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Vialinin B, a novel potent inhibitor of TNF-α production, isolated from an edible mushroom, *Thelephora vialis*

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Abstract—A novel dibenzofuran compound designated vialinin B was isolated from dry fruiting bodies of an edible mushroom, *Thelephora vialis*, and potently inhibits TNF-α production in RBL-2H3 cells (IC_{50} = 0.02 nM) and is a promising anti-allergic agent.

The incidence of an immediate hypersensitive allergy (type I) caused by immunoglobulin E, for example, food allergy, pollinosis, asthma, drug-induced allergy, etc., is increasing worldwide[1] and several therapeutic agents that inhibit the release of chemical mediators such as histamine from mast cells and basophils are currently used as therapeutic agents.[2] Mast cells and basophils, which are high-affinity receptors for IgE (FceRI), are activated by a specific antigen (IgE) through cross-linking of the IgE–FceRI complex. Cell activation induces the degranulation and release of chemical mediators such as histamine and leukotrienes, and subsequently causes the release of cytokines including TNF-α, which have important roles in the late phase inflammation of type I allergy. Tumor necrosis factor (TNF)-α is a potent multifunctional cytokine that mediates a variety of biological actions with a central role in the pathogenesis of many inflammatory diseases.[3] Thus, inhibitors of TNF-α production in the activated mast cells and basophils are promising candidates for a new type of anti-allergic agent.

In our search for bioactive compounds from edible Chinese mushrooms, we isolated the potent antioxidants vialinin A and ganbajunin B (**1**), and an inseparable mixture of ganbajunins D and E, from the dry fruiting bodies of *Thelephora vialis*.[4,5] Further studies of the functional fungal components led to the discovery of a novel dibenzofuran compound, vialinin B (**2**), together with a known cycloleucomelone (**3**) (Fig. 1). In this paper, we describe the isolation and structure elucidation of **2**. The potent inhibitory activity of **2** against TNF-α production in rat basophilic leukemia cell lines RBL-2H3, which is highly dependent on its chemical structure, is also evaluated.

The dry fruiting bodies[6] (420 g) of the edible mushroom *Thelephora vialis* were extracted with 8.0 L of 80% acetone for 48 h at room temperature. The aqueous concentrate after evaporation of the solvent in vacuo was adjusted to pH 3.0 and extracted with the same volume of ethyl acetate (EtOAc). The organic layer was concentrated in vacuo and gave 36.5 g of the EtOAc extract. A part of the extract (10.0 g) was applied to a Sephadex LH-20 column using a mixture of 40% methanol in chloroform as the eluent, to give two major fractions, A (6.04 g) and B (0.76 g). Fraction A was further subjected to silica gel column chromatography (2% methanol in chloroform), and subsequently rechromatographed using a reverse-phase MPLC (YMC ODS-AQ 120) column to yield three independent fractions (A-a, A-b, and A-c), eluted with 0.15% KH_2PO_4 (pH 3.5)/acetonitrile (1:1). Finally, 5.8 mg of vialinin B (**2**) was purified as a brownish amorphous powder from fraction A-a by reverse-phase preparative HPLC.[7]

Keywords: Mushroom; Vialinin B; TNF-α; *Thelephora vialis*.
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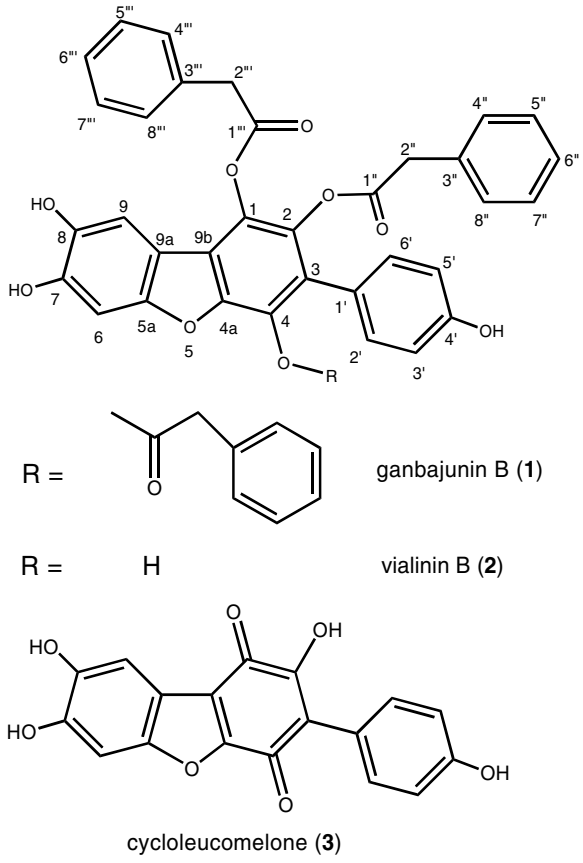

Figure 1. Structures of ganbajunin B (**1**), vialinin B (**2**), and cycloleucomelone (**3**).

