Tigliane Diterpene Esters from the Leaves of *Croton tiglium*

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Three new tigliane-type diterpene esters, **1**–**3** with unusual 7-oxo-5-ene or 7-hydroxy-5-ene moieties in their skeletons, were isolated from the leaves of *Croton tiglium*. Their structures were unambiguously elucidated on the basis of spectroscopic data.

Introduction. – Tigliane diterpenes constitute a group of interesting natural products with diverse biological properties. Early attentions toward tigliane diterpenes focused on their irritant and tumor-promoting activities, which were found to result from the activation of protein kinase C [1][2]. In addition, some tigliane-type diterpenes were shown to possess beneficial features such as antitumor and anti-HIV-1 activities [3–5]. It was observed in recent years that the 12-deoxyphorbol derivative prostratin [6][7] and some phorbol-13-monoesters [8] were capable of reactivating the HIV virus in latently infected CD4+ T-cells. These findings indicated the possibility to eradicate the latent viral reservoirs in CD4+ T-cells by treatment with phorbol esters in combination with other antiretroviral medications. Thus, these diverse biological effects rendered tigliane diterpenes as attractive research targets. *Croton tiglium* is a plant of the family Euphoriaceae. The seed of *C. tiglium* is traditionally used in the treatment of parasitic and infective skin diseases or as a purgative agent in P. R. China [9]. Phytochemical studies revealed that *C. tiglium* is a rich source of tigliane diterpenes [4][10]. In our efforts to isolate novel tigliane diterpenes from plants, the leaves of *C. tiglium* were investigated. Three new tigliane diterpenes, **1**–**3** with unusual 7-oxo-5-ene or 7-hydroxy-5-ene moieties in their skeletons, were isolated, along with three known compounds. The structures of the new compounds were unambiguously determined by spectroscopic analysis (*Fig.*). The known compounds were identified as 12-*O*-(2-methylbutyryl)phorbol-13-yl acetate [4][10][11], 12-*O*-tigloylphorbol-13-yl isobutyrate [4][10][11], and 12-*O*-tigloylphorbol-13-yl 2-methylbutyrate [4][11]. Herein, we describe the isolation and structural elucidation of the new compounds.

Results and Discussion. – Air-dried leaves of *C. tiglium* were extracted with 95% EtOH. The crude extract was suspended in H_2O and extracted with petroleum ether (PE). Repeated chromatography of the PE-soluble fraction over SiO_2 and *Sephadex LH-20*, and further separation by preparative HPLC afforded six pure compounds.

