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Total Syntheses of (−)-Spirooliganones A and B and Their Diastereoisomers: Absolute Stereochemistry and Inhibitory Activity against Coxsackie Virus B3

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Supporting Information

ABSTRACT: To investigate the effects of configuration on bioactivity, spirooliganones A and B and their six diastereoisomers (**1**−**8**) were synthesized in 11 steps. The key benzopyran core was assembled by intermolecular [4 + 2] hetero-Diels−Alder cycloaddition between (−)-sabinene and *o*-quinone methide, which was generated from the corresponding *o*-hydroxybenzyl alcohol. After establishing the absolute configuration, the inhibitory activities of spirooliganones **1**−**8** against Coxsackie virus B3 were evaluated, and the primary structure−activity relationships were analyzed. Compound **3** was the most potent compound, with an IC_{50} of 0.41 μM.

Coxsackie virus B (CVB) is a nonenveloped, single positive-stranded RNA virus, which belongs to the Picornaviridae family[1] and often causes chronic infections in adults, such as chronic pancreatitis, dilated cardiomyopathy, and central nervous system infection.[2a−c] In addition, serious neonatal diseases with high fatality rates from 11.1% to 40.0% (such as encephalitis, myocarditis and hepatitis) can be caused by CVB.[3a,b] No vaccine or specific drugs are available for CVB. Therefore, new antiviral drugs that exhibit good bioactivities against CVB are needed.

Spirooliganones A (**1**) and B (**2**) represent a pair of epimers that were isolated from the roots of *lllicium ligandrum*. Their unprecedented structure and potent bioactivities against Coxsackie virus B3 (IC_{50} 3.70−11.11 μM) excited our interest.[4] Structures of **1** and **2** contain a dioxaspiro skeleton and five chiral centers, which are entirely different from existing antiviral drugs. Thus, these compounds exhibit potential as new antiviral leads that possess novel mechanisms of action. The activity of spirooliganone B (**2**) against Coxsackie virus B3 was more potent than that of spirooliganone A (**1**) due to the configuration at C17. To investigate the effects of configuration on bioactivity, we planed to synthesize spirooliganones A (**1**) and B (**2**) and their stereoisomers (Figure 1).

While our research was still in progress, Xie and co- workers reported the first synthetic route of spirooliganones A (**1**) and B (**2**). The syntheses were accomplished in 8 steps without the use of any protecting group, and the C4-epimers of **1** and **2** were also obtained (the NMR spectra for the C4-epimer of **1** were not presented).[5] Soon after, Tong and co-workers developed another synthetic route of spirooliganones A (**1**) and B (**2**) involving 12 steps.[6] For the syntheses of spirooliganones A and B, both of these research groups adopted (−)-sabinene as starting material. The use of sabinene as starting material is an efficient method to install the stereocenters at C18 and C20. To obtain the stereoisomers of **1** and **2**, both (+)-sabinene and (−)-sabinene were required (Figure 1). However, according to an Internet search, (+)-sabinene is difficult to obtain. When limited to the starting material sabinene, only the monoterpene moiety of spirooliganones **1**−**8** stemming from (−)-sabinene may be synthesized.

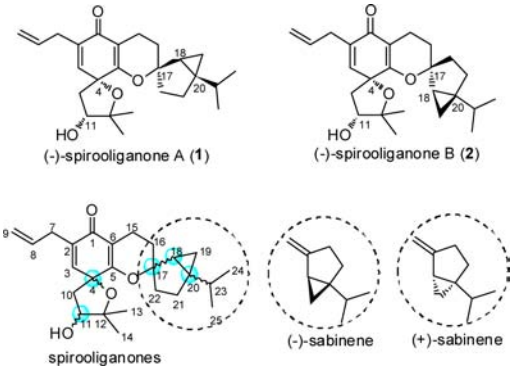

Figure 1. Structures of spirooliganones and sabinene.

