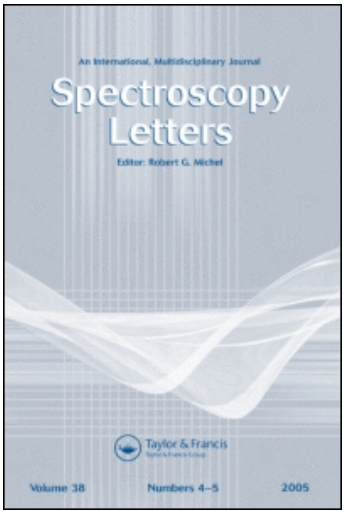

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ASSIGNMENT OF THE ^{1}H AND ^{13}C NMR SPECTRA OF A NEW ANTIBACTERIAL BISABOLANE SESQUITERPENE BY TWO-DIMENSIONAL NMR TECHNIQUES

Key Words : *Ligularia thyrsoidea*; Compositae; bisabolane; sesquiterpene; antibacterial activity.

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ABSTRACT

A new antibacterial bisabolane sesquiterpene, 1β-acetoxy-2β,8-diangeloyloxy-3β-hydroxy-4α-chloro-10,11-expoxybisabol-7(14)-ene (**1**), was isolated from *Ligularia thyrsoidea* and its structure was elucidated by 2D NMR techniques.

INTRODUCTION

Ligularia species (Compositae) have been used as folk remedies with antibiotic, antiphlogistic and antitumor activities [1]. In continuation of our research on the genus *Ligularia* in northwestern China [2,3], now we have examined the whole plants of *L. thyrsoidea* which afforded a new chlorine-bearing

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bisabolane sesquitepenes. A combination of ^{1}H-^{1}H COSY, ^{1}H-^{1}H NOESY, HMQC and HMBC spectra enabled us to deduce its structure and to assign completely its ^{1}H and ^{13}C NMR spectra. Antibacterial bioassay indicated that this compound inhibited the growth of human pathogenic bacteria *Estherichia coli*, *Bacillus subtilis*,and especially *Pseudomonas aeruginosa.*

EXPERIMENTAL

The plant material was collected in Tiaoshan, Xingjiang province in August 1994. It was identified by Prof. Guo-Liang Zhang of Lanzhou University and a voucher specimen(No: 94961) is deposited in the Herbarium of our institute. IR spectra were recorded on a **Nicolet** 170SX FTIR spectrometer. EIMS were obtained on HP-5988A MS. NMR experiments were run on a Bruker AM-400 FT-NMR with TMS as internal standard. The air-dried whole plants of *L. thyrsoidea* (4.0 Kg) were powdered and extracted with petrol (60-90°)-Et_2O-MeOH (1:1:1) at room temp. and gave 130 g of residue after removal of the solvent. The residue was separated on a silica gel column over 1.2 kg silica gel (200-300 mesh) eluting with a gradient of petrol-actone (20:1-2:1, 200 ml each eluent). From the fraction of petrol-actone 6:1, **1** (100 mg) was obtained by repeated CC on silica gel with petrol-EtOAc (6:1).

Antibacterial activity:

Compound **1** was screened for its antibacterial activity. Four pathogenic bacterial viz., *Escherichia coli*, *Bacillus subtilis*, *Staphyloccus aureus*, and *Pseudomonas aeruginosa* were employed for antibacterial activity. Cup-plate method was followed for the screening. The antibacterial activity was compared with standard chloramphenicol.

The results of antibacterial activity indicate that compound **1 has** shown strong activity against *Ps. aeruginosa* which was superior to the standard chloramphenicol and also shown considerable activity against *E. coli* and *B. subtilis*. But compound **1** has shown no inhibition against *Staph. aureus.*

RESULTS AND DISCUSSION

Compound **1** was isolated as colorless gum and had IR absorptions indicative of a hydroxyl group (3559cm^{-1}) , ester groups (1750 1718cm^{-1}) and double bond (1648cm^{-1}). The EI mass spectrum of **1** showed that one chlorine atom presented in **1** for a series of characteristic isotopic ion peaks at *m/z* = 528, 526[M]$^{+}$; 510, 508; 468, 466; 457, 455,etc. (their relative abundance ratios were about 1:3). The molecular formula of **1** could be determined as $C_{27}H_{39}O_8Cl$ in combination with NMR data, one acetoxy group and two angeloyloxy groups [4,5] existed in **1** also discerning from ^{1}H and ^{13}C NMR data (see FIG 1). The signals of oxygen-bearing carbons at δ 57.7 (C) and 60.7 (CH) together with the proton signal at δ 2.70 (1H, t, J = 5.5 Hz) indicated an epoxy group existed in compound **1** [6]. Except for all these groups and to accommodate 8 degrees of unsaturation, compound **1** was proposed to be a monocyclic sesquiterpene skeleton with a terminal double bond (δ_H = 5.28, 5.03 each 1H, br s; δc = 144.7 C, 116.1 CH_2).

