Journal of Chemistry, Vol. 45 (3), P. 382 - 385, 2007

CHEMICAL TRANSFORMATION OF *ENT*-KAURANE-TYPE DITERPENOIDS FROM *CROTON TONKINENSIS* GAGNEP.

I - HYDROLYSIS, ACETYLATION, AND OXIDATION OF *ENT*-18-ACETOXY-7β-HYDROXYKAUR-16-EN-15-ONE

Received 28 August 2006

PHAN MINH GIANG, LE THI HONG DUNG, PHAN TONG SON

Laboratory of Chemistry of Natural Products, Faculty of Chemistry

College of Natural Science, Vietnam National University, Hanoi, Vietnam

SUMMARY

*In our studies on the phytochemistry and biological activities of Croton tonkinensis Gagnep. (Euphorbiaceae) oriented by traditional medicine interesting biological activities of the plant were demonstrated in correlation with the ent-kaurane-type diterpenoid constituents. The high accumulation of the active principle ent-18-acetoxy-7β-hydroxykaur-16-en-15-one (**1**) led to our recent study on the chemical modification of this lead compound. In this paper the transformations of **1** into several derivatives by hydrolysis, acetylation, and oxydation were reported. The cytotoxic activity against of the transformation products Hep-G2 and LU cells was evaluated.*

Keywords: *Croton tonkinensis*; Euphorbiaceae; *ent*-kaurane-type diterpenoid; chemical transformation; cytotoxicity

I - INTRODUCTION

Croton tonkinensis Gagnep. (Euphorbiaceae) is a small plant of 1 - 2 m high and known in Vietnamese as *Kho sam Bac Bo* or *Kho sam cho la* [1 - 3]. The plant occurs widely and also cultivated as a medicinal plant in northern Vietnam. Its dried leaves (*Folium Tonkinensis*) have been used in Vietnamese traditional medicine to treat boils, abscesses, impetigo, abdominal pain, dyspepsia, dysentry, gastric and duodenal ulcers. Moreover, it is a component of recipes applied to cure urticaria, leprosy, psoriasis, vaginitis due to trichomonas and genital organs prolapse. The studies on phytochemistry and biological activities of *C. tonkinensis* were oriented by traditional medicine. The anti-inflammatory activity of the plant was assessed using the nuclear factor kappa B (NF-κB) reporter gene assay and nitric oxide (NO) production assay. For the first time the accumulation of *ent*-kaurane-type diterpenoids in *C. tonkinensis* leaves was revealed [4, 5] and they were demonstrated to be responsible for the activity. Previously it was believed that the alkaloidal and flavonoidal constituents were responsible for the biological activities of the plant [6]. The genus *Croton* L. (Euphorbiaceae) consists of 800 species mainly distributed in tropical regions, among which 32 species grow in Vietnam [1]. Among the *Croton* species *ent*-kaurane-type diterpenoids were isolated from *C. argyrophylloides* [7], *C. lacciferus* [8], *C. argyrophylliides* [9], *C. sublyratus* [10], and *C. kongensis* [11], therefore the disclosure of this type of diterpenoids in *C.*

tonkinensis is of high interest from chemotaxonomic point of view. Further, the assumption on the presence of *ent*-kaurane-type diterpenoids as main active principles of *C. tonkinensis* was confirmed by integrated phytochemical and biological studies of *C. tonkinensis* [12 - 15, 20]. In addition phytosterols [16], long chain alkyl alcohols [17], and flavonoid glucosides [18] were isolated from the plant. On removal of the sterols, alcohols, and flavonoids, the antimicrobial [16], antiplasmodial [16, 19], antistaphylococcal [14], and cytotoxic [13] activities studied were selective for the diterpenoids. The main diterpenoid constituent of *C. tonkinensis* leave extract was found to be *ent*-18-acetoxy-7β-hydroxykaur-16-en-15-one (**1**) [4]. In the "lead optimization" process focusing on **1** we designed chemical transformations to yield several oxygenated derivatives of **1**.

II - EXPERIMENTAL

1. General Procedure

Melting points were recorded on a Boetius melting point apparatus. FT-IR spectra were recorded on a Impact 410-Nicolet FT-IR spectrometer. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were obtained on a Bruker Avance 500 spectrometer with tetramethyl silane as reference. EI-MS (70 eV) were measured on a Hewlett Packard 5989B mass spectrometer. Silica gel (63 - 100 μm, Merck) was used for open column chromatography (CC). TLC was performed on precoated DC Alufolien 60 F_{254} plates (Merck) and detected by UV light (254 nm) or by spraying with 1% vanillin in concentrated H_2SO_4.

