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The Granulation-Inhibiting Principles from *Eucalyptus globulus* LABILL. II.[1] The Structures of Euglobal -Ia$_1$, -Ia$_2$, -Ib, -Ic, -IIa, IIb and -IIc[2]

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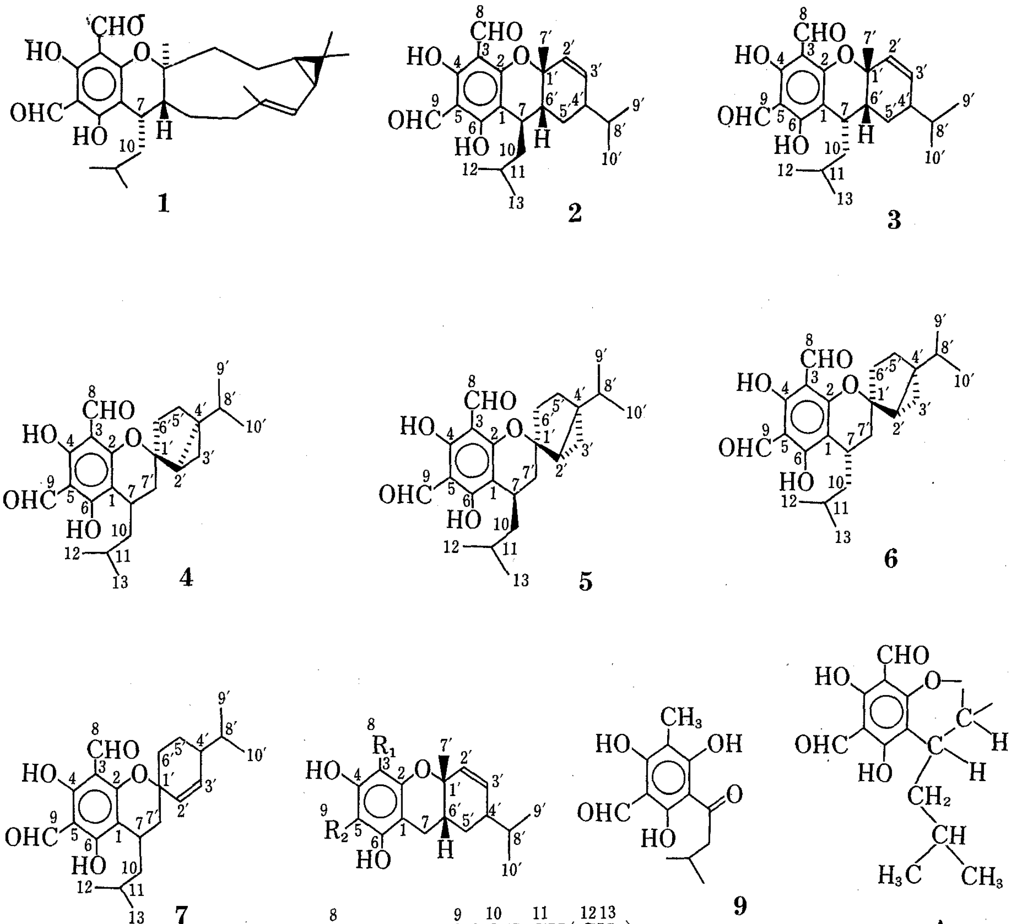
The structures of seven novel potent granulation-inhibiting principles isolated from the buds of *Eucalyptus globulus* LABILL., were examined on the basis of physicochemical data, especially of ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR, mass spectrum (MS) and circular dichroism (CD). The structures (but not the complete stereochemistry) of euglobal-Ia$_1$, -Ia$_2$, -Ib, -Ic, -IIa, -IIb and -IIc were proposed to be **2**—**7** and **8**, respectively.

Keywords——euglobal-Ia$_1$; euglobal-Ia$_2$; euglobal-Ib; euglobal-Ic; euglobal-IIa; euglobal-IIb; euglobal-IIc; acetogenin-monoterpene structure; granulation-inhibiting principle; *Eucalyptus globulus*

As part of our continuing search[3] among 1850 crude drugs and plants for agents with potential granulation-inhibiting activity, we have investigated the plant *Eucalyptus globulus* LABILL. A preliminary report[4] describes the isolation and structural determination of a novel active principle, euglobal-III, which has an acetogenin-sesquiterpene structure (**1**). Further investigation[1] of the other active constituents of this plant led to the isolation of ten new compounds, which showed remarkable activity in the fertile egg test,[5] by preparative reversed-phase liquid chromatography. In this paper, the structure elucidations of seven new compounds, euglobal-Ia$_1$ (**2**), -Ia$_2$ (**3**), -Ib (**4**), -Ic (**5**), -IIa (**6**), -IIb (**7**), and -IIc (**8**), are reported.

These seven compounds have the same composition, $C_{23}H_{30}O_5$ (mass spectra and elemental analyses),[1] and showed ultraviolet (UV), infrared (IR) and mass spectra (MS) similar to those of euglobal-III (**1**). The UV spectra [$\lambda_{\max}^{\text{EtOH}}$ nm(ε): 277 (32000—38000), 340 (3600—4500, inflection)] of these compounds were virtually superimposable upon that of grandinol (**9**) isolated from *Eucalyptus grandis*[6] and suggested the existence of a similarly substituted phloroglucinol chromophore. These compounds contained two aldehydic groups (one aldehydic and one ketonic group in euglobal-IIc), as evidenced by the proton magnetic resonance (PMR) spectra and the ^{13}C-nuclear magnetic resonance (CMR) spectra (data in Tables I and II). Since the IR absorption at around 1620 cm^{-1} was only observed in the carbonyl stretching band region of these compounds, the carbonyl groups should be conjugated and hydrogen-bonded. These compounds also contained two hydrogen-bonded hydroxyl groups, as evidenced by IR bands at around 3400 cm^{-1}, and the disappearance of two singlet signals at δ 13.32—15.35 in the PMR spectra on addition of deuterium oxide. The MS of these seven compounds exhibited the same fragmentation pattern as shown in Chart 2, although slight differences in the relative abundances of the peaks were observed. The peaks at m/z 251 and 195 with the compositions $C_{13}H_{15}O_5$, and $C_9H_7O_5$, respectively, are in common with the MS of the other euglobals.[1] It was also apparent that these seven compounds were split into two fragments, m/z 251 ($C_{13}H_{15}O_5$) and 136 ($C_{10}H_{16}$), which were supposed to be formed by retro-Diels–Alder cleavage of the molecular ions. These two fragments were assigned from their compositions to an aromatic part and a monoterpene moiety. The ion at m/z 251 suffered further cleavage to give the m/z 195 fragment with the loss of C_4H_8. Similarly, the ion at m/z 136 gave a peak at m/z 93 by loss of a saturated group, C_3H_7. The CMR spectra (Tables I and II) of these



