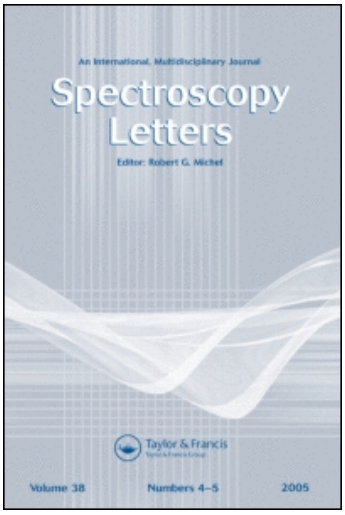

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

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To cite this Article Zheng, W. F. , Tan, R. X. , Yang, L. and Liu, Z. L.(1996) 'A New Antimicrobial Sesquiterpene Lactone from *Artemisia giraldii*', Spectroscopy Letters, 29: 8, 1589 — 1597
To link to this Article: DOI: 10.1080/00387019608007150
URL: http://dx.doi.org/10.1080/00387019608007150

SPECTROSCOPY LETTERS, 29(8), 1589-1597 (1996)

A NEW ANTIMICROBIAL SESQUITERPENE LACTONE FROM *ARTEMISIA GIRALDII*

Key Words: *Artemisia giraldii*, Compositae, eudesmanolide, antimicrobial activity

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ABSTRACT

A new antimicrobial eudesmanolide, 1-oxo-8α-hydroxy-11αH-eudesm-4-en-12,6α-olide (1), was isolated from a medicinal plant *Artemisia giraldii* and its structure was elucidated by spectroscopic methods (IR, MS, ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY and NOESY). Antimicrobial bioassay indicated that this compound inhibited the growth of human pathogenic bacteria *Staphylococcus auerus, Bacillus subtilis* and *Escherichia coli* as well as human opportunistic pathogenic fungi *Candida tropicalis, Gecotrichum candidun, Aspergillus flavus and A. nigers.*

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INTRODUCTION

Previously, we have studied Tibetan drug *Artemisia giraldii* and resulted in the characterization of two new antimicrobial flavones [1]. The mother liquor yielded was shown to contain further minor antimicrobial non-aromatic constituent. This observation prompted us to reinvestigate this species and we here report a new susquiterpene lactone and its antimicrobial activity.

EXPERIMENTAL

NMR experiments were conducted on a Bruker AM400 FT-IR spectrometer with ^{1}H and ^{13}C spectra recorded at 400 MHz and 100 MHz, respectively. IR spectra were measured on a 5DX-FT IR spectrometer, Mass spectra were obtained on a VG ZAB-HS mass spectrometer.

The aerial parts of *A. giraldii* were collected in July 1992 in Wenfeng County, Gansu Province, P. R. China. A voucher specimen (LZU 403) identified by Prof. G. L. Zhang was deposited in the Herbarium of the Department of Biology, Lanzhou University, Lanzhou, P. R. China. The well-chopped, air-dried plant material (1.1 kg) was extracted twice for 24 h with MeOH/Et_2O/petroleum ether (1:1:1) at room temperature. The extract was defatted followed by CC separation as reported previously [2]. Further column chromatography (silica gel) of the fifth fraction yielded 4 parts (I-IV). The part I was subjected to the Sephadex LH-20 column with methanol and the flow rate was adjusted to 3 drops/min. A pure compound was obtained as an oil (100 mg).

Qualitative antimicrobial activity control

The paper disk diffusion method was used. Inocula consisted of 24 h cultures of bacteria grown on Nutrient Agar at 37 ^{0}C, or 72 h cultures of fungi grown on Potato Dextrose Agar at 25 ^{0}C. The samples were dissolved in EtOH/H_2O (2:9). Paper disks (ϕ: 5 mm) were impregnated with the solutions and placed onto Petri

dishes containing NA for *Staphylococcus aureus* (NUB* -003), *Escherichia coli* (NUB-013), *Bacillus subtilis* (NUB-017) and PDA for *Candida tropicalis* (NUB-8868), *Geotrichum candidun* (NUB-9137), *Aspergillus flavus* (NUB-8721) and *A. nigers* (NUB-9122). After incubation for 18-24 h for bacteria or 72 h for fungi, the inhibition zones (mm) were measured and the microorganisms which were inhibited were tested for minimal inhibitory concentration (MIC).

