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Scopariusicides, Novel Unsymmetrical Cyclobutanes: Structural Elucidation and Concise Synthesis by a Combination of Intermolecular [2 + 2] Cycloaddition and C−H Functionalization

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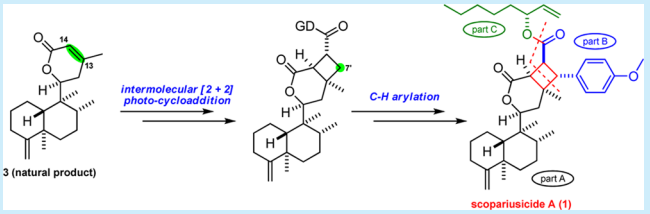
Supporting Information

ABSTRACT: Scopariusicides A (**1**) and B (**2**), two novel immunosuppressive unsymmetrical cyclobutane derivatives, were isolated from the aerial parts of *Isodon scoparius*. Moreover, based on the results of phytochemical investigation, a concise stereocontrolled synthesis of scopariusicide A and its analogues with enhanced biological activities was efficiently achieved using the main diterpenoid (**3**) isolated from this plant as a readily available starting material. A crossed intermolecular [2 + 2] photocycloaddition and a Pd-catalyzed sp^3 C−H bond β-arylation were used synergistically to access the highly congested unsymmetrical cyclobutane core with four contiguous stereocenters.



The [2 + 2] photocycloaddtion is a very useful reaction in organic synthesis because it constructs two new C−C bonds and establishes up to four new stereogenic centers in a single step.[1] It has been used effectively to perform homodimerizations and in intramolecular contexts.[2] However, the intermolecular heterodimerizations between similar yet distinct acyclic monomers are typically very inefficient.[3] Many researchers have suggested that photoexcited monomers with exocyclic double bonds can undergo rapid relaxation to the ground state through *E*/*Z* isomerization, an energy-wasting process that hinders the formation of the corresponding cyclobutanes.[3] Moreover, the formation of the desired crossed product competes with other reactions, particularly homodimerizations and modes of cyclization ('head-to-head' versus 'head-to-tail'). The desired cycloaddition may also be disfavored by the steric and electronic properties of the monomeric species.[3] Therefore, stereocontrolled synthesis of a complex asymmetrical cyclobutane ring using a photochemical approach is a hot topic but still a long-standing unsolved problem in the chemical research community.[3] Interestingly, Baran's[4] and Daugulis'[5] groups recently provided an exciting new venue for the construction of unsymmetrical cyclobutanes using an efficient C−H functionalization logic.

In our further phytochemical investigation,[6] scopariusicides A and B (**1** and **2**, Figure 1), a new class of such fully unsymmetrical cyclobutanes with immunosuppressive activity, have been isolated from the aerial parts of *Isodon scoparius* (colloquial name "Zhu-Zong-Cao"), which is used extensively as an antipyretic, analgesic, and anti-inflammatory agent by the Naxi people of Shangri-La in Yunnan Province, P. R. China. Their structures were elucidated by interpretation of the NMR, X-ray crystallographic, and CD data (see Table 1, Figure 2, and Supporting Information). Biosynthetically, scopariusicides might be formed via a crossed head-to-head intermolecular [2 + 2] cycloaddition between an *ent*-clerodane diterpenoid (part A) and an unusual ester of trans-4-hydroxycinnamic acid (part B) and (3*R*)-1-octen-3-ol (part C) (Scheme 1). Moreover, **1** was screened for immunosuppressive activity arising from the inhibition of human T cell proliferation *in vitro* using BD750 as a positive control agent (IC_{50} = 1.1 μM),[7] which exhibited weak

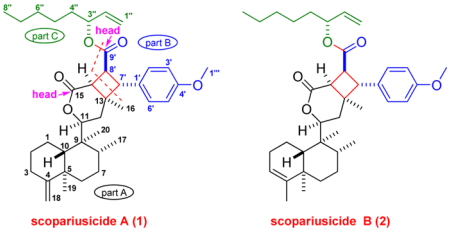


Figure 1. Structures of compounds **1** and **2**.

