Unusual Tigliane Diterpenes from *Euphorbia grandicornis*

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S *Supporting Information*

ABSTRACT: Phytochemical study of the aerial parts of *Euphorbia grandicornis* led to the isolation of two new tigliane diterpenes, 16-angeloyloxy-13α-isobutanoyloxy-4β,9α,20-trihydroxytiglia-1,5-diene-3,7-dione (**1**) and 16-angeloyloxy-13α-isobutanoyloxy-4β,9α,7β-trihydroxytiglia-1,5-dien-3-one (**2**). The structures and relative configuration of the new compounds were elucidated on the basis of extensive spectroscopic analysis, including 1D and 2D NMR experiments (^{1}H NMR, JMOD, [1]H−^{1}H COSY, NOESY, HSQC, and HMBC), mass spectrometry, and comparison with literature data. The biogenesis of **1** and **2** with respect to the unusual 5-en-7-one and 5-en-7-ol moieties is also discussed.
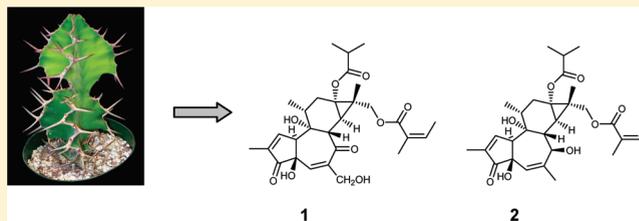
Tigliane derivatives belong to a group of tetracyclic diterpenes that occur in plants of the Euphorbiaceae and Thymelaeaceae families as acylated polyhydroxy derivatives.[1] These compounds are well-known tumor-promoting and pro-inflammatory agents and are responsible for the skin irritant and toxic effects of the milky latex, roots, herbs, and seeds of these plants. Since the 1960s, esters of phorbol (= 4β,12β,13α,20,9α-pentahydroxy-tiglia-1,3-dien-3-one), especially 12-*O*-tetradecanoylphorbol 13-acetate (TPA), have been utilized as pharmacological and biochemical tools for provoking inflammation and investigating the mechanism of tumor promotion in both in vivo and in vitro test systems.[2,3] More recently, phorbol derivatives have gained attention in potential HIV therapy because these reactivate HIV-1 latency by protein kinase C-dependent NF-κB activation and downregulate the expression of the HIV-1 receptor CD4 and the co-receptors CXCR4 and CCR5, thus avoiding new infections of CD4+ cells. Treatment with phorbol esters (e.g., prostratin) in combination with other antiretroviral drugs acting at different steps of the viral cycle has been suggested as a potential strategy to activate viral reservoirs and eradicate the pool of latent HIV-infected CD4+ T-cells.[4,5] Moreover, also the TRPV (transient receptor potential vanilloid) receptor affinity makes phorbol and daphnane esters promising candidates for drug development, since most recently the activation of the TRPV4 channel by 4α-phorbol esters has been reported.[6]

The aim of the present work was to search for new promising phorbol derivatives of natural origin, and therefore the diterpene constituents of *Euphorbia grandicornis* Goebel (Euphorbiaceae) were studied. *E. grandicornis* (syn. *E. breviarticulata* Pax and *E. breviarticulata* var. *breviarticulata* Pax) is a succulent cactiform plant native to southern Africa and is used as an ornamental plant in many countries. It produces a white milky latex, which causes irritation on the skin and a burning sensation in the mouth and throat. Its extract has been used traditionally to treat various

infectious diseases (chlamydia, syphilis, and gonorrhea).[7] Previous phytochemical studies of the latex of *E. grandicornis* revealed the presence of triterpene alcohols euphol, tirucallol, and euphorbol.[8] The present paper reports the isolation and structure determination of two new phorbol-type diterpenes (**1** and **2**). Their structures differ from all previously known phorbol derivatives, since a 5-en-7-one or 5-en-7-ol functionality is present in the molecule instead of the usual 6,7-olefin group. The proposed biosynthesis of **1** and **2** is also discussed.
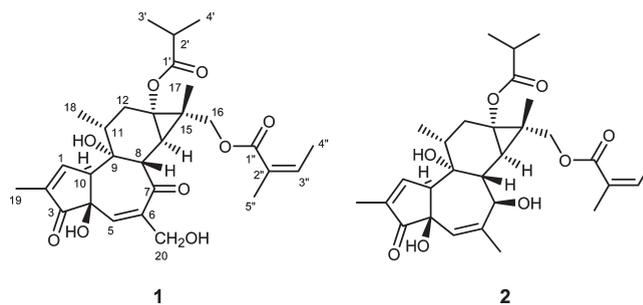
■ RESULTS AND DISCUSSION

