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Phytochemical Study of *Eryngium triquetrum*: Isolation of Polyacetylenes and Lignans

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Key words
- *Eryngium triquetrum*
- Apiaceae
- polyacetylenes
- lignans
- NMR

Abstract

Phytochemical investigation of the ethyl acetate extract from the aerial parts of *Eryngium triquetrum* Vahl resulted in the isolation of new polyacetylenes, triquetridiol (**6**) and *trans*-epoxy-triquetrol (**7a**/**7b**, diastereomeric mixture), and the lignan demethoxy carolignan Z (**8a**/**8b**, *erythro*/*threo* pair), together with a series of related known metabolites. Additionally, some already reported phenolic and flavonoid compounds were also identified in the extract. Structural elucidation of the new compounds was made by spectroscopic analysis, mainly NMR and mass spectrometry. To the best of our knowledge, this is the first report of polyacetylenes and lignans from *E. triquetrum*.

Introduction

The genus *Eryngium* belongs to the family Apiaceae (Umbelliferae) and contains about 317 species [1]. Some of them, such as *Eryngium campestre* L., *Eryngium creticum, Eryngium kotschyi, Eryngium maritimum* L., and *Eryngium trisectum*, are used as a folk remedy for the treatment of various anti-inflammatory disorders. Some other species, for instance, *Eryngium falcatum,* are known for their antinociceptive activity. Secondary metabolites isolated from plants belonging to this genus have displayed important biological activities, including antitumor, antibacterial, antimicrobial, antifungal, phototoxic, and other chemical and medicinal properties [2,3]. The phytochemical constituents of the *Eryngium* genus (23 studied species) including terpenoids, polyacetylenes, triterpenoid saponins, steroids, and phenolics such as flavonoids and coumarins, have been recently reviewed in a comprehensive article [4].

Eryngium triquetrum Vahl is an endemic North Africa plant widely distributed in all parts of Algeria along with other *Eryngium* species such as *Eryngium dichotomum* Desf, *Eryngium barrelieri* Boiss, *Eryngium ilicifolium* Lam, *E. maritimum* L., *E. campestre* L., and *Eryngium tricuspidatum* [5]. *E. triquetrum* grows particularly well in rocky pastures and it is known as "*Choukzerka*" by local people. To the best of our knowledge, only a single paper has recently been published [6] that describes the chemistry of the essential oils and flavonoids of this plant.

Here we describe the chemical investigation of the ethyl acetate extract of the aerial part of *E. triquetrum* Vahl collected in Merouana (Algeria), which showed a remarkable and complex secondary metabolite pattern, including molecules of different structural classes. This study led to the finding of 20 compounds – polyacetylenes, lignans, and phenolic metabolites – including two new C_{17} polyacetylenes, triquetridiol (**6**) and *trans*-epoxy-triquetrol (**7a**/**7b**, diastereomeric mixture), and the inseparable *erythro*/*threo* pair of unprecedented demethoxy carolignan Z (**8a**/**8b**).

Results and Discussion

The dried aerial part of *E. triquetrum* was exhaustively extracted with an hydroalcoholic solution and the resulting aqueous residue was treated with organic solvents of increasing polarity (see Materials and Methods for details). The ethyl acetate extract (3 g) of *E. triquetrum* was taken into consideration for chemical analysis, revealing the presence of a rich metabolite pattern. The extract was first subjected to Sephadex LH-20 in $CHCl_3$/MeOH, 1 : 1, to give 11 main fractions. Preliminary NMR analysis of fractions 3 and 4 showed the

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presence of signals attributable to polyacetylenes and lignans, whereas fraction 7 contained mainly flavonoids. Purification of these three fractions by SiO_2 chromatography, followed by semi-preparative HPLC (see Materials and Methods), afforded 20 compounds, 3 of them not previously reported, including polyacetylenic, lignan, and phenolic metabolites. The polyacetylene fraction, the less polar of the *E. triquetrum* extract, was considered first. The chemical analysis of this fraction resulted in the isolation of C_{17} enynols (compounds **1**–**7**; ▶ **Fig. 1**).

First of all, the main component was identified as *trans*-panaxydiol (**1**) (8*E*)-heptadeca-1,8-diene-4,6-diyne-3*S*,10-diol), a polyacetylene known from ginseng (*Panax ginseng*, Araliaceae) and from a number of further members of the Apiaceae and Arialiaceae families [7–12]. Both (−)- and (+)- stereoisomers have been found in nature even though the absolute configuration of naturally occurring panaxydiol has not been unequivocally defined. All four possible stereoisomers [(3*R*,10*R*), (3*R*,10*S*), (3*S*,10*R*), (3*S*,10*S*)] of panaxydiol have been synthesized and their optical rotation have been measured showing that the sign of $[\alpha]_D$ is mainly affected by the C-3 configuration (3*R* in levorotatory isomers, 3*S* in dextrorotatory isomers) [13]. The observed optical rotation ($[\alpha]_D = 32.8°$) for *trans*-panaxydiol (**1**) isolated in this work was very close to that reported for the (3*S*,10*R*)-stereoisomer ($[\alpha]_D = 30.3°$) [13], leading us to tentatively assign this absolute configuration. In order to further support this assignment, the modified Mosher method [14,15] was applied to compound **1**. The NMR analysis of both *S* and *R* MTPA esters of **1** substantiated the 3*S* absolute configuration (see Materials and Methods; ▶ **Fig. 2**), whereas the evaluation of $\Delta\delta$ values (δ_S ester – δ_R ester) for the protons adjacent to C-10 appeared to be quite complicated. It has been reported in the literature [11] that attempts to determine the absolute configuration of C-10 for a levorotatory panaxydiol failed due to the too small magnitude of the $\Delta\delta$ values observed for the adjacent protons at C-10. In our case, a careful analysis of the high-resolved proton spectra of the Mosher esters of **1** revealed that two set of signals (ratio ca. 1 : 1) were generated by the protons adjacent to C-10. As depicted in ▶ **Fig. 2**, both signals at $\Delta\delta$ 5.77 (d, *J* = 15.9 Hz, H-8) and $\Delta\delta$ 6.33 (dd, *J* = 15.9, 8.0, H-9) (line a) were each split into two distinct equivalent multiplets shifted at $\Delta\delta$ 5.78/5.65 and $\Delta\delta$ 6.26/6.18 (lines b and c), respectively, revealing that *trans*-panaxydiol isolated from *Eryngium* extract was not a single compound, but rather a mixture of C-10 epimers.