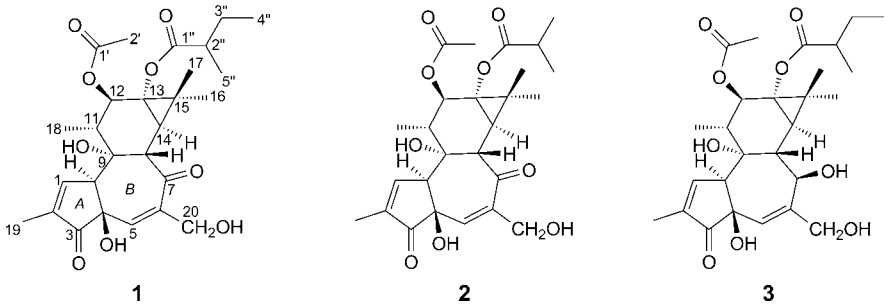

Figure. *Structures of compounds* **1**–**3**

Compound **1** was obtained as yellow oil. The *pseudo*-molecular-ion peak at *m*/*z* 503.2287 ($[M-H]^-$) in the HR-ESI-MS spectrum provided the molecular formula $C_{27}H_{36}O_9$, which requires ten C=C-bond equivalents. The IR spectrum confirmed the presence of OH (3384 cm^{-1}) and C=O groups (1680, 1714, and 1745 cm^{-1}). The UV spectrum showed a maximum at 235 nm (4.20), indicating the presence of α,β-conjugated C=O groups. The ^{13}C-NMR (*Table*) and DEPT spectra revealed signals of 27 C-atoms, including seven Me, two CH_2, eight CH groups, and ten quaternary C-atoms. Signals in the low-field region of the ^{13}C-NMR spectrum revealed the presence of two keto C=O (δ(C) 205.5, 201.1) and two ester C=O groups (δ(C) 179.1, 170.7), and two –C=C– fragments (δ(C) 159.9, 148.3, 136.8, 135.0). The FAB-MS displayed fragment-ion peaks at *m*/*z* 445 and 343, indicating the sequential loss of an Ac group and C_5-acyl group. The latter turned out to be a 2-methylbutanoyl moiety as suggested by the ^{1}H-NMR signals (δ(H) 2.39 (*m*, H–C(2″)), 1.46 (*m*, H_a–C(3″)), 1.74 (*m*, H_b–C(3″)), 0.94 (*t*, $J = 7.5$, Me(4″)), and 1.16 (*d*, $J = 7.5$, Me(5″))) and the corresponding ^{1}H,^{1}H-COSY correlations. These findings evidenced that compound **1** was the diester of a tetracyclic diterpene, which had two keto groups and two C=C bonds in the diterpene skeleton. Besides signals of the acyl moieties, the ^{1}H-NMR spectrum of **1** displayed resonances commonly found in spectra of phorbol diesters [4], such as the signals ascribable to Me(16) (δ(H) 1.21 (*s*)), Me(17) (δ(H) 1.22 (*s*)), Me(18) (δ(H) 0.96 (*d*, $J = 7.5$), Me(19) (δ(H) 1.81 (*d*, $J = 1.5$), and CH_2(20) (δ(H) 4.41, 4.28 (2*d*, $J =$ 15.0)). Two olefinic H-atom signals were observed at δ(H) 7.66 and 6.97. The signal at δ(H) 7.66 exhibited allylic coupling with Me(19) ($J = 1.5$), in the ^{1}H,^{1}H-COSY spectrum and was thus assigned to H–C(1). The three-bond long-range correlations from the H–C(1) signal to the C-atom signals ascribable to C(19) (δ(C) 10.3), C(3) (δ(C) 205.5), and C(4) (δ(C) 72.9) in the HMBC spectrum confirmed the presence of a Me-substituted five-membered *A*-ring. Different from phorbol derivatives, signals arising from the common CH_2(5) group in the seven-membered *B*-ring are missing in the NMR spectra of **1**, while an extra keto signal (δ(C) 201.1) was observed in the ^{13}C-NMR of **1**. The olefinic H-atom signal at δ(H) 6.97 showed HMBCs with those of both keto C=O groups (δ(C) 205.5, 201.1), H–C(10) (δ(C) 59.1), CH_2OH (δ(C) 62.4 C(20)), and C(4), indicating that the *B*-ring structure of compound **1** differed from that of phorbol derivatives in that the usual C(6)=C(7) group was replaced by a 5-ene-7-one moiety. The remaining ^{1}H- and ^{13}C-NMR signals of the diterpene skeleton of **1** were

Table. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of* **1**, **2**, *and* **3**. δ in ppm, J in Hz.

