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Isolation and Identification of Diterpenoids from *Euphorbia helioscopia* and Their Anti-inflammatory Activities

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Abstract: Phytochemical investigation of 70% ethanol extract of *Euphorbia helioscopia* L. led to eight diterpenoids, including four jatrophane-type (**1**–**4**), three abietane-type (**5**–**7**) and one lathyrane-type (**8**). Among them, (5*E*,11*E*)-3*β*-benzoyloxy-7*β*,14*α*,15*β*-trihydroxyjatropha-9-one (**1**) was a new jatrophane diterpenoid. An extensive array of spectral analyses, including HR-ESI-MS, IR, UV, 1D and 2D NMR, as well as quantum chemical calculations of ECD curve, were utilized for their structural elucidation. Bioassay results showed that compounds **2**–**3** and **6**–**8** could significantly inhibit NO release on LPS-induced RAW 264.7 cells, especially for jatrophane diterpenoid **3** and abietane diterpenoid **8** that showed stronger activities with the IC_{50} values of 9.16 ± 0.70 and 5.74 ± 0.27 μM.

Keywords: *Euphorbia helioscopia*; diterpenoids; anti-inflammatory. © 2025 ACG Publications. All rights reserved.

1. Introduction

Euphorbia helioscopia L., also known as “Maoyancao”, is abundant of natural resources and widely distributed around the world [1]. It is firstly found in *Divine Farmer's Materia Medica*, characterized by its slightly cold nature and bitter taste, and associated with the lung, large intestine, and small intestine meridians. According to *Compendium of Materia Medica*, *E. helioscopia* exceled in reducing swelling, eliminating phlegm and fever, dispersing nodules and killing insects [2-3]. Modern research and clinical studies have demonstrated that *E. helioscopia* exhibited antitussive, expectorant, antitumor, antibacterial, antiviral, analgesic, antioxidant and immune-regulating effects, which were largely attributed to its diverse secondary metabolites, including diterpenoids, triterpenoids, flavonoids and phenolic acids. Among them, diterpenoids was regarded as the chemotaxonomic characters of *E. helioscopia*, mainly consisting of jatrophane-type, abietane-type, and lathyrane-type [4-6].

In the present study, eight diterpenoids, consisting of four jatrophane-type (**1**–**4**), three abietane-type (**5**–**7**) and one lathyrane-type (**8**) were obtained from 70% ethanol extract of *E. helioscopia* (Figure 1). Among them, one jatrophane diterpenoid, (5*E*,11*E*)-3*β*-benzoyloxy-7*β*,14*α*,15*β*-

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trihydroxyjatropha-9-one (**1**) was previously undescribed. An extensive array of spectral analyses, comprising HR-ESI-MS, IR, UV, 1D and 2D NMR, as well as quantum chemical calculations of ECD curve, were utilized for their structural elucidation. The anti-inflammatory properties of these diterpenoids were assessed in LPS-induced RAW 264.7 cells. These findings indicated that compounds **2**–**3** and **6**–**8** could significantly inhibit NO release, especially for jatrophane diterpenoid **3** and abietane diterpenoid **8** that showed stronger activities having IC_{50} values of 9.16 ± 0.70 and 5.74 ± 0.27 μM.

