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NOVEL NEUROTROPHIC SESQUITERPENE-NEOLIGNANS FROM *MAGNOLIA OBOVATA*

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ABSTRACT: Novel sesquiterpene-neolignans, eudesobovatols A (1) and B (2) isolated from *Magnolia obovata* have been assigned structures on the basis of detailed spectroscopic analyses and chemical degradation, and eudesobovatol A has been found to exhibit neurotrophic activity at 10^{-5} M ~ 10^{-7} M on neuronal cell culture system of fetal rat cerebral hemisphere.

The bark of *Magnolia obovata* Thunb. (Magnoliaceae) has been used in Chinese and Japanese traditional medicine for neurosis and gastrointestinal complaints, and recently has been documented to have central depressant effect,[1] which are mainly due to its main constituents, magnolol and honokiol.[2,3] Our independent study on neurotrophic active substance in the title plant resulted in the isolation of novel sesquiterpene-neolignans , designated eudesobovatols A (1) and B (2), the former of which exhibited an interesting neurotrophic activity on neuronal cell culture system of fetal rat cerebral hemisphere.[4] Herein, we wish to report the structures of these unique compounds with a neurotrophic property.

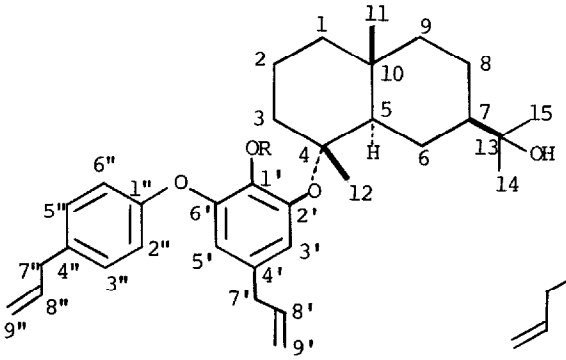
Compound **1** had the following physical data: $[\alpha]_D$ -40.3°(*c* 2.5, $CHCl_3$); IR ($CHCl_3$) 3600 (OH), 3530 (OH), 1640 (C=C), 1600 and 1500 (aromatic) cm^{-1}; UV (EtOH) 208 (ε 58000), 274 (ε 7200), 281 (ε 6700) nm. It's FDMS showed the molecular ion peak at *m/z* 504 giving the molecular formula $C_{33}H_{44}O_4$ coupled with the ^{13}C NMR data (Table). The ^1H NMR spectrum [6] of 1 indicated the presence of two sets of an allylic group [δ 3.26 (2 H, d, *J* = 6.8 Hz, H-7"), 5.03 (1 H, dd, *J* = 17.2, 2.0 Hz, H-9"), 5.10 (1 H, dd, *J* = 10.3, 2.0 Hz, H-9"), and 5.91 (1 H, ddt, *J* = 17.2, 10.3, 6.8 Hz, H-8"); 3.31 (2 H, d, *J* = 6.4 Hz, H-7'), 5.04 (1H, dd, *J* = 10.3, 2.0 Hz, H-9'), 5.08 (1 H, dd, *J* = 17.1 ,2.0 Hz, H-9'), and 6.01 (1 H, ddt, *J* = 17.1, 10.3, 6.4 Hz, H-8')], A_2B_2 type aromatic protons [δ 7.05 (2 H , d, *J* = 8.8 Hz, H-2", 6") and 7.11 (2 H, d, *J* = 8.8 Hz, H-3", 5")], and *meta* coupled aromatic protons [δ 6.84 (1 H, d, *J* = 2.0 Hz, H-5') and 7.02 (1 H, d, *J* = 2.0 Hz, H-3')], as well as of four methyl groups [δ 0.93 (s), 1.42 (total 9 H, s)].[7]

Confirmation of these proton systems were also made by the 2D DQFCOSY and C/H COSY spectra.