Position	**1**[a])		**2**[b])		**3**[a])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	7.66 (*q*, *J* = 1.5)	159.9 (*d*)	7.66 (*q*, *J* = 1.8)	159.8 (*d*)	7.61 (*s*)	160.1 (*d*)
2		135.0 (*s*)		135.0 (*s*)		134.0 (*s*)
3		205.5 (*s*)		205.1 (*s*)		206.6 (*s*)
4		72.9 (*s*)		73.0 (*s*)		72.5 (*s*)
5	6.97 (*s*)	136.8 (*d*)	6.92 (*s*)	137.2 (*d*)	6.11 (*s*)	126.4 (*d*)
6		148.3 (*s*)		148.4 (*s*)		152.9 (*s*)
7		201.1 (*s*)		201.3 (*s*)	4.79 (*d*, *J* = 9.5)	70.0 (*d*)
8	3.81 (*d*, *J* = 5.5)	54.5 (*d*)	3.76 (*d*, *J* = 5.4)	54.8 (*d*)	2.57 (*m*)	48.0 (*d*)
9		75.6 (*s*)		75.6 (*s*)		75.2 (*s*)
10	3.26 (*t*, *J* = 2.5)	59.1 (*d*)	3.29 (*t*, *J* = 2.4)	59.2 (*d*)	3.05 (*s*)	56.3 (*d*)
11	2.27 (*m*)	44.5 (*d*)	2.23 (*m*)	44.6 (*d*)	2.17 (*m*)	44.4 (*d*)
12	5.41 (*d*, *J* = 10.5)	76.5 (*d*)	5.40 (*d*, *J* = 10.2)	76.5 (*d*)	5.39 (*d*, *J* = 10.0)	76.7 (*d*)
13		65.1 (*s*)		65.2 (*s*)		65.7 (*s*)
14	1.79 (*d*, *J* = 5.5)	29.6 (*d*)	1.81 (*d*, *J* = 5.4)	29.7 (*d*)	1.51 (*d*, *J* = 5.5)	31.7 (*d*)
15		25.7 (*s*)		25.7 (*s*)		26.0 (*s*)
16	1.21 (*s*)	23.4 (*q*)	1.20 (*s*)	23.5 (*q*)	1.23 (*s*)	23.4 (*q*)
17	1.22 (*s*)	16.7 (*q*)	1.21 (*s*)	16.7 (*q*)	1.20 (*s*)	17.0 (*q*)
18	0.96 (*d*, *J* = 7.5)	14.5 (*q*)	0.95 (*d*, *J* = 6.6)	14.5 (*q*)	0.91 (*d*, *J* = 6.0)	14.6 (*q*)
19	1.81 (*d*, *J* = 1.5)	10.3 (*q*)	1.83 (*d*, *J* = 1.8)	10.3 (*q*)	1.78 (*s*)	10.3 (*q*)
20	4.41 (*d*, *J* = 15.0, H_a), 4.28 (*d*, *J* = 15.0, H_b)	62.4 (*t*)	4.39 (*d*, *J* = 14.4, H_a), 4.29 (*d*, *J* = 14.4, H_b)	63.2 (*t*)	4.32 (*s*)	66.7 (*t*)
1′		170.7 (*s*)		170.6 (*s*)		170.8 (*s*)
2′	2.09 (*s*)	20.9 (*q*)	2.08 (*s*)	20.9 (*q*)	2.08 (*s*)	21.0 (*q*)
1″		179.1 (*s*)		179.4 (*s*)		179.5 (*s*)
2″	2.39 (*m*)	41.2 (*d*)	2.59 (*m*)	34.2 (*d*)	2.38 (*m*)	41.3 (*d*)
3″	1.46 (*m*, H_a), 1.74 (*m*, H_b)	26.1 (*t*)	1.19 (*d*, *J* = 7.2)[c])	18.5 (*q*)[c])	1.46 (*m*, H_a), 1.73 (*m*, H_b)	26.2 (*t*)
4″	0.94 (*t*, *J* = 7.5)	11.6 (*q*)	1.20 (*d*, *J* = 7.2)[c])	18.6 (*q*)[c])	0.94 (*t*, *J* = 7.5)	11.7 (*q*)
5″	1.16 (*d*, *J* = 7.5)	16.1 (*q*)			1.14 (*d*, *J* = 7.0)	16.2 (*q*)
HO–C(9)	6.28 (*s*)		6.22 (*s*)		6.01 (*s*)	
HO–C(4)	3.45 (*s*)				3.54 (*s*)	

[a]) Recorded at 500 MHz. [b]) Recorded at 600 MHz. [c]) Signal assingments interchangeable.

assigned by analyzing the ^{1}H,^{1}H-COSY, HMQC, and HMBC data and are compiled in the *Table*. The downfield shifts of the C(12) (δ(C) 76.5) and C(13) (δ(C) 65.1) signals, compared to those of phorbol, suggested *O*-acylation at these two C-atoms. The HMBC from H–C(12) (δ(H) 5.41) to C(1′) (δ(C) 170.7) confirmed the connection of the AcO group to C(12), and the 2-methylbutyryl unit was thus at C(13).

The relative configuration of **1** was determined by NOESY experiment and the chemical shifts of key H-atoms. The NOE correlations H–C(10)/HO–C(9), HO–C(9)/Me(18), and Me(18)/H–C(12) indicated that these H-atoms and Me groups had the same orientation; *i.e.*, α. On the other hand, the NOE interactions H–C(11)/H–C(8) and H–C(8)/Me(17) suggested that H–C(8), H–C(11) and Me(17) were β-oriented. The NOE correlation H–C(14)/Me(16) confirmed the α orientation of H–C(14). The absence of NOE effect between H–C(10) and HO–C(4), and the chemical shifts of H–C(1) (δ(H) 7.66), H–C(8) (δ(H) 3.81), and H–C(10) (δ(H) 3.26) [12] indicated an *A*/*B* trans-ring junction. The ^{13}C-NMR signal of C(4) at δ(C) 72.9 indicated that C(4) was β-configured [11]. Based on these observations, the structure of **1** was determined as 12-*O*-acetyl-5,6-didehydro-7-oxophorbol-13-yl 2-methylbutanoate. At present, the absolute configuration of the diterpene unit and at C(2″) of the ester moiety remain undetermined.