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Table 1. ^{13}C (125 MHz) NMR Data for 1 and 2 in C_5D_5N (δ ppm)

no.	1	2	no.	1	2
1	24.3 t	20.5 t	1′	131.5 s	131.4 s
2	28.9 t	27.0 t	2′	129.5 d	129.4 d
3	33.8 t	121.7 d	3′	114.8 d	114.7 d
4	160.4 s	143.8 s	4′	159.5 s	159.5 s
5	41.0 s	38.9 s	5′	114.8 d	114.7 d
6	37.7 t	36.8 t	6′	129.5 d	129.4 d
7	28.5 t	28.2 t	7′	51.9 d	52.0 d
8	37.4 d	36.9 d	8′	41.8 d	41.7 d
9	43.5 s	42.7 s	9′	172.5 s	172.5 s
10	49.3 d	46.9 d	1″	116.8 t	116.6 t
11	85.3 d	85.3 d	2″	138.1 d	138.0 d
12	40.5 t	40.4 t	3″	76.1 d	76.0 d
13	40.0 s	39.9 s	4″	34.7 t	34.6 t
14	44.3 d	44.2 d	5″	25.4 t	25.3 t
15	172.3 s	172.3 s	6″	32.4 t	32.2 t
16	22.8 q	22.6 q	7″	23.3 t	23.2 t
17	17.3 q	16.9 q	8″	14.7 q	14.5 q
18	103.9 t	18.7 q	1‴	55.7 q	55.5 q
19	21.2 q	20.1 q			
20	14.5 q	14.5 q			

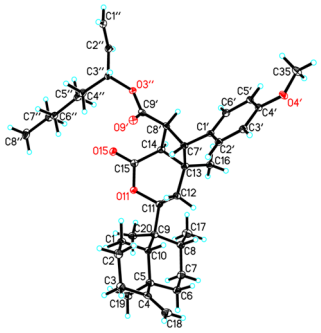
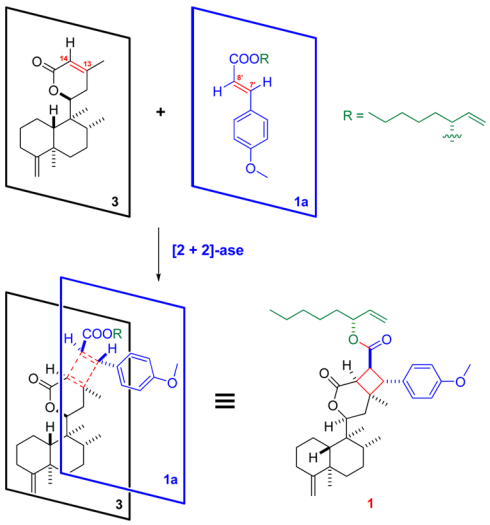


Figure 2. X-ray crystallographic structures of compound **1**.

Scheme 1. Hypothetical Biogenetic Pathway of Compound 1



immuosuppressive activity at nontoxic concentrations, with IC_{50} = 20.7 μM.

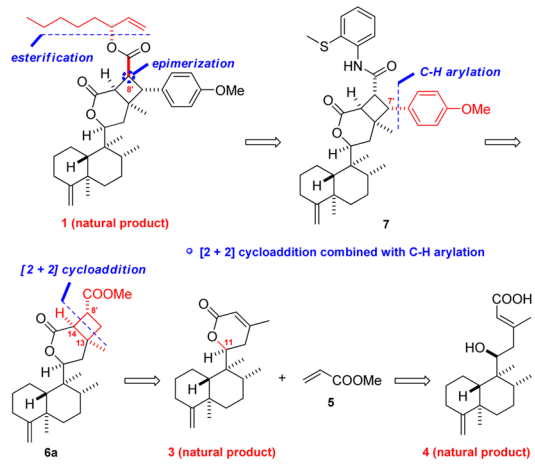
Inspired by the complex asymmetrical cyclobutane core and potential biological properties of scopariusicides, we proceeded to develop an efficient strategy to construct the unsymmetrical cyclobutane ring and explore the molecular space around the scopariusicides in the search for enhanced biological compounds.