The remaining components of the mixture, including falcarinol (**2**), heptadeca-1,8-diene-4,6-diyne-3-ol-10-one (**3**), panaxjapyne B (**4**), *cis*-panaxydiol (**5**), and the unreported triquetridiol (**6**) and *trans*-epoxy-triquetrol (**7**), are described below on the basis of their polarity.

Falcarinol (**2**), also known as panaxynol [16,17], was easily identified in the less polar polyacetylene fraction by NMR and MS data [18]. As for panaxydiol, both (−)- and (+)- enantiomers have been isolated from natural sources, and the absolute configuration has been established by different methods [19,20]. The *S* absolute configuration of C-3 was indicated by the positive $[\alpha]_D$ and confirmed by the Mosher method [19]. The NMR analysis of both *S* and *R* MTPA esters of **2** showed $\Delta\delta$ values consistent with the 3*S* absolute configuration (see Materials and Methods).

Heptadeca-1,8-diene-4,6-diyne-3-ol-10-one (**3**) [8,21] and panaxjapyne B (**4**) [22–24] were isolated in a very minor amount. The 3*S* configuration for both molecules could be suggested by biogenetical considerations. The positive $[\alpha]_D$ value of compound

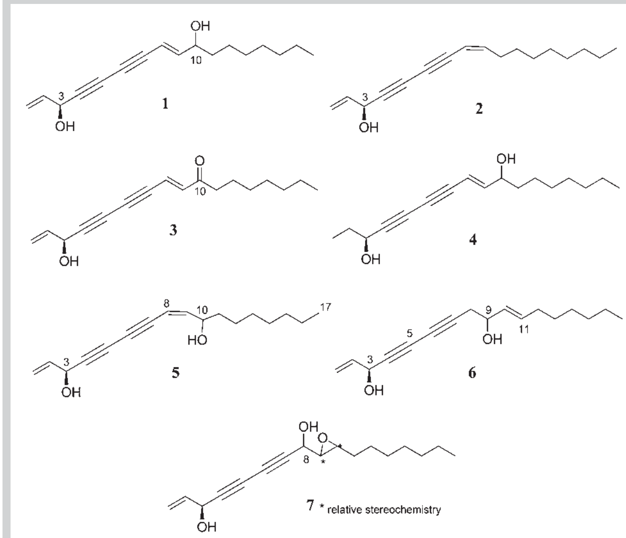

Fig. 1 Polyacetylenes isolated from *E. triquetrum*.

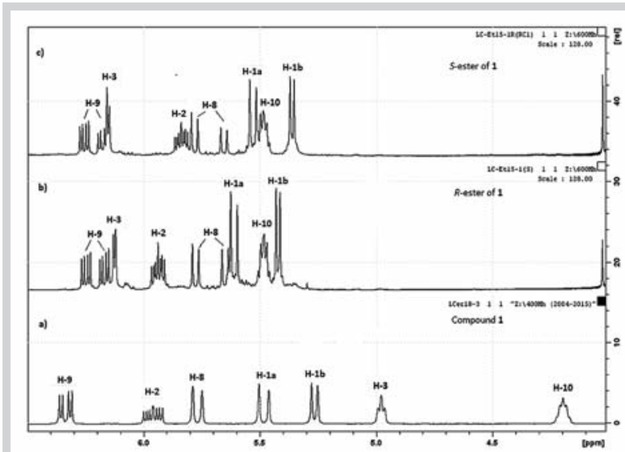

Fig. 2 Selected ^{1}H NMR regions for compound **1** (line a), *R*-MTPA (line b), and *S*-MTPA (line c) esters of **1**. It should be noted that two sets of H-8 and H-9 signals are present in the Mosher esters spectra (lines b and c).

4 supported this assumption, whereas, unfortunately, compound **3** degraded before the optical activity measurement.

cis-Panaxydiol (**5**) (8*Z*)-heptadeca-1,8-diene-4,6-diyne-3*S*,10-diol) has been previously reported to occur in two species of the Apiaceae family, but it has been only partially characterized, and, in particular, no NMR data have been reported [7,8]. Analysis of ^{1}H and ^{13}C NMR resonances of **5** (▶ **Tables 1** and **2**) revealed strong similarities with those of main co-occurring *trans*-panaxydiol (**1**), mainly differing in the coupling constant values of Δ^8 double bond protons (J_{H8-H9} = 11.0 Hz in **5**; J_{H8-H9} = 15.9 Hz in **1**, see Materials and Methods). This was consistent with a different double bond geometry, in particular, indicating the *cis*-Δ^8 stereochemistry in compound **5**. Steric effects observed between H-8 and H-9 by selected NOE difference experiments confirmed such an assignment. Unfortunately, the easy and almost quantitative isomerization of compound **5** into **1** that was observed to occur during the chromatographic workup as well as in the NMR

Table 1 ^{1}H NMR (400 MHz) data for compounds 5–7 in $CDCl_3$ (δ H in ppm, *J* in Hz). [a]Data divergent for minor isomers are shown in square brackets.