2. Plant Material

The leaves of *C. tonkinensis* were collected in Hanoi, Vietnam, in September 2004. A voucher specimen was identified by Dr Tran Ngoc Ninh, Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology, Hanoi, Vietnam.

3. Extraction and Isolation

The leave powder (1 kg) was extracted with MeOH and fractionated with solvents of increasing polarity as described in [5]. The combined *n*-hexane- and CH_2Cl_2-soluble fractions were chromatographed on a silica gel column using *n*-hexane-EtOAc (gradient) as eluent to give **1** (870 mg, 0.09% yield on the basis of dry weight of leaves).

Ent-18-acetoxy-7β-hydroxykaur-16-en-15-one (**1**): white needles, m.p. 135 - 138°C, R_f = 0.46 (silica gel TLC, Me_2CO-CH_2Cl_2, 10:1), IR, EI-MS, and ^{1}H-NMR (500 MHz, $CDCl_3$) spectroscopic data were superimposable with the reported data [4].

*Alkaline hydrolysis of **1***. Compound **1** (47 mg) was hydrolyzed with 10 drops of 5% aqueous KOH in 400 μl Me_2CO for 15 hr at 80°C. After removal of solvent by evaporation, the residue was purified using preparative (octadecyl silica gel) ODS-HPLC (MeOH-H_2O, 3:2) to give **2** (yield 47.4%).

Ent-7β,18-dihydroxykaur-16-en-15-one (**2**): White amorphous powder, analytical HPLC R_t = 21.83 min (ODS gel, CH_3OH-H_2O, 3:2). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.75 (3H, s, Me-19), 1.13 (3H, s, Me-20), 3.06 (1H, d, *J* = 10.8 Hz, H-18a), 3.08 (1H, s, H-13), 3.49 (1H, d, *J* = 10.8 Hz, H-18b), 4.15 (1H, dd, *J* = 11.1 Hz, 4.2 Hz, H-7), 5.27 (3H, s, H-17a), 5.96 (3H, s, H-17b).

*Acetylation of **1***. Compound **1** (100 mg) was acetylated with a mixture of acetic anhydride (0.4 ml) and pyridine (0.5 ml) at room temperature for 3 days. The reaction mixture was extracted with CH_2Cl_2 and the resultant solution was evaporated till dryness under reduced pressure. The residue was separated on a silica gel column (*n*-hexane-acetone, 6:1) to give **3** (yield 89.5%).

Ent-7β,18-diacetoxykaur-16-en-15-one (**3**): White needles, m.p. 139 - 141°C, R_f = 0.63 (silica gel TLC, *n*-hexane-EtOAc, 2:1). IR ν_{max} (KBr) cm^{-1}: 1736, 1642, 1459, 1369, 1250, 1038; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.82 (3H,

s, Me-19), 1.15 (3H, s, Me-20), 1.91 (3H, s, 7-OAc), 2.13 (3H, s, 18-OAc), 3.10 (1H, brs, H-13), 3.60 (1H, d, J = 11 Hz, H-18a), 3.85 (1H, d, J = 11 Hz, H-18b), 5.10 (1H, dd, J = 11 Hz, 4.5 Hz, H-7), 5.29 (1H, s, H-17a), 5.96 (1H, s, H-17b); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 17.6 (t, C-2), 17.7 (q, C-19), 17.9 (t, C-11), 18.2 (q, C-20), 21.0 (q, 7-OAc and 18-OAc), 24.4 (t, C-6), 29.2 (t, C-14), 32.6 (t, C-12), 35.7 (t, C-3), 35.7 (d, C-13), 36.4 (s, C-4), 39.0 (t, C-1), 39.7 (s, C-10), 45.9 (d, C-5), 51.9 (d, C-9), 56.2 (s, C-8), 72.0 (t, C-18), 73.1 (d, C-7), 115.2 (t, C-17), 148.8 (s, C-16), 169.5 (s, 7-OAc), 171.4 (s, 18-OAc), 207.4 (s, C-15); EI-MS (70 eV): *m/z* (%) 402 ($C_{24}H_{34}O_5$, $[M]^{+\cdot}$) (1), 360 (6), 342 (26), 299 (5), 282 (100), 269 (37), 239 (12), 225 (14), 213 (12), 187 (14), 173 (22), 147 (18), 131 (22), 121 (24), 91 (55), 67 (37), 55 (63).