Determination of MIC's by agar dilution procedure

Aqueous EtOH solutions of compound 1 were incorporated into the solid medium, with each plate containing a different concentration of the compound. The inocula prepared according to the standard methods were applied by standardized loops. After 24 h incubation at 35 ^{0}C (bacteria) and 72 h at 25 ^{0}C (fungi), the plates were examined for growth and MIC was determined as the lowest concentration that inhibited the growth. The results were shown in Table 1.

Spectroscopic features:

IR (CCl_4): 3440, 2950, 1760, 1700, 1610 cm^{-1}; EIMS *m/z* (rel. int.): 264 (28; $[M]^+$), 246 (22; $[M-H_2O]^+$), 218 (12), 95 (42), 61 (100). ^{1}H and ^{13}C NMR, see Table 2.

RESULTS AND DISCUSSION

The mass spectrum of compound 1 showed the molecular ion peak at *m/z* 264, in agreement with a molecular formula $C_{15}H_{20}O_4$ evidenced from the NMR data (Table 2 and Fig. 1). The IR bands (3400, 1750, 1710 and 1610 cm^{-1}) indicated the presence of hydroxyl, lactone and ketone carbonyl groups. In the ^{1}H NMR spectrum (Table 2), the methyl signals at δ 1.26 (s), 1.31 (d, J = 7.2 Hz), 1.69 (br

* NUB: culture collection center of microorganisms, Department of Biological Science and Technology, Nanjing University.

Table 1 Minimal inhibitory concentration ($\mu g\ ml^{-1}$) of 1 against a range of microorganisms

species	A	B	C	D	E	F	G*
MIC	<50	>200	100	<50	75	<50	75

*) A: *Staphylococcus auerus*, B: *Bacillus subtilis*
C: *Escherichia coli*, D: *Candida tropicalis*
E: *Gecotrichun candidun*, F: *Aspergillus nigers*,
G: *Aspergillus flavus*

Table 2 ^{1}H and ^{13}C NMR data of 1 ($CDCl_3$, 25 °C)

C	^{1}H (J in Hz)	^{13}C (DEPT)
1		212.66 (C)
2α	2.60 m*	32.81 (CH_2)
2β	2.35 m*	
3α	2.40 m*	35.39 (CH_2)
3β	2.50 m*	
4	---	127.92 (C)
5	---	127.53 (C)
6	4.54 d (11.5)	78.48 (CH)
7	1.75 ddd (11.5, 10.5,7.0)	58.47 (CH)
8	3.95 dt (10.4, 4.6)	68.99 (CH)
9α	1.39 dd (13.2, 10.5)	40.52 (CH_2)
9β	2.03 dd (13.2, 4.6)	
10	---	47.45 (C)
11	2.53 dq (7.2, 7.0)	43.96 (CH)
12	---	178.66 (C)
13	1.31 d (7.2)	14.04 (CH_3)
14	1.26 s	24.55 (CH_3)
15	1.69 br s	19.47 (CH_3)

* Not the first order

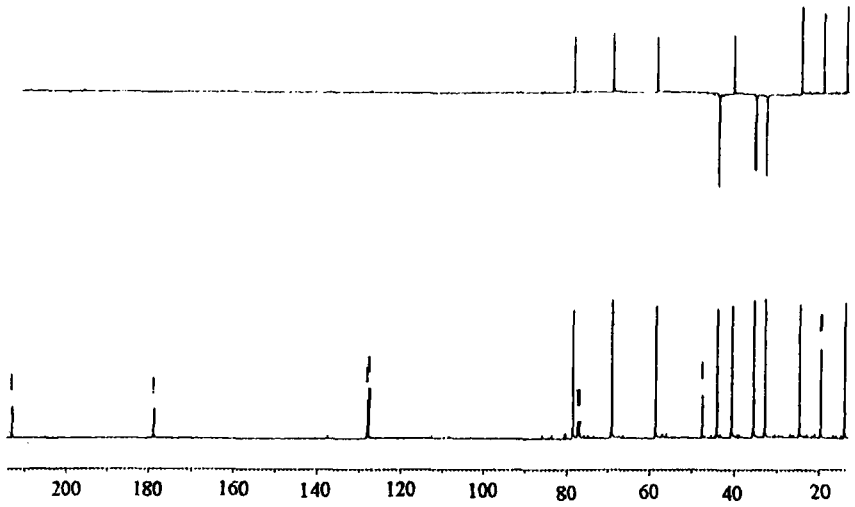
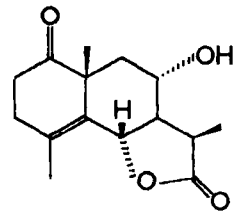