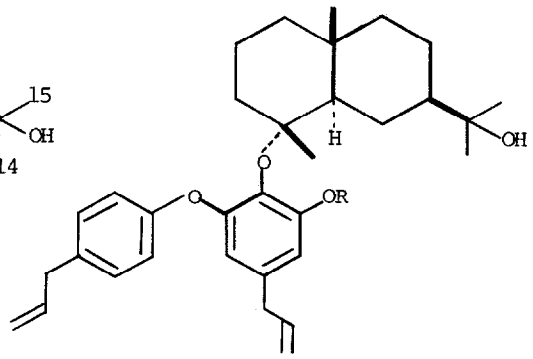
The ^{13}C NMR spectrum summarized in Table contained thirty one carbon signals which could be unambigously assigned to eudesmol-type sesquiterpene and neolignan, obovatol moieties, respectively, by analyses of the C/H and long-range C/H COSYs, and the NOESY. In addition, the presence of obovatol as an aromatic unit was substantiated by the observation of a base ion peak at *m/z* 282 in the EIMS. The above spectral evidence disclosed that 1 was made up of eudesmol and obovatol linked to each other *via* an ether bond. In fact, treatment of 1 with CF_3COOH in benzene yielded γ-eudesmol [$[\alpha]_D$ 55.5°(*c* 0.08, $CHCl_3$)][8], and an aromatic product, the spectral data and TLC behavior of which were completely identical with those of obovatol[9] occurring in the title plant, indicating that the one of the two hydroxyl groups on the obovatol ring was linked to the C-4 position in eudesmol. The linkage position and configuration of both the units were clarfied by a combination of the NOESY and difference NOE experiments of a methylated derivative **1a** readily available on treatment of 1 with CH_2N_2 in ether. Namely, selective irradiation of the methyl signal (H-12) at δ 1.21 caused NOE interaction not only for the methyl signal (H-11) at δ 0.92 accounting for a 1, 3-diaxial relationship of the both methyl groups, but also for the *meta* coupled aromatic proton signal at δ 6.61, whereas no NOE was detected for any aromatic proton signals upon irradiation of the methoxy signal at δ 3.74 and even in the NOESY. This clearly indicates that eudesmol should be linked to the C_2'-OH on the obovatol ring through an ether bond, and the obovatol unit takes an equatorial configuration on a chair-form cyclohexane ring. These results, thus, fully corroborated the structure 1 for eudesobovatol A.

Eudesobovatol B (**2**), $[\alpha]_D$ -26.1° (*c* 1.15, $CHCl_3$), exhibited the quasi-molecular ion peak due to $[M^+ - 1]$ at *m/z* 503 and a prominent fragment ion peak due to obovatol at m/z 282 in the FABMS, and its ^1H-NMR spectrum[10] was found to be quite similar to that of 1 except for a methyl signal at δ 1.58 and *meta* coupled aromatic resonances at δ 6.61 and 6.99 (each d, J = 1.5 Hz). These spectral analogies imply that the structure of 2 is closely related to that of eudesobovatol A. On the other hand, the ^{13}C NMR data of 2 (Table) well corresponded to those of 1 except for the signals due to the C-4 in an eudesmol unit and the C-1', 2', 3', 4', 5', and 6' in the obovatol unit. Moreover, NOE was not observed for any aromatic protons upon irradiation of the C_4-CH_3 at δ 1.58, although clear NOE was detected for the C_{10}-CH_3 at δ 0.92 taking account for a 1, 3-diaxial relationship as in 1. These spectral similarity and difference between 1 and **2** led to the proposal that 2 should be a regioisomer of 1 with respect to an ether linkage position between eudesmol and the one of the two hydroxyl groups on the obovatol ring. This proposal was verified by the observation of NOE for the *meta* coupled aromatic proton resonance at δ 6.52 upon irradiation of the methoxy signal at δ

3.81 in a methylated derivative **2a**, and thereby **2** turns out to involve two units of eudesmol and obovatol bonded *via* an ether linkage between the C-4 in eudesmol and the $C_{1'}$-OH in obovatol. Thus, the structure of eudesobovatol B was represented as **2**.