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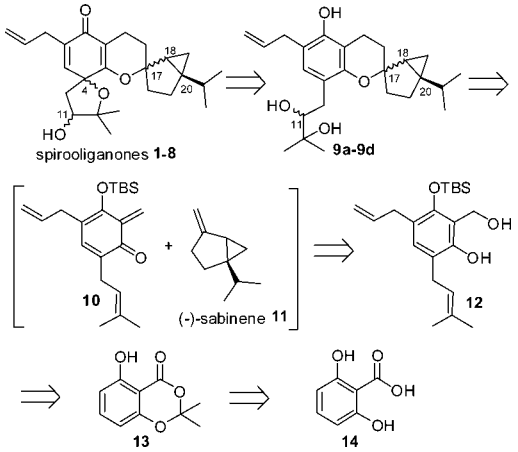
An economic plant extract that contained approximately 75% of (−)-sabinene acted as our starting material.

The retrosynthetic analysis of spirooliganones is shown in Scheme 1. Three key reactions are required: (i) an aromatic Claisen rearrangement;[7a,b] (ii) the [4 + 2] cycloaddition of *o*-quinone methide, which is generated from *o*-hydroxybenzyl alcohol;[8] and (iii) oxidative dearomatization/cyclization.[9a−f] In our strategy, the stereocenters were introduced at a late stage to minimize the problems that they cause during separation and to avoid tedious repetitive operations during the synthesis. First, the stereocenter at C4 of spirooliganones **1**−**8** could be introduced by adopting the protocol reported by Xie using diol phenols **9a**−**9d**.[5] Then, the stereocenter of **9a**−**9d** at C11 could be introduced by Sharpless dihydroxylation of the corresponding prenyl chains using different chiral catalysts.[10a−e] The last stereocenter at C17 of **9a**−**9d** could be obtained by the intermolecular [4 + 2] cycloaddition between *o*-quinone methide **10** and (−)-sabinene **11** due to poor diastereoselectivity. The *o*-quinone methide **10** could be generated from the thermolysis of the *o*-hydroxybenzyl alcohol **12**.[11a−g] The double o-alkylation/Claisen rearrangement of phenol **13** could be used to construct the allyl and prenyl chains in the *o*-hydroxybenzyl alcohol **12**.[7a,b] Phenol **13** could be obtained from the starting material 2,6-dihydroxybenzoic acid **14**.

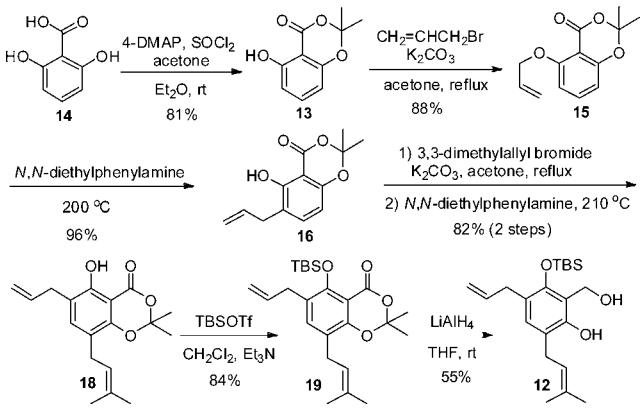
Our synthesis started from the commercially available dihydroxybenzoic acid **14** (Scheme 2), which was treated with thionyl chloride, acetone, and 4-DMAP to afford the acetonide **13**.[12] Sequentially, o-alkylation of **13** with allyl bromide led to the known compound **15** in 88% yield,[13] which was heated in *N*,*N*-diethylaniline and converted to the *o*-allylic phenol **16** with high regioselectivity in 96% yield. Then, an isopentene group was introduced to phenol **16** to afford diene **18** over two steps in 82% overall yield.[14a,b] Claisen rearrangement of the isopentene group at 200 °C generated a 1:1.2 mixture of the inseparable phenols **16** and **18**. This problem was solved by increasing the temperature of the reaction to 210 °C, and the small amount of byproduct **16** was removed during the following $LiAlH_4$ reduction step. Phenol **18** was transformed to the TBS-protected diene **19**,[15] which was treated with $LiAlH_4$ in anhydrous THF. The *o*-hydroxybenzyl alcohol **12** was obtained in 46% yield over two steps.[16]

