Macrocarpal A, a Novel Antibacterial Compound from *Eucalyptus macrocarpa*

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A novel antibacterial compound, macrocarpal A, was isolated from the leaves of *Eucalyptus macrocarpa*, and its structure was determined on the basis of an X-ray crystal structure analysis. Macrocarpal A is composed of a phloroglucinol dialdehyde and diterpene, having a 3-membered ring, a 5-membered ring and a 7-membered ring.

Plants of the *Eucalyptus* species are widely distributed throughout the world and famous for their rapid growth. Recently, several biologically active compounds have been isolated from *Eucalyptus* species as biological resources. Grandinol¹⁾ is a rooting inhibitor and was isolated from adult tissue of *E. grandis*. We have also isolated and identified grandinol as an antibacterial compound from *E. perriniana*.²⁾ Euglobals^{3–5)} have been isolated from the buds and leaves of *E. globulus* and were found to show anti-inflammatory activity. Robustadials⁶⁾ are antimalarial compounds and were isolated from *E. robusta*.

We are interested in antimicrobial compounds from plants of the *Eucalyptus* species,

and have reported antibacterial compounds from *E. perriniana*.²⁾ In this paper, we described the isolation and structural elucidation of a novel antibacterial compound from *E. macrocarpa*. It was named macrocarpal A (**1**, Fig. 1) after the species name of the plant.

Results and Discussion

Isolation and characterization

An acetone extract of the leaves of the plant was fractionated into strongly acidic, weakly acidic, alkaline and neutral fractions in the usual way. The neutral fraction was further purified by silica gel column chromatography (CHCl₃/MeOH), Sephadex LH-20 column chromatography, silica gel column chromatography (CHCl₃/AcOH) and reversed-phase HPLC. *Bacillus subtilis* was used as a test organism in each step. As a result, several active compounds, which had similar physicochemical properties and antibacterial spectra, were isolated.⁷⁾ Compound **1** was crystallized from MeOH.

Macrocarpal A (**1**), a colorless flat plate, mp 198–200 °C, $[\alpha]_D^{20} -94.7^\circ$ ($c=0.275$, EtOH), has molecular weight of 472 by negative FAB-MS (m/z 471 ($M-1$)⁻) and the composi-

Fig. 1. Structure of Macrocarpal A.

tion $C_{28}H_{40}O_6$, which were finally determined by the results of an X-ray crystal structure analysis. The UV spectrum of **1** ($\lambda_{\max}$ (EtOH) nm (ϵ): 275 (13,000), 393 (5600)) is in good agreement with the data reported for grandinol and euglobals. Fragmentation peaks of EI-MS at m/z 454, 397, 251, 204, 203, 195 and 161 were similar to euglobals III, IV, V and VII. These data suggest that **1** has a similarly substituted phloroglucinol chromophore. The 270 MHz 1H -NMR spectrum of **1** showed two hydrogen-bonded phenolic hydroxyls (δ 13.57), a phenolic hydroxyl (δ 6.00), two aldehyde groups (δ 10.13, 10.19), two methine protons (δ 0.54, 0.65) on a cyclopropane ring, a methine proton (δ 3.67) adjacent to the phloroglucinol and isobutyl side chain, four singlet methyls (δ 0.75–1.15) and two doublet methyls (δ 0.83, 0.80). The ^{13}C -NMR spectrum of **1** showed six methyl carbons, five methylene carbons, six methine carbons, two quaternary carbons and a quaternary carbon bearing a oxygen atom in the region of the sp^3 carbons.

These results show that **1** has a similar structure to euglobals III, IV, V and VII, which are composed of a phloroglucinol part and a diterpene part, but that **1** is different from euglobals III, IV, V and VII.

Structure

The structure of **1** was determined by an X-ray crystal structure analysis. The crystal data are as follows: $C_{28}H_{40}O_6$, MW 472.62, monoclinic, space group $P2_1$, $a = 8.578$ (3), $b = 21.800$ (7), $c = 7.233$ (2) Å, $\beta = 103.34$ (2)°, $V = 1316.0$ (7) Å³, $Z = 2$, $D_x = 1.19$ g·cm⁻³. The final R value was 0.060 for 3133 reflections. The final atomic parameters for the non-hydrogen atoms are given in Table I.⁸⁾ The molecular structure of **1** with the numbering of the atoms is shown in Fig. 2. Macrocarpal A has seven asymmetric carbon atoms, whose relative configurations were determined. The bond distances and angles are listed in Table II, and the torsion angles in Table 3. The three-membered ring condensed with a seven-membered ring is an approximately equiangular triangle (59.8, 60.1 and 60.1°). The seven-