8 : R_1 = CHO, R_2 = $\overset{9}{C}O\overset{10}{C}H_2\overset{11}{C}H(\overset{12}{C}\overset{13}{H_3})_2$
13 : R_1 = $COCH_2CH(CH_3)_2$, R_2 = CHO

Chart 1

seven compounds exhibited six aromatic carbons bearing no hydrogen, and the chemical shifts were very similar to those of grandinol (**9**) and euglobal-III (**1**). The calculated ^{13}C-chemical shifts$^{7)}$ of carbons in a benzene ring substituted by two hydroxyls, two aldehydes, an isopropyl and a methoxyl group are given in Table III. When the calculated values in Table III were compared with the experimental values for the seven euglobals, the values of the first column showed the best conformity. Therefore, the substitution pattern on the aromatic rings was suggested to be as shown in [A]. Each compound contained an isobutyl group as evidenced by the characteristic PMR and CMR spectral signals listed in Tables I and II, and by the diagnostic MS cleavage with the loss of C_4H_8 or C_4H_9. The CMR spectra showed the presence of two methyls (δ 20.9—21.8 and 23.5—24.0), a methine (δ 25.3—25.9) and a methylene (δ 42.2—42.7) which were assigned to the isobutyl group. These CMR data were very similar to those of euglobal-III (**1**). From the chemical shifts in the PMR spectra and similarities in the splitting pattern to that of euglobal-III (**1**), the methine proton signal at δ

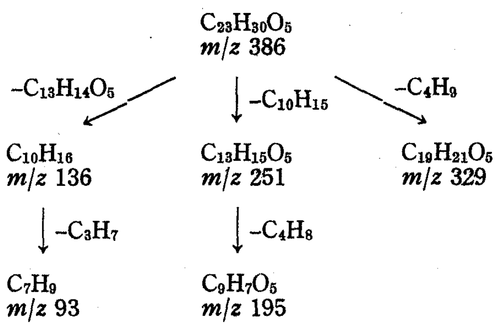


Chart 2. The Fragmentation of Euglobal-Ia$_1$ (**2**), -Ia$_2$ (**3**), -Ib (**4**), -Ic (**5**), -IIa (**6**), -IIb (**7**) and -IIc (**8**) in MS

Table I. PMR and CMR Spectral Data for Euglobal-Ia_1 (**2**), -Ia_2 (**3**) and -IIc (**8**)

Carbon	Euglobal-Ia_1 (2)			Euglobal-Ia_2 (3)			Euglobal-IIc (8)		
	M[a]	δ ^{13}C	δ ^1H	M[a]	δ ^{13}C	δ ^1H	M[a]	δ ^{13}C	δ ^1H
1	s	107.2		s	104.9		s	100.4	
2	s	162.2		s	163.3		s	160.6	
3	s	104.5		s	104.4		s	103.6	
4	s	167.3		s	167.8		s	167.8	
5	s	104.0		s	103.9		s	103.3	
6	s	168.7		s	169.8		s	171.1	
7	d	29.4	2.78 (dt $J=9$, 3.5, 3.5 Hz)	d	29.7	3.17 (dt $J=9$, 5.5, 5.5 Hz)	t	20.4[e]	2.39 (dd $J=16$, 7 Hz) 2.65 (dd $J=16$, 6 Hz)
8	d	191.7[b]	10.19[d] (s)	d	192.1[b]	10.17[c] (s)	d	191.6	10.04(s)
9	d	191.3[b]	10.08[d] (s)	d	191.4[b]	10.06[c] (s)	s	206.0	
10	t	44.7		t	36.8	1.57 (dd, $J=9.4$, 11.4 Hz) 1.80(m)	t	52.5	2.97 (d $J=7$ Hz)
11	d	25.9		d	25.3		d	25.0	
12, 13	q	23.7, 21.5	0.8—1.1	q	23.5, 21.8	0.8—1.1	q	22.7, 22.7	0.98 (d $J=7$ Hz)
1′	s	78.6		s	78.0		s	78.3	
2′	d	130.6[e]	5.51 (dd $J=10$, 2 Hz)	d	130.5	5.83 (dd $J=10$, 1.5 Hz)	d	130.4[b]	5.71(m)
3′	d	134.7[e]	5.83 (dd $J=10$, 3.5 Hz)	d	134.3	5.96 (dd $J=10$, 3.5 Hz)	d	132.9[b]	5.71(m)
4′	d	39.2[c]	2.04	d	40.7	2.04	d	38.7	
5′	t	31.5		t	22.4		t	27.6	
6′	d	38.9[c]		d	35.9		d	33.2	
7′	q	28.0	1.56(s)	q	25.7	1.40(s)	q	27.4	1.49(s)
8′	d	31.0		d	31.9		d	31.8	1.49(s)
9′, 10′	q	20.0, 20.0	0.8—1.1	q	20.6, 20.9	0.8—1.1	q	19.9, 19.8	0.93, 0.95 (d $J=7$ Hz)
OH			13.36(s) 13.32(s)			13.65(s) 13.44(s)			15.35(s) 14.45(s)
			1.2—2.4(8H)			1.1—2.5(8H)			1.4—2.4(6H)

Chemical shifts are expressed in δ (ppm) values.
a) Multiplicity.
b, c, d) Assignments may be interchangeable among the signals with the same superscripts in any one spectrum.
e) These δ values were assigned by selective proton decouplings.

2.78—3.17 (m) in these six compounds exclusive of euglobal-IIc (**8**), was assigned to the methine adjacent to the aromatic ring and bearing the isobutyl group, as observed in euglobal-III (**1**). Thus the six euglobals exclusive of euglobal-IIc (**8**) are considered to have the same aromatic part [A] found in the previously described euglobal-III (**1**).

On the other hand, the presence of an isopropyl group in the monoterpene moiety of these seven compounds was assumed on the basis of the diagnostic MS peak (m/*z* 136—43) and the characteristic NMR signals. The NMR spectra indicated the absence of a triplet methyl proton signal and of a methyl carbon signal due to a propyl moiety at around δ 14.0. Instead, two carbon signals which were assigned to the two methyls in the isopropyl group from their chemical shifts (δ 19.1—20.9) were observed. Further structural study on the monoterpene portion of each of these seven compounds will be described below.

