• 方药研究 •

• 特邀稿件 •

人工种植帚状香茶菜中木脂素类成分的研究 *

陈素萍[1,2]，胡　坤[1]，孙汉董[1]，普诺·白玛丹增[1△]

（1. 中国科学院昆明植物研究所 植物化学与西部植物资源持续利用国家重点实验室，云南 昆明 650201；
2. 中国科学院大学，北京 100049）

摘要：从人工种植帚状香茶菜的地上部分中分离得到了 1 个新的苯并二氢呋喃类木脂素 scopariunan（**1**）以及 5 个已知的木脂素类化合物，其中包括骈双四氢呋喃类、四氢呋喃类及降木脂素等多种结构类型。化合物 **1** 的结构通过光谱学方法结合量子化学 ECD 计算确定。

关键词：帚状香茶菜；木脂素；量子化学计算

中图分类号：R284.1　　**文献标志码**：A　　**文章编号**：1000－2723(2022)06－0071－05

DOI：10.19288/j.cnki.issn.1000-2723.2022.06.015

Lignans from Artificially Cultivated *Isodon scoparius*

CHEN Suping[1,2], HU Kun[1], SUN Handong[1], PUNO Pema-Tenzin[1]

(1. State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China;
2. University of Chinese Academy of Sciences, Beijing 100049, China)

ABSTRACT: One new dihydrobenzofuran lignan, scopariunan (**1**), along with five known lignans comprising furofuran, tetrahydrofuranoid and norlignan types, were isolated from the leaves and stems of artificially cultivated *Isodon scoparius*. The structure of **1** was determined by spectroscopic analyses and quantum chemical ECD calculation.

KEY WORDS: *Isodon scoparius*; lignans; quantum chemical calculation

1　Introduction

Isodon scoparius is mainly distributed in northwestern Yunnan Province, China, and it is commonly used as an antipyretic agent by local inhabitants. Previous phytochemical investigations of *I. scoparius* collected in Shangri-La indicated that its major chemical constituents were *ent*-clerodane diterpenoids[1-2]. Noteworthily, the discovery of cyclobutane-bearing meroditerpenoids and complicated dimeric *ent*-clerodanoids from *I. scoparius*, along with their fascinating immunosuppressive activities and the completion of their organic synthesis, marked one of the most fantastic breakthroughs in research on *Isodon* species in recent years[3-9]. To achieve sustainable utilization of plant resources, *I. scoparius* was artificially cultivated in Kunming Botanical Garden with seeds collected from Shangri-La. Subsequently, phytochemical research on this plant material was undertaken by our research group. Previous study on the cultivated plants has led to the identification of two cyclobutane-bearing meroditerpenoids, including a novel one characterized by an oxygen insertion reaction in its proposed biosynthesis[10]. In this work, we continued to investigate the chemical constituents of artificially cultivated *I. sco-*

收稿日期：2022－11－09

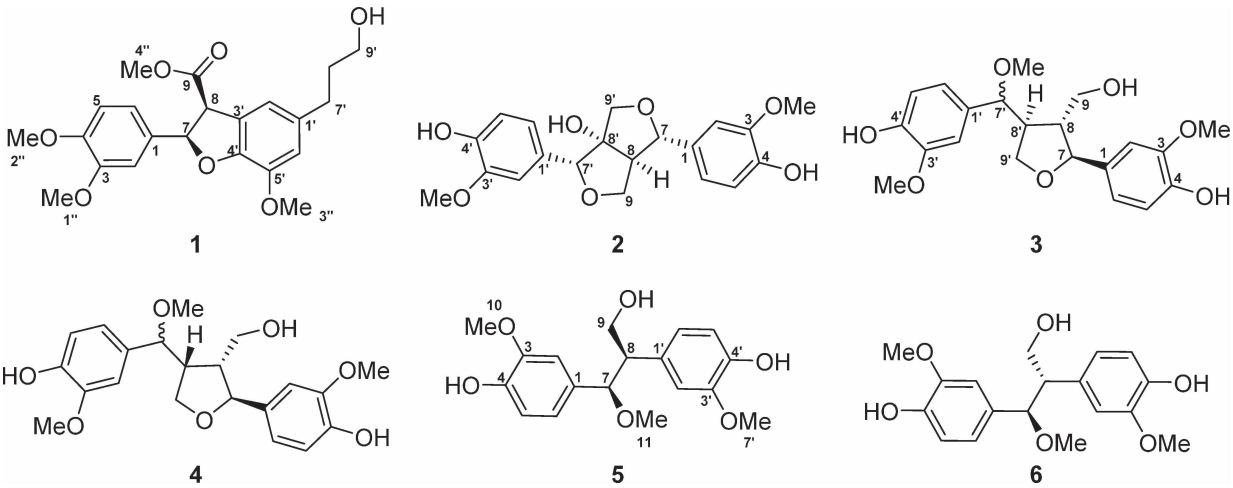
* **基金项目**：国家自然科学基金项目(81903520, 81673329)

