文章编号:1001-6880(2015)11-1843-04

长序三宝木中的降二萜成分研究

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摘　要:从长序三宝木(*Trigonostemon howii*)茎的化学成分研究中发现 4 个降二萜化合物,其中新化合物 trigohowilone A(**1**)经波谱数据包括核磁、质谱、比旋光等鉴定为 trigoxyphin P 的对映异构体。已知化合物结构确定为 trigoxyphin Q(**2**),trigohowilol H(修订后的俗名)(**3**)和 9-*O*-demethyltrigonostemone(**4**)。所有化合物均为首次从该植物中分离得到。

关键词:大戟科;长序三宝木;降二萜;trigohowilone A;(－)-trigoxyphin P

中图分类号:R284.1;Q946.91　　**文献标识码**:A　　DOI:10.16333/j.1001-6880.2015.11.001

Degraded Diterpenoids from *Trigonostemon howii*

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Abstract: Phytochemical investigation on the stems of *Trigonostemon howii* resulted in the isolation of four degraded diterpenoids. A new compound, trigohowilone A (**1**), was defined as the enantiomer of trigoxyphin P based on the analyses of its spectroscopic data including NMR, MS, and the specific rotation data. The structures of known compounds were identified as trigoxyphin Q (**2**), trigohowilol H (revised trivial name) (**3**), and 9-*O*-demethyltrigonostemone (**4**). All compounds were obtained from the plant for the first time.

Key words: Euphorbiaceae; *Trigonostemon howii*; degraded diterpenoids; trigohowilone A; (－)-trigoxyphin P

Introduction

Trigonostemon howii Merrill & Chun (Euphorbiaceae), an evergreen shrub growing in dense montane forests, is distributed in Hainan Province of China and Vietnam [1]. Previous research on this plant collected in China has revealed the occurrence of 14 daphnane-type diterpenoids [2] and 10 degraded diterpenoids [3], while phytochemical investigation of the Vietnamese plant species *T. howii* only obtained a tigliane diterpenoid, four coumarins, and two penylpropanoids [4]. As a part of our ongoing research on this plant [3], four degraded diterpenoids (Fig. 1), including the enantiomer of trigoxyphin P, trigohowilone A (**1**), and three known ones, trigoxyphin Q (**2**), trigohowilol H (**3**), and 9-*O*-demethyltrigonostemone (**4**) were isolated from the stems of *T. howii* collected from Hainan Province of China. Compounds **1**-**4** were obtained from *T. howii* for the first time.

Materials and Methods

Instruments

1D and 2D NMR spectra were recorded on Bruker AM-400 and Bruker DRX-600 spectrometers using TMS as an internal standard. ESI-MS analyses were carried out on an API Qstar Pulsar 1 instrument. HR-EI-MS analyses were carried out on a Waters Autospec Premier P776 mass spectrometer. Silica gel (80-100 and 300-400 mesh, Qingdao Makall Group Co., Ltd.), C_8 silica gel (20-45 μm, Fuji Silysia Chemical Ltd.), and SephadexLH-20 (GE Healthcare Bio-Xciences AB) were used for column chromatography (CC) and silica gel

Received: July 28, 2015　　Accepted: September 21, 2015

Foundation item: This work was supported by the National Natural Science Foundation of China (21432010); The Science Foundation of Yunnan (2014A050); The Technological leading talent project of Yunnan, and the Xibuzhiguang Project (grant to Y. T. D.)

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GF_{254} (Qingdao) was used for preparative TLC in the form of precoated plates. TLC spots were visualized under UV light and by dipping into 5% H_2SO_4 in EtOH followed by heating.

Plant material

The stems of *T. howii* were collected from Sanya, Hainan Province, China, in October 2011. The plant was identified by one of the authors (Gui-hua Tang), and a voucher specimen (H20101201) was deposited at State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany.

Extraction and isolation

The air-dried stems of *T. howii* (32.0 kg) were powdered and extracted with MeOH for three times (4, 3, and 3 h, respectively) under reflux. After evaporating the solution under reduced pressure, the crude residue was re-suspended in water and then partitioned successively with EtOAc and *n*-BuOH to give two corresponding portions. The EtOAc extract (196.6 g) was subjected to CC over silica gel (80-100 mesh) using petroleum ether (PE)-Me_2CO (50∶1→0∶1) to yield five fractions (A-E). Fr. B was subjected to CC over C_8 silica gel eluting with a gradient of increasing MeOH in H_2O (30%-100%) to gain 10 fractions (B_1-B_{10}). Subsequently, Fr. B_2 was chromatographed on a Sephadex LH-20 column (MeOH) and a silica gel column (300-400 mesh, $CHCl_3$-Me_2CO, 20∶1 + 1% formic acid) to obtain **3** (5.6 mg). Fr. D was subjected to CC over C_8 silica gel eluting with a gradient of increasing MeOH in H_2O (50%-100%) to gain nine fractions (D_1-D_9). Fr. D_8 was chromatographed on a Sephadex LH-20 column (MeOH) to gain four subfractions (D_{8a}-B_{8d}). After filtration the insoluble material from subfraction D_{8b}, the stock was subjected to CC over a silica gel column (300-400 mesh, $CHCl_3$-Me_2CO, 10∶1) and a Sephadex LH-20 column (Me_2CO) to afford **1** (4.5 mg). Subfraction D_{8d} was purified by a silica gel column (300-400 mesh, $CHCl_3$ - Me_2CO, 15∶1) to get **2** (1.3 mg) and **4** (11.8 mg).