Euglobal-Ia_1 (**2**) and -Ia_2 (**3**) were isolated as colorless oily substances and showed great somilarities in their PMR and CMR data as shown in Table I. As the characteristic signals in the PMR of the monoterpene moiety, two olefinic proton signals (**2**, δ 5.51 and 5.83; **3**, δ 5.83 and 5.96) and a singlet methyl signal (**2**, δ 1.56; **3**, δ 1.40) were observed. The CMR exhibited the presence of two olefinic carbons (**2**, δ 134.7 and 130.6; **3**, δ 134.3 and

Table II. PMR and CMR Spectral Data for Euglobal-Ib (4), -Ic (5), -IIa (6) and -IIb (7)

Carbon	Euglobal-Ib (4)			Equglobal-Ic (5)			Euglobal-IIa (6)			Euglobal-IIb (7)		
	M$^{a)}$	δ ^{13}C	δ ^{1}H	M$^{a)}$	δ^{13} C	δ ^{1}H	M$^{a)}$	δ ^{13}C	δ ^{1}H	M$^{a)}$	δ ^{13}C	δ ^{1}H
1	s	105.4		s	105.7		s	105.7		s	105.4	
2	s	165.2		s	162.9		s	163.1		s	163.4	
3	s	104.5		s	104.2		s	104.3		s	104.5	
4	s	167.7		s	167.7		s	167.8		s	167.8	
5	s	104.0		s	103.7		s	103.8		s	103.9	
6	s	169.5		s	169.2		s	169.3		s	169.5	
7	d	26.0	3.08 (m)	d	26.1	2.92 (m)	d	26.1	3.00 (m)	d	25.3	2.96 (m)
8	d	192.1$^{b)}$	10.15$^{c)}$ (s)	d	191.7$^{b)}$	10.16 (s)	d	191.9$^{b)}$	10.18$^{c)}$ (s)	d	192.0$^{b)}$	10.18$^{c)}$ (s)
9	d	191.5$^{b)}$	10.02$^{c)}$ (s)	d	191.3$^{b)}$	10.05 (s)	d	191.3$^{b)}$	10.05$^{c)}$ (s)	d	191.4$^{b)}$	10.03$^{c)}$ (s)
10	t	43.0		t	42.4		t	42.2		t	43.0	
11	d	25.5$^{d)}$	1.74	d	25.8$^{d)}$		d	25.5		d	25.5	
12, 13	q	20.9, 24.0	0.8—1.1	q	21.1, 23.9	0.8—1.1	q	20.9, 23.9	0.8—1.1	q	21.0, 23.9	0.8—1.1
1′	s	88.5		s	89.7		s	90.1		s	76.6	
2′	d	28.2		d	31.2$^{d)}$	1.24	d	31.5		d	129.0	5.72 (m)
3′	t	12.3	0.37 (dd J=8, 5 Hz) 0.75 (dd J=5, 3.5 Hz)	t	13.4$^{d)}$	0.37 (dd J=5.5, 4 Hz) 0.53 (dd J=8, 5.5 Hz)	t	13.1	0.37 (dd J=5.5, 4 Hz) 0.52 (dd J=8, 5.5 Hz)	d	136.9	5.94 (m)
4′	s	34.3		s	36.0		s	33.6		d	42.0	
5′	t	24.3$^{d)}$	1.74 (s)	t	24.0$^{d)}$	1.94 (m), 1.62 (m)	t	25.8		t	21.0	
6′	t	33.9$^{d)}$	1.74 (s)	t	35.0$^{d)}$	1.82 (m), 1.39 (m)	t	34.2		t	32.0	
7′	t	38.0		t	31.9$^{d)}$	1.93 (m)	t	32.3		t	38.7	
8′	d	32.5		d	32.5$^{d)}$	1.37 (m)	d	32.2		d	31.6	
9′, 10′	q	19.5, 19.5	0.8—1.1	q	19.5, 20.0	0.8—1.1	q	19.6, 20.0	0.8—1.1	q	19.1, 19.5	0.8—1.1
OH			13.53 (s)			13.46 (s)			13.47 (s)			13.46 (br s 2H)
			13.40 (s)			13.41 (s)			13.45 (s)			
			1.1—2.4(7H)			1.2—2.4(11H)			1.1—2.4(11H)			1.1—2.4 (11H)

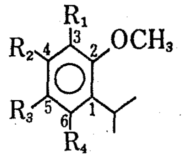
Chemical shifts are expressed in δ (ppm) values.

a) Multiplicity.

b, *c*) Assignments may be interchangeable among the signals with the same superscripts in any one spectrum.

d) These δ values were assigned by selective proton decouplings.

TABLE III. Calculated δ Value of Each Carbon in a Benzene Ring substituted by Two Hydroxyl, Two Aldehyde, an Isopropyl and a Methoxyl Groups



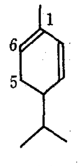
	R_1, R_3=CHO R_2, R_4=OH	R_1, R_3=OH R_2, R_4=CHO	R_1, R_2=OH R_3, R_4=CHO	R_1, R_4=CHO R_2, R_3=OH	R_1, R_4=OH R_2, R_3=CHO	R_1, R_2=CHO R_3, R_4=OH
C (1)	114.9	143.8	129.3	129.3	129.4	129.4
C (2)	167.4	138.5	153.0	152.9	153.0	152.9
C (3)	102.9	148.8	134.4	117.1	134.3	117.0
C (4)	157.0	110.9	142.2	142.6	118.1	125.3
C (5)	104.5	150.6	118.9	136.2	118.9	136.2
C (6)	162.3	116.1	130.5	130.6	147.8	147.9

130.5), a methyl carbon corresponding to the singlet methyl signal (**2**, δ 28.0; **3**, δ 25.7), a quaternary carbon bearing an oxygen atom (**2**, δ 78.6; **3**, δ 78.0), three methines (**2**, δ 39.2, 38.9 and 31.0; **3**, δ 40.7, 35.9 and 31.9), a methylene (**2**, δ 31.5; **3**, δ 22.4) and two methyls of the isopropyl group (**2**, δ 20.0 and 20.0; **3**, δ 20.9 and 20.6).

The (*Z*)-configuration was suggested for the double bonds of both **2** and **3** by the fact that the two olefinic proton signals (each dd)were converted to an AB quartet (J=10 Hz) when the frequency at δ 2.04 was irradiated. Since the olefinic proton at the higher field (**2**, δ 5.51 J=2 Hz; **3**, δ 5.83 J=1.5 Hz) showed no further coupling, it was assigned to the proton attached to the olefinic carbon adjacent to the quaternary carbon. The other olefinic proton at lower field (**2**, δ 5.83; **3**, δ 5.96; J=3.5 Hz each) was concluded to be attached to the olefinic carbon adjacent to a tertiary carbon atom. As already described, these compoounds have only one quaternary carbon, and it was thus clear that the singlet methyl (**2**, δ 1.56; **3**, δ 1.40) and this olefin group should be attached to the same quaternary carbon which, in turn, is connected to an oxygen atom in the phenyl ethereal position.