*Oxidation of **1***. To compound **1** (100 mg) in $CHCl_3$ (2 ml) a solution of $K_2Cr_2O_7$ (100 mg) in H_2O (5 ml) and concentrated H_2SO_4 (0.3 ml) was added. The solution was stirred under reflux at 50 - 60°C for 3 days. The reaction mixture was extracted with $CHCl_3$ and the resultant solution was evaporated till dryness under reduced pressure. The residue was separated on a silica gel column (*n*-hexane-acetone, 6 : 1) to give **4** (yield 82.5%).

Ent-18-acetoxykaur-16-en-7,15-dione (**4**): White needles, m.p. 105-107°C, R_f=0.69 (silica gel TLC, *n*-hexane-EtOAc, 2 : 1). IR ν_{max} (KBr) cm^{-1}: 1734, 1696, 1643, 1450, 1382, 1235, 1040; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.85 (3H, s, Me-19), 0.94 (3H, s, Me-20), 2.11 (3H, s, 18-OAc), 2.40 (1H, dd, J = 18.5 Hz, 11.5 Hz, H-6a), 2.71 (1H, dd, J = 18.5 Hz, 8 Hz, H-6b), 3.02 (1H, dd, J = 8.5 Hz, 4.5 Hz, H-13), 3.64 (1H, d, J = 11 Hz, H-18a), 3.82 (1H, d, J = 11 Hz, H-18b), 5.39 (1H, s, H-17a), 5.92 (1H, s, H-17b); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 14.8 (q, C-20), 17.2 (q, C-19), 17.5 (t, C-2), 17.7 (t, C-11), 21.0 (q, 18-OAc), 27.7 (t, C-6), 30.2 (t, C-14), 35.1 (d, C-13), 35.8 (t, C-3), 36.8 (s, C-4), 38.2 (s, C-10), 38.4 (t, C-12), 39.2 (t, C-1), 44.0 (d, C-5), 54.9 (d, C-9), 63.8 (s, C-8), 72.2 (t, C-18), 116.9 (t, C-17), 151.2 (s, C-16), 171.5 (s, 18-OAc), 203.2 (s, C-15), 210.7 (s, C-7); EI-MS (70 eV): *m/z* (%) 358 ($C_{22}H_{30}O_4$, $[M]^{+\cdot}$) (5), 330 (4), 298 (100), 283 (24), 255 (14), 241 (7), 227 (10), 213 (12), 189 (15), 173 (10), 159 (14), 149 (18), 134 (33), 91 (65), 79 (55), 67 (37), 55 (48).

III - RESULTS AND DISCUSSION

The interesting biological activities of **1** were shown in our recent studies [5, 13, 14, 16, 19]. HPLC analysis revealed the presence of **1** as the main diterpenoid constituent in the MeOH extract from the leaves of *C. tonkinensis*. Following by the "lead discovery", we prepared in this study several derivatives from **1** for the "lead optimization" process. By using systematic extraction and chromatographic isolation schemes pure **1** could be obtained in the yield of 0.09% of dry weight of leaves (see Experimental). Treating **1** with KOH/MeOH, Ac_2O/pyridine, and $K_2Cr_2O_7$/ concentrated H_2SO_4, gave diol **2**, diacetate **3**, and dione **4** from **1**, respectively.

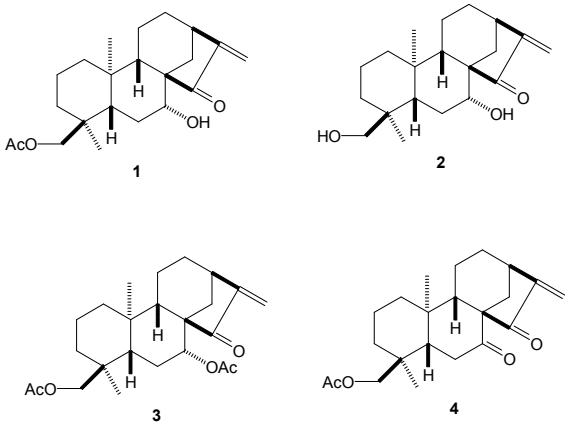

Fig. 1: Chemical structures of **1** and its derivatives (**2**, **3** and **4**)