Table I. FINAL ATOMIC COORDINATES ($\times 10^4$) AND EQUIVALENT ISOTROPIC TEMPERATURE FACTORS FOR NON-HYDROGEN ATOMS OF MACROCARPAL A

Atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i> (eq)
O (1)	1867 (3)	8262 (1)	1349 (3)	4.1
O (2)	−94 (3)	7407 (1)	−232 (4)	5.0
O (3)	676 (4)	6542 (1)	4724 (4)	5.1
O (4)	3653 (4)	7151 (1)	9664 (4)	5.4
O (5)	3991 (3)	8157	7937 (4)	4.5
O (6)	923 (4)	11440 (1)	2704 (4)	4.6
C (1)	2946 (3)	8250 (1)	4653 (4)	2.9
C (2)	2024 (3)	7974 (1)	3032 (4)	3.1
C (3)	1222 (3)	7405 (1)	3052 (4)	3.4
C (4)	1410 (4)	7087 (1)	4752 (5)	3.5
C (5)	2384 (4)	7327 (1)	6424 (4)	3.5
C (6)	3101 (3)	7916 (1)	6335 (4)	3.2
C (7)	190 (4)	7161 (2)	1346 (6)	4.5
C (8)	2784 (4)	6971 (2)	8156 (6)	4.4
C (9)	3777 (3)	8874 (1)	4681 (4)	2.8
C (10)	4883 (4)	8890 (1)	3282 (5)	3.4
C (11)	6084 (4)	8351 (1)	3481 (6)	4.4
C (12)	7085 (5)	8415 (3)	1970 (7)	6.3
C (13)	7172 (5)	8294 (2)	5473 (7)	5.8
C (14)	2566 (3)	9422 (1)	4534 (4)	3.0
C (15)	1441 (4)	9522 (2)	2589 (5)	4.3
C (16)	740 (4)	10166 (2)	2691 (6)	4.7
C (17)	2014 (3)	10526 (1)	4133 (4)	2.9
C (18)	3415 (3)	10063 (1)	4843 (4)	2.5
C (19)	4309 (3)	10209 (1)	6853 (4)	3.2
C (20)	5102 (4)	10826 (2)	7343 (5)	4.0
C (21)	5158 (4)	11292 (2)	5845 (6)	4.4
C (22)	3530 (5)	11545 (1)	4861 (6)	4.5
C (23)	2498 (4)	11138 (1)	3359 (4)	3.6
C (24)	6112 (4)	10248 (2)	7589 (6)	5.2
C (25)	7259 (4)	10140 (2)	6343 (9)	7.1
C (26)	6708 (8)	10042 (4)	9655 (9)	10.1
C (27)	1572 (4)	9319 (2)	6029 (6)	4.6
C (28)	3207 (5)	11071 (2)	1610 (5)	5.3

membered ring is bent at the C(18)···C(21) and C(17)···C(22) axes, and the atoms of C(18)–C(19)–C(20)–C(21) and C(17)–C(18)···C(21)–C(22) lie fairly well on a plane, respectively. It resembles a chair form. The five-membered ring is bent at the C(15)···C(18) axis, and the atoms of C(15)–C(16)–C(17)–C(18) are planar. The bond distances of C(14)–C(18) (1.567 Å), C(17)–C(18) (1.563 Å) and C(9)–C(14) (1.571 Å) are significantly longer than the other bonds, which are considered to be caused by steric repulsion between the bulky substituents. The aromatic