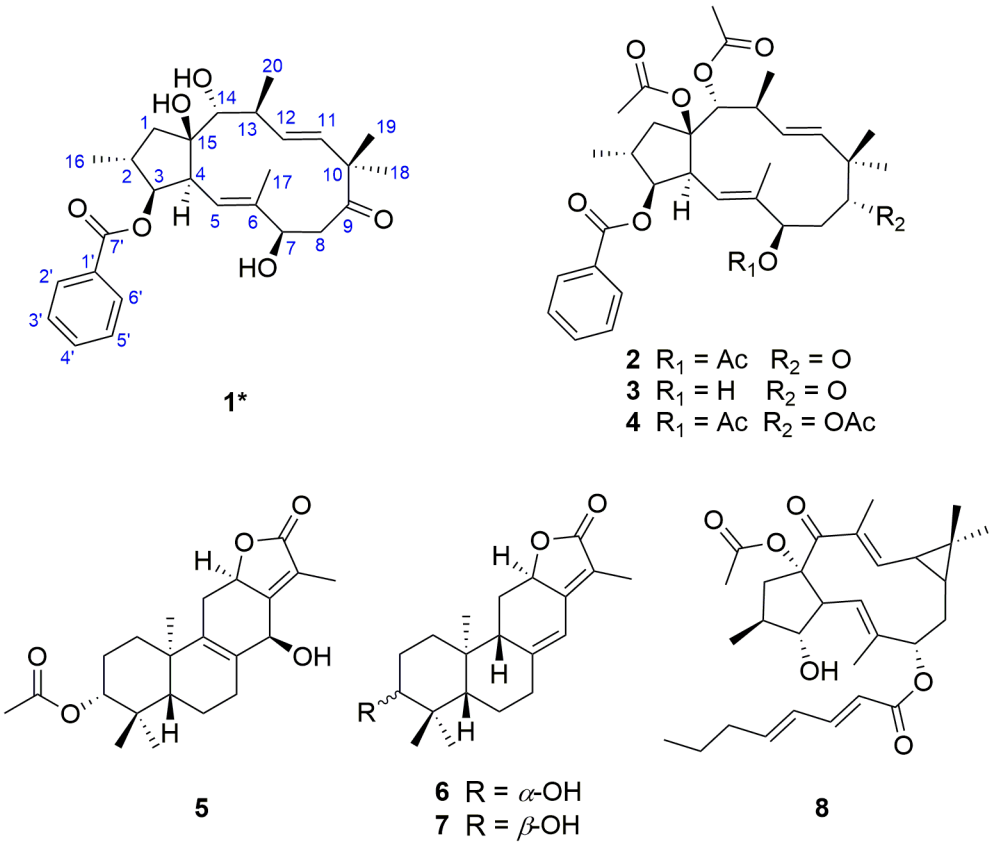

Figure 1. Chemical structures of compounds **1**-**8**

2. Materials and Methods

2.1. Apparatus and Reagents

HR-ESI-MS analysis was carried out using a Thermo UPLC/Orbitrap-Exploris-120. Optical rotation was measured with an Anton Paar MCP 5100. UV-Vis spectrum was obtained on a Shimadzu 210A. IR spectrum was recorded by a Thermo Nicolet IS5. NMR spectra, encompassing 1D and 2D techniques, were acquired on Bruker Avance III 500. ECD curve was recorded using an Applied Photophysics Chirascan V100. Chromatographic separations utilized silica gel (100-200 and 200-300 mesh, Qingdao Marine Chemical), ODS-C18 (50 μm, YMC), and a *semi*-preparative HPLC system (QBH LC-52, Beijing Qingbohua) with a YMC column (250 mm × 10 mm, 5 μm I.D.).

2.2. Plant Material

Euphorbia helioscopia L. was harvested in September 2020 from Henan Province, China, and was authenticated via Prof. Liping Dai of Henan University of Chinese Medicine. This specimen (voucher number: 20200910) was archived at the Engineering Technology Research Center for Integrated Development and Utilization of Genuine Medicinal Materials in Henan Province.

