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Scopariusic Acid, a New Meroditerpenoid with a Unique Cyclobutane Ring Isolated from *Isodon scoparius*

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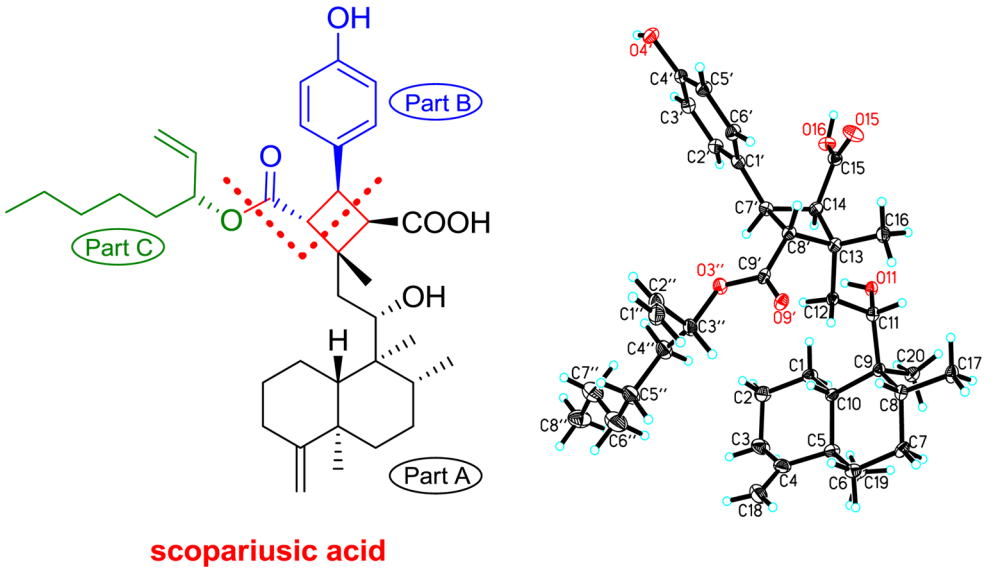
ABSTRACT



scopariusic acid

Scopariusic acid (1), a new *ent*-clerodane-based meroditerpenoid with a unique cyclobutane ring and an unusual 1-octen-3-ol substituent, together with its biosynthetic related compound 2, were isolated from the aerial parts of *Isodon scoparius*. The structures of 1 and 2, including their absolute configurations, were determined by spectroscopic methods, single-crystal X-ray diffraction analysis, and chemical methods. Compound 1 showed weak cytotoxicity and moderate immunosuppressive activity.

In 1908, the first photochemical [2 + 2] cycloaddition was reported by Ciamician and involved the intramolecular cyclobutanation of carvone on exposure to intense sunlight for one year.[1] After confirmation of this finding in the late 1950s, the [2 + 2] photocycloaddition reactions involving α,β-unsaturated cyclic enones, cycloalkenyl esters, and lactones have been established to be one of the most useful and stereoselective methods in the synthesis of cyclobutane derivatives.[2]

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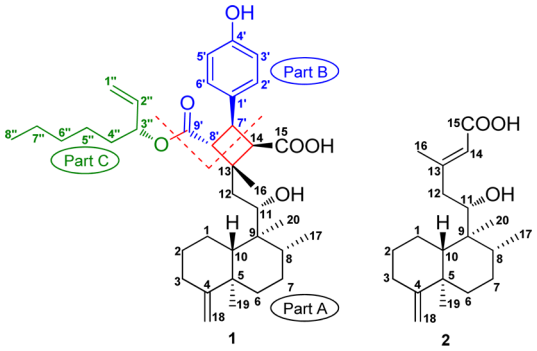
Meanwhile, cyclobutane-containing natural products were also found in plants or microorganisms as a small yet diverse family with a variety of biological activities.[3] Interestingly, structure−activity relationship studies performed on several monoterpene alkaloids suggested that the cyclobutyl motifs play a crucial role in the expression of their antinociceptive activity in a formalin-induced pain model in mice.[4] Moreover, some of them have been synthesized by using a photochemical [2 + 2] approach as the key step, such as incarvillateine,[3b,5] an antinociceptive monoterpene alkaloid characterized by a tetrasubstituted cyclobutyl moiety, biyouyanagin A,[3c,6] a unique hydrophobic compound exhibiting significant activity against HIV and inhibiting cytokine production, and littoralisone,[3d,7] a neuritogenic iridolactone having a novel heptacyclic skeleton. Therefore, such naturally occurring cyclobutanes often provoke interesting questions and draw widespread attention of the scientific community.[3a,8]

