Tetrahedron 71 (2015) 9638–9644

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

# Cytotoxic tigliane-type diterpenoids from *Croton tiglium*

Dong-Dong Zhang, Bin Zhou, Jin-Hai Yu, Cheng-Hui Xu, Jian Ding, Hua Zhang *, Jian-Min Yue *

*State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, People's Republic of China*

ARTICLE INFO

*Article history:*
Received 14 September 2015
Received in revised form 22 October 2015
Accepted 26 October 2015
Available online 5 November 2015

*Keywords:*
*Croton tiglium*
Diterpenoid
Tigliane
Cytotoxicity

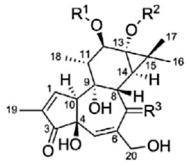
ABSTRACT

Chemical study on the twigs and leaves of *Croton tiglium* has led to the isolation of 11 new tigliane-type diterpenoids, crotignoids A–K (**1**–**11**), along with eight known analogues (**12**–**19**). The structures of these compounds were elucidated on the basis of detailed spectroscopic analysis. In vitro cytotoxic assays revealed moderate inhibition of compounds **1**–**10** toward human HL-60 and A549 tumor cells, with **1** exhibiting the strongest activity against HL-60 cell line with an $IC_{50}$ value of 1.61 μM.

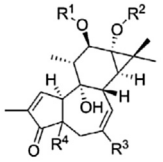


## 1. Introduction

The genus *Croton* (Euphorbiaceae) comprises more than 1300 species mainly distributed in the tropical and subtropical regions of the world.[1] Many species of this genus are used as folk medicines for the treatment of stomachache, abscesses, malaria, inflammation, and so on.[2–4] The *Croton* plants are rich sources of terpenoids displaying a broad spectrum of biological activities, such as acetylcholinesterase inhibitory,[2] neurite outgrowth-promoting,[5] antimycobacterial,[6] antifungal,[7] antiplasmodial,[8] cytotoxic,[9,10] antiviral,[11] and antiinflammatory activities.[12,13] *Croton tiglium* L. is a shrub or small tree that grows in southeast of China such as Zhejiang and Fujian Provinces.[14] Phorbol esters, the typical metabolites of *C. tiglium*, were found to possess tumor-promoting function by Van Duuren and co-workers in 1963.[15] Fifteen years later, Castagna and co-workers discovered the functional mechanism that the protein kinase C (PKC) is the receptor of phorbol esters.[16] In continuing our efforts to identify structurally diverse and biologically important compounds from medicinal plants, 11 new diterpenoids crotignoids A–K (**1**–**11**) and eight known analogues were isolated from *C. tiglium* L. Subsequent in vitro cytotoxic evaluation revealed that most new compounds displayed cytotoxic activities against two human tumor cell lines A549 and HL-60. Of these compounds, crotignoid A (**1**) showed the best activity against HL-60 cell line with an $IC_{50}$ value of 1.61 μM. We herein present the isolation, structural elucidation, and biological tests of these diterpenoids.



| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **1** | tiglyl | 2-methylbutyryl | H,β-OOH |
| **2** | tiglyl | isobutyryl | H,β-OOH |
| **3** | tiglyl | 2-methylbutyryl | H,β-OH |
| **4** | tiglyl | isobutyryl | H,β-OH |
| **5** | tiglyl | isobutyry | O |
| **6** | tiglyl | propionyl | O |
| **7** | H | decanoyl | O |



| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| **8** | tiglyl | isobutyryl | CHO | β-OH |
| **9** | 2-methylbutyryl | 2-methylbutyryl | $CH_2OH$ | β-OH |
| **10** | benzoyl | isobutyryl | $CH_2OH$ | β-OH |
| **11** | benzoyl | acetyl | $CH_2OH$ | α-H |
| **12** | tiglyl | 2-methylbutyryl | $CH_2OH$ | β-OH |
| **13** | isobutyryl | acetyl | $CH_2OH$ | β-OH |
| **14** | tiglyl | propionyl | $CH_2OH$ | β-OH |
| **15** | tiglyl | isobutyryl | $CH_2OH$ | β-OH |
| **16** | 2-methylbutyryl | acetyl | $CH_2OH$ | β-OH |
| **17** | tiglyl | acetyl | $CH_2OH$ | β-OH |
| **18** | 2-methylbutyryl | isobutyryl | $CH_2OH$ | β-OH |
| **19** | tiglyl | acetyl | $CH_2OH$ | α-H |

* Corresponding authors. Tel.: +86 21 50806718; fax: +86 21 50807088; e-mail addresses: h.zhang@simm.ac.cn (H. Zhang), jmyue@simm.ac.cn (J.-M. Yue).