	5	6	7	7 (C_6D_6)
1	5.49, d (17.0) 5.28, d (10.2)	5.47, d (17.2) 5.25, d (10.2)	5.48, d (17.0) 5.28 d (10.1)	5.20, d (17.0) 4.86, d (10.2)
2	5.97, ddd (17.0, 10.2, 5.5)	5.95, ddd (17.2, 10.2, 5.3)	5.95, ddd (17.0, 10.1, 5.4)	5.62, ddd (17.0, 10.2, 5.4)
3	5.00, app t (5.5)	4.92, app t (5.7)	4.94, app t (5.0); OH 1.9, d (6.0)	4.47, dd (5.6, 5.4); OH 1.17 overlapped
8	5.59, d (11.0)	2.55, d ABq (6.0, 17.5)	4.66, app t (5.0); OH 2.12, d (5.0) [4.41, dd (3.8, 7.1)]; [OH 2.15, d (7.1)]	4.16, dd (3.3, 5.0); OH 1.52 d (5.0) [4.01], dd (3.7, 7.0); OH 1.59 d (7.0)
9	6.08, dd (11.0, 8.6)	4.26, ddd (6.8, 6.0, 4.6)	3.02, m [3.00, m]	2.65, dd (3.3, 1.4) [2.68], m
10	4.64, m	5.52, dd (15.3, 6.8)	3.10, dt (2.0, 5.6) [2.98, dt (2.0, 5.6)]	2.88, br t (5.5) [2.67], m
11	1.63, m 1.51, m	5.74, dt (15.3, 6.8)	1.59, m [1.58]	1.28, m [1.27]
12	1.37, m	2.05, m	1.44, m	1.22, m
13	1.31, m	1.31, m	1.29, m	1.15, m
14	1.30, m	1.30, m	1.32, m	1.15, m
15	1.27, m	1.27, m	1.28, overlapped	1.17, m
16	1.29, m	1.29, m	1.28, overlapped	1.26, m
17	0.89, t (6.9)	0.89, t (7.1)	0.89, t (7.1)	0.89, t (6.9)

[a]Assignments aided by COSY, HSQC, and HMBC experiments

tube prevented an accurate measurement of the $[\alpha]_D$ value and further stereochemical analysis. In this regard, some considerations should be made. It is reasonable to assume that *trans*-panaxydiol (**1**) isolated from *Eryngium* extract derives, in part, by the isomerization of co-occurring *cis*-panaxydiol (**5**). This implies that both isomers have the same 3*S* absolute configuration, as inferred by analysis of the Mosher derivatives of **1** (see above), while the stereochemistry at C-10 remains undetermined. Similar to the *trans*-isomer, *cis*-panaxydiol could be a C-10 epimeric mixture even though the possibility that the two naturally occurring isomers **1** and **5** display opposite configuration at C-10 should not be ruled out. In such a case, the transformation of **5** into **1** should explain the existence in the extract of C-10 epimers of **1**.

Compound **6** was obtained as a yellowish oil and its molecular formula was assigned as $C_{17}H_{24}O_2$ from the sodiated molecular peak at *m/z* 283.1665 $[M + Na]^+$ observed in the HRESIMS spectrum. The ^{1}H and ^{13}C NMR spectra of this compound displayed signals very similar to those observed for known co-occurring polyacetylenes **1**–**5**, in particular, indicating the presence of the same partial structure C-1/C-7 (**Tables 1** and **2**). The ^{1}H NMR spectrum also showed multiplets that were consistent with the presence of a *trans* double bond [δ_H 5.52 (1H, dd, *J* = 15.3, 6.8 Hz, H-10) and δ_H 5.74 (1H, dt, *J* = 15.3, 6.8 Hz, H-11)], a carbinolic methine [δ_H 4.26 (1H, ddd, *J* = 6.8, 6.0, 4.6 Hz, H-9)], a downfield shifted methylene linked to a quaternary carbon [δ_H 2.55 (2H, d ABq, *J* = 6.0, 17.5 Hz, H_2-8)], an allylic methylene [δ_H 2.05, (2H, m, H_2-12)], and an aliphatic chain with a terminal methyl group [δ_H 0.89 (3H, t, *J* = 7.1 Hz, H_3-17)]. Analysis of the ^{1}H-^{1}H COSY experiment led us to define the proton sequence from H-8 to terminal H_3-17, whereas the heterocorrelations observed in the HSQC and HMBC spectra aided us in defining the remaining part of the molecule and to complete the structure. In particular, the H-1/H-3 and H-8/H-17 spin systems were connected through a conjugated diyne moiety by diagnostic HMBC correlations observed between H_2-8 (δ_H 2.55) and both C-7 (δ_C 78.2) and C-6 (δ_C 66.5) as well as H-3 (δ_H 4.92) and C-4 (δ_C 81.5). Thus, the structure was suggested to be (10*E*)-heptadeca-1,10-diene-4,6-diyne-3,9-diol (**6**), to which the trivial name triquetridiol was given. The absolute configuration of C-3 and C-9 chiral centers could not be determined by the Mosher method due to the scarce amount of sample. However, the 3*S* configuration was suggested analogous with co-occurring compounds **1**-**5** on the basis of the positive $[\alpha]_D$ value, which was also observed for this member of *Eryngium* polyacetlenes.

Table 2 ^{13}C NMR (75 and 150 MHz) data for compounds 5–7 in $CDCl_3$ (δ C in ppm). [a]Data divergent for minor isomers are shown in square brackets.