第一作者简介：陈素萍(1996－)，女，在读博士生，药物化学专业。

△通信作者：普诺·白玛丹增，E-mail: punopematenzin@mail.kib.ac.cn

parius, which led to the isolation and identification of a series of lignans, including a new compound, scopariunan (**1**), along with five known ones, 1-hydroxypinoresinol (**2**)[11], tanegool-7′-methylether (**3**)[12], 7′-methoxylariciresinol (**4**)[12], *erythro*-2,3-bis-(4-hydroxy-3-methoxyphenyl)-3-methoxy-propanol(**5**)[13], *threo*-2,3-bis-(4-hydroxy-3-methoxyphenyl)-3-methoxy-propanol (**6**)[14]. Herein, we report the details of the isolation and structure determination of these compounds.

2 Results



Scopariunan (**1**) was obtained as colorless oil and its molecular formula was determined as $C_{22}H_{26}O_7$ by a negative HRESIMS ion peak at *m/z* ([M-H]$^-$, 401.1609, calcd for 401.1606), implying ten degrees of unsaturation. The ^{1}H NMR data together with HSQC spectrum showed four methoxy groups at δ_H 3.88 (3H, overlapped, H_3-1"), 3.88 (3H, overlapped, H_3-2"), 3.89 (3H, s, H_3-3") and 3.82 (3H, s, H_3-4"), five aromatic hydrogens at δ_H 6.98 (1H, dd, *J*=8.2, 2.0 Hz, H-6), 6.95 (1H, d, *J*=2.0 Hz, H-2), 6.84 (1H, d, *J*=8.2 Hz, H-5), 6.80 (1H, s , H-2′) and 6.70 (1H, s , H-6′). The ^{13}C NMR and DEPT spectra exhibited 22 resonances, which can be categorized into four methyls, three methylenes, seven methines (including five aromatic carbons and one oxygenated carbon), and eight non-protonated carbons (including one carbonyl and seven aromatic carbons). Through the analysis of 2D NMR spectra, compound **1** was inferred to be a dihydrobenzofuran lignan with ester group and four methoxy groups. The HMBC correlations from H-7 (δ_H 6.05), H-8 (δ_H 4.32) and H_3-4" (δ_H 3.82) to C-9 (δ_C 171.5) indicated that C-9 was oxidated to a methyl ester. Besides, the HMBC correlations between H_3-1" (δ_H 3.88) to C-3 (δ_C 149.4), H_3-2" (δ_H 3.88) to C-4 (δ_C 149.4) and H_3-3" (δ_H 3.89) to C-5′ (δ_C 144.5) revealed the substitutional positions of the remaining methoxy groups (Figure 1). As for the relative configuration of compound **1**, the coupling constants between H-7 and H-8 (8.6 Hz) revealed that these two protons adopted *cis* arrangement. Then, quantum chemical calculations were run on (7*S*,8*R*)-**1T** (**1Ta**), with **1T** being the truncated structure of **1** (to be specific, the -CH_2-CH_2-CH_2-OH chain in **1** was replaced by a methyl in **1T** to avoid redundant conformers). The calculated $^3J_{H-7/H-8}$ of 9.8 Hz at B972-SCRF/pcJ-1(chloroform)//M06-2X-D3/6-311G(d,p) level of theory verified the established relative configuration of compound **1**. The calculated ECD spectrum of **1Ta** at CAM-B3LYP-SCRF/6-31+G(2d,p) (methanol)//M06-2X-D3/6-311G(d,p) level of theory appeared as nearly image of the experimental ECD spectrum (Figure 2), indicating that the

