Chinese Chemical Letters Vol. 16, No. 7, pp 867-870, 2005 867
http://www.imm.ac.cn/journal/ccl.html

Synthesis of Boswellic Acid Derivatives and Primary Research on their Activities

Yan Qiu MENG[1,2], Lin Xiang ZHAO[1]*, Zan WANG[2], Dan LIU[1], Yong Kui JING[3]

[1]School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110015
[2] Teaching and Research Section of Pharmaceutical-engineering, Shenyang Institute of Chemical Technology , Shenyang 110142
[3] Mount Sinai School of Medicine, New York, USA

Abstract: In order to search for new potent anti-cancer agents, a series of boswellic acid derivatives were designed and synthesized. Six of them were identified by IR, NMR and MS as new compounds and biologic assay of anti-cancer is underway.

Keywords: Boswellic acid, design, synthesis.

Boswellic acid and its acetates, isolated from the gummy exudates of *Boswellia serrata* Roxb and *Boswellia carterri* Birdw, belong to the ursane-type pentacyclic triterpene class of compounds[1]. It has been found that acetyl-11-keto-β-boswellic acid (AKBA, 3-acetyl-11-oxo-ursa-12-en-24-oic acid) is an inhibitor of topoisomerase I and 5-lipoxygenase, and induces apoptosis in human leukemia, colon, hepatoma, and other malignant cell lines also[2-7]. Recently, it was found that α- and β-boswellic acid acetates without the 11-keto group can induce apoptosis in several leukemia cell lines also[8]. These data suggested that a group existing both in AKBA and boswellic acid acetates is required for its apoptosis induction ability.

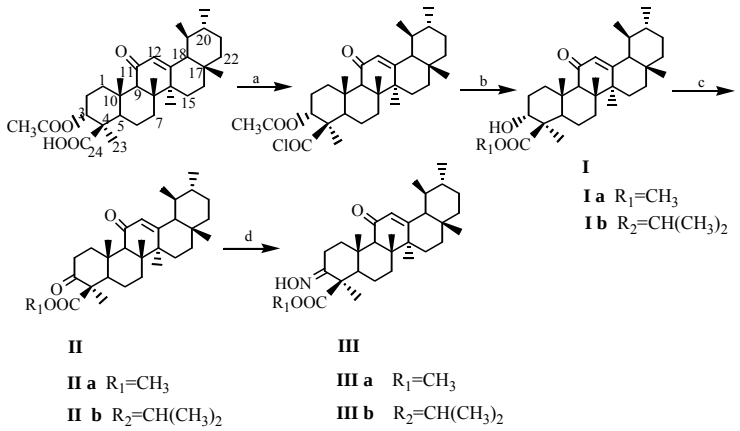
In this paper, AKBA was utilized as the parent compound from which six novel boswellic acid derivatives were designed and synthesized with substitutions at the positions 3 and 24, in order to find new anti-cancer drugs with novel action-mechanism and low toxicity. The synthetic route is outlined in **Scheme 1**. The apoptosis-induced ability of these compounds was evaluated in human NB4 leukemia cells.

Experimental

Infrared (IR) spectra were taken on DJ-IR-27G infrared spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker ARX-300MHz spectrometer using tetramethylsilane as an internal standard. Mass spectra were determined on an Agilent-1100 spectro-meter. Melting points were recorded on Büchi melting point apparatus (B-545).

* E-mail: zhaolx@mail.sy.ln.cn

Scheme 1



I

I a $R_1=CH_3$

I b $R_2=CH(CH_3)_2$

II

II a $R_1=CH_3$

II b $R_2=CH(CH_3)_2$

III

III a $R_1=CH_3$

III b $R_2=CH(CH_3)_2$

a)$SOCl_2$, 90 ℃, 2.5 hrs; b)R_1ONa, R_1OH, r.t; c)PCC/CH_2Cl_2 ,r.t; d)$H_2NOH{\cdot}HCl$, pyridine, 50℃, 2.5 hrs.