Compound **2** was obtained as yellow oil. The molecular formula was determined as $C_{26}H_{34}O_9$ by the $[M-H]^-$ peak at *m*/*z* 489.2134 in the HR-ESI mass spectrum. The ^{1}H- and ^{13}C-NMR spectra (*Table*) of **2** showed patterns similar to those of compound **1**, except for the replacement of the 2-methylbutanoyl signals by those of an isobutyryl (2-methylpropanoyl) group (δ(H) 2.59 (*m*), 1.19 (*d*, *J* = 7.2) and 1.20 (*d*, *J* = 7.2), and δ(C) 34.2, 18.5, 18.6 and 179.4). The HMBCs from H–C(12) (δ(H) 5.40) to C(1′) (δ(C) 170.6) and C(2′) (δ(C) 20.9) confirmed the connection of the Ac group with C(12) and of the isobutyryl with C(13). The ^{1}H- and ^{13}C-NMR data and NOESY correlations were comparable to those of **1**, suggesting that it had the same configuration as **1**. The structure of **2** was thus deduced as 12-*O*-acetyl-5,6-didehydro-7-oxophorbol-13-yl 2-methylpropanoate.

Compound **3**, a yellow oil, had the molecular formula $C_{27}H_{38}O_9$, *i.e.*, with two more H-atoms than **1**, based on the HR-ESI-MS (*m*/*z* 529.2393 ($[M+Na]^+$)). Comparison of the ^{1}H- and ^{13}C-NMR spectra of **3** with those of **1** revealed close similarities (*Table*). Notable differences were found in the seven-membered *B*-ring. The C(7)=O (δ(C) 201.1) in the *B*-ring of **1** was apparently replaced by a CH–O group (δ(H) 4.79 (*d*, *J* = 9.5, H–C(7); δ(C) 70.0 C(7)) in **3**. The upfield shift of C(5), C(8), and C(10) signals, and the downfield shift of the C(6) signal supported this change at C(7). The location of H–C(7) was established by ^{1}H,^{1}H-COSY correlation between H–C(7) and H–C(8) (δ(H) 2.57 (*m*)). The NOESY correlations between H–C(7) and H–C(10) indicated a β-orientation for HO–C(7). The configurations of the remaining stereogenic centers were considered to be identical with those of compound **1** according to the NOESY spectrum. On the basis of these evidences, the structure of **3** was determined as 12-*O*-acetyl-5,6-didehydro-6,7-dihydro-7-hydroxyphorbol-13-yl 2-methylbutanoate.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Inc.*, P. R. China) and *Sephadex LH-20* (*GE Healthcare Bio-Science AB*, Sweden). Semi-prep. HPLC: *Waters 600* system with UV detector and *RP-18* semi-prep. column (250 × 20 mm, 5 μm; *YMC Corporation*, Japan). Optical rotation: *PE-243B* spectrometer (*PerkinElmer*, USA). UV Spectra: *Cintro-20* spectrometer (Australia); λ_{max} (log ε) in nm. IR: *Nicolet Manga* spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Varian INOVA-500* or *Varian INOVA-600* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. FAB-MS: *Micromass ZabSpec* spectrometer; in *m*/*z*. HR-ESI-MS: *Micromass-LCT* spectrometer, in *m*/*z* (rel. %).

Plant Material. The leaves of *Croton tiglium* L. (Euphoriaceae) were collected from Nanjing County in Fujian Province of China in October 2000, and they were authenticated by Prof. *Zhong Tao Wang* at the Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 02000) has been deposited with the Laboratory of Natural Product Chemistry, Beijing Institute of Pharmacology and Toxicology, P. R. China.