Table II. BOND DISTANCES (Å) AND ANGLES (deg) FOR MACROCARPAL A

Bond	Distance	Bond	Angle
O (1)-C (2)	1.349 (4)	C (2)-C (1)-C (6)	115.4 (3)
O (2)-C (7)	1.233 (5)	C (2)-C (1)-C (9)	124.5 (2)
O (3)-C (4)	1.342 (5)	C (6)-C (1)-C (9)	120.0 (2)
O (4)-C (8)	1.235 (5)	O (1)-C (2)-C (1)	118.6 (3)
O (6)-C (23)	1.479 (4)	O (1)-C (2)-C (3)	117.9 (3)
C (1)-C (2)	1.391 (4)	C (1)-C (2)-C (3)	123.5 (3)
C (1)-C (6)	1.398 (4)	C (2)-C (3)-C (4)	119.2 (3)
C (1)-C (9)	1.533 (4)	C (2)-C (3)-C (7)	121.1 (3)
C (2)-C (3)	1.420 (4)	C (4)-C (3)-C (7)	119.8 (3)
C (3)-C (4)	1.388 (5)	O (3)-C (4)-C (3)	118.1 (3)
C (3)-C (7)	1.444 (5)	O (3)-C (4)-C (5)	121.7 (3)
C (4)-C (5)	1.403 (5)	C (3)-C (4)-C (5)	120.2 (3)
C (5)-C (6)	1.432 (4)	C (4)-C (5)-C (6)	118.1 (3)
C (5)-C (8)	1.445 (5)	C (4)-C (5)-C (8)	121.5 (3)
C (9)-C (10)	1.539 (5)	C (6)-C (5)-C (8)	120.1 (3)
C (9)-C (14)	1.571 (4)	C (1)-C (6)-C (5)	123.5 (3)
C (10)-C (11)	1.547 (6)	O (2)-C (7)-C (3)	125.6 (4)
C (11)-C (12)	1.544 (7)	O (4)-C (8)-C (5)	124.7 (4)
C (11)-C (13)	1.530 (7)	C (1)-C (9)-C (10)	111.5 (2)
C (14)-C (15)	1.527 (5)	C (1)-C (9)-C (14)	112.1 (2)
C (14)-C (18)	1.567 (4)	C (10)-C (9)-C (14)	115.9 (2)
C (14)-C (27)	1.540 (5)	C (9)-C (10)-C (11)	114.8 (3)
C (15)-C (16)	1.535 (6)	C (10)-C (11)-C (12)	109.4 (4)
C (16)-C (17)	1.540 (5)	C (10)-C (11)-C (13)	113.5 (3)
C (17)-C (18)	1.563 (4)	C (12)-C (11)-C (13)	110.8 (4)
C (17)-C (23)	1.541 (4)	C (9)-C (14)-C (15)	116.0 (3)
C (18)-C (19)	1.513 (4)	C (9)-C (14)-C (18)	113.0 (2)
C (19)-C (20)	1.513 (4)	C (9)-C (14)-C (27)	107.8 (3)
C (19)-C (24)	1.517 (5)	C (15)-C (14)-C (18)	100.3 (2)
C (20)-C (21)	1.494 (6)	C (15)-C (14)-C (27)	109.5 (3)
C (20)-C (24)	1.517 (6)	C (18)-C (14)-C (27)	110.2 (3)
C (21)-C (22)	1.517 (6)	C (14)-C (15)-C (16)	104.9 (3)
C (22)-C (23)	1.519 (5)	C (15)-C (16)-C (17)	105.9 (3)
C (23)-C (28)	1.532 (6)	C (16)-C (17)-C (18)	105.4 (2)
C (24)-C (25)	1.498 (8)	C (16)-C (17)-C (23)	113.9 (3)
C (24)-C (26)	1.530 (9)	C (18)-C (17)-C (23)	115.3 (2)
		C (14)-C (18)-C (17)	103.4 (2)
		C (14)-C (18)-C (19)	116.1 (2)
		C (17)-C (18)-C (19)	111.2 (2)
		C (18)-C (19)-C (20)	120.8 (2)
		C (18)-C (19)-C (24)	126.8 (3)
		C (20)-C (19)-C (24)	60.1 (2)
		C (19)-C (20)-C (21)	121.5 (3)
		C (19)-C (20)-C (24)	60.1 (2)
		C (21)-C (20)-C (24)	122.5 (3)
		C (20)-C (21)-C (22)	114.1 (3)
		C (21)-C (22)-C (23)	116.4 (3)
		O (6)-C (23)-C (17)	101.5 (2)
		O (6)-C (23)-C (22)	107.9 (3)
		O (6)-C (23)-C (28)	106.6 (3)
		C (17)-C (23)-C (22)	114.1 (3)
		C (17)-C (23)-C (28)	114.1 (3)
		C (22)-C (23)-C (28)	111.6 (3)
		C (19)-C (24)-C (20)	59.8 (2)
		C (19)-C (24)-C (25)	122.5 (4)
		C (19)-C (24)-C (26)	114.2 (4)
		C (20)-C (24)-C (25)	119.8 (4)
		C (20)-C (24)-C (26)	114.3 (4)
		C (25)-C (24)-C (26)	115.1 (5)

Fig. 2. Perspective View of Macrocarpal A.
The absolute configuration is tentative.

Fig. 3. Crystal Structure of Macrocarpal A.

The *a* axis is down from the plane of the paper. ---, hydrogen bonds: O(1)---O(2), 2.593 Å; O(4)---O(5), 2.571 Å; O(6)---O(2), 2.748 Å; O(6)---O(3), 2.563 Å.