In our further study of the chemical constituents of *Isodon scoparius*,[9] scopariusic acid (**1**), a new *ent*-clerodane-based meroditerpenoid with a unique cyclobutane ring, together with its biosynthetic related compound (**2**), have been isolated from the aerial parts of this plant. Interestingly, compound **1** might be biosynthetically formed via an intermolecular [2 + 2] cycloaddition between the acyclic side chains of two absolutely different units, one moiety possessing an *ent*-clerodane diterpenoid nucleus (part A) and the other derived from 1′-octen-3′-yl-4-hydroxycinnamate, an unusual ester of *trans*-4-hydroxycinnamic acid (part B) and (3*R*)-1-octen-3-ol (part C). To the best of our knowledge, compound **1** represents a new class of meroditerpenoid with a unique cyclobutane ring and a novel 1-octen-3-ol substituent. Herein, we report the isolation and structure determination of compounds **1** and **2** as well as their cytotoxic and immunosuppressive activities.



Isoscoparin P (**2**) was obtained as colorless crystals. Its molecular formula $C_{20}H_{32}O_3$ was determined on the basis of the HREIMS at m/z 343.2244 $[M + Na]^+$ (calcd 343.2249), corresponding to 5 degrees of unsaturation. The ^{1}H NMR spectrum of compound **2** exhibited three olefinic protons (δ_H 4.66, 4.66, and 6.32), an oxymethine (δ_H 3.97), two tertiary methyl groups (δ_H 1.11 and 1.13), one secondary methyl group (δ_H 0.85), and one olefinic methyl group (δ_H 2.50) (Table S1, Supporting Information). The HSQC spectrum resolved the 20 carbon signals as four methyls, seven methylenes (including one sp^2 methylene), four methine (of which one was oxygenated), and five quaternary carbons (including two sp^2 carbons and one carbonyl) (Table 1), which was consistent with a skeleton of a clerodane diterpenoid.[9] One oxymethine proton at δ_H 3.97 (1H, br d, J = 10.0 Hz) observed in the ^{1}H NMR spectrum, was located at C-11, which was proven by the HMBC correlations from H-11 to C-8, C-10, and C-13 (Figure 1). These features indicated the gross structure of **2** as 11-hydroxy-clerodane-4(18),13-dien-15-oic acid. However, the relative configuration at C-11 in **2** could not be determined from spectroscopic data alone. Finally, its absolute configuration was established by single-crystal X-ray diffraction analysis (Figure 3). Thus, compound **2** was identified as (11*S*,13*E*)-11-hydroxy-*ent*-clerodane-4(18),13-dien-15-oic acid and named isoscoparin P.

Scopariusic acid (**1**) was isolated as colorless needles. Its molecular formula $C_{37}H_{54}O_6$ was determined on the basis of the HREIMS at m/z 594.3907 $[M]^+$ (calcd 594.3920), corresponding to 11 degrees of unsaturation. The ^{1}H NMR spectrum of compound **1** exhibited nine olefinic protons (δ_H 4.46, 4.64, 5.40, 5.50, 6.01, 7.08, 7.08, 7.39, and 7.39), two oxymethines (δ_H 4.22 and 5.59), three tertiary methyl groups (δ_H 1.12, 1.17, and 1.80), and two secondary methyl groups (δ_H 0.80 and 0.88) (Table S1, Supporting Information). The ^{13}C NMR and DEPT spectra resolved the 37 carbon signals (Table 1) as 5 methyls, 12 methylenes (including two sp^2 methylenes), 12 methines (of which five were sp^2 methines and two were oxygenated), and 8 quaternary carbons (including three sp^2 carbons and two carbonyls). Among them, two carbonyl carbons and 10 olefinic carbons occupied seven degrees of unsaturation. Extensive analyses of 1D and 2D NMR data prompted us to consider that **1** was a meroterpenoid, which might be assembled by three subunits: one moiety possessing a diterpenoid nucleus (part A), one phenol or phenylpropanoid

