Dammarane Triterpenes from *Gardenia aubryi* VIEILL.

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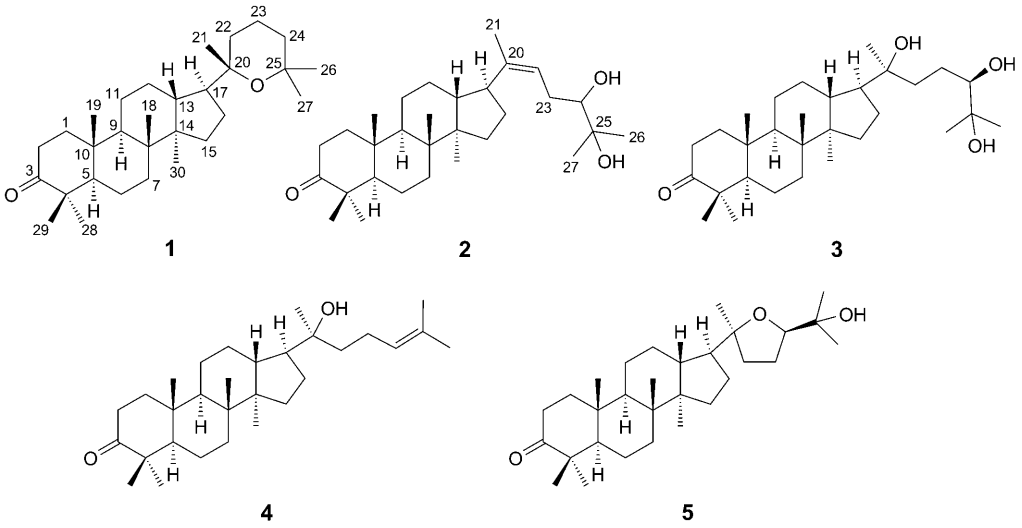
Three new dammarane triterpenoids, gardaubryones A–C (**1**–**3**), were isolated from the gum collected on the aerial parts of *Gardenia aubryi* VIEILL., together with the known compounds hydroxydammarenone II (**4**), ocotillone (**5**), cabraleone, and hollongdione. The structures of the novel compounds were established on the basis of mass spectrometry, NMR experiments, and chemical-correlation reactions.

Introduction. – The genus *Gardenia* (Rubiaceae) includes some two hundred species of trees and shrubs widely distributed in the warm and tropical regions of the Old World. Six endemic species occur in New Caledonia [1][2]. The leaves and flower buds of two of these, *Gardenia aubryi* VIEILL. and *Gardenia urvillei* MONTROUZ., are covered with an exudate locally used for chewing, especially by children. In a continuation of our chemical studies on secondary metabolites of New Caledonian *Gardenia* species [3], we report here the structure elucidation of three new dammarane triterpenoids, gardaubryone A–C (**1**–**3**), isolated from *Gardenia aubryi* gum, along with the known hydroxydammarenone II (**4**), ocotillone (**5**), cabraleone, and hollongdione.

Results and Discussion. – Repeated column chromatography on silica gel of *G. aubryi* exudate resulted in the isolation of the three novel dammarane triterpenoids, gardaubryones A–C (**1**–**3**), together with four known dammaranes, which were identified as hydroxydammarenone II (=20-hydroxy-dammar-24-en-3-one; **4** (20*S*)) [4][5], ocotillone (=(24*R*)-20,24-epoxy-25-hydroxydammaran-3-one; **5** (20*S*)) [6–8], cabraleone [6–8], and hollongdione [9][10] by comparison with literature data.