Guided by the biosynthetic hypothesis, we initially hypothesized that the cyclobutanes core of scopariusicides could be constructed by a direct intermolecular [2 + 2] photocycloaddition. Unfortunately, all of our attempts to prepare the cyclobutane core by crossed intermolecular [2 + 2] coupling between the natural or modified precursors under photochemical irradiation failed despite the use of diverse substrates, solvents, and additives (including CuOTf, $BF_3{\cdot}Et_2O$, and 2′-acetonaphthone) (Scheme S2). These failures were tentatively attributed to the steric constraint imposed by the large aromatic substituent and *Z*/*E* isomerization of the acyclic double bonds in the precursor compounds.

We therefore investigated alternative ways of achieving the pivotal crossed intermolecular [2 + 2] coupling. It was suggested that the reaction might proceed more smoothly if the monomeric precursors were simplified by removing the bulky aryl substituents. If the modified photoheterodimerization could be successfully accomplished, the crucial question would become how to install the aromatic group at the appropriate position of the newly formed 1,2,3-trisubstituted cyclobutane ring. Fortunately, we anticipated that this could be achieved by means of a directed regioselective Pd-catalyzed C−H bond β-arylation using chemistry of the sort reported by Daugulis and co-workers[5] and used in natural product synthesis by authors such as Baran,[4] Maimone,[8a] and Chen.[8b] The revised synthetic plan was thus to prepare the partially substituted cyclobutane core of **1** using a crossed intermolecular [2 + 2] photocycloaddition and then append the *p*-methoxybenzene ring by C−H arylation, with the configuration of the new arylated stereocenter being governed by that of the preexisting stereocenter at C-8′. Such a novel combined strategy should provide access to these complex unsymmetrical cyclobutane derivatives. After achieving promising preliminary results using model substrates (Scheme S3), we applied the new strategy to the synthesis of the scopariusicides.

Our retrosynthetic analysis of **1** is outlined in Scheme 2. With the above-mentioned strategy in mind, we envisioned that compound **1** would be obtained from compound **7** by means of a selective epimerization at C-8′ followed by an esterification at C-9′. The pre-existing carboxylate functionality in compound **6a** was to be used as a latent directing group for C−H arylation, so the *p*-methoxybenzene group at C-7′ on the cyclobutane ring of **7**

Scheme 2. Retrosynthetic Analysis of Compound 1



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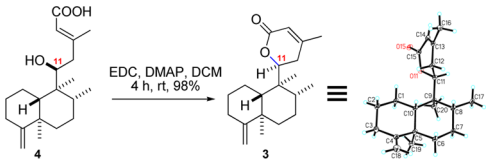
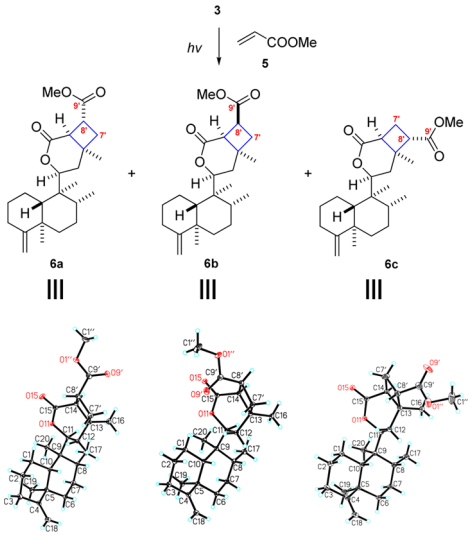
was to be preceded by a simple C−H bond. Furthermore, guided by our postulated biosynthetic pathway, we hoped that it would be possible to prepare the cyclobutane core with three desired stereogenic centers (the initial configuration of the C-8′ center should be the opposite of that in **1**) via a crossed intermolecular [2 + 2] photocycloaddition between a possible precursor **3** and a simplified precursor **5**. Finally, it was expected that intermediate **3** would be easily prepared from compound **4** (Scheme 3), an *ent*-clerodane derivative that is very abundant in *I. scoparius* (a yield of 5.0 g/kg of dry plant material).