2.3. Extraction and Isolation

The whole plant of *E. helioscopia* (20.0 kg) was extracted by refluxing with 70% EtOH at around 80°C (2 × 100 L, 2 h each). After solvent evaporation, the crude extract (3.3 kg) was suspended in water and partitioned into petroleum ether, EtOAc, and *n*-butanol fractions, resulting in 260.0 g, 255.0 g, and 360.0 g, respectively. The petroleum ether fraction was chromatographed on silica gel (100-200 mesh) with a DCM-MeOH gradient (1:0→0:1), yielding six fractions (Fr.A ~ Fr.G). Fr.D (45.8 g) was further chromatographed on a Toyopearl HW-40C column using DCM-MeOH (1:1) to obtain Fr.D-1 ~ Fr.D-7. Subsequently, Fr.D-3 (15.4 g) was separated on silica gel (100 ~ 200 mesh) using a petroleum ether-EtOAc gradient (20:1→1:5) to yield Fr.D-3-a ~ Fr.D-3-i. Fr.D-3-f (2.3 g) was chromatographed on Sephadex LH-20 (DCM-MeOH, 1:1), and further purified by *semi*-preparative HPLC (3 mL/min, 63% acetonitrile-H_2O) to obtain compounds **2** (6.2 mg, t_R = 55.8 min), **3** (5.5 mg, t_R = 59.2 min), **4** (6.3 mg, t_R = 66.3 min) and **8** (3.6 mg, t_R = 75.6 min). Fr.D-3-h (1.5 g) was separated on an ODS column (MeOH-H_2O, 50:50→90:10) to obtain Fr.D-3-h-1 ~ Fr.D-3-h-15. Subsequently, Fr.D-3-h-8 (85.1 mg), Fr.D-3-h-10 (232.1 mg) and Fr.D-3-h-11 (109.7 mg) were respectively purified by semi-preparative HPLC (3 mL/min), resulting in compounds **1** (5.2 mg, 60% acetonitrile-H_2O, t_R = 73.9 min); **6** (8.2 mg, 65% acetonitrile-H_2O, t_R = 95.2 min) and **7** (7.2 mg, 65% acetonitrile-H_2O, t_R = 98.3 min); and **5** (5.1 mg, 70% acetonitrile-H_2O, t_R = 45.2 min).

Table 1. ^1H and ^{13}C NMR (500 and 125 MHz, $CDCl_3$) data of compound **1** (δ in ppm, J in Hz)

Positions	**1**	
	δ_H	δ_C
1	1.86 (t, 12.9)	42.1
	1.71 (dd, 12.9, 6.3)	
2	2.56 (ddd, 17.2, 12.9, 6.3)	35.2
3	4.65 (dd, 10.7, 8.8)	83.1
4	3.50 (dd, 11.5, 8.8)	42.5
5	5.38 (d, 11.5)	119.5
6	-	139.8
7	4.00 (brd, 6.2)	73.6
8	3.12 (dd, 15.0, 2.1)	38.9
	2.39 (dd, 15.0, 6.4)	
9	-	214.2
10	-	51.7
11	5.15 (d, 15.5)	129.5
12	5.57 (dd, 15.5, 10.0)	134.2
13	2.17 (dtd, 9.7, 6.5, 3.2)	44.2
14	3.26 (d, 9.4)	79.4
15	-	84.0
16	1.06 (d, 6.3)	16.5
17	1.58 (s)	15.9
18	1.13 (s)	19.3
19	1.21 (s)	24.2
20	1.16 (d, 6.5)	20.6
-OBz	-	-
1'	-	130.8
2',6'	7.96 (d, 7.6)	129.6
3',5'	7.41 (d, 7.6), 7.39 (d, 7.6)	128.4
4'	7.52 (t, 7.6)	132.8
7'		167.1

2.4. Spectral Data

(5*E*,11*E*)-3*β*-Benzoyloxy-7*β*,14*α*,15*β*-trihydroxyjatropha-9-one (**1**): White amorphous powder; $[\alpha]_D^{25}$ − 44.001(*c* 0.1, MeOH); IR (KBr) λ_{max}: 3467, 2927, 1714, 1452 and 1277 cm^{-1}; UV (MeOH) λ_{max} (log ε): 201 (4.05), 218 (3.72) and 228 (3.74) nm; HR-ESI-MS at *m/z* 479.2399 $[M + Na]^+$ (calcd for $C_{27}H_{36}O_6Na$, 479.2404); ^1H and ^{13}C NMR (500 and 125 MHz, MeOD) data, see Table 1.

2.5. ECD Calculation

A CD spectrum of compound **1** was acquired using a chirascan spectropolarimeter. This Dreiding force field within MarvinSketch was employed to generate Optimized low-energy conformers. Subsequent re-optimization of the geometry was conducted using a TDDFT technique at B3LYP/6-31+G(d) level with Gaussian 16. ECD calculations for compound **1** were carried out utilizing RB3LYP/6-31G(d,p) strategy. The ECD curve was produced via SpecDic software and OriginPro 8 with UV correction.