General procedures [9]:

A solution of AKBA (500 mg, 0.98 mmol) in thionyl chloride (2 mL) was refluxed for 3.0 h, and evaporated to dryness. The residue was treated with methanol-sodium methylate (5 mL) or isopropanol-sodium isopropoxide (5 mL), then the mixture was stirred for about 1.0 h at room temperature. The reaction mixture was isolated in the usual way, the residue was chromatographed on a silica gel column (eluent: petroleum /acetone, 15/1 or 30/1) to give a white powder **I_a** or **I_b** (yield 71.5% or 60.5%). To the CH_2Cl_2(5 mL) solution of **I_a** or **I_b** (500 mg) was added pyridinium chlorochromate (320 mg or 300 mg). After stirring for 2.5 h at room temperature, the mixture was diluted with CH_2Cl_2 and filtered, and chromatographed on silica gel column (eluent: petroleum/acetone, 60/1) to yield white powder of **II_a** or **II_b**(yield 40.3% or 35.1%). The CH_2Cl_2(5 mL) solution of **II_a** or **II_b**(500 mg) and hydroxylamine hydrochloride (300 mg or 320 mg) in pyridine (2 mL) was heated for 2.5 h at 50℃. After cooling to room temperature, the reaction mixture was isolated in the usual way, the residue was chromatographed on silica gel column (eluent: petroleum/acetone, 60/1) to yield white powder **III_a** or **III_b** (yield 38.8% or 31.0%).

The phrase "in the usual way" implies, dilution with water, extraction with methylene chloride, washing with aqueous HCl and water, drying over Na_2SO_4, filtration and evaporation to dryness under vacuum.

Biological assay

NB4 cells were treatment for 24 h at 30 μmol/L. The viable cells were determined after staining with trypan blue and the percentage of apoptotic cells was determined after staining with AO and EB. The data showed here is average of two independent experiments each with three triple samples. The primary results were summarized in **Table 1**.

Table 1 The percentages of apoptotic and viable cells after treatment with AKBA or the derivative of AKBA

Compounds	Viability (%)	Apoptosis (%)
AKBA	82.5	61.2
$\mathbf{I_a}$	86.5	76.6
$\mathbf{I_b}$	82.3	81.9
$\mathbf{II_a}$	98.0	1.5
$\mathbf{II_b}$	98.5	0.5
$\mathbf{III_a}$	77.0	35.1
$\mathbf{III_b}$	96.5	2.5

Discussion

Based on our finding that hydroxy and carboxyl group of ursolic acid or betulinic acid were modified, the activities of these derivatives can be increased[10,11]. The result demonstrates that the hydroxy and carboxyl group are active groups[12,13].

Compound $\mathbf{I_a}$ or $\mathbf{I_b}$ was obtained directly from AKBA through the hydrolysis of the 3-acetyl group as well as the chlorination and esterification of 24-carboxyl group, the intermediates were not purified. This procedure is an improvement on previous ones in terms of convenient operation[14,15].

The structure of compounds $\mathbf{II_a}$ or $\mathbf{II_b}$ contains two carbonyl groups (at position C-3 and position C-11). But under the experimental condition, only the carbonyl group at position C-3 was converted into corresponding oxime by treatment with hydroxylamine hydrochloride. By 1,4-nucleophilic addition the α,β-unsaturated carbonyl at position C-11 did not react with $NH_2OH{\cdot}HCl$.

Acknowledgment

Financial Supported by The G&P Foundation for Cancer Research; by Scientific Research Foundation for the Excellent Young Teacher from State Education Ministry (2002), and by Natural Science Foundation of Liaoning Province (No. 20022017) are gratefully acknowledged.