Scheme 3. Preparation of Compound 3



After extensive optimization of the reaction conditions (Scheme S4), we were pleased to find that irradiating a mixture of **3** and **5** with UV light led to two major head-to-head cyclobutane products, **6a** and **6b**, plus a minor head-to-tail cyclobutane **6c** in a ratio of 5:2:0.2 and a combined yield of 98% (Scheme 4). The ROESY correlations of H-11/H_3-16 and H-11/

Scheme 4. Photochemical Irradiation of Compounds 3 and 5[a]

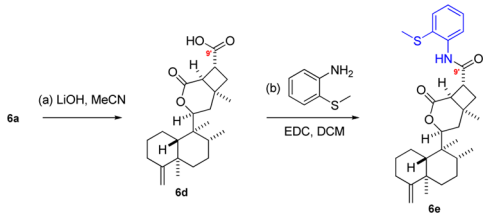


[a]Reagents and conditions: photoreactor with 16 × 60 W mercury lamps, *hv* = 313 nm, 10 or 100 mL quartz tubes, MeCN/$(CH_3)_2CO$ = 5:1, **3** (1.0 equiv), **5** (15.0 equiv), rt, 5 h, 98%, **6a**:**6b**:**6c** = 5:2:0.2.

H-14 in **6a**−**6c** demonstrated that H-11, H_3-16, and H-14 were all α-oriented, as is the case in the naturally occurring products **1** and **2**. However, the relative configurations at the C-8′ position of **6a** and **6b** or **6c** were very difficult to determine by spectroscopy alone due to the flexibility of the cyclobutane ring. Fortunately, the structures of **6a**−**6c** were unambiguously established by single-crystal X-ray diffraction experiments, which confirmed that the major product **6a** was the desired cyclobutane. The highly diastereoselective formation of **6a** under these conditions is presumably due to the steric and electronic properties of **3**.

With the key intermediate **6a** in hand, attention was turned to the regioselective β-arylation of the unactivated sp^3 C−H bond at C-7′. The C-9′ methyl ester in **6a** was selectively hydrolyzed using LiOH to give carboxylic acid **6d**, which was converted into the desired C−H arylation precursor **6e** by EDC coupling with 2-aminothioanisole, a general auxiliary for Pd-catalyzed C−H activation (Scheme 5). When **6e** was heated in a sealed tube

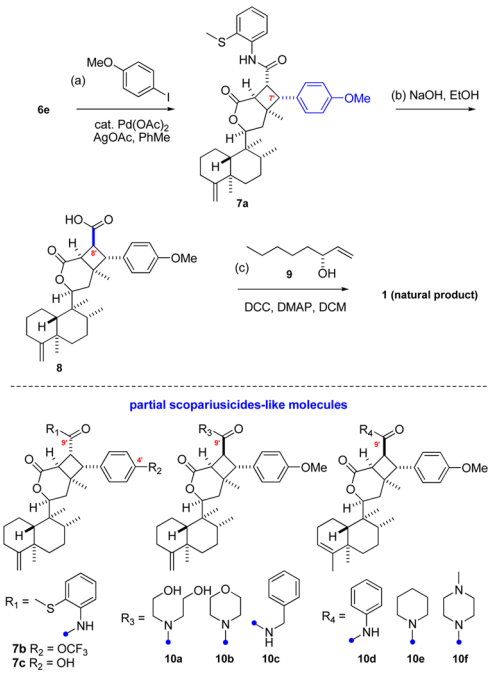
Scheme 5. Synthesis of Compound 6e[a]



[a]Reagents and conditions: (a) **6a**, 1 M LiOH/MeCN = 1:1, rt, 24 h, 95%; (b) **6d** (1 equiv), 2-aminothioanisole (1.5 equiv), EDC (2.0 equiv), DCM, rt, 2 h, 95%.

containing a catalytic amount of $Pd(OAc)_2$, excess *p*-methoxy-iodobenzene, Ag_2CO_3, and *t*-BuOH as the solvent, the monoarylated product **7a** was obtained as the major product (58% yield) (Scheme 6). Having demonstrated the feasibility of the desired carbon−carbon coupling, we optimized the conditions for the C−H arylation reaction (Scheme S5). Eventually we found that the desired product **7a** could be achieved in a very reproducible yield of 82% by heating **6e** with excess *p*-methoxyiodobenzene under the $Pd(OAc)_2$−AgOAc−PhMe conditions at 115 °C for 5 h. With the key intermediate **7a**

Scheme 6. Synthesis of Compound 1[a] **and Its Analogs**



[a]Reagents and conditions: (a) palladium(II)acetate (0.1 equiv), *p*-methoxy-iodobenzene (3.0 equiv), AgOAc (2.2 equiv), PhMe, 115 °C, 5 h, 82% **7a**; (b) NaOH (5 equiv), EtOH, 100 °C, 2 h, 85%; (c) **8** (1 equiv), **9** (1 equiv), DCC (2.0 equiv), DMAP (0.2 equiv), DCM, 0 °C to rt, 2 h, 85%.