	5	6	7	7 (C_6D_6)
1	117.4, CH_2	117.2, CH_2	117.2, CH_2	116.1, CH_2
2	135.8, CH	135.8, CH	135.8, CH	136.0, CH
3	63.7, CH	63.5, CH	63.3, CH	63.0, CH
4	81.1, C	81.5, C	nd	79.2, C
5	70.9, C	Nd	nd	70.3, C
6	nd	66.5, C	nd	nd
7	75.2, C	78.2, C	77.2, C	nd
8	108.4, CH	28.6, CH_2	61.3, CH [62.3]	61.7, CH [62.5]
9	150.0, CH	70.8, CH	58.8, CH [59.3]	59.0, CH [59.6]
10	70.4, CH	130.4, CH	56.0, CH [56.1]	55.7, CH [55.5]
11	36.6, CH_2	133.9, CH	31.2, CH_2	31.0, CH_2
12	25.0, CH_2	32.1, CH_2	25.7, CH_2	25.8, CH_2
13	29.2, CH_2	28.9, CH_2	28.5, CH_2	29.3, CH_2
14	29.5, CH_2	29.0, CH_2	29.0, CH_2	29.3, CH_2
15	31.8, CH_2	31.7, CH_2	31.7, CH_2	31.8, CH_2
16	22.6, CH_2	22.6, CH_2	22.6, CH_2	22.8, CH_2
17	14.1, CH_3	14.1, CH_3	13.9, CH_3	13.8, CH_3

trans-Epoxy-triquetrol (diastereomeric mixture **7a**/**7b**) exhibited a sodiated molecular peak at *m/z* 299.1629 in the HRESIMS spectrum, consistent with the molecular formula $C_{17}H_{24}O_3$, with a difference of 16 mass units with respect to panaxydiol (**1**) and triquetridiol (**6**). The NMR spectra (**Tables 1** and **2**) resembled those of the other co-occurring polyacetylenes, indicating the presence in the structure of the same terminal portions. The main difference was in the lack of the internal double bond being the olefinic resonances replaced by signals due to oxygenated carbons. However, a careful analysis of both ^{1}H and ^{13}C NMR spec-

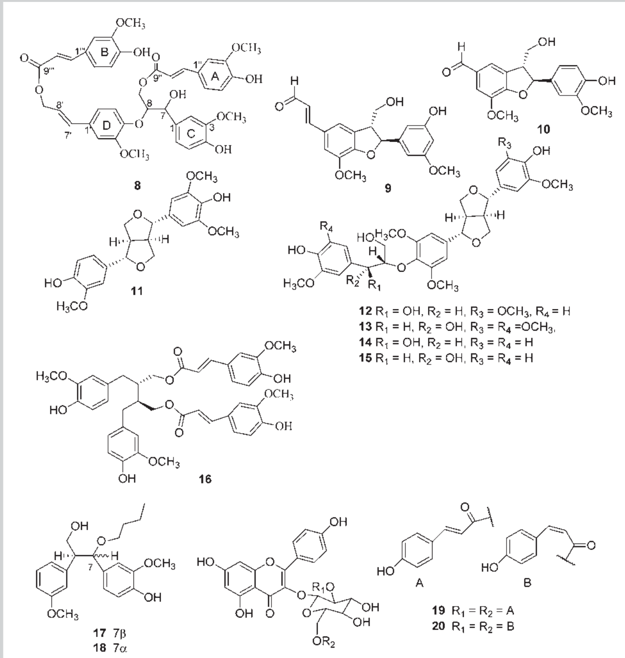

Fig. 3 Lignans and flavonoids isolated from *E. triquetrum*.

tra revealed that we were dealing with a mixture of two diastereoisomers (**7a**/**7b**). Unfortunately, every attempt to separate the two isomers failed, thus, they were characterized spectroscopically as a mixture. In the NMR spectra (recorded in $CDCl_3$ and C_6D_6), along with the expected series of signals due to terminal C-1/C-7 and C-11/C-17 fragments, resonances due to oxygenated methines were observed (**Tables 1** and **2**). In particular, two sets of signals (ratio 1 : 0.8) were recognized in the proton and carbon spectra, and were connected by detailed analysis of ^{1}H-^{1}H COSY and HSQC experiments. Two distinct spin systems accounting for the C-8/C-10 fragment of both isomers were defined, whereas the remaining portions of the molecules gave indistinguishable signals in the spectra as reported in **Tables 1** and **2**. For the main isomer (**7a**), the sequence was constituted by the proton resonating at δ_H 4.66 (1H, app. t, J = 5.0 Hz, H-8), which had a cross-peak with the multiplet at δ_H 3.02 (1H, m, H-9), which, in turn, correlated with the signal at δ_H 3.10 (1H, dt, J = 2.0 and 5.6 Hz, H-10). An analogous sequence connecting sequentially H-8 (δ_H 4.41, dd, J = 3.8 and 7.1 Hz), H-9 (δ_H 3.00, m), and H-10 (δ_H 2.98, dt, J = 2.0 and 5.6 Hz) was deduced for the minor component (**7b**). These data along with the ^{13}C NMR values [δ_C **7a**/**7b** 61.3/62.3 (C-8), δ 58.8/59.3 (C-9), and δ 56.0/56.1 (C-10)] strongly suggested the presence of a hydroxyl group at C-8 and the epoxy ring, including C-9 and C-10. An NMR assignment was also made in C_6D_6 (**Tables 1** and **2**) due to the observation that the signals of the two isomers were better distinguished in this solvent. The relative configuration of the chiral centers C-9 and C-10 was deduced by comparing the proton and carbon value pattern for the hydroxy epoxide fragment in both **7a** and **7b** with literature NMR data for model compounds exhibiting a *cis* or *trans* epoxide [25–28]. In particular, the small coupling constants of epoxide protons ($J_{H\text{-}H}$ = 1–2 Hz) measured by homo-decoupling experiments, the NOE effects observed between H-8 and H-10, and the epoxide carbon values were consistent with a *trans* geometry, analogous to the synthetic C-10 epimer of oploxyne A [28]. Having fixed the *trans* stereochemistry of the epoxide moiety, it was reasonable to suppose that the two isomers differed in the relative configuration of the carbinol carbon adjacent to the epoxide. Unfortunately, due to the very scarce amount of the sample, a further stereochemical investigation could not be made and, thus, this aspect remained undetermined. On the other side, the absolute configuration of C-3 was supposed to be the same as the co-occurring polyacetylenes by biogenetic considerations.