Trigohowilone A (**1**) Yellow amorphous powder; $[\alpha]_D^{25}$ −21.5 (*c* 0.27, $CHCl_3$); UV (MeOH) λ_{max} (logε) 366 (3.77), 312 (3.99), 283 (4.08), 208 (4.51) nm; IR (KBr) υ_{max} 3442, 1707, 1689, 1657, 1640, 1629, 1549, 1511 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) δ 7.86 (1H, s, H-14), 6.44 (1H, s, H-6), 6.27 (2H, s, H-2′and H-6′), 4.56 (1H, br. s, H-7′), 3.67 (6H, s, 3′-and 5′-OMe), 3.17 (1H, q, J = 7.2 Hz, H-8′), 2, 37 (3H, s, H-17), 1.35 (3H, s, H-18), 1.33 (3H, s, H-19), 1.27 (3H, d, J = 7.2 Hz, H-9′); ^{13}C NMR (CD_3OD, 150 MHz) δ 207.9 (C, C-3), 187.2 (C, C-7), 159.9 (C, C-12), 159.6 (C, C-5), 150.2 (C, C-8), 149.1 (C × 2, C-3′and C-5′), 143.9 (C, C-1), 135.8 (C, C-4′), 135.4 (C, C-1′), 131.8 (C, C-13), 128.9 (CH, C-14), 128.0 (C, C-9), 124.1 (C, C-11), 123.4 (C, C-10), 121.1 (CH, C-6), 105.2 (CH × 2, C-2′and C-6′), 56.6 (CH_3 × 2, 3′-and 5′-OMe), 48.2 (C, C-4), 46.4 (CH, C-7′), 36.7 (CH, C-8′), 24.4 (CH_3, C-19), 22.6 (CH_3, C-18), 20.7 (CH_3, C-9′), 17.7 (CH_3, C-17); ESI-MS *m/z* 469 [M + Na]$^+$, HR-EI-MS *m/z* 446.1715 [M]$^+$ (calcd for $C_{27}H_{26}O_6$, 446.1729).

Trigoxyphin Q (**2**) Yellow amorphous powder; UV (MeOH) λ_{max} (logε) 542 (3.30), 408 (2.97), 373 (3.02), 317 (3.62), 291 (3.65), 208 (4.11) nm; IR (KBr) υ_{max} 3442, 1711, 1689, 1630, 1552 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 8.26 (1H, s, H-14), 6.89 (1H, s, H-6), 6.53 (2H, s, H-2′and H-6′), 3.84 (6H, s, 3′-and 5′-OMe), 2.47 (3H, s, H-19), 2, 20 (3H, s, H-17), 1.47 (6H, s, H-18 and H-19); ^{13}C NMR (CD_3OD, 100 MHz) (seven carbon resonances not visible) δ 209.0 (C, C-3), 156.3 (C, C-5), 150.3 (C × 2, C-3′and C-5′), 148.2 (C, C-1), 136.4 (C, C-4′), 135.3 (C, C-7′), 135.2 (CH, C-14), 132.7 (C, C-8′), 126.3 (C, C-9), 118.5 (CH, C-6), 106.5 (CH × 2, C-3′and C-5′), 57.0 (CH_3 × 2, 3′-and 5′-OMe), 50.3 (C, C-4), 24.4 (CH_3 × 2, C-18 and C-19), 17.7 (CH_3, C-17), 15.1 (C, C-9′); ESI-MS *m/z* 467 [M + Na]$^+$, 911 [2M + Na]$^+$, HR-EI-MS *m/z* 444.1581 [M]$^+$ (calcd for $C_{27}H_{24}O_6$, 446.1573).