http://dx.doi.org/10.1016/j.tet.2015.10.070
0040-4020/

## 2. Results and discussion

Compound **1** was assigned a molecular formula of $C_{30}H_{42}O_{10}$ based on $^{13}C$ NMR data and the (+)-HRESIMS ion peak at *m/z* 585.2660 $[M+Na]^+$ (calcd 585.2670), requiring 10 indices of hydrogen deficiency. The IR spectrum showed absorption bands for hydroxyl (3389 $cm^{-1}$), carbonyl (1713 $cm^{-1}$) and vinyl (1651 $cm^{-1}$) groups. Analysis of the NMR data (Tables 1 and 2) revealed characteristic signals for a 2-methylbutyryl, a tiglyl, and an α,β-unsaturated carbonyl groups, as well as a trisubstituted double bond, six methines (two oxygen bonded), an oxygenated methylene, and four methyls. These observations accounted for six out of 10 indices of hydrogen deficiency supportive of a tetracyclic carbon skeleton for **1**. Further interpretation of 2D NMR especially HMBC data (Fig. 1A) established that compound **1** was a diterpenoid of the tigliane family as briefly characterized below. The HMBC correlations of H-19/C-1, C-2 and C-3; H-5/C-3, C-4 and C-10; H-10/C-1; and H-1/C-4 confirmed the presence of the methylsubstituted five-membered ring-A incorporating an α,β-conjugated ketone moiety. Similarly, the cyclopropyl ring-D with gem-dimethyl substitution was also established via the mutual correlations between the two methyl groups both also showing correlations with C-13, C-14 and C-15. Subsequent observations of the correlations of H-10/C-9; $H_3$-18/C-9, C-11 and C-12; H-12/C-13; H-8/C-9 and C-14; H-7/C-8; and $H_2$-20/C-5, C-6, and C-7, facilitated the construction of ring-B and ring-C as shown. The 12-*O*-tiglyl group was evidenced by the HMBC correlation from H-12 ($\delta_H$ 5.52) to its carbonyl carbon ($\delta_C$ 167.8), while the presence of 4-OH, 9-OH, 20-OH and 13-*O*-(2-methylbutyryl) groups was indicated by the chemical shifts of C-4, C-9, C-20 and C-13 ($\delta_C$ 72.6, 75.1, 66.6 and 65.9, respectively) and comparison with known tigliane analogues possessing the same substitution patterns.[17] The structural characterization presented above explained all but the elements of $O_2H$ which was identified to be a hydroperoxyl group at C-7 based the on the chemical shift of the latter ($\delta_C$ 83.6) and excellent comparison to 12-*O*-decanoyl-7-hydroperoxy-phorbol-5-ene-13-acetate having the same 7-$O_2H$ functionality.[18]

The relative configuration of **1** was characterized mainly by NMR comparison to 12-*O*-decanoyl-7-hydroperoxy-phorbol-5-ene-13-acetate[18] and examination of NOESY data (Fig. 1B). The only differences between the two compounds were attributable to the *O*-substituents at C-12 and C-13 and the remaining structural parts displayed highly comparable NMR data, which indicated that the two molecules possessed the same relative configuration. Moreover, the NOESY correlations of H-10/H-7, H-7/9-OH, and 9-OH/H-11 suggested that they were *pseudo*-axially bonded and α-oriented, while those of H-8/H-11, H-8/$H_3$-17, and H-7/H-14 supported the β-direction of H-8, H-11 and the cyclopropyl moiety. The structure of **1** was thus unequivocally characterized and was named crotignoid A.

Compound **2** displayed a sodiated molecular ion peak at *m/z* 571.2507 $[M+Na]^+$ in the (+)-HRESIMS spectrum, consistent with a molecular formula of $C_{29}H_{40}O_{10}$ (calcd 571.2514). Analysis of the NMR data (Tables 1 and 2) revealed a tigliane scaffold for **2** showing high similarity to **1**. Compared with **1**, the absence of NMR signals for the 2-methylbutyryl group and the presence of those for an isobutyryl residue ($\delta_H$ 2.59 (m), 1.19 (d, 7.0) and 1.17 (d, 7.0)) supported a 13-*O*-isobutyl substitution in **2**. The HMBC correlation from H-12 ($\delta_H$ 5.50) to the tiglyl carbonyl carbon ($\delta_C$ 167.9) further supported the above-mentioned assignment. The remaining NMR resonances for the two compounds were almost superimposable indicating a common relative stereochemistry. Thus the structure of **2** was elucidated as shown. The presence of the hydroperoxyl group in **1** and **2** is interesting, and this functionality has also been found in other types of natural products.[19–21] However, the possibility that **1** and **2** might be handling artifacts could not be totally excluded, since the formation of hydroperoxyl derivative from phorbol esters was once reported.[22,23]