Gardaubryone A (**1**) was obtained as a colorless amorphous solid, whose empirical formula was determined as $C_{30}H_{50}O_2$ by HR-EI-MS. Inspection of the ^{13}C-NMR spectrum revealed the presence of a C=O *s* and 29 sp^3 C-atoms (six quaternary C, four CH, eleven CH_2, and eight Me). In the ^{1}H-NMR spectrum, the eight Me signals appeared as *s*s. A typical set of five Me resonances, correlated in the HMQC plot, at δ(C)/δ(H) 15.1/0.98, 16.1/0.91, 16.5/0.84, 21.0/1.01, and 26.7/1.13, was associated with



1 **2** **3** **4** **5**

the Me(18), Me(19), Me(30), Me(29), and Me(28) groups, respectively, of a dammarane skeleton bearing a C=O group at C(3) (*Tables 1* and *2*) [5][11][12]. Indeed, these data were closely related to those previously reported for the dammarane core present in ocotillone (**5**) [7] and in cabraleone [7], (24*S*)-20,24-epoxydammar-25(26)-en-3-one [7], 20-hydroxydammaran-3-one [5], and hydroxydammarenone II (**4**) [5]. The ^{13}C-NMR resonances of the side chain at C(17), associated with a 2,2,6-trimethyltetrahydro-2*H*-pyran moiety, were observed at δ(C) 76.5 (C(20)), 26.0 (C(21)), 31.2 (C(22)), 16.4 (C(23)), 37.0 (C(24)), 70.9 (C(25)), 28.2 (C(26)), and 32.9 (C(27)). They were similar to the corresponding signals present in the spectrum of (20*S*)-panaxadiol (=(3β,12β)-20,25-epoxydammarane-3,12-diol (20*S*)) [13] and strongly differed from those of (20*R*)-panaxadiol (=(3β,12β)-20,25-epoxydammarane-3,12-diol) [13–15]. These data permitted to depict the structure of gardaubryone A as 20,25-epoxydammaran-3-one (20*S*) (**1**).

Gardaubryone B (**2**) was found to possess a molecular formula $C_{30}H_{50}O_3$ by HR-EI-MS. The ^{13}C-NMR spectrum indicated the presence of a C=O, two sp^2 C-atoms (one quaternary C, one CH) corresponding to the C=C bond, and 27 sp^3 C-atoms (five quaternary C, four CH, ten CH_2, and eight Me). The signals observed were typical for a dammaran-3-one derivative and closely related to those of **5**, except for the resonances associated with the side chain at C(17) (*Table 2*). In the ^{1}H-NMR spectrum, the signal of the Me(21) group at the side chain was strongly deshielded (δ(H) 1.56), whereas those of the two geminal ones, Me(26) and Me(27), were only slightly deshielded. Typical ^{1}H,^{13}C-HMBC cross-peaks, associated with a 6-substituted 2-methylhept-5-ene-2,3-diol moiety were noticed between Me(21) and the quaternary and tertiary sp^2 C(20) and C(22) (δ(C) 141.8 and 120.5, resp.) on the one hand, and between Me(26) and Me(27) and the quaternary and tertiary oxygenated C(25) and C(24) (δ(C) 72.6 and 78.2, resp.) on the other hand. The side-chain structure was confirmed by the observation of a *t* at δ(H) 5.21 and a *dd* at δ(H) 3.38, attributable to H–C(22) and

Table 1. *^{1}H-NMR Data* (400 MHz, $CDCl_3$) *of Compounds* **1**–**3**. δ in ppm, J in Hz. In the case of overlapped signals or H-atoms observed as *m*, only the δ(H) is presented. The δ(H) of overlapped H-atom signals is derived from HSQC-DEPT data.

