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Kansuinone, a novel euphane-type triterpene from *Euphorbia kansui*

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ABSTRACT

Kansuinone (**1**), a rearranged euphane triterpenoid containing a spiro [5,6] ring system, was isolated from the roots of *Euphorbia kansui*. Its structure and stereochemistry were elucidated on the basis of spectroscopic, CD, and computational methods. Kansuinone (**1**) exhibited inhibitory activity against human and mouse 11β-HSD1 (11β-hydroxysteroid dehydrogenase type 1), with IC_{50} values of 1.12 and 1.08 μM, respectively.

Plants of the *Euphorbia* genus produce structurally unique and diversified diterpenoids and triterpenoids, which have attracted great interest from biogenetic, synthetic, biological, and toxicological points of view.[1] Some of these compounds have shown potent antitumor, cytotoxic, antiviral, NADH oxidase inhibitory, and multidrug resistance-reversing activities.[2]

Euphorbia kansui is a vivacious herb distributed in the central and western parts of China, from which a novel euphane-type triterpenoid, kansuinone (**1**), and its presumed bioprecursor, kansenone (**2**) were isolated during our continuous search for biologically active compounds from the genus *Euphorbia*.

Compound **1** possessed a rearranged backbone involving a spiro [5,6] ring system that may have an origin from euphane-type triterpenoids. Previously, spirotriterpenoids have been found only as lanostane-type triterpenoids derivative.[3] Compound **1** exhibited inhibitory activity against human and mouse 11β-HSD1, with IC_{50} values of 1.12 and 1.08 μM, respectively. Reported here is the isolation, structure elucidation, and biological activity assays of **1**.

The roots of *E. kansui* used in this investigation were collected at Kuitun, Gansu Province, P.R. China, in December 2007. The dried and powdered roots of *E. kansui* (20 kg) were extracted with 95% EtOH. A crude residue (760 g) was obtained after evaporating the solvent and the residue was partitioned between petroleum ether and H_2O. The petroleum ether fraction (280 g) was separated by a combination of silica gel, amino silica gel, and Sephadex LH-20 columns. Final purification by silica gel column chromatography yielded kansuinone (**1**, 18 mg) and a known euphane-type triterpene, kansenone (**2**, 20 mg).[4]

Kansuinone **1**[5] was obtained as colorless and optically active oil. Its molecular formula was determined to be $C_{30}H_{50}O_3$ by HR-TOF-MS (*m/z* $[M+Na]^+$ 481.3657, calcd 481.3657) with six units of unsaturation. The IR absorption bands indicated the presence of ketone (1675 cm^{-1}) and hydroxyl (3432 cm^{-1}) groups. The hydroxyl group was also implied by the observation of a fragment ion at *m/z* 440 $[M-H_2O]^+$ in the EI-MS. The 1D NMR spectra exhibited resonances for seven quaternary, nine methylene, six methine, and eight methyl carbons, which were assigned to a trisubstituted double bond, a ketone, two oxygenated methines, one secondary methyl, seven tertiary methyl, and two hydroxyl groups. This compound should be a tetracyclic triterpene since one olefinic group and one ketone accounted for two of the six units of unsaturation indicated by the molecular formula.

Comparison of the ^1H and ^{13}C NMR data of **1** with those of kansenone (**2**), a euphane-type triterpene isolated from the same plant, revealed that both compounds were characterized by similar chemical shifts attributed to rings A and D and a monounsaturated side chain, suggesting a common structural motif they shared. The presence of the monounsaturated side chain was suggested by MS fragment ions at *m/z* 305 [M−side chain−D ring]$^+$, 369 $[M-2H_2O-69-2H]^+$, and 69 $[CH_2CH{=}C(Me)_2]^+$.[6] Two-dimensional NMR analysis confirmed the presence of these motifs (Fig. 1). Two hydroxyls, as required by its molecular formula and IR spectra, were located at positions C-3 and C-7 based on their chemical shifts and HMBC correlations from H-3 to Me-28 and Me-29, and

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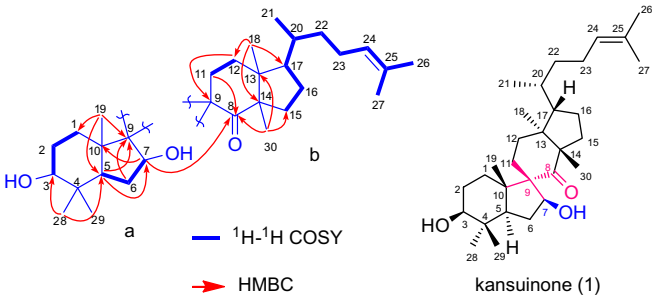

Figure 1. Selected two-dimensional NMR correlations of kansuinone (**1**).