The HMQC spectrum of **1** (see FIG 2) revealed the cross peaks between the carbon signals at δ 73.4(C-1, CH), 70.0(C-2,CH), 64.4(C-4, CH), 29.4(C-5, CH_2), 34.4(C-6, CH), 74.5(C-8, CH), 32.8(C-9, CH_2), 60.7(C-10, CH), and the corresponding proton signals at δ 5.48(H-1), 5.25(H-2), 4.16(H-4), 2.61(H-5β), 1.77(H-5α), 3.10(H-6), 5.50(H-8), 2.01(H-9a), 1.85(H-9b), 2.70(H-10), respectively. In the ^{1}H-^{1}H COSY spectrum (see FIG 3), strong cross peaks were found: H-1 (δ 5.48, dd, J = 3.3, 3.0Hz) with H-2 (δ 5.25, d, J = 3.3Hz) and H-6(δ 3.10, ddd, J=13.2, 3.0, 2.9Hz), H-6 with H-5β (δ 2.61, ddd, J=14.9, 13.2, 2.9Hz) and H-5α (δ 1.77, m), H-4(δ 4.16, t, J = 2.9Hz) with 5β and H-5α, 5β with H-5α, H-8 (δ 5.50, t, J = 7.0Hz) with H-9a(δ 2.01, m) and H-9b (δ 1.85, m), H-10 (δ 2.70, t, J = 5.5Hz) with H-9a and H-9b. Thus two main structure sequences of compound **1** were determined: -H_8-H_{9a},H_{9b}-H_{10}- and -H_2-H_1-H_6-$H_{5\alpha}$,$H_{5\beta}$-H_4-. Its skeleton was determined by connection of HMBC (FIG 4) due to the ^{2}J and ^{3}J couplings: C-1/H-2, C-2/H-1, 4, 15; C-3/H-4, 15; C-7/H-6, 8, 14; C-10/H-8, 9, 12,