FIG. 1. ^{13}C NMR and DEPT of compound 1

s) and the doublet at δ 4.54 (br d, J = 11.5 Hz) suggested an eudesmanolide framework similar to those of 8α–hydroxytaurin [3, 4] and ψ–santonin [5, 6]. The downfield angular methyl singlet at δ 1.26 (s) revealed the presence of 1-ktone group while the existence of the 4,5-double bond was indicated by the broadened methyl singlet of H-15 at δ 1.69 [7, 8] which, as illustrated by the ^1H-^1H COSY spectrum (Fig.2), showed a clear homoallylic coupling



The molecular structure of 1

with H-6 at δ 4.54. Further scrutiny of the COSY spectrum showed that H-6 signal coupled with the signal of H-7 at δ 1.75 (ddd, J = 11.5, 10.5, 7.0 Hz) which were actually split by a double quarlet of H-11 at δ 2.53 and a double triplet of H-8 at 3.95. The signals at 1.39 (dd, J = 13.2, 10.5 Hz) and 2.03 (dd, J = 13.2, 4.6 Hz) coupled simultaneously with H-8, were assigned to H-9α and H-9β, respectively. A long range coupling was also observed between H-6 signal at δ

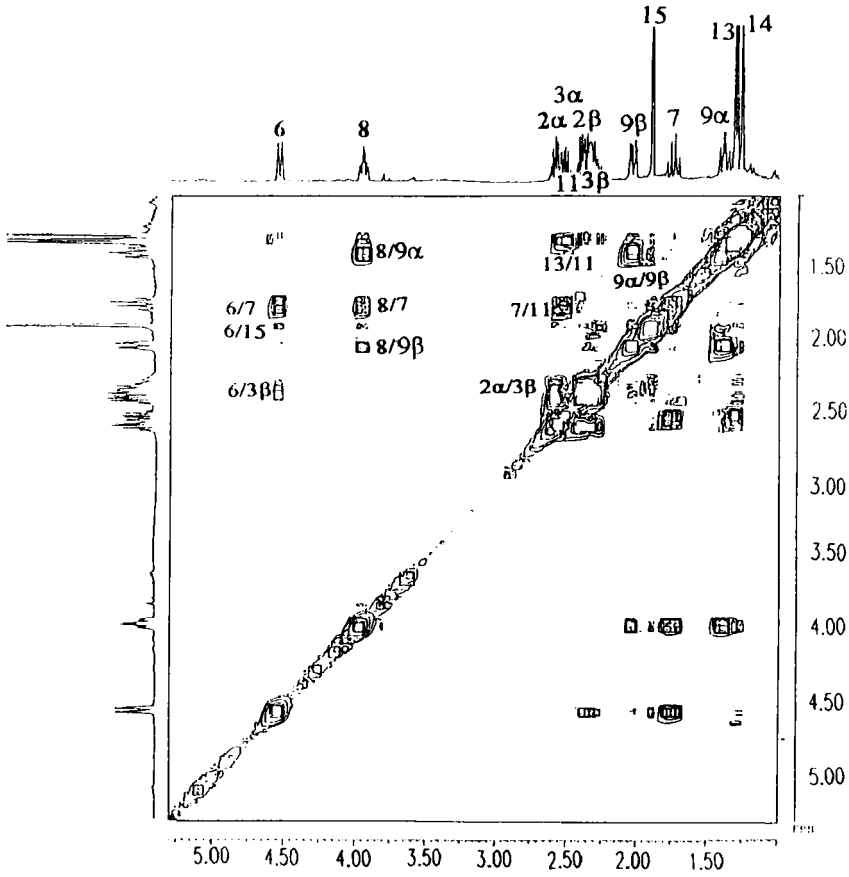


FIG. 2. ^{1}H-^{1}H COSY spectrum of compund 1 with several important correlations indicated on the map.