2.6. Bioassay for Anti-inflammatory Activity

2.6.1 RAW 264.7 Cell Viability

RAW 264.7 Cell viabilities were detected via the MTT assay. Cells were plated at a density of 1×10^5 cells/mL in 96-well plates and incubated for 12 hours. Subsequently, the cells were exposed to compounds **1**, **2**-**3** and **6**-**8** at concentrations ranging from 25 to 150 μM for 24 hours. MTT solution (20 μL, 5 mg/mL) was then added to each well, followed by an additional 4-hour incubation. After discarding the supernatant, 150 μL of DMSO was added to dissolve the formazan crystals by shaking for 10 minutes. Absorbance at 490 nm was measured via a spectrophotometer.

2.6.2 NO Production

Nitrite levels within the culture supernatant were assayed to determine NO production using the Griess reagent. Cells (2×10^6/well) were seeded in 12-well plates and incubated for 12 h. After treatment with different concentrations of tested compounds for 1h (dexamethasone as positive control), LPS (1 μg/mL) was added, followed by an additional 24 h of culture. 50 μL of supernatant was mixed with an equal volume of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% NED in water) at 37 °C for 10 min. Absorbance at 540 nm was measured to calculate NO inhibition rates.

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was isolated as a white amorphous powder with a molecular formula of $C_{27}H_{36}O_6$ determined by HR-ESI-MS. The pseudomolecular ion peak at *m/z* 479.2399 $[M + Na]^+$ matched the calculated value for $C_{27}H_{36}O_6Na$ (*m/z* 479.2404). These IR data at 3467, 1714 and 1452 cm^{-1} revealed inclusion of hydroxyl, carbonyl, and aromatic benzene ring moieties. These ^1H NMR and HSQC spectra showed signals for a monosubstituted benzene ring [δ_H 7.96 (d, *J* = 7.6 Hz, H-2', 6'), 7.52 (t, *J* = 7.6 Hz, H-4'), 7.41 and 7.39 (d, *J* = 7.6 Hz, H-3', 5')], three olefinic protons [δ_H 5.57 (dd, *J* = 15.5, 10.0 Hz, H-12), 5.38 (d, *J* = 11.5 Hz, H-5) and 5.15 (d, *J* = 15.5 Hz, H-11)], three oxymethine protons [δ_H 4.65 (dd, *J* = 10.7, 8.8 Hz, H-3), 4.00 (brd, *J* = 6.2 Hz, H-7) and 3.26 (d, *J* = 9.4 Hz, H-14)], and five methyl groups [δ_H 1.21 (s, H_3-19), 1.58 (s, H_3-17), 1.13 (s, H_3-18), 1.16 (d, *J* = 6.5 Hz, H_3-20) and 1.06 (d, *J* = 6.3 Hz, H_3-16)]. ^{13}C NMR and HSQC spectra demonstrated 27 carbon resonances, attributable to one keto carbonyl (δ_C 214.2), one ester carbonyl [δ_C 167.1 (C-7')], six aromatic carbons [δ_C 132.8 (C-4'), 130.8 (C-1'), 129.6 (C-2', 6') and 128.4 (C-3', C-5')], two double bonds [δ_C 139.8 (C-

6), 134.2 (C-12), 129.5 (C-11) and 119.5 (C-5)], two quaternary carbons [one oxygenated at δ_C 83.1 (C-3)], seven sp^3 methines [three oxygenated at δ_C 83.1 (C-3), 79.4 (C-14) and 73.6 (C-7)], two sp^3 methylenes and five methyls. These above-mentioned data suggested that compound **1** possessed this jatrophane diterpenoid structure, frequently present in *E. helioscopia* [4], and substituted by a benzoyl group.