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Table 1. ^{13}C NMR Spectroscopic Data (δ in ppm)[a] of Compounds **1**, **2**, and **4** in Pyridine-d_5

no.	1	2	4	no.	1	4
1	24.7 t	24.4 t	24.7 t	1′	132.1 s	132.7 s
2	29.2 t	29.0 t	29.4 t	2′	129.0 d	129.2 d
3	34.1 t	34.0 t	34.1 t	3′	116.2 d	116.2 d
4	161.1 s	160.9 s	161.2 s	4′	157.5 s	157.4 s
5	41.2 s	41.1 s	41.1 s	5′	116.2 d	116.2 d
6	38.0 t	37.9 t	38.0 t	6′	129.0 d	129.2 d
7	29.2 t	29.1 t	29.1 t	7′	38.0 d	38.4 d
8	37.5 d	37.8 d	37.5 d	8′	50.4 d	50.5 d
9	45.5 s	45.0 s	45.5 s	9′	172.6 s	176.0 s
10	49.1 d	49.3 d	49.4 d	1″	118.3 t	
11	74.6 d	75.7 d	74.5 d	2″	137.9 d	
12	38.5 t	44.6 t	38.7 t	3″	75.5 d	
13	42.2 s	158.8 s	41.7 s	4″	34.9 t	
14	53.5 d	119.8 d	54.1 d	5″	25.5 t	
15	175.9 s	169.6 s	175.5 s	6″	32.1 t	
16	23.7 q	19.7 q	23.6 q	7″	23.1 t	
17	17.5 q	17.3 q	17.5 q	8″	14.5 q	
18	103.4 t	103.4 t	103.3 t			
19	21.2 q	21.1 q	21.1 q			
20	14.9 q	15.1 q	14.8 q			

[a] Data of compounds **1**, **2**, and **4** were recorded at 125 MHz.

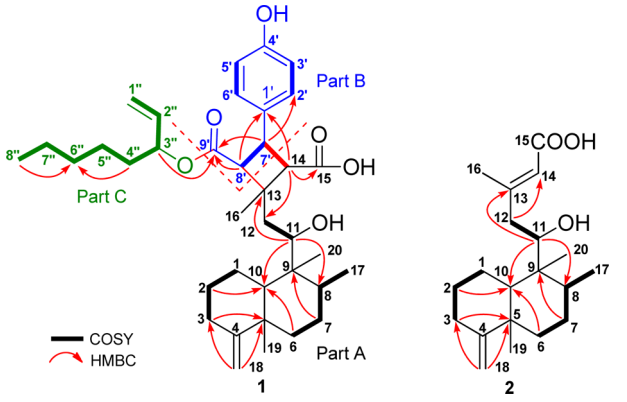


Figure 1. Key ^{1}H−^{1}H COSY and selected HMBC correlations of **1** and **2**.

derivative (part B), and a substituent group of a medium-chain fatty acid or alcohol (part C).