The CHCl₃-soluble fraction of the concentrated MeOH extract prepared from the aerial parts of *E. grandicornis* was subjected to polyamide column chromatography using mixtures of MeOH and H₂O as eluents. A diterpene-containing chlorophyll-free fraction was obtained with MeOH−H₂O (3:2), which was separated on silica gel using vacuum-liquid chromatography, centrifugal planar chromatography, and HPLC and finally purified by RP-HPLC, to yield pure compounds **1** and **2**.

Table 1. NMR Data of Compounds 1 and 2 [500 MHz (^{1}H), 125 MHz (^{13}C), CDCl$_3$, δ (ppm) (J = Hz)]

position	1 ^{1}H	1 ^{13}C	2 ^{1}H	2 ^{13}C
1	7.65 s	159.9	7.58 s	159.7
2		134.8		133.9
3		204.8		206.9
4		72.8		71.9
5	6.90 s	137.3	6.04 s	126.8
6		147.9		150.0
7		201.4	4.65 d (9.4)	83.1
8	3.53 d (5.5)	54.6	2.60 dd (9.4, 5.4)	45.6
9		73.2		72.6
10	3.32 brs	58.6	3.07 s	56.7
11	2.08 m	38.2	2.08 m	37.8
12β	2.10 m	31.5	2.18 dd (11.8, 4.3)	31.5
12α	1.62 dd (14.0, 11.0)		1.60 dd (14.9, 11.8)	
13		62.7		63.3
14	1.95 d (5.5)	20.8	1.71 d (5.5)	27.0
15		25.8		25.4
16a	4.17 d (11.5)	69.0	4.51 d (11.3)	70.3
16b	4.07 d (11.5)		3.99 d (11.3)	
17	1.16 s	11.1	1.13 s	11.2
18	0.96 d (6.4)	18.5	0.94 d (6.5)	18.5
19	1.83 d (1.5)	10.0	1.80 d (1.4)	9.8
20a	4.37 d (14.2)	63.7	2.04 s	22.3
20b	4.25 d (14.2)			
isobutanoyl				
1′		178.8		178.8
2′	2.54 sept (7.0)	34.2	2.56 sept (7.0)	34.0
3′	1.15 d (7.0)	18.5	1.13 d (7.0)	18.5
4′	1.15 d (7.0)	18.5	1.14 d (7.0)	18.5
angeloyl				
1″		167.7		168.0
2″		127.5		127.0
3″	6.09 q (7.3)	137.8	6.08 q (7.2)	139.0
4″	1.97 dd (7.3, 1.5)	15.8	1.96 dd (7.2, 1.1)	15.7
5″	1.88 s	20.4	1.87 s	20.0
OH-4	2.67 s		2.23 s	
OH-7			8.52 s	
OH-9	6.09 s		5.73 s	
OH-20	2.37 s			

Compound **1** was isolated as a colorless, amorphous solid. Its positive ion ESIMS displayed a quasimolecular ion peak at m/z 553 [M + Na]$^+$, indicating a molecular mass of 530, corresponding to the formula C$_{29}$H$_{38}$O$_9$, which was supported by HR-ESIMS (m/z 553.2425, calcd for 553.2408, C$_{29}$H$_{38}$O$_9$Na). The ^{1}H and JMOD NMR spectra exhibited typical resonances for isobutyrate [δ_H 2.54 sept (1H), 1.15 d (6H); δ_C 178.8, 34.2, 2 × 18.5] and angelate [δ_H 6.09 q (1H), 1.97 dd (3H), 1.88 s (3H); δ_C 167.7, 137.8, 127.5, 15.8, 20.4] ester groups (Table 1). In addition, with the aid of the JMOD, ^{1}H–^{1}H COSY, and HSQC spectra, eight quaternary carbons, six methines, three methylenes, and three methyl groups were detected, accounting for a 20-carbon-containing diterpene skeleton. Analysis of the ^{1}H–^{1}H COSY spectrum provided only a little more information on the