H-7 to C-5. A mutually coupled system that comprised C-5, C-6, and C-7 was suggested by ^{1}H–^{1}H COSY thus determined the structure of ring A, which fused with a contracted five-membered ring B at C-5 and C-10, forming substructure **a**, as suggested by the observation of HMBC correlations from H-5, H-6, and Me-19 to C-9 and from H-7 to C-10. Ring C, containing a ketone at C-8, was assembled by ^{1}H–^{1}H COSY correlations between H_2-11/H_2-12 as well as HMBC correlations of H-12/C-9, H-11/C-8, Me-30/C-8, and Me-18/C-12. It fused with ring D connected by the side chain constituted the substructure **b**. The two substructures should join at C-9, forming a unique spiro [5,6] ring system, as confirmed by the HMBC cross peaks between H-7 and C-8 and the chemical shift of C-9 (δ_C 61.8). Therefore, a planar structure of **1** was derived, as shown in Figure 1.

The relative configuration of **1** was determined by ROESY experiments (Fig. 2) and computational methods. The large coupling constant ($J_{2,3}$ = 9.2 Hz) of H-3 indicated that the hydroxyl group was oriented equatorially (β) at C-3.[7] The relative configurations of the methyl groups and other protons in the rings A–D were ascertained on the basis of the ROESY correlations. The significant ROESY correlations of H-3/H-5, H-3/Me-28, and H-5/H-7 indicated that H-3, H-5, H-7, and Me-28 were cofacial, adopting an α-orientation. The relative configuration of C-9 was assigned by computational methods. As shown in Figure 3, the close fitting correlations between predicted and experimental chemical shifts for 9*S* (R = 0.995) than 9*R*-kansuinone (R = 0.983) (excluding that of C-8 with large chemical shift and lead to the leveling effect) indicate 9*S*-kansuinone is the preferred one. The 9*S* configuration was also consistent with its NOESY correlations, the cross-peaks of H-7/CH_3-

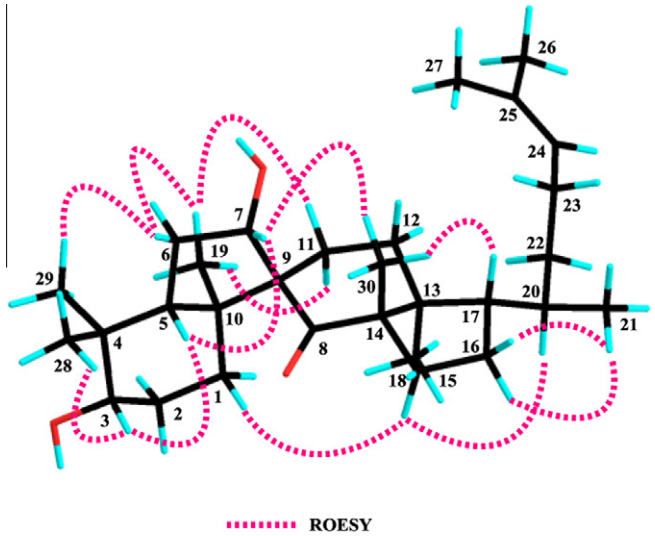

Figure 2. Key ROESY correlations of kansuinone (**1**).