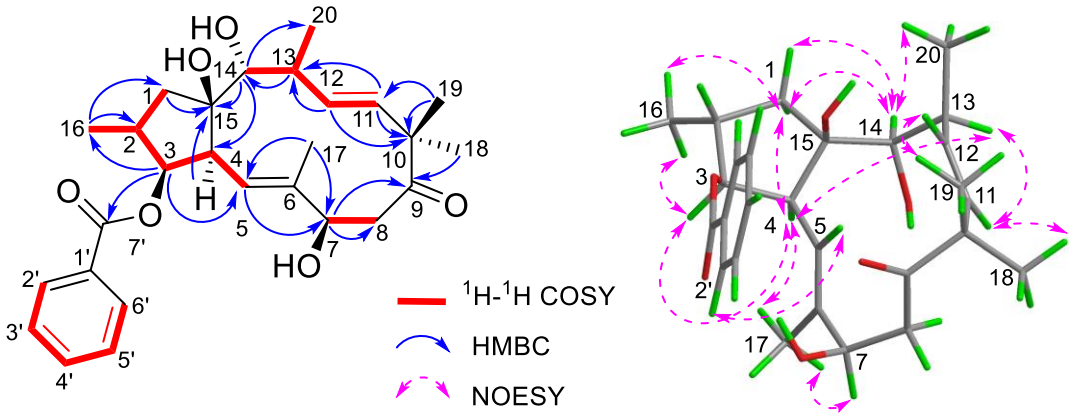

Figure 2. Key ^{1}H-^{1}H COSY, HMBC and NOESY correlations of compound **1**

The jatrophane structure was verified via its 2D NMR experiments. The ^{1}H-^{1}H COSY spectrum (Figure 2) reflected spin–spin coupling fragments of H_2-1/H-2(/H_3-16/H-4/H-5/, H-7/H_2-8, H-11/H-12/H-13 (H_3-20)/H-14 and H-2'/H-3'/H-4'/H-5'/H-6'. The obvious HMBC correlations (Figure 2) from olefinic protons H-5 to C-7 and Me-17, H_3-17 to C-5, C-6 and C-7, H-11 to C-9, C-10, C-13, Me-18 and Me-19, and H-12 to C-10, suggested two olefinic bonds at $\Delta^{5,6}$ and $\Delta^{11,12}$. This keto carbonyl at C-9 was diminished via HMBC corporations from H-7, H_2-8, H-11, H_3-18 and H_3-19 to C-9 (δ_C 214.2). The benzoyloxy group was linked to C-3 through key HMBC cross-peak from oxymethine proton H-3 to C-7' (δ_C 167.1), and three hydroxyl groups were respectively located at C-7, C-14, and C-15 according to their chemical shifts and HMBC correlations from H-7 to C-6 and C-9; H-14 to C-4, C-13, C-15 and Me-20; and H_2-1 and H-14 to C-15.

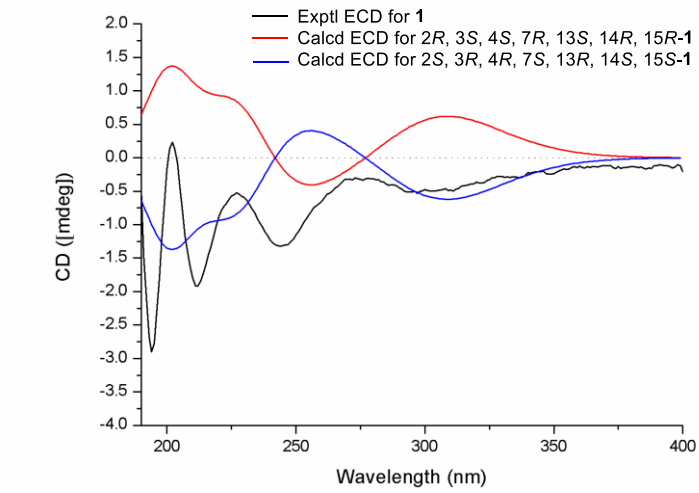

Figure 3. Experimental and calculated ECD spectra of compound **1**

The relative configuration of compound **1** was additionally established through analyzing NOESY correlations (Figure **2**). According to these reported jatrophane diterpenoids in *Euphorbia* plants, the angular proton H-4 was exclusively α-oriented and the hydroxyl group 15-OH was β-oriented [7]. These crucial NOESY friendships of H-4 and H-1α (δ_H 1.86) /H-3/H-13/H_3-17, H_3-16 and H-1α/H-3, H-11 and H-13/H_3-18, and H_3-7 and H-17, suggested these protons were all α-oriented, and 3-OBz group, 7-OH and Me-20 were β-oriented. A correlation for Me-20 and H-14 further assigned

