Department of Pharmacy[1], Fujian Medical University, Fuzhou, Department of Biology[2], and Department of Chemistry[3], National Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou, P. R. China

Diterpenoids from the Chinese herb *Caryopteris terniflora* and their antibacterial and antitumor activity

YONG-HONG ZHANG[1], YAN-LIN WANG[2], QING-YI WEI[3], YU-JUN CAI[3], QIN WANG[2], ZHONG-LI LIU[3]

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Dr. Yong-Hong Zhang, Department of Pharmacy, Fujian Medical University, Fuzhou 350004, P.R. China
zhangyh@mail.fjmu.edu.cn

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Two *ent*-kaurene type diterpenoids, diterpenoids A (**1**) and B (**2**) were isolated from the Chinese herb *Caryopteris terniflora* and defined as *ent*-7β,11α,14-trihydroxy-18-aldehyde-11β-20-epoxy-kaur-16-en-15-one and *ent*-7β,14-dihydroxy-11α-methoxy-18-aldehyde-11β-20-epoxy-kaur-16-en-15-one respectively. Compounds **1** and **2** showed significant antibacterial and antitumour activity.

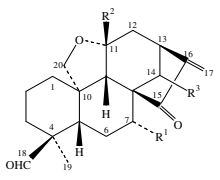
1. Introduction

Caryopteris terniflora Maxim. (Verbenaceae) has been used in China as a folk medicine for the relief of colds, headache, cough, scrofula and rheumatic pains (Jiangsu New Medical College 1977). Up to now, there is no report about its chemical constituents. During studies on the biologically active substances of the plants belonging to the genus *Caryopteris* (Gao and Han 1997; Zhang et al. 2000), we examined the constituents of the whole plants of *C. terniflora* and isolated two *ent*-kaurene type diterpenoids. The present paper reports the isolation, structural elucidation and antitumor and antibacterial activity of two diterpenoids from this herb.

2. Investigations, results and discussion

The chopped dry whole plant of *Caryopteris terniflora* was extracted with ethanol followed by careful column chromatographic separation to give compounds **1** and **2**.

Compound **1**, $C_{20}H_{26}O_6$, has a pentacyclic structure in which a ketone group is conjugated with an α-methelene group, as shown by the following spectral data: λ_{max} (MeOH) 238 nm; ν_{max} 1711 and 1639 cm^{-1}; ^1H NMR δ 6.21 and 5.39 (each 1 H, br s); ^{13}C NMR δ 152.79 (s), 116.69 (t) (exo-methylene) and 208.14 (s) (ketone). The ^1H NMR spectrum of **1** showed the presence of three hydroxyl groups [δ 8.26, 7.97 and 7.60 (each 1 H, s)] of which the ^1H NMR signals at δ 5.39 (s) and 4.94 (dd, J = 12.1, 3.3 Hz) coupled with the ^{13}C NMR signals at δ 73.89 and 73.83 (each d) showed that two were secondary. This fact suggested that, out of the three hydroxy groups in **1**, two are secondary and the third is tertiary. The ^1H NMR signals at δ 9.35 (1 H, s), 4.16 (1 H, d, J = 8.7 Hz) and 4.13 (1 H, dd, J = 8.7, 1.9 Hz), together with ^{13}C NMR signals at δ 205.27 (d) and 68.84 (t) showed the presence of an aldehyde group and a $-CH_2-O-$ group. The ^{13}C NMR spectrum of **1** also showed the presence of a methyl group, six methylenes, five methines, and four tetrasubstituted carbon atoms together with two olefinic, one aldehyde and one carbonyl carbon atom. These data, together with the consideration of the structure of diterpenoids previously isolated from the genus *Isodon* (Labiatae) (Fujita et al. 1976), suggested that compound **1** has the basic skeleton *ent*-kaur-16-en-15-one. Acetylation of **1** with acetic anhydride and pyridine gave the triacetylated product **3**. In the ^1H NMR spectrum of **3**, the signal of H-14 underwent a downfield shift to δ 6.09 (br s), suggesting that it was assignable to H-14α, which is affected anisotropically by the 15-carbonyl group and has an angle of *ca* 90° to the C-13-proton. Treatment of **1** with acetone and anhydrous copper (II) sulphate gave the acetonide **4**. This confirmed the presence of an α-oriented hydroxyl group at C-7 in **1**. From comparisons of the ^{13}C NMR spectra of **1** with those of other kaurene derivatives, it was assumed that no oxygenated group was present on the A-ring. The ^{13}C NMR signals of **1** at δ 68.84 (t) and 103.64 (s) coupled the movement of the ^1H NMR signals of H-9β and H-12α to lower field at δ 2.32 (1 H, s) and 3.21 (1 H, dd, J = 14.1, 9.0 Hz) and the movement of the ^{13}C NMR signals of C-9 and C-12 to lower field at δ 61.99 (d) and 45.99 (t), respectively, suggested the presence of a hemiacetal ring attached to the C-11 position (Li and Chen 1992; Wang et al. 1995). The CHO-4β was assigned by comparing its ^{13}C NMR spectral data with those of similar diterpenoids (Wang et al. 1986). Thus, the structure of compound **1** is elucidated as *ent*-7β,11α,14-trihydroxy-18-aldehyde-11β-20-epoxy-kaur-16-en-15-one. It is the first



1 $R^1=R^2=R^3=OH$

2 $R^1=R^3=OH$, $R^2=OCH_3$

3 $R^1=R^2=R^3=OAc$

4 $R^2=OH$, $R^1R^3=$

report of *ent*-kaurene type diterpenoid from *Caryopteris* plants (Hosozawa et al. 1973; 1974).