partial structures. Two isolated methylenes [δ_H 4.17 and 4.07 d (J = 11.5 Hz), 4.37 and 4.25 d (J = 14.2 Hz)] and the following short structural fragments were elucidated on the basis of the correlated proton sequences: CH$_3$−CH−CH$_2$− (unit A, δ_H 0.96 d, 2.08 m, 2.10 m, and 1.62 dd), −CH−CH− (unit B, δ_H 3.53 and 1.95 d), and −CH−CH= (unit C, δ_H 3.32 brs and 7.65 s). The carbon resonances at δ_C 201.4 and 204.8 demonstrated the presence of two keto groups in the molecule. Furthermore, two trisubstituted olefins were evident from the carbon resonances at δ_C 137.3, 147.9, 159.9, and 134.8 and the proton resonances at δ_H 7.65 and 6.90 s. One of these units, together with a methyl (C-19), a keto group (C-3), a methine (C-10), and a O-substituted quaternary carbon (C-4), comprised a methyl-substituted five-membered ring, characteristic of 4-hydroxyphorbol esters, as proved by the HMBC correlations detected between H-19/C-1, H-19/C-2, and H-19/C-3. The other trisubstituted olefin was placed at positions C-5−C-6, since heteronuclear long-range correlations were detected between H-5 and C-10 and between H-5 and C-3 in the HMBC spectrum and allylic coupling between H-5 and H-20 in the ^{1}H−^{1}H COSY spectrum. The quaternary carbon at δ_C 73.2 (C-9) showed two- and three-bond correlations to the protons of unit A [δ_H, 2.08 m (H-11) and 0.96 d (H-18)], suggesting that this structural fragment represents the C-18−C-11−C-12 part of a 9,13-substituted tigliane diterpene. The two methine groups containing unit B were assigned as the C-8−C-14 part of the molecule and confirmed from the HMBC correlations between H-8 and C-9, H-14 and C-13, H-14 and C-15, and H-8 and C-15. The presence of a keto group (δ_C 201.4) at C-7 was concluded on the basis of the H-8/C-7 and H-14/C-7 long-range correlations, and the 16- and 20-methylenes were corroborated by the HMBC cross-peaks between H-5/C-20, H-16/C-15, H-16/C-17, and H-17/C-13. The position of the angeloyl group at C-16 was evident from the three-bond correlation between the ester carbonyl carbon (δ_C 167.7) and the H$_2$-16 protons (δ_H 4.07 and 4.17 d). Hydroxy groups at C-9 and C-20 were deduced from the NOESY correlation between the OH group (δ_H 6.09) and H-10 and H-12b and the ^{1}H−^{1}H COSY correlation between OH group (δ_H 2.37) and H-20 protons, respectively. The location of the isobutanoyl group at C-13 was determined on the basis of the chemical shift value of C-13 (δ_C 62.7 ppm), which was in the usual range of 13-acyl-substituted phorbol esters (δ_C 62.7−63.6 ppm).[9−11]

The relative stereochemistry of **1** was elucidated by analyzing the correlations detected in a NOESY spectrum (Figure 1). NOE interactions between H-8 and H-11, H-8 and H$_3$-17, H$_3$-17 and H-12β, and H-12β and H-11 indicated the β-position of all these protons and the methyl group. On the other hand, NOESY cross-peaks between H-12α and OH-9, OH-9, and H-10; H-10 and H-1; H-1 and H-18,; and H-16a,b and H-14 dictated an α-arrangement of H-10, OH-9, H-14, and the C-16-methylene and C-18-methyl groups. The *trans* A/B ring junction with H-10α and OH-4β and the 13-acyl group in an α-position were concluded on the basis of biogenetic considerations, since all phorbol-type diterpenes isolated so far have such functionalities and because the C-4, C-10, and C-13 chemical shift values of **1** were in good agreement with those of structurally related compounds.[10,12,13] All of the above data were compatible with the structure of **1** being proposed as 16-angeloyloxy-13α-isobutanoyloxy-4β,9α,20-trihydroxytiglia-1,5-diene-3,7-dione.