Table 1
^{1}H, ^{13}C NMR data for kansuinone (**1**) in $CDCl_3$

	δ_H (multi, J in Hz)	δ_C
1α	2.10 (1H, m)	29.7 (t)
1β	1.20 (1H, m)	
2	1.67 (2H, m)	28.2 (t)
3	3.49 (1H, dd, 6.4, 9.2 Hz)	79.3 (d)
4		37.8 (s)
5	2.68 (1H, dd, 14.4, 6.0 Hz)	47.8 (d)
6α	2.16 (1H, m)	34.7 (t)
6β	1.43 (1H, m)	
7	4.26 (1H, t, 7.2 Hz)	76.4 (d)
8		219.3 (s)
9		61.6 (s)
10		49.0 (s)
11a	2.13 (1H, m)	24.2 (t)
11b	1.64 (1H, m)	
12a	1.88 (1H, m)	31.5 (t)
12b	1.79 (1H, m)	
13		45.9 (s)
14		61.8 (s)
15α	1.76 (1H, m)	29.7 (t)
15β	1.30 (1H, m)	29.7 (t)
16α	1.31 (1H, m)	26.8 (t)
16β	1.88 (1H, m)	
17	1.62 (1H, m)	49.6 (d)
18	0.69 (3H, s)	16.8 (q)
19	0.90 (3H, s)	17.6 (q)
20	1.46 (1H, m)	35.2 (d)
21	0.86 (3H, d, 6.4 Hz)	18.6 (q)
22α	1.55 (1H, m)	35.3 (t)
22β	1.13 (1H, m)	
23α	1.88 (1H, m)	24.6 (t)
23β	2.00 (1H, m)	
24	5.07 (1H, dd, 7.0, 1.2 Hz)	124.8 (d)
25		131.5 (s)
26	1.68 (3H, s)	25.7 (q)
27	1.60 (3H, s)	17.7 (q)
28	0.99 (3H, s)	29.7 (q)
29	0.88 (3H, s)	16.6 (q)
30	1.16 (3H, s)	22.4 (q)

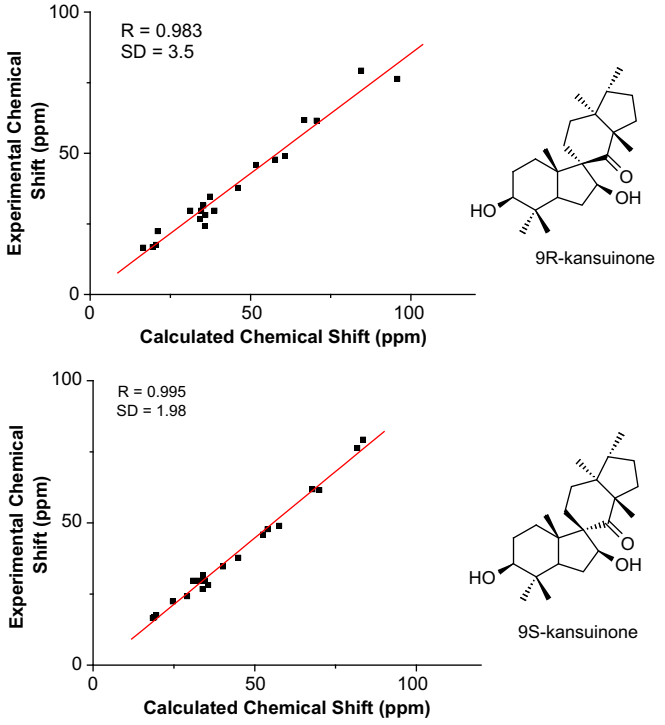

Figure 3. The correlations between the calculated and experimental chemical shifts for 9*R* and 9*S*-kansuinone by removing the point with large chemical shift (>200 ppm).