14-OH as α-orientation. The *E* configurations of $\Delta^{5,6}$ and $\Delta^{11,12}$ were accordingly confirmed by the NOESY cross-peaks of H-4 and H_3-17, and H-11 and H-13, as well as the $^3J_{11,12}$ value of 15.5 Hz. Furthermore, ECD spectra of compound **1** were discovered within MeOH to resolve its stereochemistry. Form Figure 3, its experimental ECD curve exhibited a comparable trend to helioscopnin B [8] and matched the calculated ECD curve for 2*R*, 3*S*, 4*S*, 7*R*, 13*S*, 14*R*, 15*R* configuration, confirming the absolute structure of compound **1** as 2*R*, 3*S*, 4*S*, 7*R*, 13*S*, 14*R*, 15*R*. Thus, structure of compound **1** was elucidated as (5*E*,11*E*)-3β-benzoyloxy-7β,14α,15β-trihydroxyjatropha-9-one.

In addition, seven diterpenoids, including epieuphoscopin B (**2**) [9], 2-*epi*-euphornin I (**3**) [10], euphornin L (**4**) [11], eupheliosco pnoid N (**5**) [12], helioscopinolide A (**6**) [13–14], helioscopinolide B (**7**) [13, 15] and euphohelioscopin A (**8**) [16], were isolated from *E. helioscopia* by comparison of the ^{1}H and ^{13}C NMR data with those reported.

3.2. Anti-inflammatory Activity

Table 2. Anti-inflammatory of different compounds on LPS-induced RAW 264.7 cell

Compounds	**Cell viabilities** [a]		**IC_{50} (μM)**[b]
	c (μM)	**Viability** (%)	
Dexamethasone	-	-	8.59 ± 1.22
1	150	22.35 ± 0.09	> 50
	100	70.23 ± 0.04	
	75	79.28 ± 0.02	
	50	94.51± 0.04	
	25	98.91± 0.07	
2	150	121.83 ± 0.04	16.79 ± 1.29
	100	127.21 ± 0.05	
	75	130.36 ± 0.06	
	50	127.21 ± 0.03	
	25	125.07 ± 0.05	
3	150	106.21 ± 0.03	9.16 ± 0.70
	100	106.94 ± 0.02	
	75	113.36 ± 0.02	
	50	113.93 ± 0.02	
	25	114.12 ± 0.01	
6	150	58.86 ± 0.03	28.16 ± 1.60
	100	84.88 ± 0.02	
	75	97.01± 0.09	
	50	98.57 ± 0.04	
	25	104.29 ± 0.07	
7	150	14.47 ± 0.24	13.38 ± 0.39
	100	24.15 ± 0.08	
	75	55.01 ± 0.08	
	50	90.88 ± 0.07	
	25	96.05 ± 0.09	
8	200	89.23 ± 0.19	5.74 ± 0.27
	150	97.31 ± 0.05	
	100	96.11 ± 0.15	
	75	102.49 ± 0.08	
	50	108.80 ± 0.07	

[a] RAW 264.7 cell viabilities in the presence of tested compounds at different concentrations;
[b] NO inhibitory concentration at 50**%.**

According to cell viability tests, compounds **2**–**3**, **6** and **8** at ≤ 75 μM, and **1** and **7** at ≤ 50 μM exhibited no cytotoxicity (Table 2) against RAW 264.7 cells. RAW 264.7 cells stimulated with LPS were employed for assessing these anti-inflammatory activities. These outcomes indicated that compounds **2**–**3** and **6**–**8** could significantly reduce NO release *in vitro*, and jatrophane diterpenoid **3** and abietane diterpenoid **8** that displayed powerful NO inhibition actions with IC_{50} values of 9.16 ± 0.70 and 5.74 ± 0.27 μM.

4. Conclusion

Eight diterpenoids, comprising three skeletons including jatrophane (**1**-**4**), abietane (**5**-**7**) and lathyrane-type (**8**), were isolated from *E. helioscopia*. (5*E*,11*E*)-3β-benzoyloxy-7β,14α,15β-trihydroxyjatropha-9-one (**1**) was one new jatrophane diterpenoid. Most of these diterpenoids showed significantly anti-inflammatory capacities, especially for jatrophane diterpenoid **3** and abietane diterpenoid **8** that showed stronger NO inhibition actions with the IC_{50} values of 9.16 ± 0.70 and 5.74 ± 0.27 μM.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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