A NOVEL HEXAHYDRODIBENZOFURAN DERIVATIVE WITH POTENT INHIBITORY ACTIVITY ON MELANIN BIOSYNTHESIS OF CULTURED B-16 MELANOMA CELLS FROM *LINDERA UMBELLATA* BARK

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A novel cinnamoyl-hexahydrodibenzofuran derivative (**1**) was isolated from the bark of *Lindera umbellata*. The structure was determined by extensive spectroscopic analysis to be (5a*R**,6*R**,9*R**,9a*S**)-4-cinnamoyl-3,6-dihydroxy-1-methoxy-6-methyl-9-(1-methylethyl)-5a,6,7,8,9,9a-hexahydrodibenzofuran. Compound **1** showed potent inhibitory activity on melanin biosynthesis of cultured B-16 melanoma cells without causing any cytotoxicity in the cultured cells or skin irritation in guinea pig.

KEY WORDS *Lindera umbellata*; Lauraceae; dibenzofuran derivative; B-16 melanoma cell; melanin biosynthesis inhibition

Melanin biosynthesis inhibitory compounds are useful not only for the material used in cosmetics as skin-whitening agents but also as the remedy for disturbances in pigmentation such as chloasma, ephelis and melanosis Riehl, which are treated by glutathion injection and administration of ascorbic acid in large quantities; however, no prominent curative value can be expected, and there is no certain cure for them yet. During our search for new melanin biosynthesis inhibitory compounds from natural sources, we isolated a novel hexahydrodibenzofuran derivative (**1**) with potent inhibitory activity on melanin biosynthesis of cultured B-16 melanoma cells from the bark of *Lindera umbellata* (Lauraceae). This paper reports the structural elucidation of **1** and its melanin biosynthesis inhibition.

Fresh bark of *L. umbellata* (1.7 kg) was extracted with hot MeOH. The extract was partitioned between $CHCl_3$ and water. The $CHCl_3$-soluble phase was chromatographed on silica-gel eluting with hexane - Me_2CO (5 : 1) and hexane - EtOAc (5 : 2) to give compound **1** (20 mg).

Compound **1**, $[\alpha]_D$ -22.7° ($CHCl_3$), was obtained as a yellow solid. The molecular formula was determined to be $C_{26}H_{30}O_5$ by the ^{13}C-NMR spectrum with 26 signals and high-resolution EI-MS showing an accurate $[M]^+$ ion at *m/z* 422.2093 (+Δ 0.6 mmu). The IR spectrum was consistent with the presence of hydroxyl group(s) at 3450 cm^{-1}, a conjugated carbonyl group at 1635 cm^{-1} and aromatic ring(s) at 1585, 1555 and 1495 cm^{-1}. The ^1H-NMR spectrum in $CDCl_3$ displayed signals arising from a *trans*-olefinic group at δ 8.09 and 7.86 (each d, J = 15.6 Hz), aromatic rings at δ 7.61 (2H), 7.39 (3H) and 6.08 (1H), two exchangeable protons of hydroxyl groups at δ 13.97 (s) and 1.58 (br s), a methoxyl group at δ 3.84 (3H, s), a tertiary methyl group at δ 1.61 (3H, s), two

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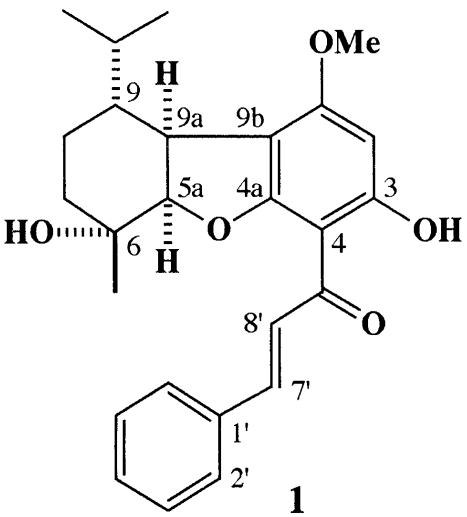