Extraction and Isolation. The air-dried leaves (4.5 kg) were extracted three times with 95% EtOH (10 l) at reflux. The combined EtOH extracts were filtered and then concentrated in a rotary evaporator under reduced pressure. The oily residue (437 g) was dispersed in 4 l of H_2O and extracted with petroleum ether (PE; 3 × 1.5 l). The PE-soluble part (158 g) was subjected to CC (SiO_2; hexane/AcOEt stepwise gradient, 9 : 1 → 0 : 1): *Frs. 1–7. Fr. 7* (28 g) was further separated by CC (SiO_2; $CHCl_3$/MeOH 50 : 1 → 4 : 1): *Frs. 71–76. Fr. 76* was refractionated by CC (*Sephadex LH-20*; CH_2Cl_2/MeOH 1 : 2), followed by reversed-phase semi-prep. HPLC (MeCN/H_2O 55 : 45; 2 ml/min) to give compounds **1** (13.4 mg), **2** (28.3 mg), and **3** (8 mg). Separation of *Fr. 72* by CC (*Sephadex LH-20*; CH_2Cl_2/MeOH 1 : 2), followed by prep. HPLC (MeCN/H_2O 55 : 45; 2 ml/min) afforded compounds 12-*O*-(2-methylbutyryl)-phorbol-13-yl acetate (15 mg), 12-*O*-tigloylphorbol-13-yl isobutyrate (12 mg), and 12-*O*-tigloylphorbol-13-yl 2-methylbutyrate (18 mg).

12-O-*Acetyl-5,6-didehydro-7-oxophorbol-13-yl 2-Methylbutanoate* (= rel-(*1a*R,*1b*S,*4a*R,*7a*S,*7b*S,*8*R,*9*R,*9a*S)*-9-(Acetyloxy)-1,1a,1b,2,4a,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-2,5-dioxo-9a*H*-cyclopropa[3,4]benzo[1,2-*e*]azulen-9a-yl 2-Methylbutanoate*; **1**). Yellow oil. $[\alpha]_D^{25} = +44.2$ ($c = 1.42$, $CHCl_3$). UV (MeOH): 235 (4.20). IR (KBr): 3384, 2976, 2933, 2879, 1745, 1714, 1680, 1572, 1407, 1340, 1273, 1232, 1194, 1157, 1111, 1084, 1020, 982, 922, 891, 806, 650. ^{1}H- and ^{13}C-NMR: see the *Table*. FAB-MS: 505 (3, $[M+H]^+$), 446 (20), 445 (75), 427 (5), 409 (3), 403 (3), 343 (18), 325 (32), 307 (18), 279 (12). HR-ESI-MS: 503.2287 ($[M-H]^-$, $C_{27}H_{35}O_9^-$; calc. 503.2281).

12-O-*Acetyl-5,6-didehydro-7-oxophorbol-13-yl 2-Methylpropanoate* (= rel-(*1a*R,*1b*S,*4a*R,*7a*S,*7b*S,*8*R,*9*R,*9a*S)*-9-(Acetyloxy)-1,1a,1b,2,4a,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-2,5-dioxo-9a*H*-cyclopropa[3,4]benzo[1,2-*e*]azulen-9a-yl 2-Methylpropanoate*; **2**). Yellow oil. $[\alpha]_D^{25} = +32.5$ ($c = 0.67$, $CHCl_3$). UV (MeOH): 237 (4.19). IR (KBr): 3386, 2976, 2927, 1743, 1714, 1678, 1574, 1408, 1342, 1234, 1199, 1161, 1111, 1082, 1020, 981, 924, 806, 650. ^{1}H- and ^{13}C-NMR: see the *Table*. FAB-MS: 491 (33, $[M+H]^+$), 432 (26), 431 (100), 403 (2), 343 (20), 325 (24), 307 (14), 279 (7). HR-ESI-MS: 489.2134 ($[M-H]^-$, $C_{26}H_{33}O_9^-$; calc. 489.2125).

12-O-*Acetyl-5,6-didehydro-6,7-dihydro-7-hydroxyphorbol-13-yl 2-Methylbutanoate* (= rel-*(1a*R,*1b*R,*2*R,*4a*R,*7a*S,*7b*R,*8*R,*9*R,*9a*S)*-9-(Acetyloxy)-1,1a,1b,2,4a,5,7a,7b,8,9-decahydro-2,4a,7b-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-9a*H*-cyclopropa[3,4]benzo[1,2-*e*]azulen-9a-yl 2-Methylbutanoate*; **3**). Yellow oil. $[\alpha]_D^{25} = +53.0$ ($c = 0.40$, $CHCl_3$). UV (MeOH): 231 (4.15). IR (KBr): 3400, 2970, 2927, 1744, 1714, 1652, 1457, 1375, 1236, 1080, 1019. ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS: 507 ($[M+H]^+$). HR-ESI-MS: 529.2393 ($[M+Na]^+$, $C_{27}H_{38}NaO_9^+$; calc. 529.2414).

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