Since the signals due to the methines adjacent to the aromatic rings were seen as double triplets at δ 2.78 (J=9, 5.5, 5.5 Hz) in **2**, and at δ 3.17 (J=9, 5.5, 5.5 Hz) in **3**, a coupling with another methine proton was assumed in addition to the coupling with the methylene of the isobutyl group. The C(7)-methine, therefore, should be attached to a tertiary carbon. These observations allowed expansion of the partial structure [A] to [B].

From the index of hydrogen deficiency of euglobal-Ia$_1$ (**2**) and -Ia$_2$ (**3**), the monoterpene moiety must be monocyclic or bicyclic. On the other hand, among hitherto known naturally occurring monocyclo- and bicyclomonoterpenes, α-phellandrene (**10**) is the only compound having an isopropyl group and the partial structure [B]. Thus, it was most



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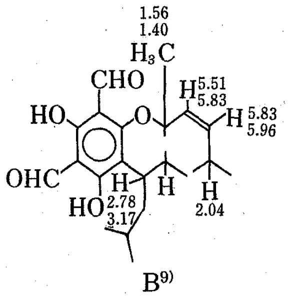
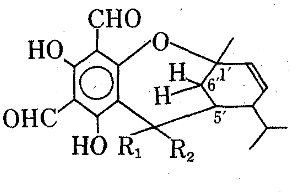
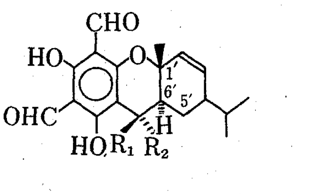


B[9]



R_1, R_2=H, $CH_2CH(CH_3)_2$

11



R_1, R_2=H, $CH_2CH(CH_3)_2$

12

Chart 3

probable that C(1) of α-phellandrene (**10**) was connected to the aromatic ring through the ethereal oxygen. From biogenetic considerations discussed later, the carbon attached to the benzylic methine was suggested to be C(5) or C(6) of α-phellandrene (**10**).

Among the possible structures, **2**, **3**, **11**, and **12**, deduced from the above evidence, the following consideration with the aid of a Dreiding model led to the most probable structures **2**, **3** and their enantiomers; these are consistent with the physical data. The structure **11** was constructed when the benzylic methine was attached to C(5) of **10**. In this case, one of the hydrogen atoms of C(6′) methylene was forced to lie above the aromatic ring and the NMR signal of the proton should be shielded by approximately 1.6—2 ppm according to calculations based on the Dreiding model. However, there was no such signal higher than δ 1.1 in the PMR spectra of euglobal-Ia_1 (**2**) or -Ia_2 (**3**). Consequently, the structures, **2**, **3** and **12** were further considered for these compounds.

Similarities of the chemical shifts in the CMR spectra of the compounds **2** and **3** suggested that both compounds possessed the same C(1′)/C(6′) *cis*-fused or *trans*-fused structure as shown by the structures **2** and **3**, or **12**. A comparison, however, revealed that the only differences between **2** and **3** were in the signals due to C(10) and C(5′) (Table I). The methylene carbons at C(10) and C(5′) of **3** were 7.9 and 9.1 ppm upfield from those of **2**, respectively, and the data could be interpreted in terms of a steric compression shift attributable to a difference in configuration of the isobutyl group. A consideration with the aid of a Dreiding model established that these steric shifts were only possible in the case of the *cis*-fused structure **3** or its enantiomer. Therefore, the structures and relative configurations of euglobal-Ia_1 and -Ia_2 were assigned as formulas **2** and **3**, respectively, exclusive of the configuration at C(4′).

Euglobal-Ic (**5**), was isolated as colorless needles, mp 119—121 °C, and had the composition $C_{23}H_{30}O_5$ on the basis of high resolution MS(M^+ at m/z 386.2053)[1] and elemental analysis.[1] The CMR spectrum (Table II) of the terpene moiety in this compound revealed the presence of a quaternary carbon atom adjacent to an oxygen atom (s, δ 89.7), another quaternary carbon (s, δ 36.0), two methines (d, δ 32.5 and 31.2), four methylenes (t, δ 13.4, 24.0, 31.9 and 35.0) and two methyls of the isopropyl group (q, δ 20.0 and 19.5). The higher field in the 360 MHz PMR spectrum (Fig. 1) showed two double doublets at δ 0.37 (J=5.2, 3.9 Hz) and δ 0.53 (J=5.2, 8.6 Hz) assignable to the protons on a cyclopropane ring. Both signals were converted to an AB quartet (J=5.2 Hz) on irradiation at the double doublets of δ 1.28 (J=8.6, 3.9 Hz). When the center (δ 0.45) of both signals at δ 0.37 and 0.53 and the signal at δ 1.28 were

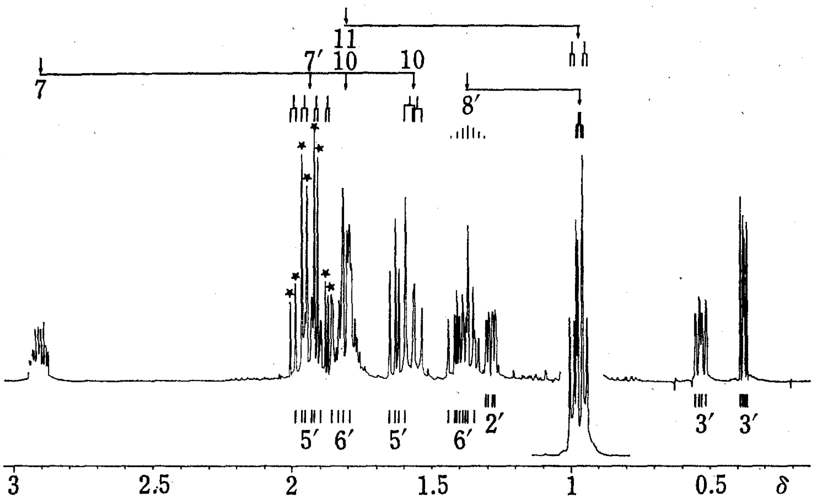
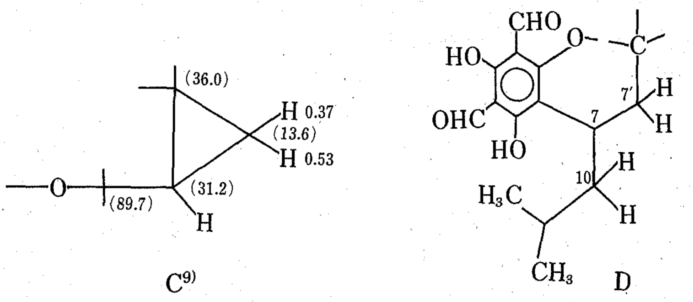


Fig. 1. The Higher Field Region of the 360 MHz PMR Spectra of Euglobal-Ic (**5**)

irradiated, the carbon signals at δ 13.4(t) and 31.2(d), rsepectively, were decoupled. The presence of a methine and a methlyene group in the cyclopropane ring was thus confirmed. Since there was no coupling between these proton signals and the other protons, the presence of one quaternary carbon in the three-membered ring and the other one attached to the methine in the cyclopropane ring was indicated. The quaternary carbon at δ 36.0 was assigned to the carbon in the three-membered ring from the chemical shifts, which led the quaternary carbon adjacent to an oxygen atom to be connected to the methine in the cyclopropane ring. These observations provided the partial structure [C] in euglobal-Ic(**5**).