The lignan fraction was purified as reported in Materials and Methods to give an inseparable *erythro*/*threo* pair of unprecedented demethoxy carolignan Z (**8a**/**8b**) along with a series of known compounds. Previously reported lignans included two epoxy-neolignans, balanophonin (**9**) [29] and ficusal (**10**) [30], the diepoxy-lignan (+)-medioresinol (**11**) [31], four sesquilignans, buddlenol C (**12**) [32], buddlenol D (**13**) [32], and *threo*- and *erythro*-buddlenol E (**14, 15**) [32, 33], and secolignan 9,9′-diferuloyl-secoisolariciresinol (**16)** [34] (**Fig. 3**).

Demethoxy carolignan Z was isolated and characterized as an *erythro*/*threo* pair (**8a**/**8b**). The molecular formula $C_{40}H_{40}O_{13}$ was deduced from the sodiated molecular peak at 751.2360 in the HRESIMS spectrum. ^{1}H and ^{13}C NMR data (**Table 3**) showed strong similarities with those reported in the literature for carolignans [35–37], suggesting the presence of a carolignan framework for **8a**/**8b**. The ^{1}H NMR spectrum (**Table 3**) showed a series of aromatic protons undistinguishable for the two isomers and attributable to two units of feruloyloxy group (A and B rings), two sets of signals for the 1,3,4-trisubstituted benzene units (C and D rings), two *trans* olefinic protons (H-7′ and H-8′), and two methylene protons (H_2-9′). Additional signals due to protons linked to oxygen-bearing carbons were also present in the spectrum (H-7, H-8, and H_2-9). Careful analysis of these latter signals and their ^{1}H-^{1}H COSY correlations clearly indicated that they constituted two distinct set of signals due to the 1,2,3-propanetriol moiety for each isomer. A spin system consisted in the proton at δ_H 4.93 (1H, m, H-7) that had a cross-peak with the methine proton at δ_H 4.30 (H-8), which was, in turn, coupled to the methylene protons at δ_H 4.14 (1H, dd, J = 12.0, 3.6 Hz, H-9a) and δ_H 4.32 (1H, dd, J = 12.0, 3.6 Hz, H-9b). In the other spin system, the proton at δ_H 4.91 (1H, m, H-7) was coupled with the methine at δ_H 4.52 (1H, m, H-8), which was correlated with the methylene protons at δ_H 4.50 (1H, dd, J = 12.0, 3.6 Hz, H-9a) and δ_H 4.32 (1H, dd, J = 12.0, 3.6 Hz, H-9b). The HSQC spectrum showed two sets of carbon values for the two sequences: δ_C 72.3 (C-7), δ_C 84.4, (C-8), and δ_C 62.6 (C-9) for the *erythro*-isomer (**8a**) and δ_C 74.4 (C-7), δ_C 86.1, (C-8), and δ_C 63.2 (C-9) for the *threo*-isomer (**8b**), according to literature data reported for *erythro*/*threo* carolignans isolated from *Ochroma lagopus* [38]. Diagnostic HMBC correlations aided us in linking all these moieties. The carbonyl C-9″ showed cross-peaks with H-8″ and H-9a connecting one of the two feruloyloxy groups (ring A) to the propanetriol moiety, whereas the second carbonyl C-9‴ had long-range correlations with both H-8‴ and H_2-9′, linking the second feruloyloxy group (ring B) to ring D. Phenolic carbons C-1 and C-4′ were correlated with H-7 and 7-OH and with H-8 and H-2″/H-6″, respectively, thus linking both 1,3,4-trisubstitued rings C and D to the propanetriol moiety. By these data, the proposed structure for the *erythro*/*threo* pair **8a**/**8b** was diferuloyloxy4,8-dihydroxy-3,3′-dimethoxy-4′,8-oxyneolignan-7′-en-9,9′-dioate, corresponding to the 5′-demethoxy derivative of carolignan Z, which has been very recently described from an *Euphorbia* species [37].

Finally, four known phenolic compounds were identified in the extract: *threo*-2,3-bis(4-hydroxy-3-methoxyphenyl)-3-butoxypropan-1-ol (**17**) [39], *erythro*-2,3-bis(4-hydroxy-3-methoxyphenyl)-3-butoxypropan-1-ol (**18**) [39], kaempferol-3-*O*-(2,6-di-*Z*-*p*-coumaroyl)-glucoside (**19**) [40], and kaempferol-3-*O*-(2,6-di-*E*-*p*-coumaroyl)-glucoside (**20**) [40] (**Fig. 3**).

In conclusion, the chemical study on *E. triquetrum* from Algeria resulted in the isolation of 20 compounds, polyacetylenes, lignans, and flavonoids, including previously undescribed metabolites. It is the first report of polyacetylenes and lignans from this plant. The secondary metabolite pattern is, however, in agreement with the literature data for other *Eryngium* species.