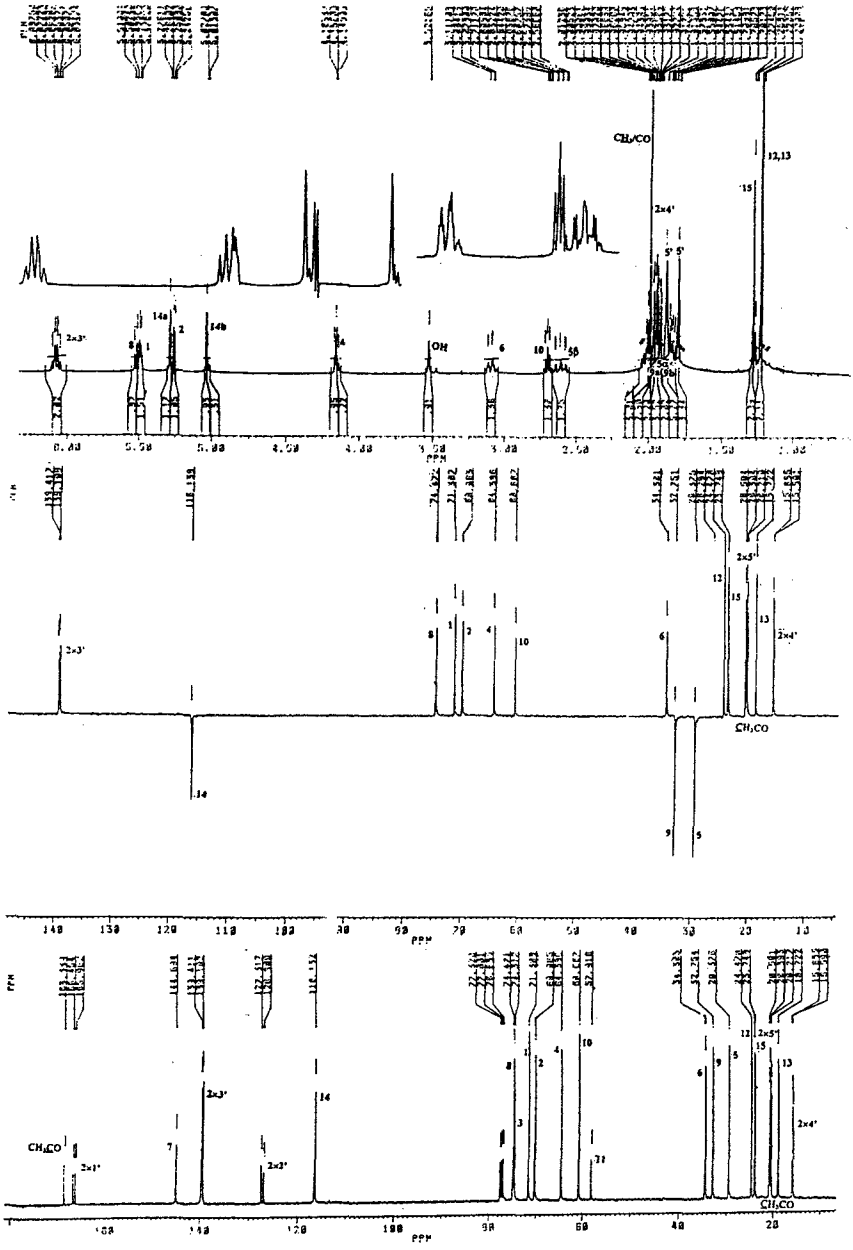

FIG 1. ^{1}H, ^{13}C NMR and DEPT spectra of compound **1**

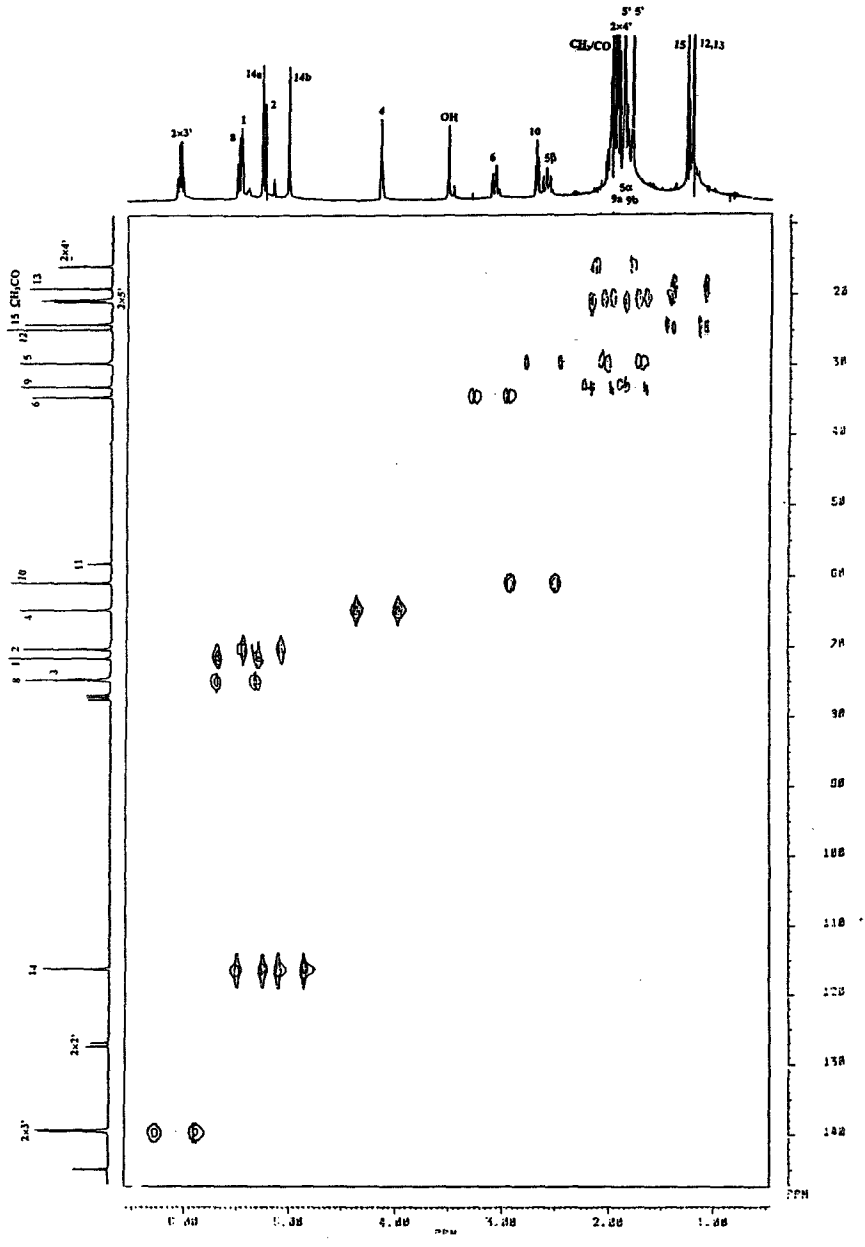

FIG 2. HMQC spectrum of compound **1**

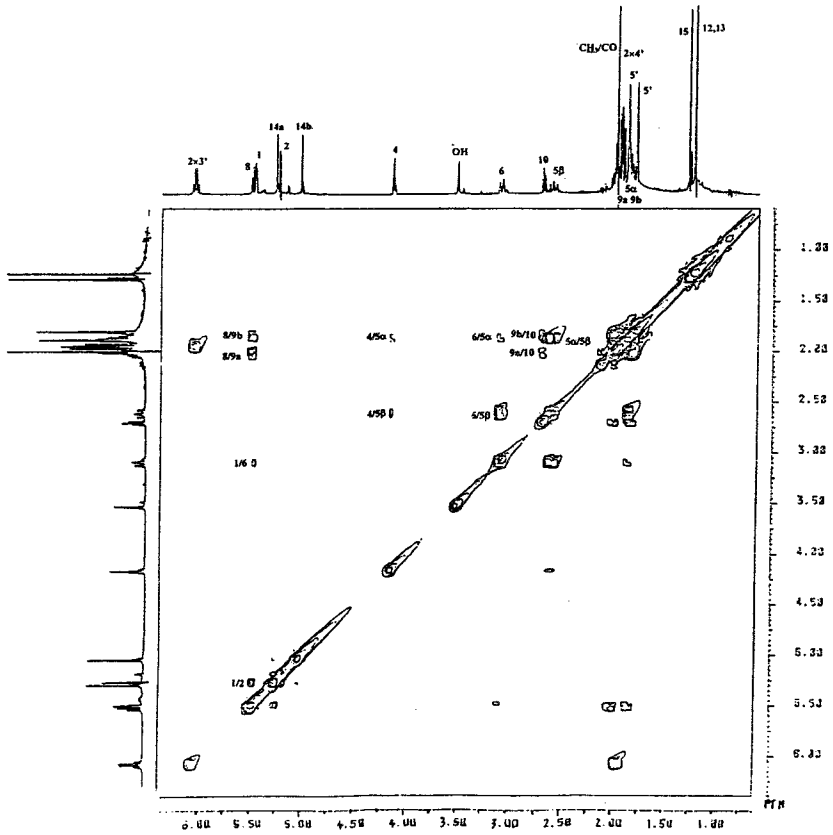

FIG 3. ^{1}H-^{1}H COSY spectrum of compound **1**

13; C-11/H-9, 10, 12, 13. Thus compound **1** was determined as a bisabolane sesquiterpene [6,7] with an epoxy group at C-10 and C-11. The locations of the three ester groups were also deduced from HMBC spectrum (FIG 4) : δ 168.3/H-1 and $COC\underline{H}_3$ (1.99, 3H, s), δ 166.5/H-2 and δ 166.0/H-8, so designing the acetoxy group to C-1 and two angeloyloxy groups to C-2 and C-8. The HMBC spectrum

