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Euglobal-III, a Novel Granulation Inhibiting Agent from *Eucalyptus globulus* Labill.

The structure of euglobal-III (**1**), a novel granulation inhibiting agent from *Eucalyptus globulus* Labill., has been determined on the basis of physicochemical data and X-ray crystallographic analysis.

Keywords——euglobal-III; acetogenin-mevalonate metabolite; granulation inhibiting agent; *Eucalyptus globulus*; Myrtaceae; isolation; NMR spectra; X-ray analysis; structure determination; fertile egg method

A search[1] among 1850 crude drugs and plants for agents with potential granulation inhibiting activity has led us to the isolation from *Eucalyptus globulus* Labill. of a novel active principle, euglobal-III (**1**). We report herein the isolation and the structure of this principle.

Hexane extract of buds of the plant was chromatographed on silica gel, and the fractions were screened by the fertile egg method.[2] The active principles were eluated with benzene–hexane (5: 95, v/v), and the eluate was submitted to preparative liquid chromatography using a LiChroprep RP-8 column with acetonitrile to give euglobal-III (**1**) in 0.01% yield from the buds. The compound **1** was also isolated in 0.001% yield from chloroform extract of leaves of this plant.

Euglobal-III (**1**), colorless needles from ethanol, mp 169—171°; $[\alpha]_D$ +229° (c=1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm (ε): 278 (32200), 345 (4880, inflection); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1620 (conjugated H-bonded CHO), has the composition $C_{28}H_{38}O_5$ (M^+, m/e 454.2739). The ultraviolet (UV) spectrum of **1** matched very closely the data reported for grandinol (**3**)[3] isolated from *Eucalyptus grandis* and betrayed the presence of a similarly substituted phloroglucinol chromophore. The circular dichroism (CD) curve of **1** in ethanol showed a positive Cotton effect at 278—280 nm which implied that an asymmetric carbon atom is adjacent to the benzene ring. The 100 MHz ^{1}H-NMR spectrum ($CDCl_3$, ppm from tetramethylsilane (TMS) of **1** exhibited two hydrogen-bonded phenolic hydroxyls (13.20, 13.36, s), two aldehyde groups (10.03, 10.18, s),[3] an olefinic proton (4.84, d, J=9 Hz), a methine proton adjacent to an aromatic ring (3.06, m.), two methine protons on a cyclopropane ring (0.60, m; 1.25, dd.), six methyls including an olefinic methyl and two methyls on the cyclopropane ring (0.84—1.70, 2 d and 4 s, 18H), ten methylene protons and two methine protons (0.70—2.30, highly complicated splitting pattern). The off-resonance ^{13}C NMR ($CDCl_3$, ppm from TMS) demonstrated the presence of six methyls (28.6, 26.2, 24.6, 21.0, 16.8, 15.1), five methylenes (42.3, 39,9, 39.6, 26.5, 19.2), five methines (39.5, 30.3, 27.7, 26.2, 24.6), two olefinic carbons (136.0, s; 121.9, d), two quaternary carbons (88.8, 20.3), two aldehydic carbons (191.6, 191.3) and six aromatic carbons (167.9, 167.2, 164.4, 109.6, 104.0, 103.5, each s). The high resolution mass spectrum (MS) of **1** showed the fragmentation pattern of the isobutyl side chain ($M-CH_3$, $M-C_3H_7$, $M-C_4H_9$). It also showed that the compound was split into two fragments yielding two strong peaks at m/e 251.0923 and 203.1818 with the compositions of $C_{13}H_{15}O_5$ and $C_{15}H_{23}$, respectively. These two fragments, supposed to be formed by a retro-Diels–Alder cleavage of the molecular ion, were assigned from their compositions to an aromatic part and sesquiterpene moiety.

1) H. Otsuka, M. Tsukui, T. Toyosato, S. Fujioka, T. Matsuoka, and H. Fujimura, *Takeda Kenkyusho Nempo*, **31**, 238 (1972).
2) P.F. D'Arcy and E.M. Howard, *Brit. J. Pharmacol. Chemother.*, **29**, 378 (1967); H. Otsuka, M. Tsukui, T. Matsuoka, M. Goto, H. Fujimura, Y. Hiramatsu, and T. Sawada, *Yakugaku Zasshi*, **94**, 796 (1974).
3) W.D. Crow, T. Osawa, D.M. Paton, and R.R. Willing, *Tetrahedron Lett.*, **1977**, 1073.

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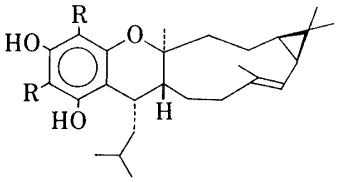
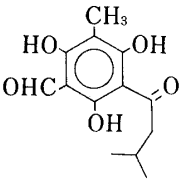
The latter further cleaved to give a typical fragmentation pattern of terpenes. The base peak (*m/e* 195.0301) was formed from the former with the loss of C_4H_8.