1: R = H
1a: R = CH_3



2: R = H
2a: R = CH_3

Table. ^{13}C-NMR data[a] (100 MHz, C_5D_5N) of **1** and **2**

	1	2		1	2		1	2
1	40.4	40.6	1'	143.6	132.4	7"	38.8	39.4
2	19.6	20.2	2'	144.7	152.2	8"	137.5	137.5
3	38.6	38.5	3'	122.0	110.9	9"	115.1	115.8
4	84.4	87.5	4'	129.6	136.7			
5	51.9	53.0	5'	116.2	112.4			
6	22.0	21.8	6'	145.0	150.4			
7	49.8	49.5	7'	39.1	39.8			
8	22.4	22.5	8'	137.6	136.9			
9	44.9	44.9	9'	115.1	116.0			
10	34.5	35.1	1"	156.7	155.8			
11	18.7	19.1	2"	116.7	117.5			
12	19.7	21.2	3"	129.4	129.4			
13	70.9	72.6	4"	133.4	134.2			
14	27.3[b]	26.5[c]	5"	129.4	129.4			
15	27.5[b]	27.2[c]	6"	116.7	117.5			

a. Assignments were based on the C/H and Long-range C/H COSYs.

b, c. may be interchangable.

Eudesobovatols A (1) and B (2) are unique in having the structure linked by the two parts of eudesmol and obovatol, and are the first reported sesquiterpenoidneolignans, though the both units and a monoterpenoidneolignan occurred in *Magnolia obovata*. Finally, it is worthy of note that eudesobovatol A (1) can cause not only interesting neurotrophic actions morphologically such as accelerating neurite sprouting and neuronal cell network formation, but also enhance choline acetyltransferase activity at 10^{-5} M ~ 10^{-7} M in fetal rat cerebral hemisphere.[5]

References and Notes

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6. ^{1}H-NMR (400 MHz, C_5D_5N) data expect for those in the text ; δ 0.93 (3 H, s, H-11), 1.01 (1 H, ddd, J = 11.7, 11.7, 4.8 Hz, H-10α), 1.23 (1 H, ddd, J = 12.7, 12.7, 3.9 Hz, H-9α), 1.29 (1 H, ddd, J =11.7, 4.8, 4.8 Hz, H-1β), 1.48 (1 H, ddd, J = 13.1, 12.2, 11.7 Hz, H-6β), 1.53 (1 H, m, H-8β), 1.66 (1 H, dddd, J = 12.2, 12.2, 3.4, 3.4 Hz, H-7), ..83 (1 H, m, H-8α), 1.94 (1 H, dd, J = 11.7, 3.4 Hz, H-5), 2.00 (1 H, m , H-3α), 2.14 (1 H, m, H-3β), 2.95 (1 H, ddd, J = 13.1, 3.4, 3.4 Hz, H-6α).
7. The ^{1}H NMR spectrum of 1 in $CDCl_3$ showed well separated signals due to the four methyl groups at δ 0.93 (3H, s), 1.23 (6 H, s), and 1.28 (3 H, s).
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10. ^{1}H NMR (400 MHz, C_5D_5N) for 2: δ 0.92 (3 H, s, H-11), 1.04 (1 H, ddd, J = 11.0, 11.0, 4.8 Hz, H-10α), 1.19 (1 H, ddd, J = 12.4, 12.4, 3.6 Hz, H-9α), 1.29 (6 H, s, H-14, 15), 1.38 (1 H, ddd, J = 12.4, 11.7, 9.5 Hz, H-6β), 1.50 (1 H, m, H-7α), 1.54 (1 H, m. H-8β), 1.58 (3 H, s, H-12), 1.86 (1 H, m, H-8α), 2.06 (1 H, dd, J = 11.7, 2.2 Hz, H-5), 2.10 (1 H, m, H-3α), 2.12 (1 H, m, H-3β), 2.65 (1 H, ddd, J = 12.4, 2.2, 2.2 Hz, H-6α), 3.25 (2 H , d, J = 6.6 Hz, H-7'), 3.33 (2 H, d, J = 6.6 Hz, H-7"), 4.96 ~ 5.06 (4 H, m, H-9', 9"), 5.94 (1 H, ddt, J = 15.4, 12.3, 6.6 Hz, H-8'), 5.98 (1 H, ddt, J = 16.8, 10.2 , 6.6 Hz, H-8"), 6.61 (1 H, d, J = 1.5 Hz, H-3'), 6.99 (1 H, d, J = 1.5 Hz, H-5'), 7.10 (2 H, d, J = 8.0 Hz, H-2", 6"), and 7.17 (2 H, d, J = 8.0 Hz, H-3", 5").

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