Trigohowilol H (**3**) Yellow amorphous powder; UV (MeOH) λ_{max} (logε) 434 (4.27), 305 (3.97), 293 (3.90), 248 (4.10), 220 (4.30), 201 (4.24) nm; IR (KBr) υ_{max} 3428, 1734, 1662, 1626, 1574, 1446, 1403, 1344, 1300 cm^{-1}; ^1H NMR (acetone-d_6, 600 MHz) δ 8.36 (1H, s, H-11), 8.04 (1H, s, H-14), 6.69 (1H, s, H-6), 2.34 (3H, s, H-11), 1.41 (6H, s, H-18 and H-19); ^{13}C NMR (acetone-d_6, 150 MHz)

δ 207.9 (C, C-3), 184.0 (C, C-7), 166.9 (C, C-1), 166.2 (C, C-5), 161.4 (C, C-12), 133.5 (C, C-9), 129.0 (C, C-13), 126.4 (CH, C-14), 124.5 (C, C-8), 119.5 (C, C-10), 101.9 (CH, C-6), 107.6 (CH, C-11), 43.9 (C, C-4), 25.0 ($CH_3 \times 2$, C-18 and C-19), 17.0 (CH_3, C-17); ESI-MS *m/z* 293 $[M + Na]^+$, 563 $[2M + Na]^+$.

9-*O*-Demethyltrigonostemone (**4**) Yellow amorphous powder; ^{1}H NMR (acetone-d_6, 400 MHz) δ 9.22 (1H, s, 7-OH), 8.00 (1H, s, H-14), 7.58 (1H, s, H-11), 7.56 (1H, s, H-1), 6.94 (1H, s, H-5), 4.02 (3H, s, 12-OCH_3), 3.84 (3H, s, 2-OCH_3), 2.34 (3H, s, H-17), 1.43 (6H, s, H-18 and H-19); ^{13}C NMR (acetone-d_6, 100 MHz) δ 198.7 (C, C-3), 158.9 (C, C-12), 153.9 (C, C-7), 148.5 (C, C-2), 144.0 (C, C-5), 132.6 (C, C-9), 127.4 (C, C-13), 124.4 (CH, C-14), 119.7 (C, C-8), 115.4 (C, C-10), 113.5 (CH, C-1), 105.9 (CH, C-6), 101.0 (CH, C-11), 55.9 (CH_3, 2-OCH_3), 55.8 (CH_3, 12-OCH_3), 49.6 (C, C-4), 28.4 ($CH_3 \times 2$, C-18 and C-19), 16.8 (CH_3, C-17); ESI-MS *m/z* 335 $[M + Na]^+$.

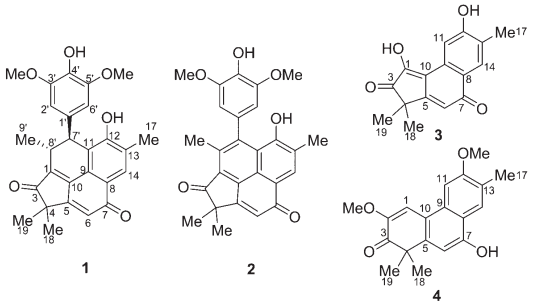

Fig. 1 Chemical structures of compounds 1-4

Structural identification

Trigohowilone A (**1**), yellow amorphous powder, had the molecular formula $C_{27}H_{26}O_6$ with 15 degrees of unsaturation based on the $[M]^+$ at *m/z* 446.1715 (calcd 446.1729) in its HR-EI-MS. The IR absorptions at 3442, 1707, 1689, 1657, 1640, 1629, 1549, 1511 cm^{-1} indicated the presence of hydroxyl, carbonyl and phenyl functionalities. The ^{1}H NMR spectrum showed four aromatic or olefinic protons [δ_H 7.86 (1H, s), 6.27 (2H, s) and 6.44 (1H, s)], two methine protons [4.55 (1H, br. s) and 3.17 (1H, q, *J* = 7.2 Hz)], three singlet methyls [δ_H 2.36 (3H, s), 1.35 (3H, s) and 1.32 (3H, s)], one methyl [δ_H 1.26 (1H, d, *J* = 7.2 Hz)] coupled with the proton at 3.17 ppm, and two *O*-methyl groups [δ_H 3.67 (6H, s)]. 27 signals consistent with six methyls, six methines, and 15 quaternary carbons were observed in the ^{13}C NMR and DEPT spectra.

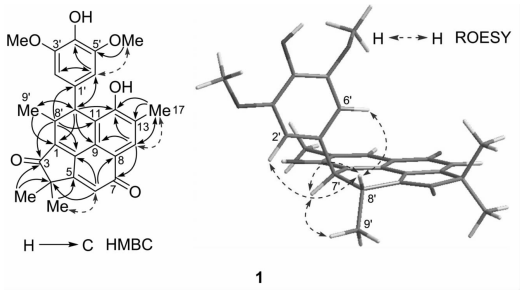

Fig. 2 Key 2D correlations of trigohowilone A (1)