The molecular formula of vialinin B (**2**) was established as $C_{34}H_{24}O_9$ on the basis of HRESI-MS [*m*/*z* 599.13040 $(M+Na)^+$, 599.13180 for $C_{34}H_{24}O_9Na$]. The UV and visible spectra showed λ_{max} (ε, methanol) at 223 (103600), 244 (sh, 86000), 265 (62300), 300 (55100), 318 (sh, 6000), and 328 (65600) nm, which were very similar to those of dibenzofuran compounds such as ganbajunin B (**1**)[8] and thelephantins H and M.[9] The IR spectrum of **2** had the following characteristic absorption bands ν_{max} (ATR) at 3400, 1750, 1730, 1210, 1110, and 1145 cm^{-1}, indicating the presence of hydroxy and ester carbonyl groups. The ^1H NMR spectral data[10] indicated 2 singlet methylene groups (δ 3.88 and 3.19) and 16 aromatic methine protons, including two characteristic singlet protons at δ 7.04 (1H) and at δ 7.06 (1H). The analyses of the ^1H–^1H coupling patterns in the aromatic proton regions suggested the presence of a 1,4-disubstituted [δ 7.07 (2H) and 6.76 (2 H)] and two-monosubstituted [(i) δ 7.44 (2H), 7.40 (2H), and 7.33 (1H), and (ii) δ 7.23 (2H), 7.22 (1H), and 6.92 (2H)] phenyl groups. The ^{13}C NMR spectrum[10] of **2** included characteristic signals of two ester (δ 171.08 and 170.85) and two sp^2 methylene (δ 41.53 and 41.01) carbon signals. Based on the chemical shifts, the remaining signals were deduced to be sp^2 quaternary or sp^2 methine carbons. The HMQC data revealed all one-bond ^1H–^{13}C correlations for C-6, C-9, C-2′(6′), C-3′(5′), C-2″, C-4″(8″), C-5″(C-7″), C-6″, C-2‴, C-4‴(8‴), C-5‴(C-7‴), and C-6‴. The fundamental structure of **2** was elucidated by comparing

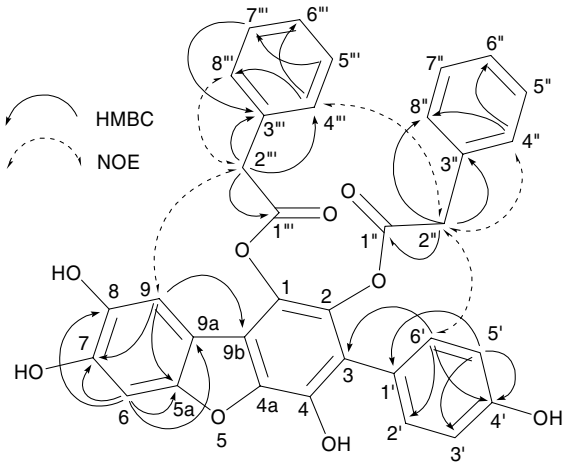

Figure 2. HMBC and NOESY correlations of vialinin B (**2**).

the UV, IR, and NMR spectral data of **2**, ganbajunin B (**1**),[8] and thelephantins H and M,[9] which revealed that the structure of **2** is similar to that of ganbajunin B and thelephantins H and M: a 3-(4-hydroxylphenyl)-1,2,4,7,8-pentaoxygenated dibenzofuran framework, derived from 2′,3,3′,4,4″,5′,6′-hexahydroxy[1,1′:4′,1″-terphenyl]. Further structural information was obtained by HMBC and NOESY experiments (Fig. 2). The long-range correlations from 2″-H to C-1″, C-3″, and C-4″ (8″), from 2‴-H to C-1‴, C-3‴, and C-4‴ (8‴) in the HMBC spectrum supported the presence of two phenylacetyl groups, and the NOEs between 9-H and 2‴-H, between 4‴-H and 2-H″, and between 2″-H and 6′-H in the NOESY spectrum indicated that the phenylacetyl groups were bonded to C-1 and C-2, respectively. Four hydroxy groups, which were deduced from the number of invisible protons in the ^1H NMR spectrum, were expected to be located at C-4, C-7, C-8, and C-4′. On the basis of the spectral analyses, the structure of **2** was determined to be 3-(4-hydroxyphenyl)dibenzo-