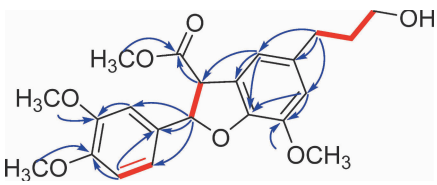


Fig.1 ^{1}H-^{1}H COSY (red bold) and selected HMBC (blue arrow) correlations of **1**.

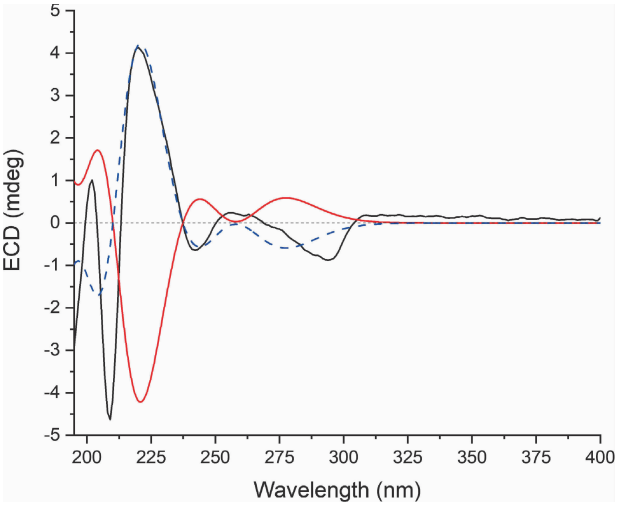


Fig.2 Experimental ECD spectrum of **1** (black). Calculated ECD spectra of (7*S*,8*R*)−**1T** (**1Ta**) (red solid line) and (7*R*,8*S*)−**1T** (**1Tb**) (blue dotted line) at CAM−B3LYP−SCRF/6−31+G(2d,p) (methanol)//M06−2X−D3/6−311G(d,p) level of theory (shift = +11 nm, full width at half maximum = 0.6 eV).

Tab.1 NMR data (δ in ppm, J in Hz) of 3′,4−*O*−dimethylcedrusin methyl ester (**1**) [a]

No.	δ_H (mult, *J*)	δ_C, type	No.	δ_H (mult, *J*)	δ_C, type
1	−	132.7 C	3′	−	125.3 C
2	6.95 (d, 2.0)	109.5 CH	4′	−	146.3 C
3	−	149.4 C	5′	−	144.5 C
4	−	149.4 C	6′	6.70 (s)	113.1 CH
5	6.84 (d, 8.2)	111.2 CH	7′	2.68 (dd, 8.6, 6.1)	32.2 CH_2
6	6.98 (dd, 8.2, 2.0)	119.1 CH	8′	1.90 (m)	34.9 CH_2
7	6.05 (d, 8.6)	86.9 CH	9′	3.71 (t, 6.4)	62.5 CH_2
8	4.32 (d, 8.6)	56.3 CH	1"	3.88 (s)	56.2 CH_3
9	−	171.5 C	2"	3.88 (s)	56.2 CH_3
1′	−	135.8 C	3"	3.89 (s)	56.1 CH_3
2′	6.80 (s)	116.7 CH	4"	3.82 (s)	52.9 CH_3

[a]Recorded on a 500 MHz NMR spectrometer with $CDCl_3$ as the solvent.

absolute configuration of **1** was 7*R*, 8*S*. Thus, the structure of compound **1** was established.