Preliminary analyses of 2D NMR data showed that two singlet methyls at δ_H 1.35 (H-18) and 1.32 (H-19) were the *gem*-dimethyl groups located at the quaternary carbon at δ_C 48.2 (C-4), and the methyl at δ_H 2.36 (H-17) was the aromatic methyl group. These information together with two ketone carbonyl [δ_C 207.9 (C-3) and 187.2 (C-7)] indicated that **1** possessed a moiety resembled the skeleton of trigohowilol H (3)[5], which was confirmed by the HMBC and ROESY correlations (Fig. 2). In addition, analyses of 2D NMR data determined the presence of a symmetrical phenylpropanoid moiety [δ_H 6.27 (H-2′/6′), 4.55 (H-7′), 3.17 (H-8′), 1.26 (H-9′), 3.67 (3′/5′-OCH_3); δ_C 149.1 (C-3′/5′), 135.7 (C-4′), 135.4 (C-1′), 105.2 (C-2′/6′), 46.4 (C-7′), 36.7 (C-8′), 20.7 (C-9′), 56.6 (3′/5′-OCH_3)]. HMBC correlations of H-9′/C-1, H-8′/C-3, C-10, and C-11, and H-7′/C-1, C-9, and C-12 (Fig. 2) confirmed the two above-mentioned moieties via C-1 – C-8′ and C-11 – C-7′ carbon bonds to construct the planar structure of **1**, which was identified as trigoxyphin P, isolated from the same genus species *T. xyphophylloides*[6]. The relative configuration of **1** was also determined as the same as trigoxyphin P by analyses of the coupling constant of H-7′ and H-8′ and the ROESY correlation of H-2′ or H-6′/H-8′ (Fig. 2). However, the specific rotation of **1** ($[\alpha]_D^{20} = -21.5$) was opposite to that of trigoxyphin P ($[\alpha]_D^{20} = +32.6$), which indicated that **1** and trigoxyphin P were a pair of enantiomers. Thus, the structure of compound **1** was defined as shown and named

trigohowilone A or (−)-trigoxyphin P.

Analyses of the 2D NMR spectra of compounds **2** and **3** determined their structures, which were identical to trigoxyphin Q [6] and trigoxyphin U [5], respectively. However, there is another different structure named trigoxyphin U [7], so the trivial name of compound **3** was revised as trigohowilol H. Moreover, the known compound, 9-*O*-demethyltrigonostemone (**4**) [8], was identified by comparison of its spectroscopic data with literature data.

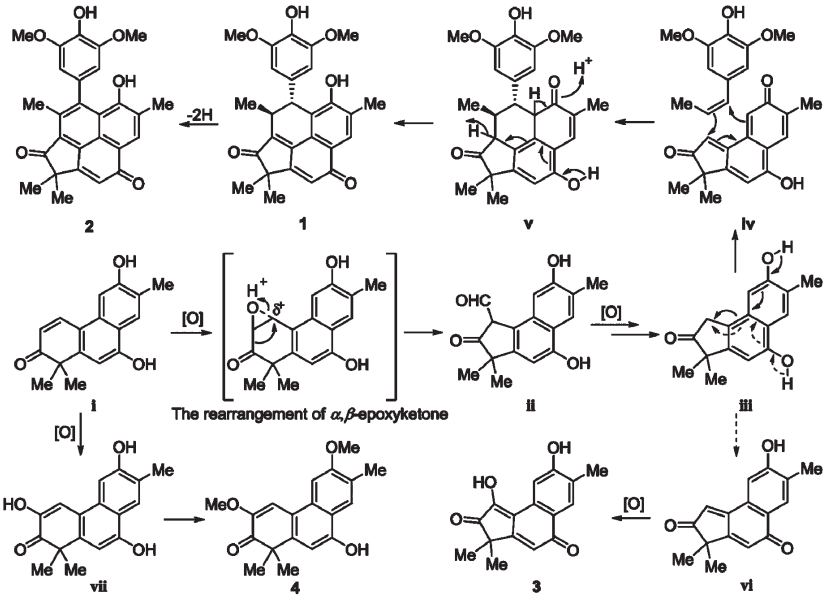

Fig. 3 **Scheme** 1 **Proposed biogenetic pathways of compounds 1-4**

The intriguing discovery of these four degraded diterpenoids (**1-4**) in the same plant led us to further study the possible biogenetic relationships among these compounds. As shown in Scheme 1, the postulated precursor (**i**) could be modified through different biosynthetic pathways involving ring contraction based on the rearrangement of α,β-epoxyketone[9] and oxidation to form the intermediates **ii** and **vii**, respectively. Oxidative decarboxylation of the intermediate (**ii**) would yield **iii**, which was then converted into **iv** or **vi** by keto-enol tautomerism. The heterodimers, compounds **1** and **2**, could be visualized as cycloadducts of **iv** and phenylpropanoid via intermolecular Diels-Alder reaction, whereas **3** could be derived from the oxidation of **vi**. Oxidation of the intermediate (**i**) followed by methyl esterification would produce compound **4**.

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