Compound **2** was isolated as a colorless oil. Its molecular formula was assigned as C$_{29}$H$_{40}$O$_8$ on the basis of the HRESIMS and NMR data. The ^{1}H NMR and *J*-modulated ^{13}C NMR
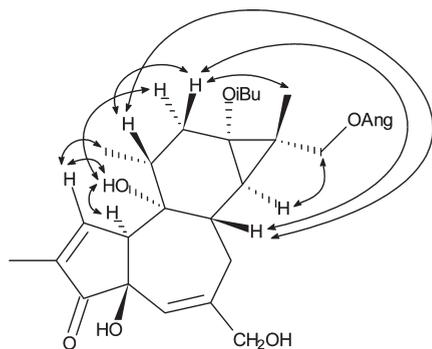
Figure 1. Key NOESY correlations for compound **1**.

spectra, analyzed with the aid of 1H−1H COSY, HSQC, and HMBC experiments, showed the same ester groups, one iso-butyroyl and one angeloyl as in the case of **1** (Table 1). The diterpene core of **2** was found to be constructed by structural elements [−CH−CH=C(CH$_3$)− (C-10−C-1−C-2−C-19) and CH$_3$−CH−CH$_2$− (C-18−C-11−C-12)] similar to those of **1**, indicating the same tigliane skeleton, but in the case of **2** only one keto group (δ_C 206.9) and one O-substituted methylene (δ_H 4.51 d, 3.99 d, δ_C 70.3) were detected. Moreover, an additional methyl (δ_H 2.04 s, δ_C 22.3) and an O-substituted sp^3 methine group (δ_H 4.65 d, δ_C 83.1) were identified. The latter comprises part of the molecular fragment, −CH−CH−CH−, as demonstrated by the 1H−1H COSY correlations between δ_H 4.65 (d, H-7), 2.60 (dd, H-8), and 1.71 (d, H-14). This structural element was deduced as the C-7−C-8−C-14 portion of the molecule with regard to the heteronuclear long-range correla-tions between H-14 and C-13, C-15, C-16, and C-17 and between H-8 and C-6 and C-15. A deshielded methyl signal (δ_H 2.04 s) displayed HMBC correlations to C-5, C-6, and C-7 and allylic coupling with H-5 and H-7 in the 1H−1H COSY spectrum, proving its assignment as C-20 and the presence of a 5,6-double bond. The locations of the ester and hydroxy groups were determined with the aid of the 2D NMR spectra. The positions of the angeloyl group at C-16, isobutanoyl group at C-13, and hydroxy groups at C-4 and C-9 were indicated by the 1H and 13C chemical shift values of **2**, similar to those of **1**, and by the HMBC and NOESY correlations. The position of a hydroxy group (δ_H 8.52 s) at C-7 in **2** was demonstrated by the 1H−1H COSY correlation between H-7 and OH-7.

The relative configuration of the stereogenic centers in **2** was investigated on the basis of the NOESY spectrum. Starting from the H-10α stereochemistry, the α-orientation of H-7, H-14, C-16-methylene, H-18, and OH-9 was concluded, since NOEs were observed between H-10 and H-7, H-7 and H-14, H-14 and H-16, H-12b and H-18, and H-14 and OH-9. On the other hand, the NOESY correlations between H-11 and H-8, H-11 and H$_3$-17, H$_3$-17 and H-8, and H-12a and H-11 pointed to these β-oriented protons and a methyl group. All of the above evidence was used to propose the structure of compound **2** as 16-angeloyloxy-13α-isobutanoyloxy-4β,9α,7β-trihydroxytiglia-1,5-dien-3-one.