C[9]

D

Chart 4

Irradiation of the one-proton multiplet at δ 2.92 [C(7)–H] caused the double doublets δ 1.57 [C(10)–H, J=9.4, 11.4 Hz] to coalesce into a doublet (J=11.4 Hz), and the overlapped one-proton signal at δ 1.80 [C(10)–H] to be simplified. In addition, the pair of double doublets centered at δ 1.93 (2H, δ 1.97, J=14.2, 6.4 Hz; δ 1.89, J=14.2, 4.3 Hz) was changed into an AB quartet (J_{gem}=14.2 Hz). In the selective proton decoupling, the carbon signal at δ 31.9 (t) was decoupled on irradiation of the protons centered at δ 1.93. These results suggested that C(7) bound to two methylene groups. Of these, one of the methylenes (δ 1.57 and 1.80) is a part of the isobutyl group, and the other one (centered at δ 1.93) is attached to a quaternary carbon since the methylene was not coupled with any proton other than the C(7) proton. Based on these observations, a partial structure [D] was assigned to euglobal-Ic (**5**). Irradiation of the septet at δ 1.37 changed each of the two methyl doublets (J=6 Hz each) at δ 0.965 and 0.960 to a singlet.

Conversely, irradiation of the signals due to these methyls converted the septet to a singlet. These results suggested that an isopropyl group was attached to a quaternary carbon. The remaining two carbon signals at δ 24.0 and 35.0 in the CMR of the terpene moiety were assigned to methylene carbon signals by off-resonance decoupling. In the 360 MHz PMR spectra, the proton signals of these methylenes were assigned as shown in Fig. 1 by the decoupling technique. These two methylenes were adjacent, forming an ethylene group, and attached to quaternary carbons at both ends, since there was no coupling except for the coupling between the ethylenic protons. The examination of the possible structures derived from the partial structures [C] and [D], together with the isopropyl group attached to a quaternary carbon and the ethylene group as described above, gave only one structure, **5**.

Further confirmation of this assignment was obtained by a single crystal X-ray analysis of euglobal-Ic (**5**).[8]

On the other hand, the observed J value (0 Hz) between C(5')-H (δ 1.62) and C(6')-H (δ 1.82) suggested that the dihedral angle of these protons is approximately 90°. The X-ray studies[8] indicated that the dihedral angle between the C(5')-H_α and C(6')-H_β is 92.4°. A long-range coupling of C(5')-H_β with C(3')-H_β was also justified as a consequence of a "W" arrangement of the four σ-bonds.

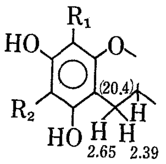
Euglobal-IIc (**8**) was isolated as an oil, and showed UV, IR and MS similar to those of the other euglobals. The signals due to the aromatic part in the NMR spectra (Table I) of this compound were also comparable with those of the other euglobals except for the signals described below. The NMR data demonstrated the presence of two hydroxyls (singlets at δ 15.35 and 14.45 in PMR), analdehyde (s, δ 10.04, 1H in PMR; d, δ 191.6 in CMR) and a ketonic carbon (s, δ 206.0 in CMR). These data suggested that the structure of the aromatic part of euglobal-IIc (**8**) is slightly different from those of the other euglobals.

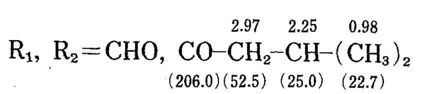
In the CMR spectrum, the six aromatic carbons were all singlets and their chemical shifts coincided with those of the other euglobals, indicating that the substitution pattern on the aromatic ring of euglobal-IIc (**8**) was similar to those of the others. This suggests that one of the two aldehyde groups present in the other six euglobals was substituted by an acyl group in euglobal-IIc (**8**). The acyl group was found to be an isovaleryl group from the following NMR experiments. Irradiation of the signal at δ 2.25 (1H, m, $J=7$ Hz) converted the signals of a methylene at δ 2.97 (2H, d, $J=7$ Hz) and two methyls at δ 0.98 (6H, d, $J=7$ Hz) to a singlet, in each case. The assignments were also confirmed by the fact that the CMR spectrum revealed the presence of a ketone (δ 206.0), a methylene (δ 52.5) adjacent to the ketone, a methine (δ 25.0) and two methyls (δ 22.7). The PMR and CMR chemical shifts of the acyl group coincided with those of the isovaleryl group of grandinol (**9**).

As the signal corresponding to that of C(7)-H observed in the PMR spectra of the other six euglobals, there was a methylene signal (δ 2.65, $J=16$, 7 Hz; δ 2.39, $J=16$, 6 Hz, a pair of double doublets) related to the carbon signal at δ 20.4(t) by selective proton decoupling. The coupling pattern suggested that the methylene group was attached to a methine group. These observations led to a partial structure [E].

The CMR of the terpene moiety of this compound demonstrated the presence of two olefinic carbons (δ 132.9 and 130.4, each d), a quaternary carbon (δ 78.3), three methines (δ 38.7, 33.2 and 31.8), a methylene (δ 27.6) and three methyls (δ 27.4, 19.9 and 19.8). Since these CMR data were very similar to those of euglobal-Ia$_1$ (**2**) and -Ia$_2$ (**3**), the formula **8** or **13** should represent euglobal-IIc.