Compound **2**, $C_{21}H_{28}O_6$, has a five membered ring system with a ketone conjugated group to an α-methylene group, as shown by the following spectral data: ν_{max} 1640 cm^{-1}; ^1H NMR δ 6.18 and δ 5.53 (each 1 H, br s); ^{13}C NMR δ 149.97 (s), δ 119.25 (t) (exo-methylene) and 206.97 (s) (ketone). The ^{13}C NMR data of compound **2** showed the presence of a methyl group, six methylenes, five methines and four tetrasubstituted carbon atoms together with two olefinic, one aldehyde and one carbonyl carbon atom. The ^1H and ^{13}C NMR spectra of **2** were similar to **1**, however, there is an extra signal for an −OMe group attached to the 11β-position, as shown by the following spectral data. In the ^1H NMR spectrum, the signal of H-1α of **2** underwent an upfield shift by 0.71 ppm relative to the corresponding signal of **1** because of the space-steric effect of the −OMe group. On the other hand, in the ^{13}C NMR spectrum, the signals assignable to C-12 and C-9 of **2** underwent an upfield shift of 6.07 ppm and 1.87 ppm, respectively, and C-11 of **2** a downfield shift of 2.21 ppm compared to **1** because of the substituent effect due to the −OMe group. Accordingly, compound **2** is established as *ent*-7β,14-dihydroxy-11α-methoxy-18-aldehyde-11β-20-epoxy-kaur-16-en-15-one.

Compound **1** and **2** exhibited significant *in vitro* cytotoxic activity against human leukemia cells (HL-60), human hepatoma cells (SMMC-7721) and lung carcinoma cells (A-549) (Table 1), as well as antibacterial activity against *B. subtilis*, *E. coli* and *S. aureus* (Table 2). The antitumour activity of compound **1** and **2** is comparable to that of etoposide (VP-16) and 5-fluorouracil (5-FU) in case of HL-60 and SMMC-7721, and the antibacterial activity of both compounds **1** and **2** is comparable to that of chloramphenicol.

3. Experimental

3.1. Apparatus

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. The IR spectra were taken on a Nicolet 170SX IR spectrometer. ^1H, ^{13}C and 2D NMR spectra were recorded on a Bruker AM400 NMR spectrometer with TMS as internal standard.

Table 1: Antitumour activity of compound 1 and 2

	HL-60	SMMC-7721	A-549
1	12.29 ± 3.3	55.80 ± 2.4	131.21 ± 4.9
2	11.78 ± 2.1	98.14 ± 1.5	78.19 ± 4.2
VP-16	2.44 ± 1.1	NA	4.59 ± 1.7
5-FU	NA*	144.6 ± 26.9	NA

Activities are expressed as IC_{50} (50% inhibitory concentration) in μM.
Data are expressed as mean ± standard deviations of triplicate assay.
* Not assayed.

Table 2: Antibacterial activity of compound 1 and 2

	B. subtilis	E. coli	S. aureus
1	+++	+++	++
2	+++	+++	++
Chloramphenicol	+++	+++	+++

Zone diameter of growth inhibition: < 10 mm (−), 10∼12 mm (+), 13∼15 mm (++) and 16∼20 mm (+++). Diameter of the cup = 8 mm.

3.2. Plant material

C. terniflora plants were collected in Gansu Province of China and identified by Guoliang Zhang, a botanist at the department of biology, Lanzhou University where a voucher specimen has been deposited.

3.3. Extraction and isolation

Dyied and powdered whole plants (2 kg) were extracted with EtOH at room temperature and the solvent was evaporated. The residue (150 g) was separated by silica gel (200–300 mesh) column chromatography with gradient elution of petroleum ether-acetone. A gummy crude extract containing **1** and **2** was obtained from the fraction eluted with petroleum ether-acetone (3 : 1) and subjected to gel filtration (Sephadex LH-20) followed by silica gel (200–300 mesh) column chromatography eluted with petroleum ether-AcOEt (2 : 1) to give **1** (35 mg) and **2** (15 mg).