Compounds **1** and **2** have unusual structural features with regard to the presence of the 5-en-7-one and 5-en-7-ol substitu-ents. Biogenetically, these structural moieties may be formed as presented in Figure 2. At first sight, it would be obvious to introduce simultaneously two hydroxy groups at C-4 and C-7, respectively, by a double allylic oxidation. However, no example is known for an allylic oxidation that would run on a saturated carbon

atom attached to a carbonyl group. Therefore, a roundabout way is preferred, in which all steps are known in the preparative organic chemistry and in the chemistry of natural products.[14,15] In the majority of the tigliane derivatives, C-4 has a similar hydroxy group, which might be introduced in the same manner. In the first step, the biosynthetic precursor tiglia-2,6,10-triene (**3**) isomerized to compound **4**; then an epoxide group can be formed (**5**), which hydrolyzed by acid or base catalysis to the vicinal diol **6**. Water elimination of **6** results in the formation of **7** and therewith the regeneration of the 5,6-olefin group. Allylic oxidation of **7** may afford the 7-hydroxy derivative (**8**), which represents the structural type of compound **2**. Dehydrogenation of the 7-hydroxy to 7-keto group forms **9**, the structural type of compound **1**. In the structural formulas in Figure 2, additional oxygen functions were omitted, as their origin is not yet clear. It should be emphasized that this and similar schemes are based on analogies and chemotaxonomical facts, rather than experimental data.

In conclusion, a phytochemical study of the aerial part of *E. grandicornis* afforded two unprecedented types of natural diter-penes of the tigliane class. The isolated compounds may have a valuable biological potential as protein kinase C activators and modulators of the TRPV4 receptor.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined in chloroform by using a Perkin-Elmer 341 polarimeter. NMR spectra were recorded on a Bruker Avance DRX 500 spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C) with TMS as internal standard. Two-dimensional data were acquired and processed with standard Bruker software. For ^{1}H−^{1}H COSY, HSQC, and HMBC experiments, gradient-enhanced versions were used. Low-resolution ESI mass spectra were recorded on an Applied Biosystems 3200QTrap instrument. Sam-ples were acquired in flow injection mode. High-resolution MS data were recorded on an Shimadzu IT-TOF mass spectrometer equipped with electrospray source. The resolution was over 10 000. Column chroma-tography was carried out on polyamide (50−160 μm, MP Biomedicals), and vacuum-liquid chromatography (VLC) on silica gel G (15 μm, Merck). Centrifugal planar chromatography (CPC) was carried out on silica gel 60 GF$_{254}$ using a Chromatotron instrument (Harrison Re-search). Separations were monitored by TLC on Merck 60 F$_{254}$ (0.25 mm) plates and visualized by staining with concentrated sulfuric acid. Preparative thin layer chromatography was performed on silica gel 60 F$_{254}$ (Merck) and RP-18 F$_{254}$ plates (Merck). HPLC was performed on a Waters instrument with detection at 254 nm on a LiChrospher RP-18 (5 μm, 250 × 4 mm, Merck) column using MeOH−H$_2$O (4:1) as mobile phase at 0.5 mL/min flow rate and on a LiChrospher Si 100 (5 μm, 250 × 4 mm, Merck) column with cyclohexane−EtOAc−EtOH (140:40:1) at 0.5 mL/min flow.

Plant Material. The aerial parts of *E. grandicornis* were cultivated as an ornamental plant in Szeged-Kecskés, Hungary, in March 2008. The plant was identified by one of the authors (Z.H.). A voucher specimen (No. 769) has been preserved in the Herbarium of the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