Materials and Methods

General experimental procedures

Optical rotations were measured on a Jasco DIP 370 digital polarimeter. FTIR spectra were obtained using a Spectrum 100 instrument from Perkin Elmer fitted with a Germanium/KBr beam splitter and a deuterated tryglycine sulfate (DTGS) wideband detector on KBr pellets. UV spectra (MeOH) were acquired on a Jasco V-650 spectrophotometer. ESIMS were performed on a Micromass Q-TOF MicroTM coupled with an HPLC Waters Alliance 2695. The instrument was calibrated by using a PEG mixture from 200 to 1000 MW (resolution specification 5000 FWHM, deviation < 5 ppm RMS in the presence of a known lock mass). High-resolution mass spectra (HRESIMS) were acquired on a Q-Exactive hybrid quadrupole-orbitrap mass spectrometer (Thermo Scientific). NMR experiments were recorded at the ICB-NMR Service Centre. Chemical shifts values are reported in ppm and referenced to the internal signals of residual protons ($CDCl_3$, ^1H δ 7.26, ^{13}C 77.0 ppm; C_6D_6, ^1H δ 7.15, ^{13}C 128.0 ppm). 1D and 2D NMR spectra were acquired on a Bruker Avance-400 operating at 400 MHz using an inverse probe fitted with a gradient along the Z-axis and a Bruker DRX-600 operating at 600 MHz using an inverse TCI CryoProbe fitted with a gradient along the Zaxis. HPLC separation was performed on a Shimadzu high-performance liquid chromatography using a Shimadzu liquid chromatograph LC-10AD equipped with an UV SPD-10A wavelength detector with a reversed-phase (RP) column (10 × 250 mm, Aventis-Supelco). Silica gel chromatography was performed using precoated Merck F254 plates (TLC) and Merck Kieselgel 60 powder (70–230 mesh). The $AgNO_3$ silica gel was prepared by adsorbing a silver nitrate solution on silica (10% w/w). The spots on TLC were visualized under UV light (254 nm) and then were sprayed with 10% H_2SO_4 in water followed by heating.

Plant material

The plant *E. triquetrum* was collected in April 2012 in Merouana (Batna, Algeria) and identified by Prof. Bachir Oudjehih, Institute of Agronomy of University of Batna (Algeria). A voucher specimen has been deposited in the herbarium of the department of the same University with code 122/ISVSA/UHL/13.

Extraction and isolation

The dried and powdered aerial part (500 g) of *E. triquetrum* was exhaustively extracted at room temperature with a hydroalcoholic solution ($MeOH/H_2O$ 8 : 2) three times (400 mL×3), filtered, combined, and concentrated under vacuum to afford an aqueous solution. The latter was sequentially partitioned with light petroleum ether (250 mL×3), ethyl acetate (250 mL×3), and *n*-butanol (250 mL×3) to give 2.4 g of light petroleum ether, 3.0 g of ethyl acetate, and 11.0 g of butanolic extracts, respectively. The extracts were analyzed by TLC chromatography, which revealed an interesting metabolite pattern in the ethyl acetate extract. A portion of this extract (2.0 g) was fractionated by Sephadex LH-20 chromatography (column diameter: 3 cm, h: 130 cm, LH-20: 200 g) using a solution of $CHCl_3/MeOH$ (1 : 1) in isocratic fashion to get 50 fractions (each with an 8-mL volume) that were combined on the basis of their TLC chromatographic behavior to give 11 fractions (1–11), two of which (fractions 4 and 7) were considered in this work. Fraction 4 (580 mg) was subjected to silica gel column chromatography (column diameter: 2.5 cm, h: 100 cm silica gel: 29 g) eluted with an increasing polarity gradient of $CHCl_3/MeOH$. A total of 70 fractions were collected (volume of each tube: 15 mL) starting from $CHCl_3$ (250 mL of solvent, collected 17 tubes), $CHCl_3/MeOH$ 98 : 2 (200 mL of solvent, 13 tubes), $CHCl_3/MeOH$ 95 : 5 (200 mL of solvent, 13 tubes), $CHCl_3/MeOH$ 9 : 1 (300 mL of solvent, 15 tubes), $CHCl_3/MeOH$ 7 : 3 (170 mL of solvent, 11 tubes), and only MeOH (150 mL, 10 tubes). After TLC chromatography, all of these fractions were combined

Table 3 ^1H and ^{13}C NMR (400 MHz) data in $CDCl_3$ for **8a** (*erythro* isomer). Data in square brackets refer to **8b** (*threo* isomer).

1	–	131.2, C
2	7.04, d (2.0)	109.2, CH
3	–	145.2, C
4	–	146.8, C
5	6.85, d (8.5)	114.2, CH
6	6.88, dd (8.5, 2.0)	119.2, CH
7	4.93, m [4.91]	72.3, CH [74.4]
8	4.30, m [4.52]	84.4, CH [86.1]
9	4.14, dd (12.0, 3.6) [4.50, dd (12.5, 3.6)] 4.32, dd (12.0, 3.6) [4.32, dd (12.0, 3.6)]	62.6, CH [63.2]
3-OMe	3.86, s	55.8, CH_3
1′	–	133.5, C
2′	6.95, d (1.5)	109.3, CH
3′	–	150.1, C
4′	–	148.1, C
5′	7.12, d (8.5)	120.2, CH
6′	7.01, dd (8.5, 1.5)	120.4, CH
7′	6.64, d (16.0)	133.6, CH
8′	6.26, dt (16.0, 6.5)	122.9, CH
9′	4.85, d (6.5)	65.1, CH_2
3′-OMe	3.89, s	55.7, CH_3
1′′	–	126.3, C
2′′	7.01, d (1.8)	110.0, CH
3′′	–	145.3, C
4′′	–	146.8, C
5′′	6.91, d (8.3)	114.7, CH
6′′	7.07, dd (8.3, 1.8)	123.7, CH
7′′	7.49, d (16.0)	145.5, CH
8′′	6.23, d (16.0)	114.9, CH
9′′	–	166.4, C
3′′-OMe	3.93, s	55.7, CH_3
1′′′	–	126.7, C
2′′′	7.01, d (1.8)	109.3, CH
3′′′	–	145.5, C
4′′′	–	147.2, C
5′′′	6.94, d (8.3)	114.3, CH
6′′′	7.08, dd (8.3, 1.8)	123.7, CH
7′′′	7.65, d (16.0)	144.9, CH
8′′′	6.33, d (16.0)	114.7, CH
9′′′	–	166.8, C
3′′′-OMe	3.92, s	55.8, CH_3

