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Chemical studies of the radical scavenging mechanism of bisorbicillinol using the 1,1-diphenyl-2-picrylhydrazyl radical

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A potent antioxidant, bisorbicillinol, which is a member of the bisorbicillinoid family isolated from the culture broth of *Trichoderma* sp. USF-2690, produces a stable radical-terminated symmetric dimer by donating two hydrogen atoms to the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

There is considerable evidence that free radicals, which induce oxidative damage to biomolecules, have a major role in the pathogenesis of various diseases.[1–5] In the course of our screening program for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavengers from the culture broth of microorganisms, we isolated 9 sorbicillin-related compounds and determined their structures, including a bisorbicillinoid, bisorbicillinol **1** (Fig. 1), that act as potent radical scavengers.[6–9]

The 'Bisorbicillinoids' were recently defined as a group of dimeric sorbicillin-related natural products.[10] Several compounds in this group have interesting biologic activities[11,12] and their complex structures make them suitable targets for chemical synthesis.[13–15] The biosynthesis of bisorbicillinoids is also of interest to many investigators from the standpoint of the biosynthetic route. We recently determined a key intermediate of bisorbicillinoid biosynthesis, sorbicillinol,[16] and a biosynthetic route from bisorbicillinol **1** to bisorbibutenolide and bisorbicillinolide.[17]

Studies of antioxidant mechanisms can provide significant information for evaluating the effectiveness and utilization of different types of antioxidants and/or individual antioxidants. In our studies of the DPPH radical scavenging mechanism of **1**, we discovered a novel radical scavenging mechanism in which bisorbicillinol **1** donates two hydrogen atoms to two DPPH radicals and then **1** is changed to a novel and stable radical-terminated symmetric dimer **2**.

Bisorbicillinol **1** scavenged the DPPH radical with an ED_{50} value of 31.4 μM in the assay system with DPPH.[6] Bisorbicillinol **1** (14.4 mg) and DPPH (24.4 mg) were dissolved in $CHCl_3$ (20 ml), and the reaction mixture was stirred under dim light and ambient temperature for 2 h. High performance liquid chromatography (HPLC) analysis† of the resulting solution detected an unidentified compound at 14.7 min. The solution was concentrated *in vacuo* and then chromatographed by preparative HPLC under the same conditions as the analytical HPLC, except that a different type of column (Capcell pak C_{18} SG120, φ 15 × 250 mm, Shiseido, Japan) and flow rate (8.0 ml min^{-1}) were used. Finally, 7.6 mg of pure compound **2** was isolated.

Compound **2** was obtained as a yellowish amorphous powder, $[\alpha]_D$ −15.2 deg cm^2 g^{-1} (CH_3OH). The UV and visible spectrum in CH_3OH exhibited an absorption maximum at 301.5 nm (ε 35 400 mol^{-1} dm^3 cm^{-1}). The IR spectrum of **2** indicated absorptions for hydroxy groups (3450 cm^{-1}), carbonyl groups (1755, 1730, and 1670 cm^{-1}), and double bonds (1630 and 1580 cm^{-1}). ESI-MS (positive) spectrum of **2** gave *m/z* 495 as a $(M + H)^+$ ion peak. Compound **2** was formulated as $C_{28}H_{30}O_8$ from HRFAB-MS data [*m/z* 495.2025 $(M + H)^+$; 495.2019 calcd for $C_{28}H_{31}O_8$].