Table III. TORSION ANGLES (deg)
FOR MACROCARPAL A

C (A)-C (B)-C (C)-C (D)	Torsion angle
C (14)-C (15)-C (16)-C (17)	28.0
C (15)-C (16)-C (17)-C (18)	-0.9
C (16)-C (17)-C (18)-C (14)	-25.6
C (17)-C (18)-C (14)-C (15)	42.1
C (18)-C (14)-C (15)-C (16)	-43.2
C (23)-C (17)-C (18)-C (19)	82.7
C (17)-C (18)-C (19)-C (20)	-58.9
C (18)-C (19)-C (20)-C (21)	-5.5
C (19)-C (20)-C (21)-C (22)	66.2
C (20)-C (21)-C (22)-C (23)	-77.9
C (21)-C (22)-C (23)-C (17)	62.9
C (22)-C (23)-C (17)-C (18)	-67.9
C (17)-C (18)-C (21)-C (22)	-3.8

ring is planar within experimental error. Hydroxyl groups O(1) and O(5) are hydrogen-bonded to oxygen atoms O(2) and O(4) of the aldehyde groups, respectively. The bond distance of C(23)-O(6) (1.479 Å) is significantly longer than the other C-O bonds. The crystal structure is shown in Fig. 3. A unit cell contains two molecules of macrocarpal A. Hydroxyl group O(6) is hydrogen-bonded to O(2) of the carbonyl group of a neighboring molecule (O(6)(X, Y, Z)···O(2) (-X, 0.5+Y, -Z), 2.748 Å) and O(3) of the hydroxyl group of another neighboring molecule (O(6)(X, Y, Z)···O(3) (-X, 0.5+Y, 1.0-Z), 2.563 Å). There are no unusually short contacts between

Fig. 4. Structures of Related Compounds.

the molecules in the crystals, except for these hydrogen bonds.

Compound **1** is a novel compound which has a phloroglucinol dialdehyde and diterpene structure. A structurally related compound, euglobal V (Fig. 4), has a phloroglucinol dialdehyde and diterpene. Euglobal V has an ether bond, C(5a)–O(5)–C(4a), between the aromatic moiety and non-aromatic moiety. On the other hand, macrocarpal A has no ether bond between C(2), O(1) and C(17), which correspond to C(5a), O(5) and C(4a) in euglobal V, respectively. Moreover, macrocarpal A has a hydroxyl group in the non-aromatic part, which is bound to C(23). The part involving a 3-membered ring, 5-membered ring and 7-membered ring is the same as that in the structure of globulol.⁹⁾ Euglobal V⁵⁾ is considered to be biogenetically derived from an isovaleroylphloroglucinol dialdehyde precursor and bicyclogermacrene, which is the common precursor of aromadendrene derivatives isolated from *E. globulus* such as α-grujunene, aromadendrene and globulol (Fig. 4). The relationship between these two compounds in biosynthesis is interesting.

Antimicrobial activity

Macrocarpal A showed antibacterial activity

against Gram-positive bacteria such as *Bacillus subtilis* PCI219 and *Staphylococcus aureus* FDA209P (minimum inhibitory concentration <0.2 μg/ml and 0.4 μg/ml, respectively) but no activity against Gram-negative bacteria such as *Escherichia coli* NIHJ and *Pseudomonas aeruginosa* P-3, yeast and fungi such as *Saccharomyces cerevisiae* and *Aspergillus niger*.

Experimental

General. The melting point (mp) was determined by a Yanagimoto micro-melting point measurement instrument. Spectroscopic measurements were carried out by using the following instruments: Hitachi 200-20 (UV), JAS IRA-1 (IR), JEOL JMS-DX300 and JEOL JMA-DA 5000 (EI-MS), JEOL JMS-SX 102 (FAB-MS), JAS DIP-181 ([α]_D) and JEOL JNM-GX270 (NMR).

Isolation of macrocarpal A (1).⁷⁾ The leaves of *E. macrocarpa* (2880 g) were freeze-dried and then extracted with 80% acetone. The acetone extract was fractionated into the strongly acidic, weakly acidic, alkaline and neutral fractions by EtOAc in the usual way. The neutral fraction was applied to a silica gel column, which was developed with CHCl₃–MeOH (10:1→2:1). The active fractions which were eluted with CHCl₃–MeOH (2:1) were collected and concentrated *in vacuo*, and then applied to a column of Sephadex LH-20. The column was developed with MeOH, and four active fractions were separated. The first fraction was concentrated *in vacuo* and applied to a column of silica gel, which was developed with CHCl₃–AcOH (200:1→50:1). The active fraction was collected and

concentrated *in vacuo*, compound **1** being crystallized in this concentrate and recrystallized from MeOH.