The five known lignans were identified by comparison of their spectroscopic data with those reported in literatures, and they were identified as 1−hydroxypinoresinol (**2**), tanegool−7′−methylether (**3**), 7′−metho−xylariciresinol (**4**), *erythro*−2,3−bis−(4−hydroxy−3−methoxyphenyl)−3−methoxy−propanol (**5**), *threo*−2,3−bis−(4−hydroxy−3−methoxyphenyl)−3−methoxy−propanol (**6**).

3　Experimental details

3.1　*General experimental procedures*　Optical rotation was measured with a JASCO P−1020 polarimeter. UV spectrum was obtained using a Shimadzu UV−2401 PC spectrophotometer. Experimental ECD spectrum was measured on a Chirascan instrument. A Tensor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. HRESIMS data was acquired on an Agilent 6540 QSTAR TOF time−of−flight mass spectrometer. 1D and 2D NMR spectra were recorded on Bruker DRX−500, 600 and 800 spectrometers with TMS as internal standard. Chemical shifts (δ) are expressed in parts per million (ppm) with reference to the solvent signals. Column chromatography was performed on silica gel (80−100 mesh and 100−200 mesh), Lichroprep RP−18 gel (40−63 μm, Merck, Darmstadt, Germany), MCI gel (75−150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), and Sephadex LH−20 (Pharmacia, Uppsala, Sweden). Semi−preparative HPLC was performed on an Agilent 1260 liquid chromatograph with a Zorbax SB−C18(9.4 mm × 250 mm) column. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

3.2　*Plant materials*　The *Isodon scoparius* was artificially cultivated in Kunming Institute of Botany and the seeds were collected from Shangri−La, Yunnan Province, China. Experimental materials were their aerial parts.

3.3　*Extraction and isolation*　The air−dried and powdered aerial parts of *Isodon scoparius* (20 Kg) were extracted with 70% aqueous acetone. With filtration and concentration, the resultant extract was partitioned with EtOAc and *n*−BuOH respectively, and then evaporated *in vacuo* to afford a crude extract (0.75 Kg in EtOAc and 0.14 Kg in *n*−BuOH). The *n*−BuOH extraction was subjected to column chro−

matography on silica gel eluted with a $CHCl_3$–Me_2CO gradient system (1∶0, 9∶1, 8∶2, 7∶3, 6∶4, 0∶1, v/v) to yield six fractions, A–F. Fraction C ($CHCl_3$–Me_2CO 8∶2, 82 g) was decolorized by MCI gel with methanol–H_2O (90∶10, v/v) and methanol (100%, v) system to afford fractions C1 and C2. Fraction C1 was subjected to RP–18 silica gel with gradient system (methanol–H_2O, from 30%∶70% to 100%∶0%, v/v) to yield subfractions C1/1–8, subfraction C1–5 was purified by repeated silica gel (eluted with petroleum ether–Me_2CO 10∶1 to 0∶1, v/v), Sephadex LH–20 (MeOH system) and semi–preparative HPLC (MeCN–H2O, 35∶65, v/v) to yield **1** (1.5 mg), **2** (1.1 mg), **3** (2.0 mg), **4** (1.2 mg), **5** (2.3 mg) and **6** (1.0 mg).

Scopariunan (***1***): Colorless oil; $[\alpha]_D^{19.4}$: −59.60 (MeOH, *c* 0.095); ECD (MeOH) λ_{max} (logε): 202 (0.25), 209 (−1.13), 220 (1.01), 242 (−0.16) and 294 (−0.21) nm; UV (MeOH) λ_{max} (logε): 204 (3.90), 226 (3.34) and 282 (2.86) nm; IR (ν_{max}): 3436, 2932, 1737, 1619, 1518, 1265, 1142 and 1026 cm^{-1}. HRESIMS at *m/z* 401.1609 ($[M-H]^-$, calcd for 401.1606). ^1H and ^{13}C NMR data, see Table 1.