The ^{1}H- and ^{13}C-NMR spectra‡ of compound **2** in $CDCl_3$ exhibited only 11 proton and 14 carbon signals, suggesting that compound **2** is a symmetrical dimer. The ^{1}H-NMR spectrum displayed the presence of an (*E*,*E*)-penta-1,3-dienyl moiety (δ_H 1.90, 6.34, 6.27, 7.37 and 7.02). The ^{13}C-NMR spectrum of **2** comprised three methyls (δ_C 24.0, 19.0 and 8.1), four olefinic methines (δ_C 147.3, 144.4, 130.6 and 124.3), an sp^3 methine (δ_C 43.2), three carbonyls (δ_C 205.5, 199.9 and 191.2), and three other quaternary carbons (δ_C 75.9, 69.2 and 67.9). The HMBC experiments on **2** led to the partial structure **3** that could be expanded to **4**, having a proper axis of symmetry, as shown in Fig. 2. The cross peaks between the two methyl peaks and carbons enabled the partial structure **3** to be constructed; the cross peaks between 10-CH_3/4-CH_3 (δ_H 8.1) and C-11/C-5 (δ_C 205.5), C-10/C-4 (δ_C 67.9 or 69.2), C-2/C-8 (δ_C 67.9 or 69.2), and C-9/C-3 (δ_C 199.9), and between 12-CH_3/6-CH_3 (δ_H 1.09) and C-11/C-5, C-12/C-6 (δ_C 75.9) and C-7/C-1 (δ_C 43.2). The advanced structure **4** was confirmed by elucidation of the $^2J_{C-H}$ or $^3J_{C-H}$ correlation between 1-H/7-H and the carbons; the cross peaks between 1-H/7-H (δ_H 3.72) and C-7/C-1 (δ_C 43.2), C-2/C-8, C-10/C-4, C-9/C-3, C-5/C-11, C-6/C-12, C-1′/C-1″

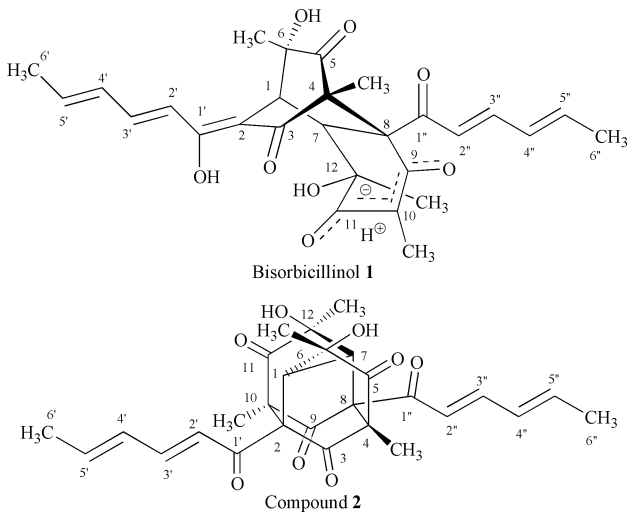

Fig. 1 Structures of bisorbicillinol **1** and compound **2**.

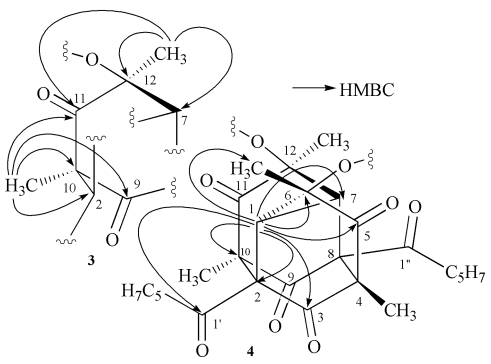

Fig. 2 Summary of the HMBC results for compound **2**.

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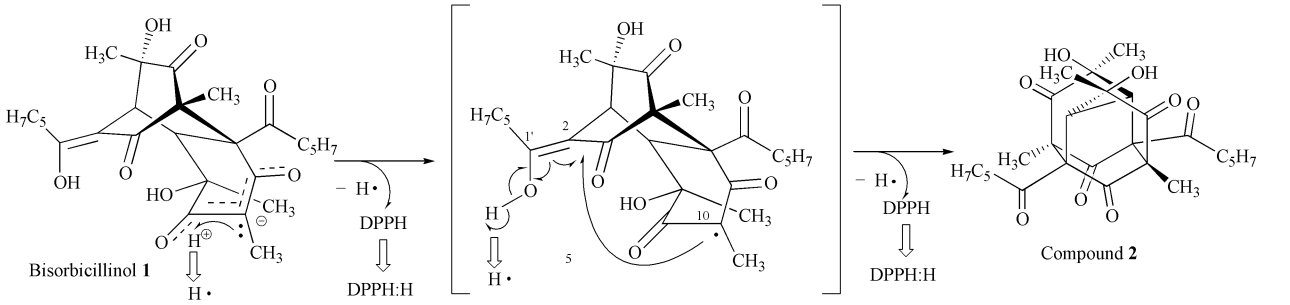

Scheme 1 Proposed mechanism for the DPPH radical scavenging activity of bisorbicillinol **1**.

