0.872	5.12ddd (4.7,10.3,10.3)	4.74d (10.3)	[12] 5.17dd (3.6,3.6)	1.976 2.052
14	1.004	0.906	1.132	0.734	0.808	0.756	0.830d,0.882d (5.6)		3.68ddd (3.9,9.8,10.3)	2.98d (9.8)	[11] 5.34ddd (2.4,3.0,4.2)	---
14a	0.862	0.992	1.198	0.726	0.798	0.758	0.826d,0.886d (6.0)		5.11ddd (4.3,10.3,11.6)	4.71d (10.3)	[11] 5.31ddd (2.4,2.7,3.7)	1.990 2.054
15	1.004	0.794	0.942	1.026	0.942	0.794	---	1.678	3.66ddd (4.4,9.6,11.4)	2.96d (9.6)	[29] 4.57dd,4.69d (1.2,2.3)(2.3)	---
15a	0.880	0.880	0.976	1.030	0.934	0.782	---	1.670	5.10ddd (4.9,10.3,10.3)	4.71d (10.3)	[29] 4.56dd,4.68d (1.2,2.3)(2.3)	1.960 2.044
16	1.014	0.810	0.940	1.072	0.734	1.014	0.940	0.940	3.70ddd (4.7,9.8,10.7)	2.98d (9.8)	[19] 4.85d (1.5)	---
16a	0.882	0.894	0.942	1.076	0.730	1.014	0.942	0.942	5.14ddd (4.5,10.3,11.0)	4.73d (10.3)	[19] 4.85d (1.7)	1.986 2.050
17	1.160	0.980	0.798	0.918	0.980	0.776	0.822d,0.886d (6.4)		3.87ddd (2.2,6.6,6.9)	3.42d (6.9)	[6] 5.63ddd (2.9,3.3,3.7)	---
17a	1.080	1.026	0.808	0.938	0.990	0.790	0.824d,0.888d (6.2)		5.02dd (2.0,3.5)	4.99bs	[6] 5.63ddd (2.2,3.4,3.7)	2.050 2.050

Compounds with **a** added to the symbol are the corresponding peracetates. Signals are singlet unless otherwise stated.

was not obtained as pure crystals because of contamination of **7**, the presence of olean-12-ene-2α,3α-diol in Frac. 14 was proved by comparison of the $[\alpha]_D$, ^{1}H-NMR and MS spectra with those of **A** and **7**. The fourth cis diol (**10**) was a new compound and its structure, neohop-12-ene-2α,3α-diol, was established by comparing its MS (m/z 218) and ^{1}H-NMR (Table II) spectra with those of neohop-12-ene.[8] Moreover, the methyl signals assignable to C-23, 24 and 25 of the four 2α,3α-diols (**7**, **8**, **9**, **10**) and their diacetates (**7a**, **8a**, **9a**, **10a**) exhibited almost the same shift values as those of the corresponding hydrocarbons.[8] The acidic part of Frac. 15 was proved to be a mixture of oleanolic (**11**) and ursolic acids (**12**) (1:2) by GC-MS and ^{1}H-NMR spectrum.

Frac. 16 (benzene-ether 4:1, 6.50 g) after acetylation was separated into acetates of three diols (**13**, **14**, **15**) by 20% $AgNO_3$-silica gel chromatography and HPLC (Bondapak A, methanol-chloroform-water 74:16:10). Frac. 17 (ether, 0.94 g) was also separated into two diols (**16**, **17**) by HPLC (Bondapak A, methanol-chloroform-water 85:5:10). The four natural diols (**13**, **14**, **15**, **16**) appeared to be the same type as 2α,3β-diols because the diols and their diacetates (**13a**, **14a**, **15a**, **16a**) demonstrated similar patterns of C-2β and C-3α diaxial protons[12,13] as shown in Table II. The carbon skeletons of each compound were determined as follows. Olean-12-ene-2α,3β-diol (**13**) as well as its diacetate (**13a**) gave a characteristic MS fragment (m/z 218), and a triplet signal of C-12 olefinic proton and methyl signals of C-26 - 30 corresponding to olean-12-ene.8) Fern-9(11)-ene-2α,3β-diol (**14**) showed a characteristic MS fragment (m/z 275 for **14**, 239 for **14a**) and ddd signal of C-11 olefinic proton as well as five methyl signals of fern-9(11)-ene.8) **14** was identified with fernenediol3) by comparison of mp, $[\alpha]_D$, and IR and ^{1}H-NMR spectra with those of the authentic sample. Lup-20(29)-ene-2α,3β-diol (**15**) and its diacetate (**15a**) demonstrated characteristic ^{1}H-NMR signals of lup-20(29)-ene (two proton signals of endo-methylene, one olefinic methyl and three methyl singlets of C-26, 27, 28). Both olean-18-ene-2α,3β-diol (**16**) and its diacetate (**16a**) gave the MS fragments (m/z 204, 189, 177, 218), and the ^{1}H-NMR signals of 18-olefinic proton and five methyls of C-26 - 30.8) Compound **16** was proved to be identical to epigermanidiol3) by methods similar to those used for **14**. The ^{1}H methyl signals of C-23, 24 and 25 of the four 2α,3β-diols and their diacetates also gave very reasonable shift values compared with those of the corresponding hydrocarbons.8)

Finally compound **17** gave a peculiar pattern of vicinal proton signals in the ^{1}H-NMR spectrum. The MS fragmentation pattern indicated that the compound is a derivative of adian-5-ene. The olefinic proton signal and six methyl signals (C-25 - 30) of **17** and its acetate (**17a**) also suggested that the compound is the 2,3-diol of adian-5-ene. Although direct comparison was not successful because the authentic sample had deteriorated, the physical constants of **17** and **17a** were very similar to those of adianenediol and its diacetate. The stereochemistry of 2,3-diol was established as follows: 1) The coupling pattern of the vicinal protons attached to C-2 and C-3 suggested that ring A had a deformed chair conformation with 2β,3β protons. 2) The fact that the two hydroxyls in **17** or the two acetoxyls in **17a** did not affect the chemical shift of C-25 methyl (9β) indicated that the hydroxyls or the acetoxyls have α-configurations. 3) Diacetate (**17a**) was treated with 4N-H_2SO_4 in benzene-acetic acid at 20°C for a few days to give 2α,3α-diol diacetate having the same vicinal proton pattern as those of **7a** - **10a** (the main product was supposed to be fern-8-ene-2α,3α-diyl diactate). Thus compound **17** was established to be adian-5-ene-2α,3α-diol.

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