1–Hydroxypinoresinol (***2***): Yellow oil; $C_{20}H_{22}O_7$; ^1H NMR (600 MHz, $CDCl_3$): δ_H 6.99 (1H, d, 10.9, H–5), 6.96 (1H, d, 10.9, H–5′), 6.95 (1H, d, 5.5, H–2), 6.92 (1H, d, 5.5, H–2′), 6.88 (1H, dd, 10.9, 5.5, H–6), 6.81 (1H, dd, 10.9, 5.5, H–6′), 4.85 (1H, d, 5.0, H–7), 4.80 (1H, s, H–7′), 4.54 (1H, t, 9.0, H–9′a), 4.11 (2H, m, H–9), 3.87 (6H, s, H–3, 3′–OCH_3), 3.75 (1H, dd, 9.0, 6.4, H–9′b), 3.13 (1H, m, H–8). δ_C (150 MHz, $CDCl_3$): 147.2 (s, C–3′), 146.9 (s, C–3), 146.2 (s, C–4), 145.7 (s, C–4′), 132.6 (s, C–1′), 127.2 (s, C–1), 119.9 (d, C–6), 119.8 (d, C–6′), 114.9 (d, C–5′), 114.5 (d, C–5), 109.6 (d, C–2), 109.2 (d, C–2′), 91.9 (s, C–8′), 88.0 (d, C–7′), 86.0 (d, C–7), 74.9 (t, C–9), 71.9 (t, C–9′), 60.3 (d, C–8), 56.2 (q, C–3–OCH_3), 56.1 (q, C–3′–OCH_3).

Tanegool–7′–methylether (***3***): Yellow oil; $C_{21}H_{26}O_7$; ^1H NMR (800 MHz, DMSO–d_6): δ_H 6.83 (1H, brs, H–2′), 6.81 (1H, brs, H–2), 6.75 (1H, d, 8.0, H–5′), 6.70 (1H, overlapped, H–5), 6.70 (1H, overlapped, H–6), 6.70 (1H, overlapped, H–6′), 4.51 (1H, d, 7.4, H–7), 3.95 (1H, d, 8.9, H–7′), 3.76 (3H, s, H–3′–OCH_3), 3.73 (3H, s, H–3–OCH_3), 3.54 (1H, overlapped, H–9′a), 3.44 (1H, overlapped, H–9′b), 3.49 (2H, overlapped, H–9), 3.02 (3H, s, H–7′–OCH_3), 2.50 (1H, m, H–8′), 2.07 (1H, m, H–8). δ_C (200 MHz, DMSO–d_6): 147.7 (s, C–3′), 147.5 (s, C–3), 146.3 (s, C–4′), 145.7 (s, C–4), 133.6 (s, C–1), 131.3 (s, C–1′), 120.4 (d, C–6′), 118.8 (d, C–6), 115.2 (d, C–5′), 115.1 (d, C–5), 111.0 (d, C–2′), 110.4 (d, C–2), 85.7 (d, C–7′), 83.3 (d, C–7), 69.1 (t, C–9′), 61.5 (t, C–9), 55.8 (q, C–7′–OCH_3), 55.7 (q, C–3′–OCH_3), 55.6 (q, C–3–OCH_3), 53.8(d, C–8), 48.3 (d, C–8′).