In order to clarify the structure of this compound, detailed analysis of the long-range ^{13}C-^1H coupling was conducted. Euglobal-Ic (**5**) was selected as a standard for the assignment of the six aromatic carbons because its structure was definitely established by the X-ray analysis study.$^{8)}$ The higher field spectrum of the high-resolution CMR of euglobal-Ic (**5**)





$R_1, R_2=CHO, \underset{(206.0)}{CO}-\underset{(52.5)}{\overset{2.97}{CH_2}}-\underset{(25.0)}{\overset{2.25}{CH}}-(\underset{(22.7)}{\overset{0.98}{CH_3}})_2$

E$^{9)}$

Chart 5

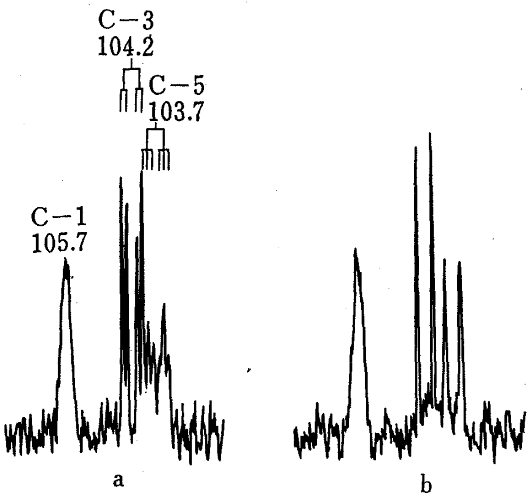


Fig. 2. The Higher Field Signals of the Aromatic Carbons in the 50.2 MHz CMR Spectra of Euglobal -Ic (**5**)

a: gated ^1H decoupled spectrum.
b: gated ^1H decoupled spectrum on addition of D_2O.

is shown in Fig. 2-a. On the basis of the CMR chemical shifts and long-range coupling patterns, the signals at δ 10.57(m), 104.2 [dd, ${}^2J(\underline{\text{C}}\text{-C}\underline{\text{H}}\text{O})=16.9$ Hz, ${}^3J(\underline{\text{C}}\text{-C-O}\underline{\text{H}})=5.9$ Hz] and δ 103.7 [dt, ${}^2J(\underline{\text{C}}\text{-C}\underline{\text{H}}\text{O})=17.6$ Hz, ${}^3J(\underline{\text{C}}\text{-C-O}\underline{\text{H}})=5.1$, 5.1 Hz] can be assigned to C(1), C(3) and C(5), respectively. The signal of C(1) appeared as an unresolved multiplet at δ 105.7 due to the coupling with C(6)-hydroxy proton, C(7)-methine, C(7′)-methine and C(10)-methylene. Since C(3) has one two-bond proton [$\underline{\text{C}}$(3)-C$\underline{\text{H}}$O] and one three-bond proton [$\underline{\text{C}}$(4)-O$\underline{\text{H}}$], it appeared as a double doublet at δ 104.2. A double triplet at δ 103.7 can be assigned to C(5), since this carbon possesses one two-bond proton [$\underline{\text{C}}$(5)-C$\underline{\text{H}}$O] and two three-bond protons [C(4) and $\underline{\text{C}}$(6)-O$\underline{\text{H}}$]. These assignments were supported by a deuterium-exchange experiment, in which the signals due to C(1), C(3), and C(5) changed into a slightly simplified multiplet and two doublets, respectively, as shown in Fig. 2-b.

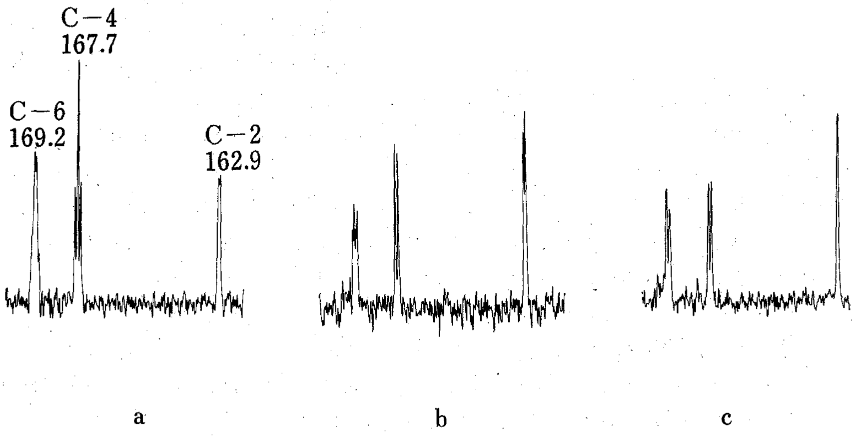


Fig. 3. The Lower Field Signals of the Aromatic Carbons in 50.2 MHz CMR Spectra of Euglobal-Ic (**5**)

a: gated ^{1}H decoupled spectrum.
b: gated ^{1}H decoupled spectrum on addition of D_2O.
c: long-range selective ^{1}H-decoupled spectrum irradiated at δ 2.92 [C(7)-H].

In the lower field of the spectrum (Fig. 3-a), the signals due to C(6) (δ 169.2, m), C(4) (δ 167.7, t, $J=5.1$ Hz) and C(2) (δ 162.9, br d) were observed, and these signals changed upon deuterium-exchange into a double doublet ($J=5.1$, 2.9 Hz), a doublet ($J=5.1$ Hz) and a doublet ($J=2.9$ Hz), respectively (Fig. 3-b). Further, in the decoupled NMR spectrum (Fig. 3-c), saturation of the proton attached to C(7) at low power caused the double doublet at δ 169.2 and the doublet at δ 162.9 to collapse into a doublet ($J=5.1$ Hz) and a singlet, respectively. The unaffected triplet at δ 167.7 was assigned to C(4). The doublet [${}^3J(\underline{\text{C}}\text{-C-C}\underline{\text{H}}\text{O})=5.1$ Hz] at δ 169.2 was assigned to C(6), and the remaining signals at δ 162.9 can be assigned to C(2).

As already described, the C(4) signal was changed to a doublet ($J=5.1$ Hz) in the deuterium-exchange experiment, showing long-range coupling with a three-bond proton [C(3)-CHO or C(5)-CHO]. The three-bond proton coupling may involve C(3)-CHO, because, as illustrated in Chart 6, the C(3)-aldehydic C-H bond adopts the *anti* conformation with respect to the C(3)-C(4) bond, while the C(5)-aldehydic C-H bond assumes a *syn* relationship with the C(4)-C(5) bond. The observations that the signal due to C(2) had no coupling with the *syn* three-bond proton of C(3)-CHO, and that the signal due to C(6) coupled with the *anti* three-bond proton of C(5)-CHO were also explicable by the geometry involving the hydrogen bonds shown in Chart 6.