1 : R=CHO
2 : R=CH=NOH



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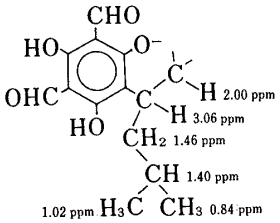
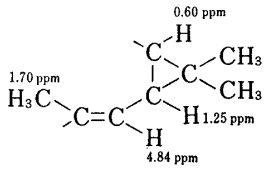


A

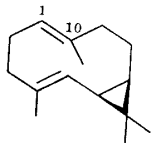
Fig. 1

In decoupled ^1H NMR spectra, saturation of the two protons at 1.46 ppm collapsed the multiplet at 3.06 ppm into a doublet. Saturation of the proton at 2.00 ppm caused the multiplet to change into double doublet-like signals. Further, saturation of the proton at 1.40 ppm caused the methyl doublets at 0.84 and 1.02 ppm to collapse into singlets. In addition to the UV, MS, CD and the decoupled ^1H NMR spectra, the additivity of the chemical shifts of the substituents on the aromatic ring in ^{13}C NMR led to the partial structure [A] for the aromatic part.

The decoupled ^1H NMR spectra also revealed that the cyclopropane ring was adjacent to the olefinic carbon. Saturation of the one proton double doublet at 1.25 ppm caused the olefinic proton doublet at 4.84 ppm to collapse into a singlet and the one proton multiplet at 0.60 ppm to be modified. Saturation of the olefinic methyl singlet at 1.70 ppm showed that it is coupled to the olefinic proton at 4.84 ppm. The (*E*)-configuration was suggested for the double bond by the fact that the olefinic proton was sharpened without any increase in the integrated intensity when the methyl signal was saturated.[4] Of the quaternary carbons in the sesquiterpene moiety, the low-field chemical shift (88.8 ppm) of the one implied that it is attached to an oxygen atom. The other quaternary carbon is assumed to be in the cyclopropane ring, because, in the MS of 1, a peak was observed at *m/e* 161.1327 ($C_{15}H_{23}$-C_3H_6), indicative of a *gem*-dimethyl group attached to the cyclopropane ring. These date revealed partial structure [B] of euglobal-III (**1**). From the index of hydrogen deficiency of the compound, the remaining sesquiterpene moiety must be bicyclic or tricyclic. On the other hand, among known bicyclo- and tricyclosesquiterpenes, bicyclogermacrene (**4**)[4] is the only compound having partial structure [B]. In the ^1H NMR spectra of **1**, however, the signals[4] due to C-10 methyl and C-1 hydrogen in **4** were not observed. This suggested that the C-1, 10 double bond in **4** is absent in **1**. Thus, a partially saturated bicyclogermacrene structure [C] for the sesquiterpene moiety was euggested for euglobal-III (**1**).



B



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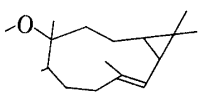

C

Fig. 2

The discussions stated above thus allow merging the partial structures [A] and [C] into the formula **1** to represent euglobal-III.

4) K. Nishimura, I. Horibe, and K. Tori, *Tetrahedron*, **29**, 271 (1973) and references cited therein.

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To confirm the structure and determine the stereochemistry, an X-ray analysis was conducted for dioxime (**2**), which was prepared by a standard method. Crystallization of **2** from benzene gave pale yellow prisms, which contained one molar equivalent benzene as a solvent of crystallization, $C_{34}H_{46}N_2O_5$, mp 137.5—138.5°. The crystal system was orthorhombic, $a=16.165$ (5) Å, $b=18.453$ (4) Å, $c=10.990$ (1) Å, space group $P2_12_12_1$, and $z=4$. Independent reflections, 3265, were collected using Mo-$K\alpha$ radiation. The structure was solved by the direct method using MULTAN[5] and refined by the block-diagonal least-squares method. All the atoms including hydrogens of **2** were determined properly. The final R value was 0.058. Thus, the structure of the dioxime (**2**) was established except for its absolute configuration.

To our knowledge euglobal-III (**1**) is the first acetogenin-mevalonate metabolite which has potent granulation inhibiting activity from *Eucalyptus* species. It is also noteworthy that the euglobal-III (**1**) has a bicyclogermacrene structure, which is the biogenetically common precursor of aromadendrane-derivatives isolated from *Eucalyptus globulus*, such as gurjunene, aromadendrene and globulol. Further studies on the other active principles are in progress.

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5) G. Germain, P. Main, and M.M. Woolfson, *Acta Crystallogr., Sect.* **A**, **27**, 368 (1971).

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Kuwanon G, a New Flavone Derivative from the Root Barks of the Cultivated Mulberry Tree (*Morus alba* L.)[1]

A new flavone derivative, containing condensed dihydrochalcone partial structure named kuwanon G, was isolated from the root barks of the cultivated mulberry tree (a variety of *Morus alba* L.). The structure was shown to be **1** on the basis of chemical and spectral data. The compound (**1**) to rabbit (1 mg/kg, *i.v.*) produced a significant hypotension.

Keywords——kuwanon G; flavone; mulberry tree; *Morus alba* L.; hypotensive action; C-13 NMR; 2′-hydroxy-2,4,4′-trimethoxychalcone

1) A part of this work was presented at the 100 th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1980.

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