7′–Methoxylariciresinol (***4***): Yellow oil; $C_{21}H_{26}O_7$; ^1H NMR (800 MHz, DMSO–d_6): δ_H 6.85 (1H, br s, H–2), 6.75 (1H, brs, H–2′), 6.72 (1H, overlapped, H–6), 6.71 (1H, overlapped, H–5′), 6.71 (1H, overlapped, H–5), 6.62 (1H, br d, 8.0, H–6′), 4.50 (1H, d, 7.4, H–7), 4.03 (1H, dd, 4.0, 8.8, H–9′a), 3.90 (1H, d, 9.3, H–7′), 3.79 (1H, br d, 8.2, H–9′b), 3.76 (3H, s, H–3′–OCH_3), 3.73 (3H, s, H–3–OCH_3), 3.05 (1H, overlapped, H–9a), 3.05 (3H, s, H–7′–OCH_3), 2.89 (1H, m, H–9b), 2.41 (1H, m, H–8′), 1.60 (1H, m, H–8). δ_C (200 MHz, DMSO–d_6): 147.7 (s, C–3′), 147.5 (s, C–3), 146.2 (s, C–4′), 145.7 (s, C–4), 133.6 (s, C–1), 130.9 (s, C–1′), 120.5 (d, C–6′), 118.7 (d, C–6), 115.2 (d, C–5′), 115.1 (d, C–5), 111.0 (d, C–2′), 110.4 (d, C–2), 85.4 (d, C–7′), 82.7 (d, C–7), 70.4 (t, C–9′), 60.4 (t, C–9), 55.8 (q, C–7′–OCH_3), 55.7 (q, C–3′–OCH_3), 55.6 (q, C–3–OCH_3), 52.2 (d, C–8), 48.0 (d, C–8′).

Erythro–2,3–bis–(4–hydroxy–3–methoxyphenyl)–3–methoxy–propanol (***5***): Yellow oil; $C_{18}H_{22}O_6$; ^1H NMR (600 MHz, CD_3OD): δ_H 6.45–6.74 (6H, m, ArH), 4.47 (1H, d, 5.5, H–7), 3.89 (2H, m, H–9), 3.69, 3.65 (each 3H, s, H–3, 3′–OCH_3), 3.15 (3H, s, H–7–OCH_3), 2.85 (1H, m, H–8). δ_C (150 MHz, CD_3OD): 148.5 (s, C–3), 148.3 (s, C–3′), 147.0 (s, C–4), 146.3 (s, C–4′), 133.3 (s, C–1), 132.4 (s, C–1′), 123.4 (d, C–6), 121.4 (d, C–6′), 115.7 (d, C–5′), 115.7 (d, C–5), 114.8 (d, C–2′), 111.9 (d, C–2), 85.1 (d, C–7), 64.5 (t, C–9), 57.2 (q, C–11–OCH_3), 56.7 (q, C–3–OCH_3), 56.4 (q, C–3′–

OCH_3), 56.2 (d, C-8).

Threo-2,3-bis-(4-hydroxy-3-methoxyphenyl)-3-methoxy-propanol (**6**): Yellow oil; $C_{18}H_{22}O_6$; ^{1}H NMR (600 MHz, CD_3OD): δ_H 6.46-6.74 (6H, m, ArH), 4.31 (1H, d, 8.4, H-7), 4.04 (1H, m, H-9a), 3.88 (1H, m, H-9b), 3.69 (3H, s, H-3-OCH_3), 3.65 (3H, s, H-3′-OCH_3), 3.25 (3H, s, H-7-OCH_3), 3.03 (1H, m, H-8). δ_C (150 MHz, CD_3OD): 148.7 (s, C-3), 148.5 (s, C-3′), 147.0 (s, C-4), 146.2 (s, C-4′), 132.8 (s, C-1), 132.8 (s, C-1′), 122.8 (d, C-6), 121.7 (d, C-6′), 115.8 (d, C-5), 115.6 (d, C-5′), 114.4 (d, C-2), 112.5 (d, C-2′), 87.5 (d, C-7), 65.1(t, C-9), 56.9(q, C-11-OCH_3), 56.7(q, C-3-OCH_3), 56.5(d, C-8), 56.3(q, C-3′-OCH_3).

