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Structures of Sideroxylonals from Eucalyptus sideroxylon

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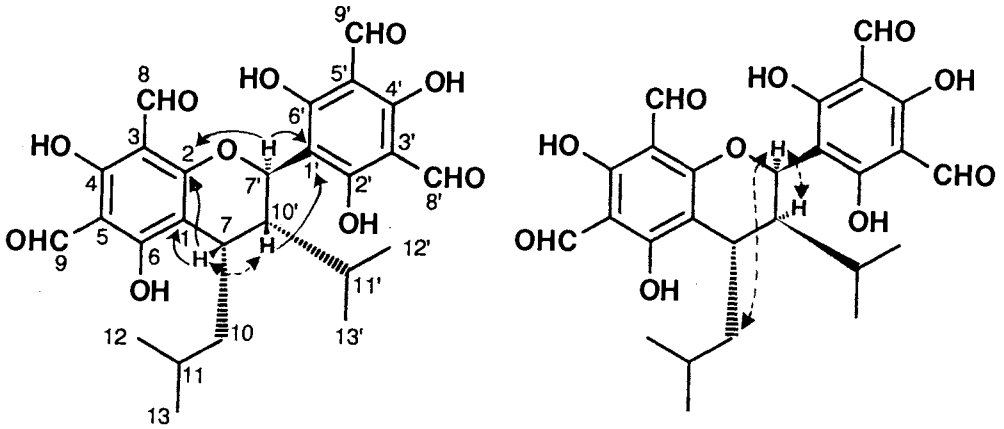
The structures of two novel isopentyl phloroglucinol dimers, designated as sideroxylonals A and B were deduced on the basis of proton magnetic resonance, ^{13}C-nuclear magnetic resonance and mass spectral evidences.

As a result of the research on bio-active components from Eucalyptus sideroxylon, two novel isopentyl phloroglucinol dimers, designated as sideroxylonals A (**1**) and B (**2**), as well as macrocarpals A[1] and B[2] were isolated. We wish to describe herein their structure elucidation.



Sideroxylonal A (**1**) Sideroxylonal B (**2**)

Fig. 1. Structures of sideroxylonals A and B.
← COLOC ◄--► NOE

Successive column chromatography of the hexane soluble portion of methanol extract by silica gel, Sephadex LH-20 (MeOH), and silica gel (chloroform-acetic acid and then methanol) followed by either HPLC with an ODS column (methanol-water=40:3) or recrystallization from methanol, yielded compounds **1** and **2**.

Sideroxylonal A (**1**)[5] was isolated in 0.0012% yield as white amorphous powder of mp 193-195 °C, [α]$_D^{25}$ ±0° (c 1.0, $CHCl_3$:MeOH=4:1) and had the composition, $C_{26}H_{28}O_{10}$ [fab mass spectra: negative, 3-nitrobenzylalcohol (3-NBA) (matrix) m/z 499 $[M-H]^-$; positive, 3-NBA m/z 501 $[M+1]^+$; high resolution fab mass spectrum: m/z 501.1748 (-1.3 mmu) (Calcd for $C_{26}H_{29}O_{10}$)]. The UV data [λ_{max}^{EtOH} nm (ε), 275 (53,000) and 341 (6000)] of **1** was very closely related to those reported for euglobals[3] isolated from Eucalyptus globulus, and indicated the presence of a similarly substituted phloroglucinol chromophore. This compound, like the other euglobals,[4] contains hydrogen-bonded conjugated carbonyl groups, as evidenced by their IR bands at 1643 cm^{-1}. ^1H NMR spectrum [δ 9.79(1H, s), 10.11 (2H, br. s) and 10.17 (1H, s)] and ^{13}C NMR spectrum [δ 191.4, 191.8, 191.8, and 192.1][5] showed that the carbonyl groups in sideroxylonal A (**1**) are four formyl ones. The EI MS of **1** showed the same fragmentation patterns as those of the other euglobals.[4] The above spectral data strongly suggested that **1** has the same aromatic part found in euglobals.[3] The fragment ion at m/z 251 ($C_{13}H_{15}O_5$) showed the retro-Diels Alder cleavage of the molecular ion. The ion at m/z 251 was suffered further losses of C_4H_8 to give one at m/z 195. The ^{13}C NMR spectrum of **1** exhibited twelve aromatic carbons. The high-field region of the ^1H NMR spectrum of **1** exhibited four methine protons [δ 5.95 (1H, d, J= 11.7 Hz), 3.38 (1H, dt, J= 3.7, 6.6 Hz), 2.40 (1H, ddd, J= 3.7, 8.1, 11.7 Hz) and 1.74 (1H, m)], four methyl group (δ 1.09, 1.04, 0.87, and 0.74, each 3H, d, J= 5.9, 7.3, 5.9, and 6.6 Hz, respectively), and one methylene and one methine proton (δ 1.46, 3H, m). The saturated carbon region of ^{13}C NMR indicated the presence of five methines (δ 74.0, 42.9, 27.7, 27.3, and 25.1). The ^1H-^1H COSY spectrum of

1 showed the correlations of between protons of a partial structure $(CH_3)_2CH\text{-}CH_2\text{-}\overline{CH\text{-}\ \ \text{-}O\text{-}CH\text{-}CH}\text{-}CH(CH_3)_2$. To establish structure **1**, detailed analysis of COLOC was carried out. Significant long range correlations were observed as shown in Fig. 1. Stereochemistry of **1** was determined by the spin coupling constant (11.7 Hz) between H-7' and H-10' and the NOE between H-7 and H-10'.