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secured, efforts were directed toward selective epimerization of the C-8′ stereocenter. Treatment with NaOMe in diverse solvents resulted in the opening of the lactone ring linking C-11 and C-15. After extensive exploration of reaction conditions, we found that **8** could be obtained in 85% yield by heating **7a** with NaOH (5 equiv) in EtOH at 100 °C for 2 h (Scheme 6). To our delight, the lactone ring of **7a** did not open under these conditions but the directing group was cleanly removed. Gratifyingly, the key ROESY cross-peaks of H-8′ with H-2′ and H-6′ suggested that both H-8′ and the *p*-methoxybenzene moiety had the same α-orientation, which is consistent with the target compound **1**. Finally, the coupling of **8** and 1-octen-3*R*-ol (**9**) with DCC and DMAP furnished synthetic **1** (85% yield, 36% total yield). The NMR and ESIMS data for this material were identical in all respects to those for the natural substance.

The synthetic route outlined above can readily modify the aromatic substructures in a manner which is independent of their electronic bias, enabling the preparation of a small library of arene-modified scopariusicide analogs (34 compounds, **7a**−**7k** and **10a**−**10w**; see Schemes S5 and S6) for biological testing. Their immunosuppressive activity was evaluated by testing their ability to inhibit human activated T-cell proliferation *in vitro*, using BD750 (IC_{50} = 1.1 μM) as a positive control (Table S3).[7] The cytotoxicity against human resting PBMCs was measured to observe whether their immunosuppressive activity was caused by cytotoxicity. Intriguingly, the results showed that partial synthetic compounds inhibited human T cell proliferation stimulated by anti-CD3/anti-CD28 mAbs with IC_{50} values in the range 3.9−9.5 μM without obvious cytotoxicity (**7b**−**7c** and **10a**−**10e**; see Table 2), implicating that these compounds exhibited significant immunosuppressive activity.

Table 2. Effect of Natural and Synthetic Molecules on Human T Cell Proliferation and Cytotoxicity on PBMC Viability

no.	IC_{50} (μM)[a]	IC_{50} (μM)[b]	no.	IC_{50} (μM)[a]	IC_{50} (μM)[b]
1	20.7	>400	**10a**	5.6	183.2
3	49.3	>400	**10b**	5.0	63.1
6e	28.6	157.4	**10c**	9.5	81.9
7a	57.9	166.3	**10d**	3.9	45.8
7b	5.5	163.4	**10e**	7.1	52.6
7c	6.2	56.7	**10f**	11.7	57.9

[a]IC_{50} of inhibition on T cell proliferation. [b]IC_{50} of cytotoxicity on PBMC viability.

In summary, scopariusicides A and B, a new class of immunosuppressive cyclobutane derivatives with a densely substituted unsymmetrical four-membered core, have been isolated from *Isodon scoparius*. Hitherto, powerful synthesis of such unsymmetrical cyclobutanes is still a long-standing unsolved problem in synthetic chemistry. Inspired by the total synthesis of piperarborenins by Baran and Gutekunst, we developed a concise, stereoselective synthesis of scopariusicide A and its analogues with enhanced activities. A crossed intermolecular [2 + 2] cycloaddition and a guided Pd-catalyzed C−H activation were used synergistically to access the complex cyclobutane ring in a stereocontrolled way. This synthesis achieves excellent chemo- and stereoselectivity, demonstrating the advantages of this combined approach to the synthesis of unsymmetrical cyclobutanes. Inevitably, the use of this comprehensive strategy will hopefully be broadly applicable to the synthesis of similar unsymmetrically substituted cyclobutanes in other molecular settings. This study also provided a new structural template for potential immunosuppressive agent discovery.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03079.

Experimental procedures, spectral and other characterization data of isolated or synthetic compounds (PDF)
X-ray data for **1** (CIF)
X-ray data for **3** (CIF)
X-ray data for **6a** (CIF)
X-ray data for **6b** (CIF)
X-ray data for **6c** (CIF)

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Notes

The authors declare no competing financial interest.

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