4 Conclusion

The genus *Isodon* contains more than 150 species worldwide. The diverse structures and potent biological activities of diterpenoids have attracted considerable attention. Among them, *I. scoparius* was special because this plant was a rich resource of diverse bicyclic diterpenoids and their interesting derivatives, such as meroditerpenoids and diterpenoid dimers. Up to now, a number of secondary metabolites involving 18 novel skeletons with significant biological activities, *e.g.* immunosuppressive activities [9], have been reported continuously from *I. scoparius* in our group. Herein, the diverse lignans of *I. scoparius* have been reported for the first time. The result suggested that *I. scoparius* is a "talented species" in the genus *Isodon*, and the cultivated *I. scoparius* is a reliable source for future in-depth research.

References:

[1] LI X R, FU Q, ZHOU M, et al. Isoscoparins R and S, two new *ent*-clerodane diterpenoids from *Isodon scoparius* [J]. J Asian Nat Prod Res, 2019, 21(10): 977-984.

[2] JIANG X C, YAN B C, LI X R, et al. 4,5-*Seco*-18-*nor*-*ent*-clerodanoids and their derivatives: structure elucidation, synthesis and resistant reversal activities against fluconazole-resistance Candida albicans [J]. Tetrahedron, 2020, 76(14): 131043.

[3] ZHOU M, ZHANG H B, WANG W G, et al. Scopariusic Acid, a new meroditerpenoid with a unique cyclobutane ring isolated from *Isodon scoparius* [J]. Org Lett, 2013, 15 (17): 4446-4449.

[4] ZHOU M, LI X R, TANG J W, et al. Scopariusicides, novel unsymmetrical cyclobutanes: structural elucidation and concise synthesis by a combination of intermolecular [2 +2] cycloaddition and C -H functionalization [J]. Org Lett, 2015, 17(24): 6062-6065.

[5] YAN B C, ZHOU M, LI J, et al. (-)-Isoscopariusin A, a naturally occurring immunosuppressive meroditerpenoid: structure elucidation and scalable chemical synthesis[J]. Angew Chem Int Edit, 2021, 60(23): 12859-12867.

[6] LI X R, HU K, YAN B C, et al. Scopariusicides D-M, *ent*-clerodane-based isomeric meroditerpenoids with a cyclobutane-fused gamma/delta-lactone core from *Isodon scoparius* [J]. Bioorg Chem, 2022, 127: 105973.

[7] LI X R, YAN B C, HU K, et al. Spiro *ent*-clerodane dimers: discovery and green approaches for a scalable biomimetic synthesis [J]. Org Lett, 2021, 23 (15): 5647-5651.

[8] LI X R, CHEN L, HU K, et al. Discovery and biological evaluation of dispirocyclic and polycyclic *ent*-clerodane dimers from *Isodon scoparius* as novel inhibitors of Toll-like receptor signaling [J]. Org Chem Front, 2022, 9(15): 4023-4033.

[9] LIU Y, LI X, WU X, et al. Sis-25, a meroditerpenoid derivative with a cyclobutane scaffold, inhibits activated T cell proliferation by targeting GSK3β in vitro and in vivo [J]. Eur J Pharmacol, 2022, 929: 175151.

[10] CHEN S P, HU K, LI X R, et al. Scopariusicide C, a novel cyclobutene-containing meroditerpenoid from artificially cultivated *Isodon scoparius*[J]. Tetrahedron Lett, 2021, 73: 153133.

[11] TSUKAMOTO H, HISADA S, NISHIBE S. Lignans from bark of the *Olea* plants[J]. Chem Pharm Bull, 1984, 32 (7): 2730-2735.

[12] SUN Y J, LI Z L, CHEN H, et al. Four new cytotoxic tetrahydrofuranoid lignans from *Sinopodophyllum emodi*[J]. Planta Med, 2012, 78(5): 480-484.

[13] HSIAO J J, CHIANG H C. Lignans from the wood of *Aralia bipinnata*[J]. Phytochemistry, 1995, 39(4): 899-902.

[14] ADLER E, BRUNOW G, LUNDQUIST K. Investigation of the acid-catalyzed alkylation of lignans by means of NMR spectroscopic methods[J]. Holzforschung, 1987, 41 (4): 199-207.