[a] Assignments aided by COSY, HSQC, and HMBC

to give 12 final fractions (F4-1→F4-12), which were analyzed by ^{1}H NMR. Subfraction F4-1 (10.0 mg) resulted in containing pure falcarinol **2** (R*f* 0.90, $CHCl_3$/MeOH 95 : 5, 8 mg). Subfraction F4-3 (60 mg) was a mixture that was further purified by HPLC (Supelco, Ascentis C18 column 1.0 × 25 cm) with a 45-min linear gradient from 60 to 100% MeOH in H_2O (flow rate 2 mL/min) to yield pure compounds **1** (t_R 34.8 min, 20 mg), **4** (t_R 35.5 min, 3.0 mg), and **3** (t_R 37.5 min, 2.0 mg), along with a mixture collected as single peak at t_R 33.5 min. This mixture (19.3 mg) was subjected to a further HPLC purification (Supelco, Ascentis C18 column, 0.46 × 25 cm) using a 40-min linear gradient starting from 70 to 100% MeOH in H_2O (flow rate 1 mL/min) to obtain pure compounds **5** (t_R 15.8 min, 4 mg) and **1** (t_R 17.2 min, 8 mg). Subfraction F4-4 (43.0 mg) was purified by HPLC using the same condition described for F4-3 (Supelco, Ascentis C18 column 1.0 × 25 cm) yielding pure compounds **11** (t_R 11.3 min, 4.0 mg), **17** (t_R 23.5 min, 3.0 mg), **18** (t_R 24.2 min, 4.0 mg), and **4** (t_R 36.6 min, 2.0 mg), along with compound **6** collected in a mixture with **5** at t_R 34 min. This mixture (3.0 mg) was purified on an $AgNO_3$-impregnated SiO_2 pipette column ($AgNO_3$ silica gel: 1 g, volume of each collected tube: 1 mL) eluted with a gradient of diethyl ether in hexane (hexane/diethyl ether 8 : 2, 10 mL; hexane/diethyl ether 7 : 3, 10 mL; hexane/diethyl ether 6 : 4, 10 mL; hexane/diethyl ether 5 : 5, 20 mL; hexane/diethyl ether 4 : 6, 10 mL) to afford pure compounds **5** (1.0 mg, eluted with hexane/diethyl ether 7 : 3) and **6** (0.8 mg, eluted with hexane/diethyl ether 6 : 4). Subfraction F4–5 (5.0 mg) was subjected to HPLC purification (Supelco, Ascentis C18 column, 0.46 × 25 cm) using a 40-min linear gradient from 70 to 100% MeOH in H_2O, (flow rate 1 mL/min) obtaining the mixture **7a/7b** (t_R 12.5 min, 1.0 mg). Subfraction F4–6 (100.0 mg) was purified by HPLC (Supelco, Ascentis C18 column, 1.0 × 25 cm) with a 60-min linear gradient from 50 to 100% MeOH in H_2O (flow rate 2 ml/min) to afford pure compounds **10** (t_R 22.1 min, 2.0 mg), **9** (t_R 26.3 min, 7.2 mg), **13** (t_R 27.1 min, 3.2 mg), **12** (t_R 29.7 min, 6.4 mg), **14** (t_R 31.1, 3.0 mg), and **16** (t_R 45.0, 2.5 mg), together with two fractions collected at t_R 28.5 (fraction A) and 43.2 min (fraction B), respectively. Both fractions were subjected to a further HLPC purification (Supelco, Ascentis C18 column 1 × 25 cm) by using an isocratic elution (CH_3CN/H_2O, 1 : 1 for fraction A; CH_3CN/H_2O, 6 : 4 for fraction B) to get pure compound **15** (t_R 8.3 min, 4.0 mg) from fraction A and the mixture **8a/8b** (t_R 12.5 min, 3.5 mg) from fraction B.

Fraction 7 (130 mg), from the starting Sephadex column, was further chromatographed on a silica gel column (column diameter: 1.5 cm; h: 60 cm; silica gel: 8 g) employing $CHCl_3$/MeOH in order of increasing polarity. A total of seven subfractions were collected (each fraction volume: 60 mL; subfraction 1 eluted with $CHCl_3$; subfraction 2 eluted with $CHCl_3$/MeOH 98 : 2; subfraction 3 eluted with $CHCl_3$/MeOH 95 : 5; subfraction 4 eluted with $CHCl_3$/MeOH 9 : 1; subfraction 5 eluted with $CHCl_3$/MeOH 8 : 2; subfraction 6 eluted with $CHCl_3$/MeOH 7 : 3; subfraction 7 eluted with MeOH). Subfraction 4 (13.5 mg), obtained from $CHCl_3$/MeOH 9 : 1, was a mixture of *cis* and *trans p*-coumaroyl-kaempferolglucosides. This mixture was further separated by HPLC (Supelco, Ascentis C18 column, 1.0 × 25 cm) by using an isocratic elution (CH_3CN/H_2O, 1 : 1, flow rate 2.0 mL/min) to give pure compounds **20** (t_R 9.8 min, 3.5 mg) and **19** (t_R 10.5 min, 4.0 mg).

(Z)-heptadeca-1,8-diene-4,6-diyne-3,10-diol (**5**), *cis*-panaxydiol: Oil; ^{1}H and ^{13}C NMR see **Tables 1** and **2**; HRESIMS *m/z* 283.1670 (calcd. for $C_{17}H_{24}O_2$ Na, 283.1674).