The higher field of the long-range ^{13}C-^{1}H coupled spectrum of euglobal-IIc (**8**) is shown in Fig. 4. In the spectrum, the double doublet at δ 103.6 [${}^2J(\underline{\text{C}}\text{-C}\underline{\text{H}}\text{O})=16.8$ Hz, ${}^3J(\underline{\text{C}}\text{-C-O}\underline{\text{H}})=$

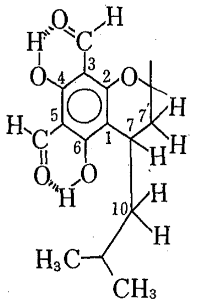
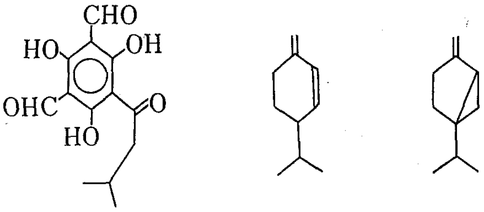


Chart 6



14 15 16

Chart 7

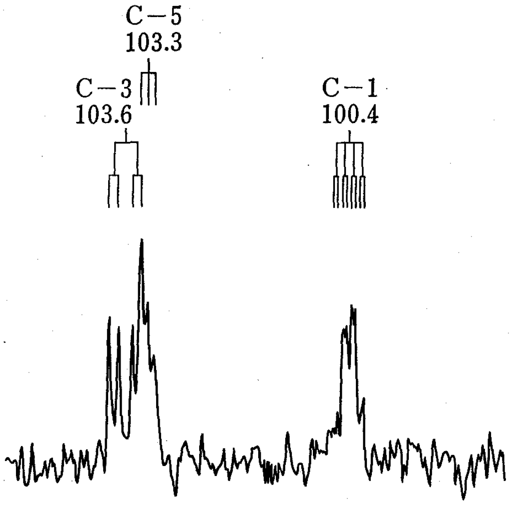


Fig. 4. The Higher Field Signals of the Aromatic Carbons in the 50.2 MHz CMR Spectra of Euglobal-IIc (**8**) with Gated ^{1}H Decoupling

5.9 Hz], the triplet at δ 103.3 [$^{3}J(\underline{C}\text{-C-O}\underline{H})=5.1$ Hz] and the double quartet-like signal at δ 100.4 [$^{2}J(\underline{C}\text{-C}\underline{H}_2)=5.9$ Hz, $^{3}J(\underline{C}\text{-C-O}\underline{H})=5.9$ Hz] were assigned to C(3), C(5) and C(1), respectively. The signal due to C(5) appeared as a triplet instead of a double triplet observed in the case of euglobal-Ic (**5**). The triplet signal was formed by coupling with both C(4) and C(6)-hydroxy protons, and partially overlapped with the signal at δ 103.6. These results indicated that the isovaleryl group was attached to C(5), and the formula **8** (excluding the absolute configuration) was assigned to euglobal-IIc.

In the lower field of the spectrum, the aromatic signals at δ 171.1(m), 167.8(t, $J=5.1$ Hz) and 160.0(m) were assigned to C(6), C(4) and C(2), respectively, by comparison with those of euglobal-Ic (**5**). The fact that the carbon signal due to C(4) was observed as the triplet similar to that of euglobal-Ic (**5**) was accounted for by the explanation already presented. Irradiation of the aldehyde proton at δ 10.04 at low power converted the double doublet at δ 103.6 [C(3), $J=16.8$ and 5.9 Hz] and the triplet at δ 167.8 [C(4), $J=5.1$ Hz] to two doublets.

Assignments of the hydroxyl groups were made possible by the following decoupling experiments. Irradiation of the low-field hydroxyl singlet at δ 15.35 changed the double quartet of C(1) at δ 100.4 to a double triplet-like signal, the triplet of C(5) at δ 103.3 to a doublet ($J=5.1$ Hz), and the multiplet of C(6) at δ 171.1 to a broad singlet. Saturation of the high-field hydroxyl singlet at δ 14.45 converted the double doublet of C(3) to a doublet ($J=16.8$ Hz), the triplet of C(4) to a doublet ($J=5.1$ Hz), and the triplet of C(5) to a doublet ($J=5.1$ Hz). Thus, the singlets at δ 15.35 and δ 14.45 were assigned to C(6)-O$\underline{H}$ and C(4)-O$\underline{H}$, respectively.

Euglobal-IIa (**6**), mp 130—132°C, was isolated as colorless prisms. The great similarities of the chemical shifts, the splitting patterns and the behavior in decoupling experiments in the PMR and CMR (Fig. 2) indicated that euglobal-IIa (**6**) and -Ic (**5**) have the same planar structure. Steric compression shifts, which will be discussed later, were observed for C(7′) in both **5** and **6**. This result indicated that compounds **5** and **6** have the same configuration of the terpene moiety, and differ only in the configuration of the isobutyl group, and that the structure of euglobal-IIa can be represented by formula **6** (excluding the absolute configuration). It was assumed that the relationship of euglobal-IIa (**6**) and -Ic (**5**) corresponds to that of euglobal-Ia$_1$ (**2**) and -Ia$_2$ (**3**).

Euglobal-Ib (**4**), mp 119—121 °C, was isolated as colorless needles. As is apparent from Table II, the CMR spectrum of **4** indicated that this compound also possessed the same planar structure as euglobal-Ic (**5**) and -IIa (**6**). The major differences among the PMR and CMR spectra of these three compounds were observed in the methylene proton signals at C(3′) and the carbon signals at C(7′). The chemical shifts of C(7′) in **5** (δ 31.9) and **6** (δ 32.3) were shifted upfield by 6.1 and 5.7 ppm, respectively, compared to those found in **4** (δ 38.0), suggesting that the steric compression shift is attributable to a difference in the configuration of the cyclopropane ring. Thus, the relative configuration of the cyclopropane ring was assigned as in structure **4** for euglobal-Ib, and as in structures **5** and **6** for euglobal-Ic and -IIa.

Euglobal-IIb (**7**) was isolated as a colorless oily substance and showed CMR spectra (Table II) similar to those of euglobal-Ib (**4**), except for the signals due to C(2′), C(3′) and C(4′). Its CMR spectrum exhibited signals due to a quaternary carbon adjacent to an oxygen atom, an isopropyl group, a methine and three methylenes. The NMR spectra of this compound did not show the signals due to the cyclopropane ring seen in euglobal-Ic (**5**), but showed two olefinic protons (δ 5.94 and 5.72) and the corresponding two olefinic carbons (δ 136.9 and 129.0, each d), suggesting the formulation **7** for euglobal-IIb.