Compounds **3** and **4** had molecular formulae of $C_{30}H_{42}O_9$ and $C_{29}H_{40}O_9$ as deduced from the HRESIMS ions at *m/z* 591.2792($[M+HCO_2]^-$, calcd 591.2811) and 555.2568 ($[M+Na]^+$, calcd 555.2565), respectively. The NMR data (Tables 1 and 2) of **3** exhibited high similarity to those of **1** with major differences occurring to signals around C-7. Compared with **1**, the markedly upfield shifted C-7 resonance ($\Delta\delta_C$ 13.3) of **3** suggested the presence of a 7-OH instead of the hydroperoxyl group in the former. The relative stereochemistry of **3** was assigned to be identical with that of **1** based on the same proton coupling patterns and close coupling constants, and this was also confirmed by acquisition of NOESY data (Fig. S20, Supplementary data). The NMR data of **4** (Tables 1 and 2)

**Table 1**
$^1H$ NMR data of compounds **1**–**6** (500 MHz, $CDCl_3$)

| No. | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | 7.65 brs | 7.64 brs | 7.61 brs | 7.61 brs | 7.67 brs | 7.67 brs |
| 5 | 6.39 d (2.4) | 6.39 d (2.4) | 6.13 brs | 6.09 d (1.6) | 6.90 brs | 6.91 brs |
| 7 | 4.78 dd (9.2, 2.4) | 4.78 dd (9.0, 2.4) | 4.78 brd (9.7) | 4.82 dd (9.6, 1.6) | | |
| 8 | 2.88 dd (9.2, 5.5) | 2.88 dd (9.0, 5.5) | 2.61 dd (9.7, 5.3) | 2.55 dd (9.6, 5.4) | 3.75 d (5.4) | 3.76 d (5.4) |
| 10 | 2.99 m | 2.99 m | 3.05 m | 3.08 m | 3.32 brs | 3.31 brs |
| 11 | 2.22 dq (10.2, 6.6) | 2.23 dq (10.2, 6.5) | 2.21 dq (10.2, 6.4) | 2.18 dq (10.3, 6.5) | 2.24 dq (10.2, 6.5) | 2.24 dq (10.3, 6.4) |
| 12 | 5.52 d (10.2) | 5.50 d (10.2) | 5.47 d (10.2) | 5.47 d (10.3) | 5.49 d (10.2) | 5.49 d (10.3) |
| 14 | 1.62 d (5.5) | 1.62 d (5.5) | 1.50 d (5.3) | 1.54 d (5.4) | 1.83 d (5.4) | 1.84 d (5.4) |
| 16 | 1.29 s | 1.28 s | 1.19 s | 1.23 s | 1.20 s | 1.19 s |
| 17 | 1.26 s | 1.25 s | 1.22 s | 1.24 s | 1.24 s | 1.23 s |
| 18 | 0.91 d (6.6) | 0.91 d (6.5) | 0.89 d (6.4) | 0.90 d (6.5) | 0.94 d (6.5) | 0.94 d (6.4) |
| 19 | 1.79 brs | 1.79 brs | 1.75 brs | 1.79 brs | 1.84 brs | 1.84 brs |
| 20 | 4.22 d (11.2) | 4.23 d (11.3) | 4.32 s | 4.31 d (12.4) | 4.40 brd (14.0) | 4.39 brd (14.0) |
| | 4.46 d (11.2) | 4.45 d (11.3) | | 4.37 d (12.4) | 4.29 brd (14.0) | 4.29 brd (14.0) |
| 9-OH | 6.15 s | 6.14 s | 6.02 s | 6.00 s | 6.29 s | 6.22 s |
| 3′ | 6.84 m | 6.84 m | 6.82 m | 6.83 m | 6.83 m | 6.84 m |
| 4′ | 1.83 brs | 1.83 brs | 1.82 brs | 1.83 brs | 1.84 brs | 1.84 brs |
| 5′ | 1.80 brd (7.0) | 1.80 brd (7.1) | 1.78 brd (7.1) | 1.80 brd (7.1) | 1.80 brd (7.2) | 1.80 brd (7.3) |
| 2″ | 2.39 m | 2.59 m | 2.37 m | 2.58 m | 2.60 m | 2.39 m |
| 3″ | 1.46 m | 1.17 d (7.0) | 1.44 m | 1.17 d (7.0) | 1.18 d (6.9) | 1.16 t (7.5) |
| | 1.74 m | | 1.71 m | | | |
| 4″ | 1.14 d (7.0) | 1.19 d (7.0) | 1.13 d (7.1) | 1.19 d (7.0) | 1.20 d (7.0) | |
| 5″ | 0.95 t (7.4) | | 0.93 t (7.4) | | | |