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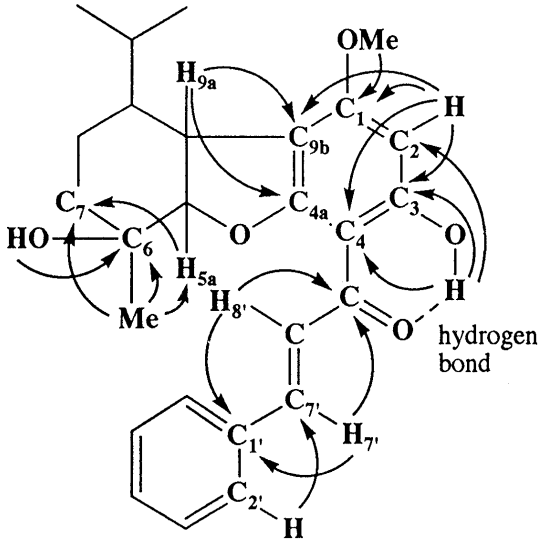
secondary methyl groups at δ 0.91 (3H, d, J = 6.8 Hz) and 0.85 (3H, d, J = 7.0 Hz), and several aliphatic groups. The ^{13}C-NMR combined with the HMQC spectrum confirmed the existence of a monosubstituted [δ 135.4 (C), 130.3 (CH), 128.9 (CH) x 2 and 128.4 (CH) x 2] and a pentasubstituted [δ 166.7 (C), 162.2 (C), 161.5 (C), 113.3 (C), 103.4 (C) and 93.1 (CH)] aromatic ring. Two sp^2 carbon signals at δ 143.4 and 125.9 showed $^1J_{C,H}$ correlations with δ_H 7.86 and 8.09, respectively, in the HMQC spectrum, allowing them to be assigned to the *trans*-olefinic group. The presence of a cinnamoyl group in **1** was revealed by $^{2 \text{ or } 3}J_{C,H}$ correlations from $\delta_{7'\text{-H}}$ 7.86 to $\delta_{C=O}$ 191.1 and $\delta_{C\text{-}2',\,6'}$ 128.9 and from $\delta_{8'\text{-H}}$ 8.09 to $\delta_{C=O}$ 191.1 and $\delta_{C\text{-}1'}$ 135.4 in the HMBC spectrum. The UV maximum at λ 340 nm (log ε = 4.35) indicated the cinnamoyl group was directly attached to another aromatic ring. Further HMBC correlations from δ_{OMe} 3.84 to $\delta_{C\text{-}1}$ 162.2, $\delta_{2\text{-H}}$ 6.08 to $\delta_{C\text{-}3}$ 166.7, $\delta_{C\text{-}1}$ 162.2, $\delta_{C\text{-}9b}$ 113.3 and $\delta_{C\text{-}4}$ 103.4, and $\delta_{Ar\text{-}(C\text{-}3)\text{-}OH}$ 13.97 to $\delta_{C\text{-}3}$ 166.7, $\delta_{C\text{-}4}$ 103.4 and $\delta_{C\text{-}2}$ 93.1 defined the location of the methoxyl group at C-1 and the hydroxyl group at C-3 of the aromatic ring.

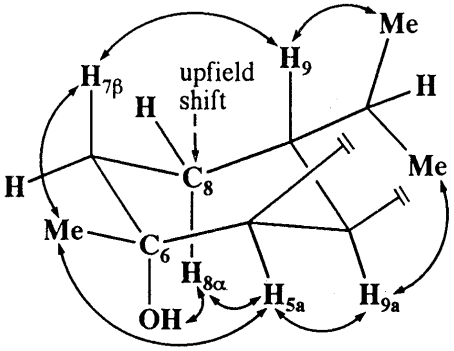
The ^{1}H-^{1}H COSY spectrum revealed the sequences of the remaining aliphatic moiety, starting from the methyl doublets at δ 0.91 and 0.85. The connectivity from C-5a to C-7 through a quaternary carbon (C-6), resulting in the formation of a cyclohexane ring, was verified by the HMBC correlations from $\delta_{5a\text{-H}}$ 4.24 to $\delta_{C\text{-}7}$ 35.4 and $\delta_{C\text{-}6}$ 69.4, and the linkage of the hydroxyl group and methyl group to the quaternary carbon was verified by the correlations between δ_{OH} 1.58 and $\delta_{C\text{-}6}$ 69.4, and δ_{Me} 1.61 and $\delta_{C\text{-}6}$ 69.4.