4.54 and multiplets at δ 2.46 and 2.37 which were thus assigned to H-3α and H-3β, respectively. The multiplets at δ 2.60 and 2.41 *ortho*-coupled with H-3α and H-3β were attributed to H-2α and H-2β. The discerned coupling sequence confirmed the assumption of ketone carbonyl group at C-1. The NOESY spectrum clearly indicated NOE effects of H-6 with H-14, H-13, H-8 establishing the formulated stereochemistry (Fig. 3). Comparison of the ^{13}C NMR data of

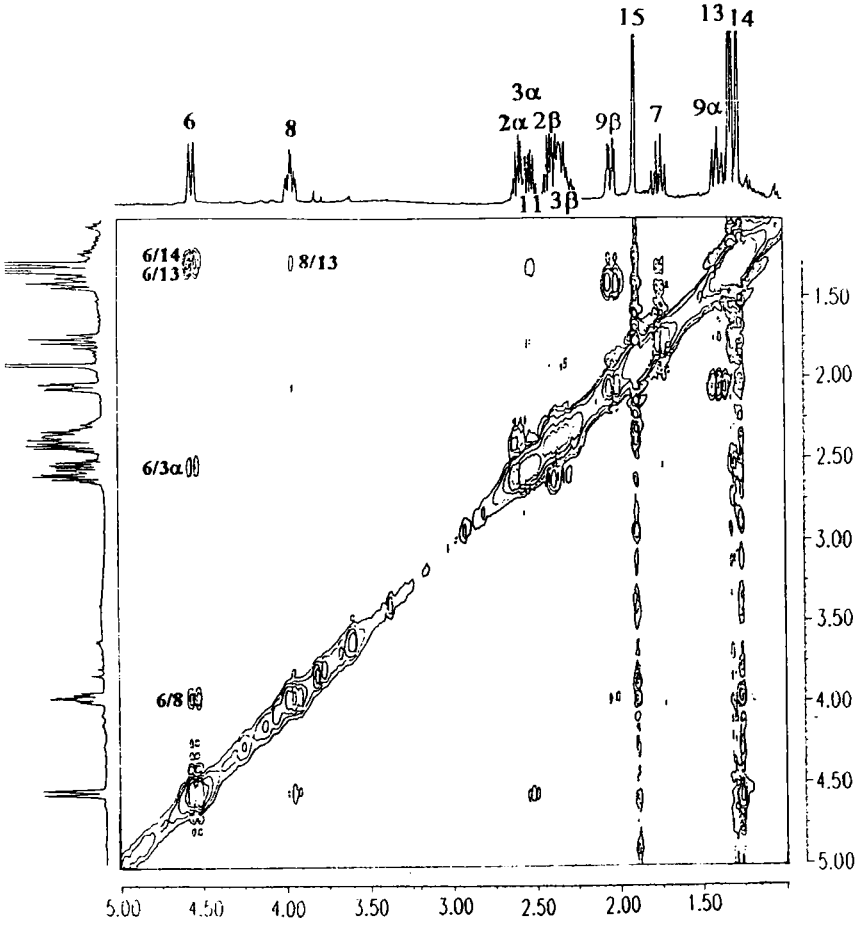


FIG. 3. NOESY spectrum of compound 1 with some decisive cross peaks marked directly on the map.

compound 1 with those of 8α-hydroxytaurin and ψ-santonin reinforced the stereochemical difference among these analogs. Particularly, the chemical shift of C-11 in 1 was shifted downfield by 3.14 ppm relative to that of 8α-hydroxytaurin [3] while the resonance line due to C-6 of 1 appeared at δ 78.48, 1.58 ppm downfield from that of ψ-santonin [5]. In conclusion, compound 1 was 1-oxo-8α-hydroxy-11αH-eudesm-4-en-12,6α-olide.

The antimicrobial bioassay of compound 1 indicated a strong inhibition to the growth of *Staphyloccus auerus* (MIC: <50 μg) , *Escherichia coli* (MIC: 100 μg), *Candida tropicalis* (MIC: <50 μg), *Geocotrichum candidun* (MIC: 75 μg), *Aspergillus nigers* (MIC: <50 μg) and *A. flavus* (MIC: 75 μg) (Table 1).

ACKNOWLEDGMENT

This work was financed by the Trans-Century Training Program Foundation for the Talents by the State Education Commission.

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Received: May 14, 1996
Accepted: June 24, 1996