	1	**2**	**3**
CH_2(1)	1.93, 1.46	1.91, 1.44	1.90, 1.44
CH_2(2)	2.47, 2.42	2.50, 2.41	2.48, 2.43
H–C(5)	1.35	1.36	1.36
CH_2(6)	1.52, 1.43	1.53, 1.44	1.53, 1.45
CH_2(7)	1.52, 1.26	1.56, 1.30	1.54, 1.29
H–C(9)	1.40	1.38	1.42
CH_2(11)	1.45, 1.26	1.49, 1.18	1.48, 1.26
CH_2(12	1.93, 1.26	1.84, 1.28	1.80, 1.27
H–C(13)	1.69	1.59	1.61
CH_2(15)	1.34, 0.98	1.47, 1.05	1.44, 1.10
CH_2(16)	1.64, 1.42	1.66, 1.44	1.74, 1.47
H–C(17)	1.67	2.20	1.79
Me(18)	0.98 (*s*)	0.99 (*s*)	0.97 (*s*)
Me(19)	0.92 (*s*)	0.92 (*s*)	0.92 (*s*)
Me(21)	1.11 (*s*)	1.56 (*s*)	1.15 (*s*)
CH_2(22) or H–C(22)	1.42, 1.32	5.21 (*t*, $J = 7$)	1.73, 1.60
CH_2(23)	1.73, 1.59	2.20	1.61, 1.41
CH_2(24) or H–C(24)	1.51, 1.34	3.38 (*dd*, $J = 8, 5.5$)	3.37 (br. *d*, $J = 8.5$)
Me(26)	1.14 (*s*)	1.17 (*s*)	1.22 (*s*)
Me(27)	1.18 (*s*)	1.22 (*s*)	1.17 (*s*)
Me(28)	1.01 (*s*)	1.02 (*s*)	1.02 (*s*)
Me(29)	1.05 (*s*)	1.06 (*s*)	1.07 (*s*)
Me(30)	0.84 (*s*)	0.86 (*s*)	0.87 (*s*)

Table 2. *^{13}C-NMR Data* (50 MHz, $CDCl_3$) *of Compounds* **1**–**3**. δ in ppm.

	1	**2**	**3**		**1**	**2**	**3**
C(1)	39.9	39.9	39.8	C(16)	25.2	27.3	25.6
C(2)	34.1	34.1	34.1	C(17)	51.4	50.6	49.9
C(3)	218.2	218.2	218.2	C(18)	15.1	15.2	15.1
C(4)	47.4	47.4	47.4	C(19)	16.1	15.8	16.0
C(5)	55.3	55.3	55.3	C(20)	76.5	140.3	75.6
C(6)	19.7	19.6	19.6	C(21)	26.0	13.3	25.2
C(7)	34.6	34.7	34.5	C(22)	31.2	120.5	36.9
C(8)	40.3	40.4	40.2	C(23)	16.4	30.4	24.9
C(9)	50.2	50.2	50.0	C(24)	37.0	78.5	78.8
C(10)	36.9	36.9	36.8	C(25)	70.9	72.6	73.2
C(11)	22.2	21.9	22.0	C(26)	28.2	23.7	23.2
C(12)	27.6	25.8	27.4	C(27)	32.9	26.6	26.6
C(13)	42.0	44.5	42.6	C(28)	26.7	26.7	26.7
C(14)	50.0	49.2	50.2	C(29)	21.0	21.0	21.0
C(15)	31.2	31.6	31.1	C(30)	16.5	16.1	16.3

H–C(24), respectively. These data permitted to depict the structure of gardaubryone B as 24,25-dihydroxydammar-20(22)-en-3-one (**2**), in good agreement with the ^{13}C-NMR

data reported for the analogous dammar-20(22)-ene-24,25-diol moiety present in the triterpene cordialin B (=(3β,11α,20*E*,24*S*)-3,19-epoxydammar-20(22)-ene-3,11,24,25-tetrol) [16]. The absolute configuration at C(24) of gardaubryone B could not be unambiguously established despite several chemical-correlation trials due to the low amount of isolated material.

It should be noted that a structure similar to that established here for gardaubryone B has been recently postulated for the triterpene ailexcelone isolated from *Ailanthus excelsa* ROXB. [17]. However, the spectral data reported for this latter compound are not consistent with the proposed structure. Specifically, the δ(C) values of the signals of the ^{13}C-NMR spectrum of ailexcelone unambiguously exclude a dammarane basic core but are in full agreement with a tirucall-7-ene (=(13α,14β,17α,20*S*)-lanost-7-ene) structure. Consequently, ailexcelone should be regarded as one of the epimers at C(24) of 24,25-dihydroxytirucall-7-en-3-one [18]. Similarly, the structure of ailexcelol, presented as the corresponding secondary alcohol at C(3), should be revised to one of the C(24) epimers of (3*S*)-tirucall-7-ene-3,24,25-triol. It should also be emphasized that (3*S*,24*S*)-tirucall-7-ene-3,24,25-triol had been previously isolated from *Ailanthus excelsa* ROXB. [19].