Physico-chemical properties of 1. Colorless flat plate; mp 198–200°C; $[\alpha]_D^{20} -94.7^\circ$ ($c=0.275$, EtOH). *Anal.* Found: C, 70.76%; H, 8.58%. *Calcd.* for $C_{28}H_{40}O_6$: C, 71.17%; H, 8.53%. UV λ_{max} (EtOH) nm (ϵ): 275 (13,000), 393 (5600); λ_{max} (0.01 N HCl in EtOH) nm (ϵ): 288 (10,000); λ_{max} (0.01 N NaOH in EtOH) nm (ϵ): 302 (16,000), 369 (2500); IR ν cm^{-1} (KBr): 3390, 2940, 1610, 1440, 1375, 1317, 1180, 760; EI-MS (m/z): 454, 397, 251, 222, 204 (100), 203 (100), 195 (100), 161; negative FAB-MS: m/z 471 ($M-1$)⁻; 1H -NMR δ ($CDCl_3$): 13.57 (2H, s), 10.19 (1H, s), 10.13 (1H, s), 6.00 (1H, br.s), 3.67 (1H, m), 2.05–1.14 (14H), 1.15 (3H, s), 1.11 (3H, s), 1.09 (3H, s), 0.83 (3H, d, $J=5.9$ Hz), 0.80 (3H, d, $J=5.6$ Hz), 0.75 (3H, s), 0.65 (1H, m), 0.54 (1H, t, $J=9.8$ Hz); ^{13}C -NMR δ ($CDCl_3$): 192.20 (2 $\times$ CHO), 170.00 (s), 167.89 (s), 164.69 (s), 106.89 (s), 104.29 (s), 103.69 (s), 75.69 (s), 53.85 (d), 48.15 (s), 44.42 (d), 44.42 (t), 35.24 (t), 34.94 (t), 34.14 (d), 28.88 (q), 27.13 (d), 27.03 (d), 25.77 (d), 24.35 (t), 24.35 (q), 22.07 (q), 20.83 (q), 20.35 (t), 20.35 (q), 19.80 (s), 17.52 (q).

Crystal-structure analysis of 1. The preliminary unit-cell dimensions and space group were determined from photographs; systematic absences were $k=2n+1$ for $0k0$. A crystal of approximate size $0.65 \times 0.45 \times 0.30$ mm was mounted on a rotating-anode Rigaku off-centered four-circle diffractometer, Mo- K_α radiation monochromated by graphite being used. Unit-cell dimensions were obtained from 2θ values of 17 reflections in the range of $19 < 2\theta < 30^\circ$. Intensities were measured up to $2\theta = 55^\circ$; ranges of h , k , l were -11 – 10 , 0 – 28 and 0 – 9 , respectively. The $\omega/2\theta$ scan technique was applied, the scanning rate and width being 4° (θ) min^{-1} and $(1.0 + 0.35 \tan \theta)^\circ$, respectively. Stationary background counts were accumulated for 5 s before and after each scan. Three standard reflections of $-2\ 10\ 1$, $-5\ 6\ 2$ and $-2\ 0\ 4$ were measured for every 50 reflections, and no significant intensity change was observed. A total of 3263 independent reflections were measured, of which 3133 with $|Fo| > 3\sigma(|Fo|)$ were used for the structural determination. Lorentz and polarization, but no absorption and extinction, corrections were applied.

The structure was solved by the direct method, using the MULTAN 78¹⁰⁾ program, and were refined by full-matrix least-squares with SHELX 76.¹¹⁾ The weighting scheme was $\omega = [\sigma(|Fo|)^2 + 0.004(|Fo|^2)]^{-1}$. Final refinement was performed with anisotropic thermal parameters for the carbon and oxygen atoms, and with isotropic ones for the hydrogen atoms.⁸⁾ The maximum values of (Δ/σ) and $\Delta\rho$ were 0.1 and $0.2e\ \text{\AA}^{-3}$, respectively. Final R and R_w values were 0.060 and 0.067, respectively, for 3133

reflections. Computation was performed on an IBM 4381-R24 in the computer center of Ochanomizu University, and graphics was carried out on an M-280H computer at the computer center of the University of Tokyo.

Assay for antimicrobial activity. The minimum inhibitory concentration was assayed by the agar dilution method. Nutrient agar was used for bacteria, and potato dextrose agar was used for yeast and fungi as test media. Bacteria were incubated at $37^\circ C$ for 24 hr, and yeast and fungi were incubated at $27^\circ C$ for 48 hr.

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