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16. $\mathbf{I_a}$: mp:216～219 ℃; LC-MS: 485.4[MH^+]; IR *v* (KBr, cm^{-1}): 1737, 1712, 1651, 1614; ^{1}H NMR (300MHz,$CDCl_3$, δ ppm): 5.55(s, 1H, H-12), 4.11(brs, 1H, H-3), 3.67(s, 3H, CH_3OCO-), 2.42(s, 1H, H-9), 1.31(s, 3H), 1.28(s, 3H), 1.17(s, 3H), 1.03(s, 3H), 0.94 (s, 3H)(5×CH_3), 0.82(3H, d, J=6.4Hz), 0.79(3H,d, J=6.4Hz)(2×CH_3). ^{13}C NMR (300MHz, $CDCl_3$, δ ppm): 199.5 (C11), 177.3 (C24), 164.9(C13), 130.6(C12), 70.8(C3), 60.4(C9), 59.1(C18), 51.3 (**C**H_3OCO-), 48.8(C5), 47.4(C4), 45.1(C8), 43.8(C14), 40.9(C22), 39.3(C19), 39.3(C20), 37.3(C10), 34.0(C1), 32.9(C7), 30.9 (C21), 29.7(C17), 28.9(C28), 27.5(C2), 27.2(C15), 26.3(C16), 24.2(C27), 21.1(C26), 20.5(C30) ,18.9(C6), 18.3(C25), 17.4(C29), 13.1(C23). $\mathbf{I_b}$: mp: 230～233 ℃; LC-MS: 513.5[MH^+]; IR *v* (KBr, cm^{-1}): 3550, 1701, 1658, 1614; ^{1}H NMR (300MHz, $CDCl_3$, δ ppm): 5.57(s,1H,H-12), 5.03(m, 1H, >CHO-), 4.12 (brs,1H, H-3), 2.48(s, 1H, H-9), 1.31(s, 3H), 1.23(s, 3H), 1.17(s, 3H), 1.09(s,3H), 0.98 (s,3H) (5×CH_3), 1.27～1.25(d, 6H, J=6.0Hz, $(CH_3)_2$CHO-, 2×CH_3), 0.82(d,3H, J=6.4Hz), 0.79(d, 3H, J=6.4Hz) (2×CH_3). ^{13}C NMR (300MHz, $CDCl_3$, δ ppm): 199.5(C11), 176.1(C24), 164.8 (C13), 130.6(C12), 70.8(C3), 67.5(-CHOCO-), 60.5(C9), 59.1(C18), 48.9(C5), 47.4(C4), 45.1(C8), 43.8(C14), 41.0(C22), 39.3(C19), 39.3(C20), 37.6(C10), 34.0(C1), 33.0(C7), 30.9(C21), 29.7(C17), 28.8(C28), 27.6(C2), 27.2(C15), 26.4(C16), 24.3(C27), 21.7, 21.6($(CH_3)_2$CHO-), 21.1(C26), 20.6(C30), 19.0(C6), 18.4(C25), 17.4(C29), 13.6(C23). $\mathbf{II_a}$: 256～258 ℃; LC-MS: 483.3 [MH^+]; IR v (KBr, cm^{-1}): 1725, 1712, 1651, 1616; ^{1}H NMR (300MHz, $CDCl_3$, δ ppm): 5.58 (s, 1H, H-12), 3.69 (s, 3H, CH_3OCO-),3.07~3.05 (t, 2H, J=7.9Hz, H-2) , 2.36 (s,1H, H-9), 1.30 (s, 3H), 1.28 (s, 3H), 1.22(s, 3H), 1.19(s, 3H), 0.95(s, 3H) (5×CH_3), 0.83(d, 3H, J=6.3Hz), 0.79 (d, 3H, J=6.3Hz) (2×CH_3). ^{13}C NMR (300MHz, $CDCl_3$, δ ppm): 208.5(C3), 198.7(C11), 173.9(C24), 165.4 (C13), 130.3 (C12), 60.0(C9), 59.1(C18), 58.4(C5), 57.5(C4), 52.1(**C**H_3OCO-), 44.8(C8), 43.8 (C14), 41.0(C22), 40.9(C1), 39.3(C19), 39.3(C20), 37.1(C10), 36.6(C2), 34.0(C17), 32.7 (C7), 30.9(C21), 29.7(C28), 28.9(C27), 27.5(C15), 27.3(C16), 21.1(C26), 20.4(C30), 19.7(C6), 18.3(C25), 17.4(C29), 13.2(C23). $\mathbf{II_b}$: mp: 188～190 ℃; LC-MS: 511.2[MH^+]; IR *v* (KBr, cm^{-1}): 1737, 1712, 1651, 1614; ^{1}H NMR (300MHz, $CDCl_3$, δ ppm): 5.58 (s,1H, H-12), 5.03~5.00 (m, 1H, >CHO-), 3.07~3.05 (t,2H, J=8.2Hz,H-2), 2.36 (s, 1H, H-9), 1.36(s, 3H), 1.34(s,3H), 1.28(s, 3H), 1.26(s, 3H), 0.94(s, 3H,) (5×CH_3), 1.24～1.22(d, 6H,J=6.0Hz, $(CH_3)_2$CHO-, 2×CH_3), 0.83(d, 3H, J=6.3Hz), 0.78 (d, 3H, J=6.3Hz) (2×CH_3). ^{13}C NMR (300MHz,$CDCl_3$, δ ppm): 208.8(C3), 199.0 (C11), 173.1(C24), 165.6(C13), 130.7(C12), 69.0(>CHO-), 60.3(C9), 59.4(C18), 58.7(C5), 58.1(C4), 45.2(C8), 44.1(C14), 41.4(C22), 41.2(C1), 39.6(C19), 39.6(C20), 37.5(C10), 37.0(C2), 34.3(C17), 33.1(C7), 31.2(C21), 29.1(C28), 27.8(C15), 27.6(C16), 21.8(C27), 21.5(C26), 21.4, 21.4 ((CH_3)$_2$-CHO), 20.7(C30), 20.0(C6), 18.6(C25), 17.7(C29), 14.0(C23). $\mathbf{III_a}$: mp: 196～200 ℃; LC-MS: 498.2[MH^+]; IR *v* (KBr, cm^{-1}): 3476, 1730, 1706, 1656, 1617; ^{1}H NMR (300MHz, $CDCl_3$, δ ppm): 5.49(s, 1H, H-12), 3.67(s, 3H, CH_3O-CO-), 3.25～3.20(d, 1H, H-2), 2.85～2.82 (d, 1H, H-2), 2.32 (s, 1H, H-9), 1.31,1.20, 1.13, 1.11, 0.87(s, 3H) (5×CH_3), 0.75(d, 3H, J=6.1Hz, CH_3), 0.71(d, 3H, J=6.3Hz, CH_3). ^{13}C NMR (300MHz, $CDCl_3$, δ pm): 199.0(C11), 175.1(C24), 165.1 (C13), 162.3 (C3), 130.5(C12), 60.2(C9), 59.1(C18), 58.0(C5), 52.1 (CH_3OCO-), 50.7(C4), 45.0(C8), 43.8(C14), 40.9(C22), 39.7(C1), 39.3(C19), 39.3(C20), 37.3(C10), 34.0(C17), 32.9(C7), 30.9(C21), 29.7(C2), 28.9(C28), 27.5(C15), 27.3(C16), 22.4(C27), 21.1(C26), 20.4(C30), 19.4(C6), 18.4(C25), 17.5(C29), 13.3(C23). $\mathbf{III_b}$: mp: 235～239 ℃; LC-MS: 526.5[MH^+]; IR *v*(KBr, cm^{-1}): 3404, 1720, 1680, 1641, 1606; ^{1}H NMR(300MHz, $CDCl_3$, δ ppm): 5.56(s, 1H, H-12), 5.03～4.95(m, 1H, >CHO-), 3.32～3.27(d, 1H, H-2), 2.90～2.86(d, 1H, H-2), 2.34(s, 1H, H-9), 1.26～1.24 (d, 6H, J=5.8Hz, $(CH_3)_2$CHO-), 1.44, 1.23, 1.20, 1.18, 0.94(s, 3H)(5×CH_3), 0.82(d, 3H, J=6.0Hz, CH_3), 0.78 (d, 3H, J=6.3Hz, CH_3). ^{13}CNMR (300MHz, $CDCl_3$, δ ppm): 199.0(C11), 174.3(C24), 165.1(C13), 162.3(C3), 130.5(C12), 68.4(>CHO-), 60.2(C9), 59.1(C18), 58.0(C5), 50.7(C4), 45.0(C8), 43.8(C14), 41.0(C22), 39.8(C1), 39.3(C19), 39.3(C20), 37.5(C10), 34.0(C17), 33.0(C7), 30.9(C21), 29.7(C2), 28.9(C28), 27.6(C15), 27.3(C16), 22.3(C27), 21.7(C26), 21.4, 21.1((CH_3)$_2$CHO-), 20.4(C30), 19.5(C6), 18.4 (C25), 17.5(C29), 13.7(C23).

Received 19 July, 2004