The empirical formula of gardaubryone C (**3**) was deduced as $C_{30}H_{52}O_4$ from the HR-EI-MS. The ^{13}C-NMR spectrum indicated the presence of a C=O and 29 sp^3 C-atoms (six quaternary C, five CH, ten CH_2, and eight Me). It exhibited, as those of **1** and **2**, the 22 signals of a dammaran-3-one tetracyclic skeleton. A set of additional resonances associated with a 6-substituted 2-methylheptan-2,3,6-triol moiety was noticed at δ(C) 75.6 (C(20)), 25.2 (C(21)), 36.9 (C(22)), 24.9 (C(23)), 78.8 (C(24)), 73.2 (C(25)), 23.2 (C(26)), and 26.6 (C(27)), similar to analogous signals in the spectrum of various glycosides of dammarane-3,12,20,24,25-pentol [20] and cyclolanostane-3,16,20,24,25-pentol [21–23]. Thus, gardaubryone C could be described as a 20,24,25-trihydroxydammaran-3-one. Catalytic OsO_4 oxidation of hydroxydammarenone II (**4**) gave a 20,24,25-trihydroxydammaran-3-one mixture of two diastereoisomers epimeric at C(24) (*Scheme*). One of these triols was identical to gardaubryone C (**3**), permitting to establish its (20*S*) configuration. Upon acid treatment, gardaubryone C was converted to a mixture of the two corresponding 20,24-epoxy derivatives epimeric at C(20) [20]. One of these compounds was identical to ocotillone (**5**), giving evidence for the (24*R*) configuration of gardaubryone C, the structure of which was therefore established as (24*R*)-20,24,25-trihydroxydammaran-3-one (20*S*).

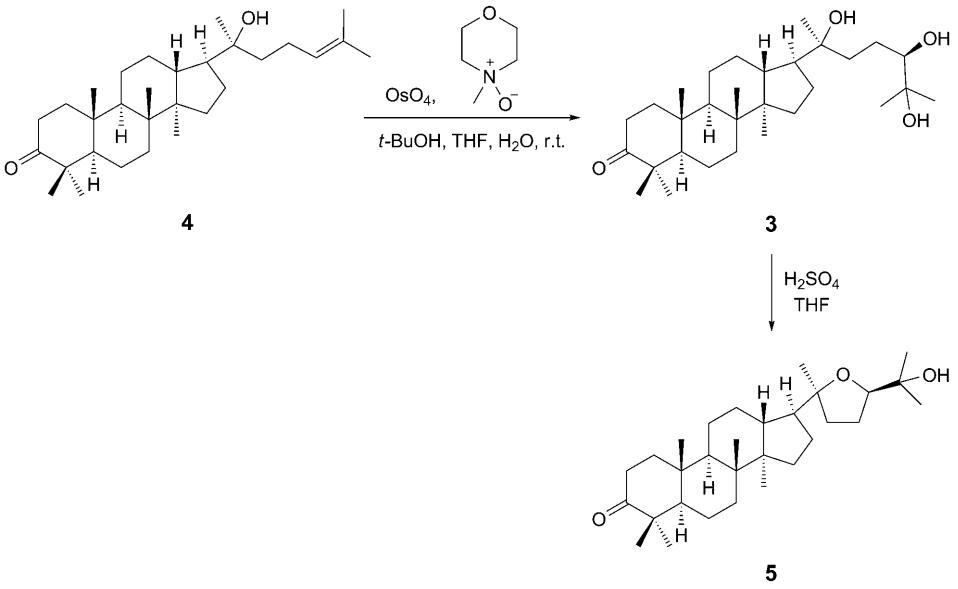
Experimental Part

General. Liquid column chromatography (CC): silica gel *60* (SiO_2, 40–63 µm, *Merck*). Optical rotations: *Perkin-Elmer-341* polarimeter. IR Spectra: *Perkin-Elmer-Paragon-500* instrument; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker-DRX-400* and *Bruker-AC-200* spectrometers; at 400 (^{1}H) and 50 MHz (^{13}C); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz; 2D-NMR experiments were performed with standard *Bruker* microprograms. EI-MS: *HP-6890* spectrometer; in *m*/*z*. HR-EI-MS: *AEI-MS-902* mass spectrometer; in *m*/*z*.