Having assembled the key intermediate *o*-hydroxybenzyl alcohol **12**, we constructed the benzopyran via a hetero-Diels−Alder cycloaddition between *o*-quinone methide and (−)-sabinene **11** (Scheme 3). Heating *o*-hydroxybenzyl alcohol **12** in sabinene (as solvent) in a sealed tube for 2 h at 170 °C resulted in two main stereoisomers **20a** and **20b** in 82% overall yield; no dimer of *o*-hydroxybenzyl alcohol **12** was observed.[11d] The two stereoisomers were separated by column chromatography from a 1:1 mixture. Following the Sharpless dihydroxylation of **20a** with an AD-mix-β at room temperature for 48 h, diols **21a** (42% yield) and **21b** (36% yield) were obtained with poor stereoselectivity.[10a,b] These compounds were separated using an HPLC instrument equipped with a chiral column. The chiral catalyst AD-mix-β did not act effectively in the reaction. When the reaction proceeded at 0 °C for 4 d, **21a** and **21b** were obtained at a ratio of 2:1 with a combined yield of 42%. At 0 °C, when the chiral catalyst was changed to AD-mix-α, **21a** and **21b** were still obtained with poor stereoselectivity (ratio 1:1.5). We speculated that the poor stereoselectivity of the reaction was due to the monoterpene moiety of the substrate. Because the separable diols **21a** and **21b** were our targets and gave similar and considerable yields, the described reaction conditions (Scheme 3) were adopted without further optimization, and the separable diols **21c** (42% yield) and **21d** (36% yield) were afforded from **20b** under the same

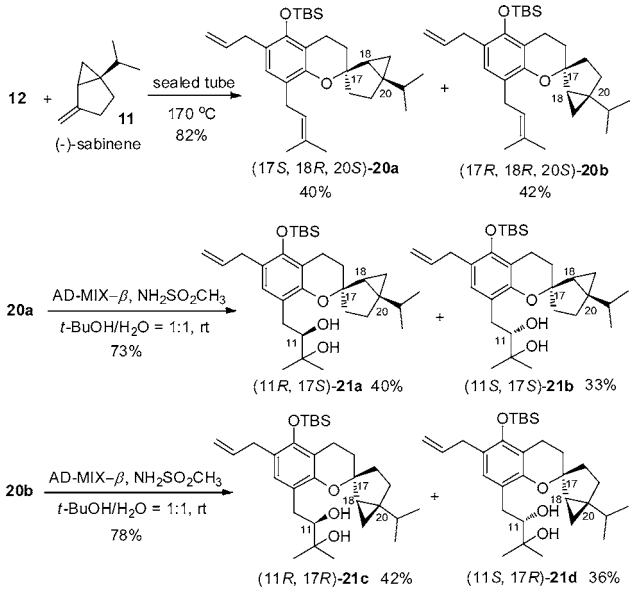
Scheme 1. Retrosynthetic Analysis of Spirooliganones



Scheme 2. Synthetic Route of *o*-Hydroxybenzyl Alcohol 12



Scheme 3. Synthesis of Diols 21a, 21b, 21c, and 21d



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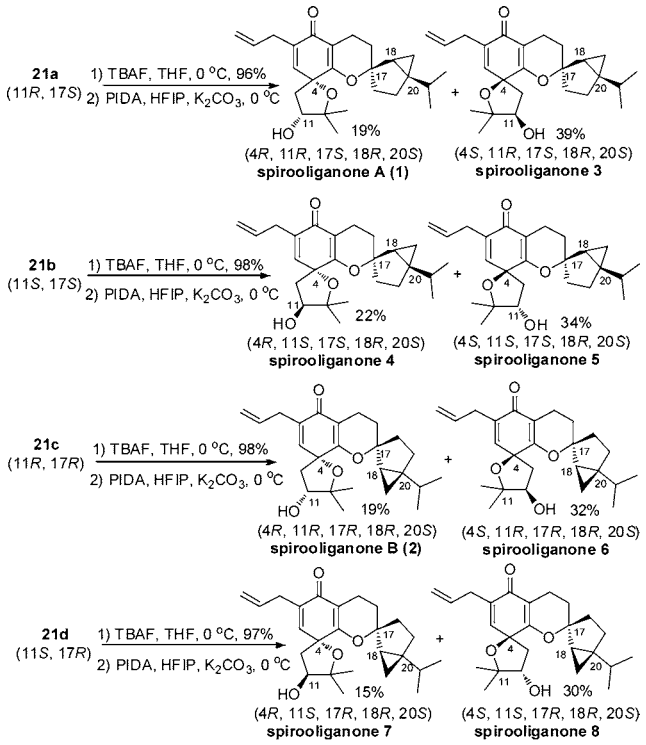
conditions as those used to prepare **21a** and **21b**. In the reaction, the isopentene in **20a** and **20b** is dihydroxylated before the allyl due to its high electron density. However, small amounts of the allyl dihydroxylated product were observed. The stereochemistry of the intermediates was verified based on the absolute configuration of the final products.