Compound **2** (47.4% yield) was isolated from a reaction mixture of **1** and **2** (ODS-HPLC), showing that longer time and harder conditions are required to complete the reaction. The ^{1}H-NMR showed the replacement of the acetoxyl group at C-18 by a hydroxy group [δ3.06 and 3.49 (1H, each d, J = 10.8 Hz]. The observed upfield shifts of the H-18a ($\Delta\delta_H$ –0.6) and H-18b ($\Delta\delta_H$ –0.38) supported the structure of **2**. On acetylation the IR band for hydroxyl

group in **1** disappeared. Accordingly, in the ^{1}H- and ^{13}C-NMR spectra of **3** H-7 (δ_H 5.1) and C-7 (δ_C 73.1) displayed downfield shift ($\Delta\delta_H$ +1.05 and $\Delta\delta_C$ +2.3). Therefore the hydroxyl group at C-7 was completely transformed into an acetoxy group (δ_C 169.5, 21.0; δ_H 1.91). The molecular formula (*m/z* 402, $C_{24}H_{34}O_5$, $[M]^{+\cdot}$, EI-MS) supported the diacetyl structure of **3**. The EI-MS spectrum of **4** showed the ion peak at *m/z* 358 ($C_{22}H_{30}O_4$, $[M]^{+\cdot}$). The IR spectrum displayed a new ketone band at ν_{max} 1696 cm^{-1} but no hydroxyl group. A ketone group appeared at C-7 (δ_C 210.7) in the ^{13}C-NMR spectrum of **4** and H-7 signal completely disappeared from the ^{1}H-NMR spectrum of **4**.

The cell culture human tumour cell line assay [21] against Hep-G2 and LU cells showed the decrease in IC_{50} on going from **1** (0.196 and 0.154 μg/ml, respectively) to **3** (0.36 and 0.454 μg/ml, respectively) and **4** (0.255 and 0.315 μg/ml, respectively).

Acknowledgements: *This research was supported by the International Foundation for Science (**IFS**, Stockholm, Sweden) through a Research Grant to Phan Minh Giang and the Basic Research Program in Natural Sciences of Vietnam.*

REFERENCES

1. Selected Medicinal Plants in Vietnam, ed. Le V. T., Nguyen G. C., P. 260 - 262, Science and Technology, Hanoi (1999).
2. Do T. L., Medicinal Plants and Herbal Remedies of Vietnam, P. 907-908, Science and Technique, Hanoi (1991).
3. Vo V. C., Dictionary of Vietnamese Medicinal Plants, P. 622-623, Medicine, Ho Chi Minh City (1997).
4. Phan T. S., Phan M. G., Taylor Walter C., Austr. J. Chem., 53, 1003 - 1005 (2000).
5. Phan M. G., Jin H. Z., Phan T. S., Lee J. H., Hong Y. S., Lee J. J., J. Nat. Prod., 66, 1217 - 1220 (2003).
6. Be T. T., Truong V. N., Vietnam Pharmaceutical J., 31, 11 - 12 (1991).
7. Kitazawa E., Ogiso A., Phytochemistry, 20, 287-289 (1981).
8. Monte F. J. Q., Andrade C. H. S., Craveiro A. A., J. Nat. Prod., 47, 55 - 58 (1984).
9. Monte F. J. Q., Dantas E. M. G., Braz F. R., Phytochemistry, 27, 3209 - 3212 (1988).
10. Ratnayake B. B. M., Wimalasiri W. R., Phytochemistry, 27, 225 - 226 (1988).
11. Thongtan J., Kittakoop P., Ruangrungsi N., Saenboonrueng J., Thebtaranonth Y., J. Nat. Prod., 66, 868 - 870 (2003).
12. Phan M. G., Phan T. S., Lee J. L., Otsuka H., Chem. Pharm. Bull., 52, 879-882 (2004).
13. Phan M. G., Phan T. S., Hamada Y., Otsuka H., Chem. Pharm. Bull., 53, 296 - 300 (2005).
14. Phan M. G., Phan T. S., Matsunami K., Otsuka H., J. Nat. Med., 60, 93 - 95 (2006).
15. Phan M. G., Otsuka H., Phan T. S., Vietnam J. Chem., 43, 263 - 264 (2005).
16. Phan T. S., Le H. T., Phan M. G., Vietnam J. Chem., 40, 53 - 57 (2002).
17. Phan M. G., Phan T. S., Vietnam J. Chem., 42, 132 (2004).
18. Phan M. G., Lee J. J., Phan T. S., Vietnam J. Chem., 42, 125 - 128 (2004).
19. Phan T. S., Van N. H., Phan M. G., Taylor W. C., Vietnam J. Chem., 37, 1 - 2 (1999).
20. Phan M. G., Lee J. J., Phan T. S., Vietnam J. Chem., 41, 1 (2003).
21. Likhitwitayawuid K., Angerhofer C. K., Cordell G. A., Pezzuto J. M., Ruangrungsi N., J. Nat. Prod., 56, 30 - 38 (1993).