Plant Material. Aerial parts of *Gardenia aubryi* were collected on July 9, 2003, in New Caledonia. A voucher sample (JWHJ-75) is kept with the Herbarium of the Center IRD of Nouméa, New Caledonia.

Extraction and Isolation. Gum (2.35 g), collected manually from fresh *Gardenia aubryi* aerial parts (1.4 kg), was dissolved in CH_2Cl_2 (35 ml) and filtered. The solvent was evaporated, and an aliquot of the

Scheme



solid residue (2.10 g) was subjected to liquid vacuum chromatography (SiO_2, stepwise gradient AcOEt/cyclohexane, 10-ml fractions). Fractions of similar composition (TLC) were pooled and further purified by CC (SiO_2 (20–45 μm), cyclohexane/CH_2Cl_2/MeOH (of increasing polarity): *gardaubryone A* (**1**; 28 mg), hollongdione (8 mg), ocotillone (**5**; 17 mg), cabraleone (24 mg), hydroxydammarenone II (**4**; 157 mg), *gardaubryone B* (**2**; 4 mg), and *gardaubryone C* (**3**; 12 mg), in this order.

Gardaubryone A (=*20,25-Epoxydammaran-3-one*; **1** ((20*S*) configuration)): Amorphous solid. $[\alpha]_D^{20} = +81$ ($c = 0.2$, MeOH). EI-MS: 442 (M^+). HR-EI-MS: 442.3817 (M^+, $C_{30}H_{50}O_2^+$; calc. 442.3811).

Gardaubryone B (=*24,25-Dihydroxydammar-20(22)-en-3-one*; **2**): Amorphous solid. $[\alpha]_D^{20} = +84$ ($c = 0.2$, MeOH). EI-MS: 458 (M^+). HR-EI-MS: 458.3758 (M^+, $C_{30}H_{50}O_3^+$; calc. 442.3760).

Gardaubryone C (=*(24*R*)-20,24,25-Trihydroxydammaran-3-one*; **3** ((20*S*) configuration)): Amorphous solid. $[\alpha]_D^{20} = +57$ ($c = 0.2$, MeOH). EI-MS: 476 (M^+). HR-EI-MS: 442.3869 (M^+, $C_{30}H_{52}O_4^+$; calc. 442.3866).

OsO_4 Oxidation of Hydroxydammarenone II (**4**). To a soln. of **4** (25 mg) in *t*-BuOH/THF/H_2O 10:3:1 (5 ml) was added a 2.5% (*w*/*v*) soln. of OsO_4 in *t*-BuOH (0.15 ml) and 4-methylmorpholine 4-oxide (30 mg). The mixture was stirred for 48 h at 25°. A sat. aq. $NaHSO_3$ soln. (20 ml) was added, and the mixture was stirred for 1 h. Then, the mixture was extracted with CH_2Cl_2 (20 ml) and H_2O (20 ml), the org. layer dried (Na_2SO_4) and concentrated, and the residue submitted to flash chromatography (SiO_2, CH_2Cl_2/MeOH 99:1) affording **3** (8 mg), identical to the natural product isolated from *Gardenia aubryi*, and a mixture of **3** with its (24*S*) epimer which could not be separated.

Cyclization of Gardaubryone C (**3**). To a soln. of **3** (8 mg) in THF (5 ml) was added H_2SO_4 (0.05 ml). The mixture was stirred for 24 h at 25°. Then, the solvent was evaporated and the residue purified by prep. TLC: ocotillone (**5**; 5 mg).

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