Sideroxylonal B (**2**)[6] was obtained in 0.0009% yield as white amorphous powder of mp 213-215 °C, $[\alpha]_D^{23}$ ±0° (c 1.0, $CHCl_3$). The molecular formula of **2** was same as that of **1**, and all the other spectral data indicated the identity of the structures of both compounds except for the relative stereochemistry; H-7 and H-7' at δ 2.98 (1H, dd, J= 1.5, 10.3 Hz) and δ 5.92 (1H, d, J=1.5 Hz), respectively, $J_{H\text{-}7'}$ and H-10 = 1.5 Hz, $J_{H\text{-}7}$ and H-10' ≈0 Hz. Compound **2** is the C-10' epimer of **1** based on the NOE analyses.

Although sideroxylonals A (**1**) and B (**2**) are found to be racemates, based on the absence of the CD, even the crude extract of methanol was found to contain **1** and **2**. Thus, compounds **1** and **2** may be biogenetically formed by Diels-Alder type coupling reaction of pentenyl phloroglucinol dialdehyde precursors as proposed by Kozuka et al.[3]

The minimum concentration giving fifty percent growth inhibition for sideroxylonals A (**1**) and B (**2**) were 62.5 and 62.5 μg/ml against HeLa S-3 cells, respectively. Compounds **1** and **2** have also anti-bacterial activity, against Gram-positive bacteria such as Staphylococcus aureus and Baccillus subtilis at 3.9 and 7.8, and 3.9 and 7.8 ug/disk (φ6 mm paper disk), respectively. Furthermore, **1** and **2** showed aldose reductase inhibitor activity at 1.25 and 2.47 μM (IC_{50}),[7] respectively.

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5) **1**: ^{1}H NMR (400 MHz, $CDCl_3$:CD_3OD=4:1) δ 0.74 (3H, d, J= 6.6 Hz, 12' or 13'-H), 0.87 (3H, d, J= 5.9 Hz, 12 or 13-H), 1.04 (3H, d, J= 7.3 Hz, 12' or 13'-H), 1.09 (3H, d, J= 5.9 Hz, 12 or 13-H), 1.46 (3H, m, 10 and 11-H), 1.74 (1H, m, 11'-H), 2.40 (1H, ddd, J= 3.7, 8.8, 11.7 Hz, 10'-H), 3.38 (1H, dt, J= 3.7, 6.6 Hz, 7-H), 5.95 (1H, d, J= 11.7 Hz, 7'-H), 9.79 (1H, s, 8-H), 10.11 (2H, br.s, 8' and 9'-H), 10.17 (1H, s, 9-H). ^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD) δ 20.5 ($C_{12'\text{ or }13'}$), 21.2 ($C_{12\text{ or }13}$), 21.6 ($C_{12'\text{ or }13'}$), 24.2 ($C_{12\text{ or }13}$), 25.1 (C_{11}), 27.3 (C_7), 27.7 ($C_{11'}$), 38.4 (C_{10}), 42.9 ($C_{10'}$), 74.0 ($C_{7'}$), 103.2 (C_5), 103.9 (C_3), 104.6 ($C_{3'\text{ and }5'}$), 106.5 ($C_{1'}$), 108.9 (C_1), 164.9 (C_2), 166.7 ($C_{6\text{ and }2'\text{ or }6'}$), 167.5 ($C_4$), 168.5 ($C_{4'\text{ and }2'\text{ or }6'}$), 191.4 ($C_9$), 191.8 ($C_{8'\text{ and }9'}$), 192.1 ($C_8$).

6) **2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.74 (3H, d, J= 6.6 Hz, 12' or 13'-H), 0.95 (3H, d, J= 6.6 Hz, 12' or 13'-H), 0.99 (3H, d, J= 6.6 Hz, 12 or 13-H), 1.09 (3H, d, J= 6.6 Hz, 12 or 13-H), 1.53 (1H, ddd, J= 2.2, 10.3, 13.2 Hz, 10a-H), 1.66 (1H, ddd, J= 2.2, 10.3, 13.2 Hz, 10b-H), 1.74 (1H, m, 11-H), 1.97 (1H, m, 11'-H), 2.02 (1H, dd, J= 1.5, 2.2 Hz, 10'-H), 2.98 (1H, dd, J= 1.5,10.3 Hz, 7-H), 5.92 (1H, d, J= 1.5 Hz, 7'-H), 8.60 (1H, s, 2' or 6'-OH), 10.03 (1H, s, 8-H), 10.17 (1H, s, 8'-H), 10.20 (1H, s, 9'-H), 10.24 (1H, s, 9-H), 13.38 (1H, s, 4-OH), 13.53 (1H, s, 6-OH), 13.56 (1H, s, 4'-OH), 13.59 (1H, s, 2' or 6'-OH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.7 ($C_{12'\text{ or }13'}$), 21.0 ($C_{12\text{ or }13}$), 23.8 ($C_{12'\text{ or }13'}$), 24.2 ($C_{12\text{ or }13}$), 25.6 (C_{11}), 26.5 ($C_{11'}$), 26.7 (C_7), 42.0 ($C_{10'}$), 44.7 (C_{10}), 77.7 ($C_{7'}$), 100.6 ($C_{1'}$), 103.6 (C_3), 104.1 ($C_{3'\text{ or }5'}$), 104.2 ($C_{3'\text{ or }5'}$), 105.3 (C_5), 108.3 (C_1), 160.8 (C_2), 165.7 ($C_{2'\text{ or }6'}$), 167.3 ($C_{2'\text{ or }6'}$), 167.6 (C_4), 168.9 ($C_{4'}$), 169.6 (C_6), 190.2 (C_8), 192.1 ($C_{8'}$), 192.3 ($C_{9'}$), 192.4 (C_9).

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