The aromatic ring was shown to be connected to the aliphatic moiety by the following data. The $^2J_{C,H}$ correlations between $\delta_{9a\text{-H}}$ 3.14 and $\delta_{C\text{-}9b}$ 113.3 and $^3J_{C,H}$ between $\delta_{9a\text{-H}}$ 3.14 and $\delta_{C\text{-}4a}$ 161.5 gave evidence for the linkage from C-9a to C-9b. Compound **1** required 12 degrees of unsaturation and two aromatic rings and a conjugated carbonyl group, and a cyclohexane ring consumed 11 degrees. Taking into account the degree of unsaturation and the shift values of the ^{13}C-NMR, C-4a (δ_C 161.5) and C-5a (δ_{CH} 92.4) were concluded to be linked through an oxygen atom to form a five-membered ring. The remaining free bond at C-4 (δ_C 103.4) was necessarily confirmed to embrace the cinnamoyl group. The aromatic hydroxyl group was considered to form an intrahydrogen bond to the carbonyl group of the cinnamoyl moiety since the hydroxy proton appeared at an extremely lower field at δ 13.97 as a sharp singlet signal.

An analysis of the phase-sensitive NOESY spectrum made the relative stereochemistry assignable. The methyl doublet at δ 0.91 showed an NOE correlation with 9a-H, which in turn showed an NOE with 5a-H. No NOE was observed between 9-H and 9a-H, and 9-H was coupled to

9a-H with a large J value of 12.1 Hz. These data were consistent with 5aR*, 9R* and 9aS* configurations. The 6-OH showed NOEs with both 5a-H and 8α-H, indicating 6R* configuration. Other NOE networks, 9-H / 7β-H and 5a-H / 8α-H, and large $^3J_{H,H}$ between 7β-H and 8α-H (12.1 Hz), and 8α-H and 9-H (12.1 Hz) confirmed the cyclohexane ring to be nearly chair-form. The C-8 carbon resonated at δ 17.2, and was shifted to upper field as a methylene carbon. This must be caused by the 1,3-diaxial interaction between 6-OH an 8α(ax)-H.





: HMBC Correlations : NOE Correlations

Accordingly, the structure of **1** was determined to be (5aR*,6R*,9R*,9aS*)-4-cinnamoyl-3.6-dihydroxy-1-methoxy-6-methyl-9-(1-methylethyl)-5a,6,7,8,9,9a-hexahydrodibenzofuran.

Compound **1** apparently inhibited melanin biosynthesis of cultured B-16 melanoma cells at a sample concentration of 3 μg/ml without any cytotoxicity toward the cultured cells. This is about 10 times more potent than that of arbutin used as a positive control. Compound **1** has no skin irritation,[1] and is stable in EtOH solution.[2] Furthermore, **1** has UV absorption maximum in the UV-A region.[3] Thus, the potentiality of **1** both as a useful skin-whitening agent with UV-A absorption and as a remedy for the disturbances in pigmentation is evident.

REFERENCES AND NOTES

1) Skin irritation of **1** was examined according to the Draize's primary skin irritation test using guinea pigs with the some modification: J. H. Draize, "Appraisal of Safety of Chemicals in Foods, Drugs and Cosmetics," Association of Food and Drug Officials of the United States, 1959.

2) The EtOH solution of **1** (0.001%) was left standing at 40°C for 7 days. No change in absorbance at λ_{max} 340 nm could be observed.

3) The longwave UV ray, UV-A (315 - 400 nm), which causes no acute sunburn and erythema, is considered to be related to dermatoheliosis as well as immediate pigment darkening. Severe dermatoheliosis leads to skin cancer.

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