The late stage of the synthesis used a tandem oxidative dearomatization/cyclization procedure to establish the final oxa-spiro ring (Scheme 4). After removal of the TBS group by

Scheme 4. Synthesis of Spirooliganones A and B and Their Diastereoisomers



treating **21a** with TBAF in THF, phenol **9a** was afforded. According to the strategy reported by Xie,[5] **9a** was treated with PIDA in HFIP in the presence of K_2CO_3, affording a 19% yield of spirooliganone A (**1**) and a 39% yield of **3** after purification by HPLC. Using the same procedure, **9c** was used to prepare spirooliganones B (**2**) (19% yield) and **6** (32% yield). Spectral data for **1** and **2** were identical in all respects to the natural spirooliganones A and B [see Tables S9 and S10 in Supporting Information (SI)], the absolute configurations of which were established by X-ray diffraction analysis of the *p*-bromobenzoyl derivatives. Because **1** and **3** shared the common precursor **21a**, according to the reaction mechanism, **1** and **3** theoretically comprise a pair of C4-epimers. Based on the structures of the spirooliganones, the configuration at C4 contributed to a Cotton effect. As expected, the CD spectra of spirooliganones A (**1**) and **3** were the inverse of each other (see Figures S41 and S42 in SI). Thus, the configuration of **3** at C4 was *S*. The configuration of **3** at C11 was confirmed as *R* using the Mosher method (^{1}H NMR analysis of the MPA esters of **3**; see Table S3 in SI). The configurations of **21a** at C17, C18, and C20 did not undergo any inversion of configuration during the following reactions. Therefore, the configurations of **3** at C17, C18, and C20 were the same as those in **1**. Herein, the absolute configuration of **3** was established as 4*S*, 11*R*, 17*S*, 18*R*, and 20*S*. Based on a comparison of the CD spectra of **2** and **6** (see Figures S41 and S43 in SI) and ^{1}H NMR analysis of the corresponding MPA esters (see Table S6 in SI), the absolute configuration of **6** was established as 4*S*, 11*R*, 17*R*, 18*R*, and 20*S* in the same way. **21b** led to **4** (22% yield) and **5** (34% yield), a pair of C4-epimers; **21d** led to another pair of C4-epimers, **7** (15% yield) and **8** (30% yield). The absolute configuration of **7** was established as 4*R*, 11*S*, 17*R*, 18*R*, and 20*S* based on an X-ray diffraction analysis of its single crystal (Figure 2). The absolute configuration of **8** was confirmed as

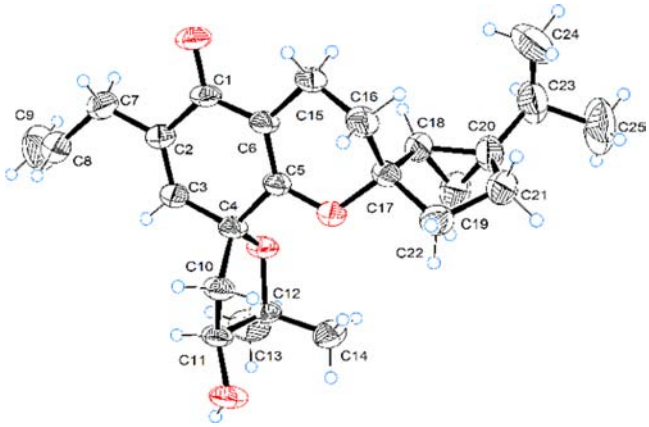


Figure 2. X-ray of the crystal structure of spirooliganone **7**.

4*S*, 11*S*, 17*R*, 18*R*, and 20*S* correspondingly (CD spectra for **7** and **8** are shown in Figure S44 in SI; regarding the ^{1}H NMR analysis of MPA esters of **8**, see Table S8 in SI).