Extraction and Isolation. The fresh plant material (10 kg) was crushed in a blender and then percolated with 38 L of MeOH at room temperature. The crude extract was concentrated in vacuo and extracted exhaustively with CHCl$_3$ (5 × 500 mL). The organic phase (29.4 g) was chromatographed on a polyamide column (180 g) with mixtures of MeOH and H$_2$O (3:2 and 4:1, each 2000 mL) as eluents. The fraction (0.6 g) obtained with MeOH−H$_2$O (3:2) was subjected to silica gel VLC using a gradient system of n-hexane−acetone (9:1, 8:2, 7:3, 6:4, 1:1, and 3:7). Fractions with similar compositions according to TLC
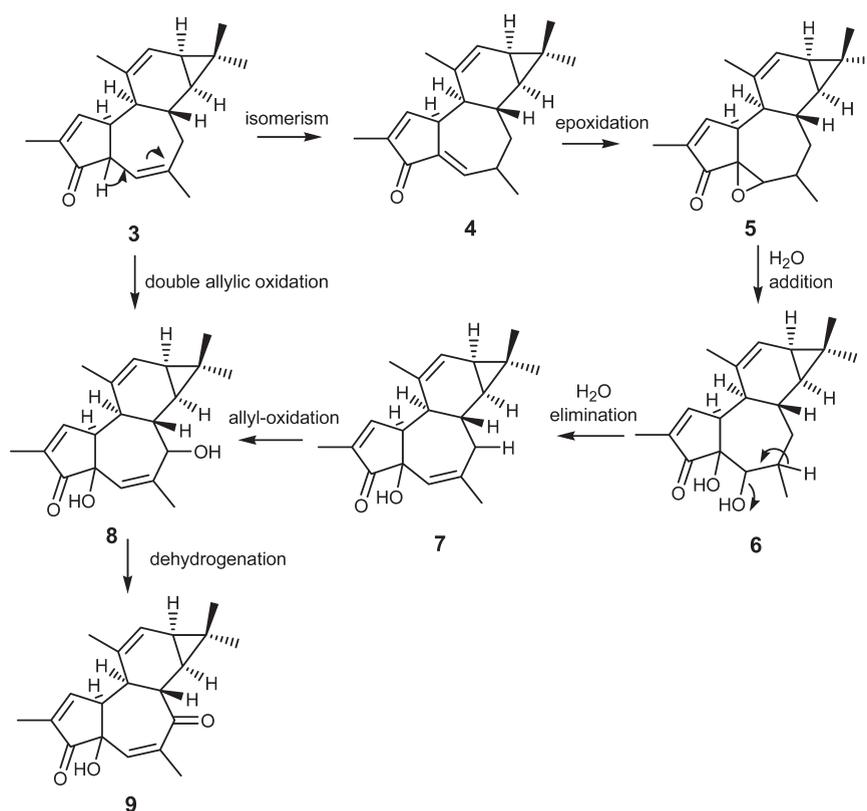
Figure 2. Proposed biogenetic derivation of structural types of **1** and **2**.

monitoring were combined, affording fractions I–XI. Fraction V, obtained with *n*-hexane–acetone (7:3), was fractionated by CPC on silica gel, using *n*-hexane–acetone mixtures with refined gradient steps [19:1 (200 mL), 9:1 (200 mL), 17:3 (200 mL), 8:2 (150 mL), 3:1 (150 mL), and 7:3 (150 mL)]. Fraction (0.118 g), eluted with *n*-hexane–acetone (19:1 and 9:1), was purified by preparative TLC using the developing system of *n*-hexane–acetone (13:7). Band 3 (R_f = 0.4), detected at UV 254 nm, was further purified by RP-HPLC using MeOH–H_2O (4:1) as mobile phase, to yield the pure compound **1** (1.8 mg). Fraction V, obtained with *n*-hexane–acetone (4:1) from the VLC separation, was purified first by normal-phase HPLC and finally by RP-HPLC to afford compound **2** (2.5 mg).

*16-Angeloyloxy-13α-isobutanoyloxy-4β,9α,20-trihydroxytiglia-1,5-diene-3,7-dione (**1**):.* colorless oil; $[\alpha]^{25}_D$ +56 (*c* 0.05, CHCl$_3$); for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Table 1; ESIMS *m/z* 553 [M + Na]$^+$, 569 [M + K]$^+$, 548 [M + NH$_4$]$^+$; HRESIMS *m/z* 553.2425 [M + Na]$^+$, calcd for 553.2414 C$_{29}$H$_{39}$O$_8$.

*16-Angeloyloxy-13α-isobutanoyloxy-4β,9α,7β-trihydroxytiglia-1,5-dien-3-one (**2**):.* colorless oil; $[\alpha]^{25}_D$ +27 (*c* 0.05, CHCl$_3$); for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS *m/z* 517.2649 [M + H]$^+$, calcd for 517.2644 C$_{29}$H$_{41}$O$_8$, 537.2484 [M + Na]$^+$ calcd for 537.2464 C$_{29}$H$_{40}$O$_8$Na.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Copies of the 1D and 2D NMR spectra of compounds **1** and **2** are available free of charge via the Internet at http://pubs.acs.org.

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