Finally, we will consider the biogenesis of the euglobals. In view of the acetogenin-monoterpene structures of the seven euglobals, these conpounds were thought to be derived biogenetically from isovaleryl phloroglucinol dialdehyde (**14**) and monoterpenes such as α-phellandrene (**10**), β-phellandrene (**15**) and sabinene (**16**), by a Diels–Alder type condensation involving the double bond of these monoterpenes. In the case of euglobal-Ia$_1$ (**2**) and -Ia$_2$ (**3**), the phloroglucinol precursor (**14**) would be condensed with the 1,6-double bond of α-phellandrene (**10**). Similarly, euglobal-Ib (**4**), -Ic (**5**) and IIa (**6**) would be derived from sabinene (**16**), and euglobal-IIb (**7**) from β-phellandrene (**15**). On the other hand, application of chiroptical methods demonstrated that euglobal-Ia$_1$ (**2**) and -IIc (**8**) exhibited negative Cotton effects while euglobal-Ia$_2$ (**3**), -Ib (**4**), -Ic (**5**), -IIa (**6**) and -IIb (**7**) were thought to be racemic. In fact, the X-ray analysis[8] of euglobal-Ic (**5**) showed that the compound was a racemate, suggesting that *dl*-sabinene (**16**) might be involved in the biosynthesis. Similarly, euglobal-Ia$_2$ (**3**), -Ib (**4**), -IIa (**6**) and -IIb (**7**) are presumably produced from the racemic monoterpenes. Euglobal-IIc (**8**) may be formed from α-phellandrene (**10**) and, in this case, one of the aldehydic carbonyls instead of the ketonic carbonyl in the phloroglucinol precursor (**14**) would be involved in the biosynthesis.

Further studies on the biosynthesis and the biological activities of these seven compounds are in progress.

Experimental

All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. The UV spectra were obtained on a Hitachi 260-10 spectrophotometer in ethanol and the IR spectra were recorded on a Perkin–Elmer model 450 recording spectrophotometer in liquid film or potassium bromide pellets. The PMR spectra were recorded on a Varian XL-100-12 spectrometer and a Bruker WM-360 spectrometer. The samples (20—40 mg) were dissolved in deuterated chloroform (0.4 ml), and measured by means of the continuous wave method (Varian XL-100-12) or the Fourier-transform method (Bruker WM-360). Chemical shifts are given in δ values downfield from tetramethylsilane (TMS) as an internal standard. The CMR spectra were determined on Varian XL-100-12 and JEOL FX-200 Fourier-transform spectrometers operating at 25.2 and 50.2 MHz with proton noise decoupling, off-resonance decoupling, selective proton decoupling, and gated decoupling techniques. The spectral width was 5000 Hz (5000—10000 Hz) for euglobal-Ic, 6000 Hz (6000—12000 Hz) for euglobal-IIc and 5000 Hz for euglobal-Ia$_1$, -Ia$_2$, -Ib, -IIa and -IIb, and 8K (32K) data points were used for 2000—10000 accumulations in the Varian XL-100-12 (JEOL FX-200). The samples (40—80 mg) were dissolved in $CDCl_3$ (0.4 or 0.8 ml) and the ^{13}C-chemical shifts were measured relative to the ^{13}C-signal of $CDCl_3$ then converted to ppm from TMS using $\delta (CDCl_3) = 76.9$. The MS were taken with a JEOL JMS-01SC mass spectrometer. The optical rotations were measured on a Perkin–Elmer model 141 polarimeter. The CD and ORD spectra were recorded on a JASCO model ORD/UV-5 spectrometer.

Euglobal-Ia$_1$ (2)——Colorless oil. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ε): 277 (32000), 340 (3700, inflection). IR $\nu_{\text{max}}^{\text{liq.film}}$ cm^{-1} 3300 (OH), 1620 (C=O). $[\alpha]_{\text{D}}^{20}$ $-216.7°$ (c=1.0, $CHCl_3$). CD (c=0.0032, ethanol) $\Delta\varepsilon^{25}$: -4.9 (340), -13.8 (288). The NMR and MS data are shown in Table I and Chart 2, respectively.

Euglobal-Ia$_2$ (3)——Colorless oil. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ε): 277 (32000), 340 (3700, inflection). IR $\nu_{\text{max}}^{\text{liq.film}}$ cm^{-1}: 3350 (OH), 1630 (C=O). $[\alpha]_{\text{D}}^{20}$ $+31.8°$ (c=1.0, $CHCl_3$). The NMR and MS data are shown in Table I and Chart 2, respectively.

Euglobal-Ib (4)——Colorless needles from ethanol, mp 119—121°C. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ε): 277 (36000), 340 (4300, inflection). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}: 3400 (OH), 1630 (C=O). $[\alpha]_{\text{D}}^{20}$ $-1.94°$ (c=1.0, $CHCl_3$). The NMR and MS data are shown in Table II and Chart 2, respectively. *Anal.* Calcd for $C_{23}H_{30}O_5$: C 71.48; H 7.82. Found: C 71.57; H 7.71.

Euglobal-Ic (5)——Colorless prisms from ethanol, mp 108—110°C. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ε): 277 (38000), 340 (4100, inflection). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}: 3350 (OH), 1630 (C=O). $[\alpha]_{\text{D}}^{20}$ $-3.12°$ (c=1.0, $CHCl_3$). The NMR and MS data are shown in Table II and Chart 2, respectively. *Anal.* Calcd for $C_{23}H_{30}O_5$: C 71.48; H 7.82. Found: C 71.56; H 7.63.

Euglobal-IIa (6)——Colorless prisms from ethanol, mp 130—132°C. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ε): 277 (38000), 340 (4100, inflection). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}: 3420 (OH), 1620 (C=O). $[\alpha]_{\text{D}}^{20}$ $+9.24°$ (c=1.0, $CHCl_3$). The NMR and MS data are shown in Table II and Chart 2, respectively. *Anal.* Calcd for $C_{23}H_{30}O_5$: C 71.48; H 7.82. Found: C 71.37; H 7.82.

Euglobal-IIb (7)——Colorless oil. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ε): 277 (36000), 340 (4500, inflection). IR $\nu_{\text{max}}^{\text{liq.film}}$ cm^{-1}: 3400 (OH), 1620 (C=O). $[\alpha]_{\text{D}}^{20}$ $+12.2°$ (c=1.0, $CHCl_3$). The NMR and MS data are shown in Table II and Chart 2, respectively.

Euglobal-IIc (8)——Colorless oil. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ε): 277 (35000), 340 (3900, inflection). IR $\nu_{\text{max}}^{\text{liq.film}}$ cm^{-1}: 3400 (OH), 1620 (C=O). $[\alpha]_{\text{D}}^{20}$ $-144°$ (c=1.0, $CHCl_3$). CD (c=0.0034, ethanol) $\Delta\varepsilon^{25}$: -1.7 (340), -5.7 (286). The NMR and MS data are shown in Table I and Chart 2, respectively.

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8) The X-ray crystallographic studies of these compounds will be reported elsewhere.
9) The figures in the structural formulas are δ^1H (δ^{13}C) in the NMR.