The ^{13}C NMR signals belong to the diterpenoid nucleus (part A) were similar to those of **2**, and the only difference was that the C-13/C-14 double bond on the side chain was replaced by a saturated bond (a methine carbon and a quaternary carbon, respectively), which was proven by the HMBC correlations from H-11 to C-13 and H-14 to C-12. This indicated that part A was linked with other moieties (part B or C) through C-13 and C-14. The structure of partial unit B was established to be a phenylpropanoid unit by the ^{1}H−^{1}H COSY correlation of H-8′ with H-7′ and the HMBC correlations from H-8′ to C-9′, C-1′ and from H-7′ to C-9′, C-2′, C-6′. The connections of part A and Part B were deduced based on the following key correlations: H-7′ with H-14 (^{1}H−^{1}H COSY); H-8′ with C-12, C-13, C-14, C-16, H-7′ with C-13, C-14, C-15, and H-14 with C-8′, C-7′, C-1′ (HMBC). Thus, the direct connections between C-8′ and C-13, C-7′ and C-14 formed a cyclobutane ring. The remaining ^{1}H and ^{13}C NMR resonances were suggestive of a C_8 fatty alcohol (part C). This aliphatic side chain was identified as 1-octen-3-ol by the ^{1}H−^{1}H COSY experiment which showed the presence of the fragment $CH_2(1'')$−$CH(2'')$−$CH(3'')$−$CH_2(4'')$−$CH_2(5'')$−$CH_2(6'')$−$CH_2(7'')$−$CH_3(8'')$ and the HMBC correlations from H-3″ to C-1″, C-5″, H-4″ to C-2″, C-6″, and H-8″ to C-5″, C-6″, as shown in Figure 1. Furthermore, the connection between part B and part C through C-9′ was supported by the HMBC correlation of H-3″ with C-9′. Therefore, the planar structure of **1** was established to be a new *ent*-clerodane-based meroditerpenoid with a unique cyclobutane ring and a 1-octen-3-ol substituent at C-9′. However, the relative stereochemistry of C-11, C-3″, and the cyclobutane ring could not be easily determined by the NOESY spectrum. Fortunately, a single-crystal X-ray diffraction experiment not only confirmed a unique cyclobutane ring as elucidated above but also determined the relative stereochemistry (the Flack parameter was too high to determine its absolute configuration) (Figure 2).

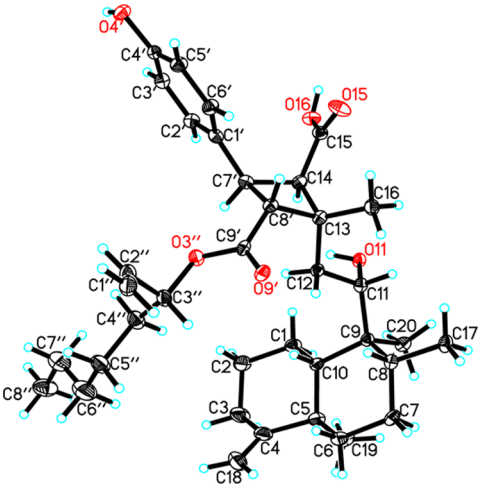


Figure 2. X-ray crystal structures of compound **1**.

In order to determine the absolute configuration, compound **1** was hydrolyzed with concentrated aq NaOH in MeOH to yield 1-octen-3-ol (**3**) and **4** (Scheme 1), which were determined by spectroscopic methods. Moreover, the absolute configuration of **3** was determined to be *R* (C-3″) by comparison of its optical rotation value with that of an authentic sample. Thus, the absolute configuration of compound **1** was established to be 11*S*,13*S*,14*R*,7′*S*,-8′*R*,3″*R* and named as scopariusic acid (**1**).

Compound **1** represents a new class of meroditerpenoid with a unique cyclobutane ring and a novel 1-octen-3-ol substituent. Although no "[2 + 2]-ase" has been identified, it is generally presumed that such cyclobutane derivatives originate from the direct coupling of the two olefinic carbon bonds in Nature.[3a,10] Our postulated biosynthetic