3.4. *ent*-7β,11α,14-Trihydroxy-18-aldehyde-11β-20-epoxy-kaur-16-en-15-one (1)

Cubic crystals from MeOH, m.p. 217–219 °C. $[\alpha]_D^{20}$-659.6° (*c* 0.10, MeOH). UV λ_{max}^{MeOH} nm: 238. IR (KBr, ν_{max}, cm^{-1}): 3422, 3196, 2921, 2741, 1711, 1639, 1463. EIMS (70eV) *m/z*: 362 $[M]^+$ (0.5), 344 $[M\text{-}H_2O]^+$ (20), 326 $[M\text{-}2H_2O]^+$ (15), 297 (21), 190 (50), 149 (45), 123 [A-ring-Me]$^+$ (33), 109 [A-ring-CHO]$^+$ (49), 91 (95), 55 (100); ^1H NMR (400 MHz, C_5D_5N): δ 0.93 (3 H, s, Me-19), 2.14 (1 H, q, J=12.4 Hz, H-6α), 2.32 (1 H, brs, H-9β), 3.21 (1 H, dd, J=14.1, 9.0 Hz, H-12α), 3.37 (1 H, brd, J = 9.5 Hz, H-13), 4.13 (1 H, dd, J = 8.7, 1.9 Hz, Ha-20), 4.16 (1 H, d, J = 8.7 Hz, Hb-20), 4.94 (1 H, dd, J = 12.1, 3.3 Hz, H-7β), 5.39 (2H, brs, Hb-17, H-14α), 6.21 (1 H, brs, Ha-17), 9.35 (1 H, s, CHO-18); ^{13}C NMR (100 MHz, C_5D_5N): δ 38.06 (t, C-1), 18.62 (t, C-2), 31.71 (t, C-3), 50.45 (s, C-4), 44.68 (d, C-5), 31.22 (t, C-6), 73.89 (d, C-7), 57.57 (s, C-8), 61.99 (d, C-9), 48.79 (s, C-10), 103.64 (s, C-11), 45.99 (t, C-12), 43.28 (d, C-13), 73.83 (d, C-14), 208.14 (s, C-15), 152.79 (s, C-16), 116.69 (t, C-17), 205.27 (d, C-18), 13.63 (q, C-19), 68.84 (t, C-20).

3.5. *ent*-7β,14-Dihydroxy-11α-methoxy-18-aldehyde-11β-20-epoxy-kaur-16-en-15-one (2)

Colorless crystals, m.p. 282–284 °C ($CHCl_3$-MeOH); $[\alpha]_D^{20}$-62.8° (*c* 0.07, MeOH); IR (KBr, ν_{max}, cm^{-1}): 3338, 1725, 1644, 1447, 1264; EIMS (70eV) *m/z*: 376 $[M]^+$ (0.5), 358 $[M\text{-}H_2O]^+$ (16), 344 $[M\text{-}OMe]^+$ (65), 297 (8), 204 (37), 192 (14), 123 (40), 109 (41), 91 (78); ^1H NMR (400 MHz, C_5D_5N): δ 0.97 (3 H, s, Me-19), 2.05 (1 H, q, J = 12.4 Hz, H-6α), 2.15 (1 H, s, H-9β), 3.17 (1 H, d, J = 9.0 Hz, H-13), 4.01 (1 H, dd, J = 8.8, 1.7 Hz, Ha-20), 3.90 (1 H, d, J = 8.8 Hz, Hb-20), 4.93 (1 H, dd, J = 8.5, 4.0 Hz, H-7β), 5.24 (1 H, s, H-14α), 5.53 (1 H, s, Hb-17), 6.18 (1 H, s, Ha-17), 9.33 (1 H, s, CHO-18), 3.13 (3 H, s, −OMe); ^{13}C NMR (100 MHz, C_5D_5N): δ 37.68 (t, C-1), 18.43 (t, C-2), 31.64 (t, C-3), 51.27 (s, C-4), 43.33 (d, C-5), 29.27 (t, C-6), 74.23 (d, C-7), 57.09 (s, C-8), 60.12 (d, C-9), 48.37 (s, C-10), 105.85 (s, C-11), 39.92 (t, C-12), 43.07 (d, C-13), 73.12 (d, C-14), 206.97 (s, C-15), 149.97 (s, C-16), 119.25 (t, C-17), 204.76 (d, C-18), 13.68 (q, C-19), 69.10 (t, C-20), 47.51 (q, −OMe).

3.6. Cytotoxicity assay

The cytotoxicity of compounds **1** and **2** was tested in three cell lines: HL-60 (human leukemia), SMMC-7721 (human hepatoma) and A-549 (lung carcinoma). Cells were cultured at 37 °C under a humidified atmosphere of 5% CO_2 in RPMI1640 medium supplemented with 10% fetal calf serum and dispersed in replicate 96-well plates with 1×10^4 cells/well for 24 h. Compound **1** (3.45–55.2 μM) or **2** (1.67–26.6 μM) and etoposide (VP-16) or 5-fluorouracil (5-FU) (positive control) were then added. After 48 h exposure to the toxins, cell viability was determined by the methylthiazolyltetrazolium bromide (MTT) colorimetric assay (Price and Mcmillan 1990) by measuring the absorbance at 595 nm with an ELISA reader.

3.7. Antibacterial assay

Compounds **1** and **2** were screened for their antibacterial activity against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* employing the cup-plate method (Xu and Bian 1982) in the nutrient agar media by measuring the inhibition zone in mm. Chloramphenicol was used as a control. The test was performed at 100 ug/ml concentration in a cup of 8 mm diameter (each cup 0.2 ml).

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