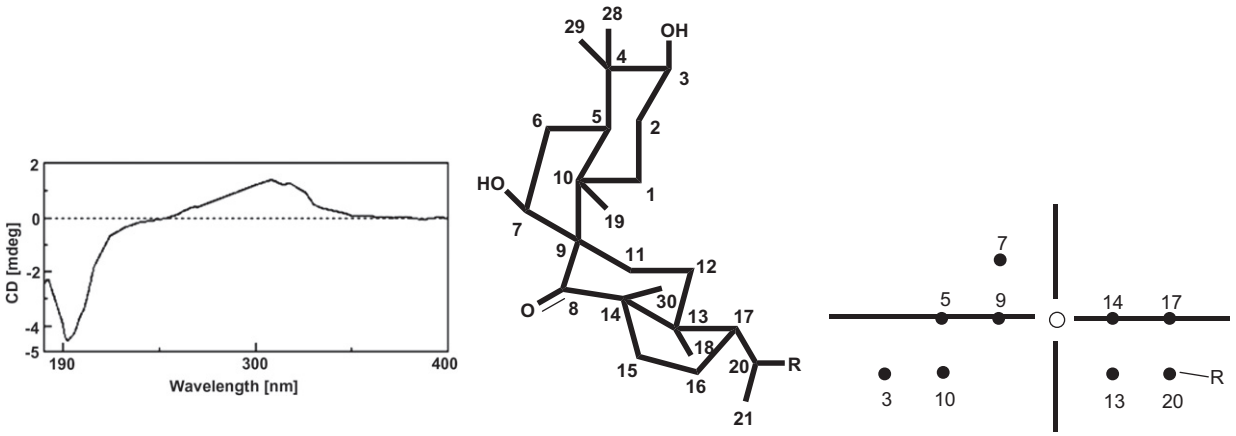

Figure 4. CD spectra and the distribution in octants of kansuinone (**1**).

30 and CH_3-19/CH_2-11. This configuration necessitated a dihedral angle near to 90° among rings A, B and C, D to maintain an anti-coplanar conformation. The ROESY cross-peaks of Me-29/6β, Me-19/H-6β, CH_2-11/Me-19, Me-30/H-7, Me-18/H-1, and Me-30/H-17 indicated that Me-19 was β-oriented, whereas Me-30 and H-17 were oriented outside the plane defined by the C/D rings and Me-18 was directed inside the plane. Furthermore, the typical chemical shift (δ_H 0.86)[6,7] of Me-21 suggested that **1** belonged to the euphane series with 20Hα, which was confirmed by the correlations between Me-18/H-20 and Me-21/CH_2-16. The stereochemistry of compound **1** was determined to be consistent with the euphane-type triterpenes.[8,9]

The absolute configuration of **1** was assigned based on circular dichroism (CD) measurements. The CD spectrum of **1** exhibited a positive Cotton effect caused by a weak $n\rightarrow\pi^*$ transition associated with the absorption of a ketone carbonyl at 305 nm (Fig. 4). Employing the octant rule for the cyclohexanone ring, the absolute configuration of **1** was assigned as 3*S*, 5*R*, 7*S*, 9*S*, 10*S*, 13*S*, 14*S*, 17*S*, and 20*R*.[9]

A plausible biogenetic pathway for **1** was proposed, as shown in Scheme 1, in which a Pinacol rearrangement reaction is the key step.

Inhibition of human and mouse 11β-HSD1 enzymatic activities by the two compounds were determined by scintillation proximity assay (SPA) using microsomes containing 11β-HSD1. Compound **1** exhibited significant dose-dependent inhibition of human 11β-HSD1 with an IC_{50} of 1.12 μM and the dose-dependent inhibition of mouse 11β-HSD1 with an IC_{50} of 1.08 μM. The selectivity for the mouse HSD2/HSD1 was higher (928) than the selectivity for human HSD2/HSD1 (2.35).[10]

Acknowledgments

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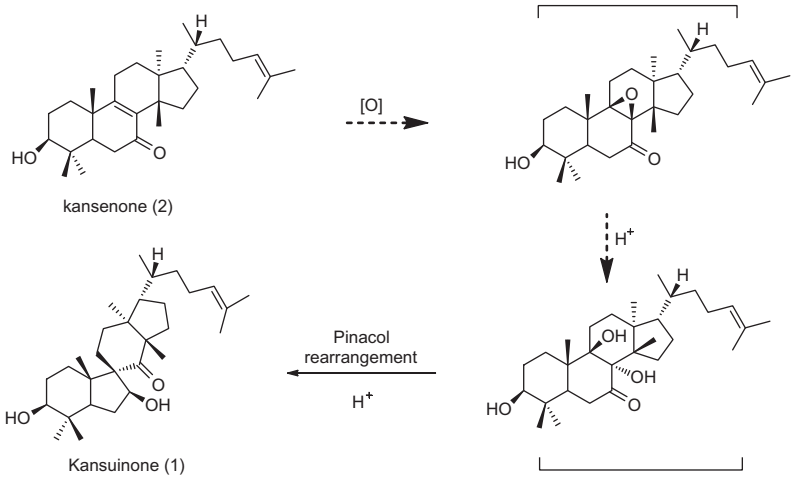
Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.09.099.



Scheme 1. Plausible biosynthetic pathway of **1**.

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