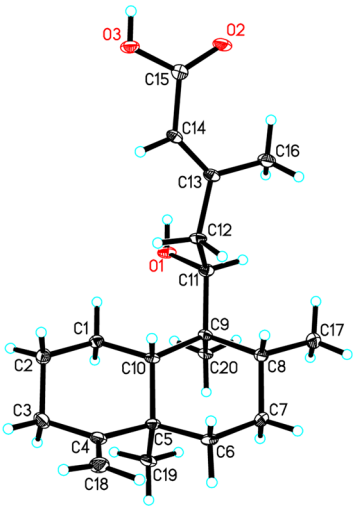
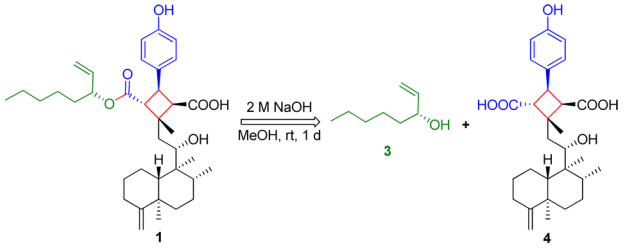


Figure 3. X-ray crystal structures of compound **2**.

Scheme 1. Alkali Hydrolysis of Compound **1**



pathway of compound **1** from the related *ent*-clerodane diterpenoid (**2**), *trans*-4-hydroxycinnamic acid (**5**), and (3*R*)-1-octen-3-ol (**3**) is shown in Scheme 2. The key step was the formation of a cyclobutane ring by an intermolecular [2 + 2] cycloaddition between (11*S*,13*E*)-11-hydroxy-*ent*-clerodane-4(18),13-dien-15-oic acid and 1′-octen-3′-yl-4-hydroxy-cinnamate (Scheme 2).

As far as we know, such a crossed intermolecular [2 + 2] photocycloaddition between acyclic side chains of two different molecules is uncommon in the natural products and is still a long-standing unsolved problem in synthetic chemistry.[11] Therefore, we suggested that the biomimetic synthesis of compound **1** could not be easily carried out in the laboratory, and enzymatic control might be essential in this case.[12]

Scheme 2. Hypothetical Biosynthetic Pathway of **1**

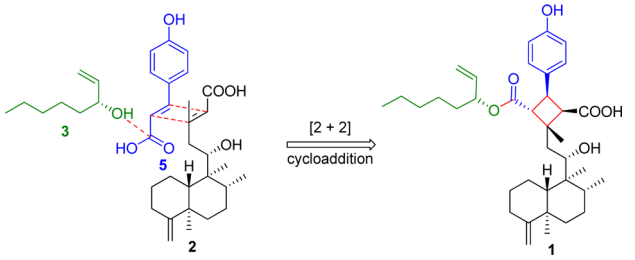


Table 2. Cytotoxic Activity of Compounds **1** and **2**

	IC_{50} (μM)				
compd	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	18.07	17.28	16.55	22.54	26.62
2	>40	>40	>40	>40	>40
DDP	2.03	13.54	12.56	18.65	19.70
Taxol	<0.008	<0.008	<0.008	<0.008	<0.008

Compounds **1** and **2** were screened for cytotoxic activities against the A-549, HL-60, MCF-7, SMMC-7721, and SW-480 human cell lines, using the MTT method, as reported previously,[13] with cisplatin and paclitaxel as the positive controls. Compound **1** exhibited weak cytotoxicity against all the five cell lines with IC_{50} values in the range of 16.6−26.6 μM (Table 2). In addtion, compounds **1** and **2** were screened for immuno-suppressive activity mediated through inhibition of mouse T cell proliferation in vitro and with BD750 as a positive control (IC_{50} = 1.5 μM).[14] The nontoxic concentrations of compound **1** exhibited moderate immunosuppressive activity (IC_{50} = 2.6 μM), while compound **2** showed no activity (Table S4 and Figure S57, Supporting Information).

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Supporting Information Available. ^{1}H NMR assignments of compounds **1**, **2**, and **4** in Table S1, immunosuppressive activity of compounds **1** and **2** in Table S4 and Figure S57, 1D, 2D NMR spectra, X-ray crystallographic data of **1** and **2**, and detailed experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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