(δ_C 191.2) and 6-CH_3/12-CH_3 (δ_C 24.0). The two remaining hydrogens of **2** are accounted for by the hydroxy groups at C-6 and C-12. The NOESY experiment indicated that there were only the cross peaks between 10-CH_3/4-CH_3 and H-2′/H-2″ and 6-CH_3/12-CH_3 and H-1/H-7 in the central rigid framework of **2**; therefore these data do not provide information on the relative configuration at C-6 and C-12. The structural relationship between bisorbicillinol **1** and the dimer **2** strongly suggests that the absolute configurations, particularly at C-6 and C-12 survived the radical scavenging process intact. Accordingly, the structure of **2** is formulated as in Fig. 1.

The DPPH radical scavenging mechanism of bisorbicillinol **1**, based on the structure **2**, is illustrated in Scheme 1. Abstraction of a hydrogen atom by the first equivalent of DPPH generates a radical at C-10, the methylated α-carbon of a β-dicarbonyl system. This transannularly attacks C-2 and terminally results in capture of the enolate hydroxy hydrogen at C-1′, as in Scheme 1. Thus, by a novel autoxidative mechanism, the unsymmetrical dimer **1** is transformed into the symmetrical dimer **2** with concomitant capture of two equivalents of DPPH. The resulting compound **2** was a non-radical compound and a stable symmetric dimer, that is, the DPPH radical scavenging process of bisorbicillinol **1** terminated the radical reaction. Quantitative study of the mechanism including side reactions[18] is currently under way.

We report that a new type of antioxidative mechanism with an asymmetric dimer **1** changed into a symmetric dimer **2** by donating two hydrogen atoms to two free radical molecules.

Notes and references

† A 1 μl aliquot of the resulting solution was injected into an analytical HPLC system under the following conditions: column, Capcell pak C_{18} SG120 (φ 4.6 × 150 mm, Shiseido, Japan); solvent system, 0.15% KH_2PO_4 (pH 3.5)–CH_3CN (1 : 1); flow rate, 1.0 ml min^{-1}; detection, 270 nm.

‡ Compound **2**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.37 (2H, dd, J = 14.9, 10.0 Hz, 3′-H and 3″-H), 7.02 (2H, d, J = 14.9 Hz, 2′-H and 2″-H), 6.34 (2H, dq, J = 15.2, 6.0 Hz, 5′-H and 5″-H), 6.27 (2H, dd, J = 15.2, 10.0 Hz, 4′-H and 4″-H), 3.72 (2H, s, 1-H and 7-H), 1.90 (6H, d, J = 6.0 Hz, 6′-H_3 and 6″-H_3), 1.12 (6H, s, 4-CH_3 and 10-CH_3), 1.09 (6H, s, 6-CH_3 and 12-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 205.5 (s, C-5 and C-11), 199.9 (s, C-3 and C-9), 191.2 (s, C-1′ and C-1″), 147.3 (d, C-3′ and C-3″), 144.4 (d, C-5′ and C-5″), 130.6 (d, C-4′ and C-4″), 124.3 (d, C-2′ and C-2″), 75.9 (s, C-6 and C-12), 69.2 (s, C-2 and C-8 or C-4 and C-10), 67.9 (s, C-2 and C-8 or C-4 and C-10), 43.2 (d, C-1 and C-7), 24.0 (q, 6-CH_3 and 12-CH_3), 19.0 (q, C-6′ and C-6″), 8.1 (q, 4-CH_3 and 10-CH_3).

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