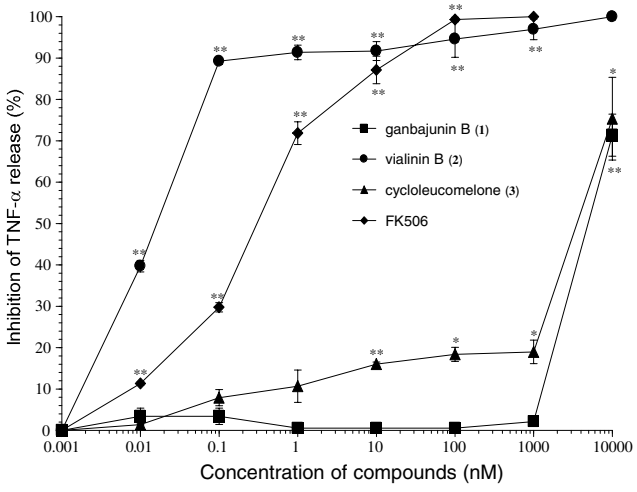

Figure 3. Effects of ganbajunin B (**1**), vialinin B (**2**), cycloleucomelone (**3**), and FK-506 on TNF-α released from RBL-2H3 cells. Each value represents means ± SD of triplicate determinations. The levels of significance of differences from the control values were estimated by Student's *t*-test (*<0.05, **<0.01).

furan-1,2,4,7,8-pentaol 1,2-*O*-diphenylacetate, designated vialinin B (Fig. 1).

Vialinin B (**2**) strongly inhibited TNF-α release induced by anti-dinitrophenyl (DNP)-specific IgE/dinitrophenylated bovine serum albumin (DNP_7–BSA) antigen, from rat basophilic leukemia (RBL-2H3) cells in a dose-dependent manner (Fig. 3).[11] The compound **2** had an IC_{50} value of 0.02 nM, indicating that **2** was approximately 2×10^5-fold more effective than the related compounds, ganbajunin B (**1**, IC_{50} = 5000 nM) and cycloleucomelone (**3**, IC_{50} = 3500 nM), and comparable to the clinical immunosuppressant FK-506[12] (IC_{50} = 0.25 nM) in this assay. Further biological investigation is in progress.

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6. The dry fruiting bodies of *T. vialis* were purchased from Kunming Edible Mushroom Institute, Yunnan, P.R. China.
7. Column: Shiseido Co. Ltd. CAPCELL PAK C18 UG120 15 mmϕ ×250 mm; mobile phase: 0.15% KH_2PO_4 (pH 3.5)/acetonitrile (4:6); detection: 254 nm; flow rate: 8.8 mL/min.
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10. Vialinin B (**2**): ^{1}H NMR (600 MHz, CD_3OD) δ 7.44 (2H, dd, J = 7.7, 1.7 Hz, 4‴-H and 8‴-H), 7.40 (2H, dd, J = 7.7, 7.1 Hz, 5‴-H and 7‴-H), 7.33 (1H, tt, J = 1.7, 7.1 Hz, 6‴-H), 7.23 (2H, m, 5″-H and 7″-H), 7.22 (1H, m, 6″-H), 7.07 (2H, d, J = 8.2 Hz, 2′-H and 6′-H), 7.06 (1H, s, 9-H), 7.04 (1H, s, 6-H), 6.92 (2H, dd, J = 7.7, 1.6 Hz, 4″-H and 8″-H), 6.76 (2H, d, J = 8.2 Hz, 3′-H and 5′-H), 3.88 (2H, s, 2‴-H_2), 3.19 (2H, s, 2″-H_2); ^{13}C NMR (150 MHz, CD_3OD) δ 171.08 (s, C-1″), 170.85 (s, C-1‴), 158.09 (s, C-4′), 152.53 (s, C-5a), 148.25 (s, C-7), 144.34, 139.17, 137.30, 129.56 (each s, C-4a#, C-4#, C-2#, C-1#) (#, interchangeable), 143.87 (s, C-8), 134.90 (s, C-3‴), 134.65 (s, C-3″), 132.95 (d, C-2′ and C-6′), 130.79 (C-4‴ and C-8‴), 130.47 (d, C-4″ and C-8″), 129.98 (d, C-5‴ and C-7‴), 129.56 (d, C-5″ and C-7″), 128.65 (d, C-6‴), 128.13 (d, C-6″), 125.01 (s, C-1′), 122.31 (s, C-3), 119.83 (s, C-9b), 114.95 (s, C-9a), 116.03 (d, C-3′ and C-5′), 107.60 (d, C-9), 99.33 (d, C-6), 41.53 (t, C-2‴), 41.01 (t, C-2″).
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