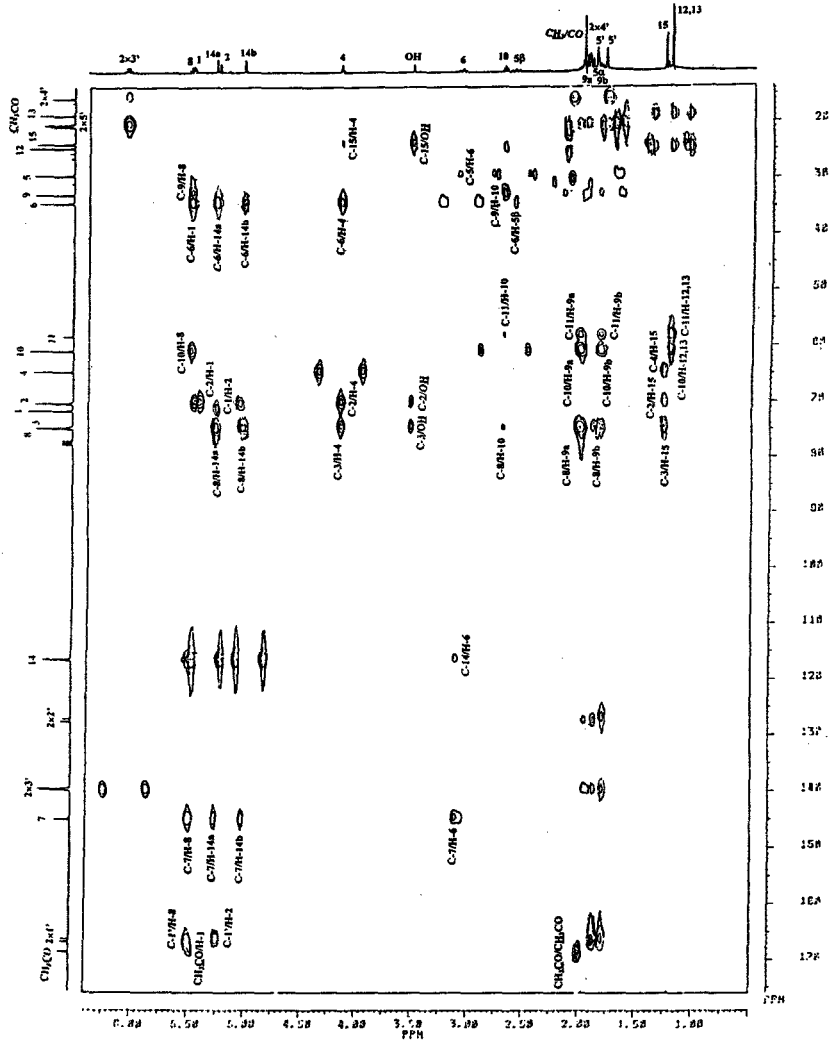

FIG. 4. HMBC spectrum of compound 1

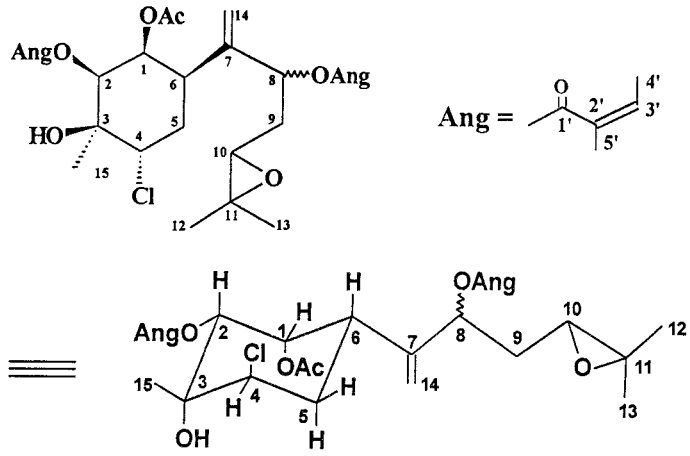

FIG 5. Structure of compound **1**

exhibited the position of hydroxyl group (δ_H = 3.52 ppm, exchanged in D_2O) by the cross peaks (FIG 4): HO/C-2, C-3 and C-15. Therefore, the hydroxyl group must substitute in C-3 (δ 74.3, C) and thus the chlorine atom in C-4 (δ 64.4, CH).

The stereochemistry of **1** was deduced on basis of the coupling magnitudes, (large vicinal couplings for axial and small couplings for equatorial protons) and ^1H-^1H NOESY spectrum. The observed triplet of the C-4 proton, with two small couplings constants ($J_{4,5\alpha}$ = $J_{4,5\beta}$= 2.9 Hz), is in agreement with the C-4 proton in equatorial position. A large coupling constants of the C-6 proton ($J_{5\beta,6}$ = 13.2Hz) indicated the C-6 proton must be in axial position. Another small coupling constants of the C-6 proton with the C-1 proton ($J_{1,6}$ = 3.0Hz) showed the C-1 proton must be in equatorial position and it was confirmed by the small coupling constants of the C-1 proton with the C-2 proton ($J_{1,2}$ = 3.3Hz). The ^1H-^1H NOESY spectrum revealed the important NOE cross peaks : H-1 with H-2 and H-6; H-2 with H-6 and H-15. That showed H-1, H-2, H-6 and H-15 in the same side of the ring (shown in FIG 5). Thus the structure of **1** was determined as 1*β*-acetoxy-2*β*,8-diangeloyloxy-3*β*-hydroxy-4*α*-chloro-10,11-epoxybisabol-7(14)-ene.

In our isolation process, we did not use chlorhydric acid and did not use chloroform as elution solvents, either. Therefore, we were convinced that compound **1** was a natural product.

ACKNOWLEDGMENT

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