We failed to obtain crystals of **4**, **5**, and their *p*-bromobenzoyl derivatives. The absolute configurations of these compounds were established as follows: The CD spectrum of **4** showed the same Cotton effect as that seen for **1**, **2**, and **7** and showed the inverse Cotton effect as that seen for **5** (see Figures S41−S44 in SI). Thus, the configuration of **4** at C4 was *R*, whereas the configuration of **5** at C4 was *S*. Second, their configuration at C11 was *S*, as determined from the ^{1}H NMR analysis of the corresponding MPA esters (see Tables S4 and S5 in SI). Finally, **4** and **5** shared the same precursor **20a** with **1** and **3**. The monoterpene moiety of **20a** did not undergo an inversion of its configuration during the following reactions. Therefore, as with **1**, **3**, and **20a**, the configurations of **4** and **5** at C17, C18, and C20 were *S*, *R*, and *S*, respectively. The absolute configuration of **4** was confirmed as 4*R*, 11*S*, 17*S*, 18*R*, and 20*S*, and that of **5** was confirmed as 4*S*, 11*S*, 17*S*, 18*R*, and 20*S*. Herein, the absolute configurations of all spirooliganones **1**−**8** were established. The ^{1}H NMR analysis of the MPA esters (see Tables S1−S8 in SI) and NOESY spectra (see Figures S93−S101 in SI) provided further proof of the absolute configurations.

The activities of spirooliganones **1**−**8** against Coxsackie virus B3 were evaluated (Table 1). Compounds **1** and **2** had similar selectivity indexes (TC_{50}/IC_{50}) to the natural spirooliganones A and B,[4] increasing the credibility of the pharmacological results. As expected, the configuration of the spirooliganones affected their bioactivities, especially the configuration at C4. When the configuration at C4 was *R*, the compounds exhibited potent inhibitory activities against Coxsackie virus B3 with IC_{50} values ranging from 1.88 to 4.29 μM. When the configuration at C4 was *S*, only **3** and **8** exhibited inhibitory activities. The bioactivities of **3** and **8** exhibited were more potent than the

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Table 1. Inhibitory Activities of Compounds 1−8 against CVB3 in Vero Cells[a,b]

compd	TC_{50} (μM)[c]	IC_{50} (μM)	SI[d]
1	7.93 ± 1.40	1.88 ± 0.37	4.22
2	16.39 ± 1.98	3.70 ± 0.00	4.43
3	3.79 ± 1.16	0.41 ± 0.00	9.24
4	7.76 ± 0.67	2.92 ± 1.10	2.63
5	7.76 ± 0.67	>3.70[e]	—[f]
6	19.12 ± 3.02	>3.70[e]	—[f]
7	23.28 ± 2.02	4.29 ± 0.83	5.43
8	16.11 ± 1.19	1.23 ± 0.00	13.10
Ribavirin[g]	2000.00 ± 0.00	275.50 ± 16.97	7.26

[a]See the Supporting Information for detailed activity assays. [b]Data represent the mean values of three independent tests. [c]Cytotoxic concentration required to inhibit Vero cell growth by 50%. [d]Selectivity index value equaled TC_{50}/IC_{50}. [e]Maximum nontoxic concentration. [f]Under the test conditions, the selectivity index (SI) could not be calculated. [g]Positive control.

other compounds with the IC_{50} values of 0.41 and 1.23 μM, respectively. The selectivity indexes of these compounds are greater than that of the positive control ribavirin. Although the development of a new antiviral drug remains distant, the pharmacological results obtained here warrant further studies, including the synthesis of analogues with more potent bioactivity and the research of structure−activity relationships (SAR) for this class of compounds.

In conclusion, the total syntheses of spirooliganones A and B and their six diastereoisomers were achieved in 11 steps from 2,6-dihydroxybenzoic acid. The benzopyran core was assembled using the intermolecular [4 + 2] hetero-Diels−Alder cycloaddition of an *o*-quinone methide, which was generated from *o*-hydroxybenzyl alcohol and (−)-sabinene. The activities of the eight spirooliganones against Coxsackie virus B3 were evaluated, and compound **3** was the most potent of the compounds tested, including the positive control ribavirin. Further studies on the SAR of structurally related spirooliganones are deserved.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures, the synthesis of MPA ester derivatives, X-ray crystal data for compound **7**, antiviral assays, and UV, CD and 1D and 2D NMR spectra for novel compounds are presented. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01419.

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Notes

The authors declare no competing financial interest.

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