(10E)-heptadeca-1,10-diene-4,6-diyne-3,9-diol (**6**), triquetridiol: Oil; $[\alpha]_D^{20}$ + 35.42 (*c* 0.5, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 257 (1.98), 243 (2.07), 230 (2.3), 203 (3.18); IR ν_{max}: 3351, 2955, 2925, 2853, 2255, 1731, 1025, 930, 705 cm^{-1}; ^{1}H and ^{13}C NMR see **Tables 1** and **2**; HRESIMS *m/z* 283.1665 (calcd. for $C_{17}H_{24}O_2Na$, 283.1674).

Heptadeca-1-ene-9,10-epoxy-4,6-diyne-3,8-diol (**7a/7b**), triquetrol: Oil; UV (MeOH) λ_{max} (log ε): 284 (2.75), 269 (2.82), 257 (2.90), 244 (2.95), 232 (2.97); IR ν_{max} 3382, 2856, 2255, 2154, 1721, 1463, 1024, 930 cm^{-1}; ^{1}H and ^{13}C NMR in $CDCl_3$ see **Tables 1** and **2**; HRESIMS *m/z* 299.1629 (calcd. for $C_{17}H_{24}O_3$ Na, 299.1623).

Diferuloyloxy-4,8-dihydroxy-3,3′-dimethoxy-9,9′-4′,8-oxyneolignan-7′-en-9,9′-di-oate (**8a/8b**), 5′-demethoxy carolignan Z: White powder; UV (MeOH) λ_{max} (log ε): 324 (4.017), 288 (3.93), 233 (4.019), 219 (4.12), 204 (4.30); IR ν_{max} 3410, 3011, 2956, 2927, 2852, 1701, 1630, 1594, 1512, 1430, 1377, 1268, 978, 819, 755 $cm^{-1.1}$H and ^{13}C NMR see **Table 3**; HRESIMS *m/z* 751.2360 (calcd. for $C_{40}H_{40}O_{13}$ Na, 751.2367).

(8E)-heptadeca-1,8-diene-4,6-diyne-3S,10-diol (**1**), *trans*-panaxydiol: Oil; ^{1}H NMR values in ppm ($CDCl_3$, 400 MHz): δ_H 6.33 (1H, dd, *J* = 15.9, 6.0 Hz, H-9), 5.95 (1H, ddd, *J* = 15.9, 10.3, 5.4 Hz, H-2), 5.77 (1H, d, *J* = 15.9 Hz, H-8), 5.47 (1H, d, *J* = 16.5 Hz, H-1a), 5.25 (1H, d, *J* = 10.3 Hz, H-1b), 4.98 (1H, app t, *J* = 5.4 Hz, H-3), 4.18 (1H, m, H-10), 1.53 (2H, m, H_2-11), 1.33 (2H, m, H_2-12), 1.29 (2H, m, H_2-13), 1.29 (2H, m, H_2-14), 1.27 (2H, overlapped, H_2-15), 1.27 (2H, overlapped, H_2-16), 0.88 (3H, t, *J* = 7.1 Hz, H_3-17); ^{13}C NMR values in ppm ($CDCl_3$, 75 MHz): δ_C 149.8 (CH, C-9), 136.0 (CH, C-2), 117.0 (CH_2, C-1), 108.1 (CH, C-8), 80.0 (C, C-4), 77.6 (C, C-7), 73.6 (C, C-6), 72.0 (CH, C-10), 70.9 (C, C-5), 63.5 (CH, C-3), 36.9 (CH_2, C-11), 31.7 (CH_2, C-15), 29.4 (CH_2,C-13) 29.2 (CH_2, C-14), 25.2 (CH_2, C-12), 22.6 (CH_2, C-16), 14.1 (CH_3, C-17).

Preparation of MTPA esters of compound 1

(*R*)- and (*S*)-MTPA-Cl (10 µL) and a catalytic amount of DMAP were separately added to two different aliquots of panaxydiol (**1**) (1.0 mg each) in dry CH_2Cl_2 (0.5 mL). The resulting mixtures were allowed to stand at rt for 12 h. After evaporation of the solvent, the mixtures were purified on a SiO_2 pipette *Pasteur* (CH_2Cl_2/MeOH, 99 : 1) affording pure (*S*)- and (*R*)-MTPA esters of **1**, respectively.

*(S)-MTPA ester of **1***: selected ^{1}H NMR values ($CDCl_3$, 600 MHz): δ_H 5.529 (1H, d, *J* = 17.0 Hz, H-1a), 5.362 (1H, d, *J* = 10.2 Hz, H-1b), 5.834 (1H, ddd, *J* = 5.5, 10.2, 17.0 Hz, H-2), 6.153 (1H, d, *J* = 5.7 Hz, H-3); ESIMS *m/z* 715 $[M + Na]^+$.

*(R)-MTPA ester of **1***: selected ^{1}H NMR values ($CDCl_3$, 600 MHz): δ_H 5.611 (1H, d, *J* = 17.0 Hz, H-1a), 5.422 (1H, d, *J* = 10.2 Hz, H-1b), 5.939 (1H, ddd, *J* = 5.5, 10.2, 17.0 Hz, H-2), 6.126 (1H, d, *J* = 5.7 Hz, H-3); ESIMS *m/z* 715 $[M + Na]^+$.

(3S,9Z)-Heptadeca-1,9-diene-4,6-diyne-3-ol (**2**), falcarinol: Oil; ^{1}H NMR values in ppm ($CDCl_3$, 400 MHz): δ_H, 5.94 (1H, ddd, *J* = 15.7, 10.1, 5.4 Hz, H-2), 5.51 (1H, m, H-10), 5.46 (1H, d, *J* = 15.7 Hz, H-1a), 5.37 (1H, m, H-9), 5.24 (1H, d, *J* = 10.1 Hz, H-1b), 4.91 (1H, app t, *J* = 5.4 Hz, H-3), 3.03 (2H, d, *J* = 7.0 Hz, H2–8), 2.02 (2H, m, H_2-11), 1.37 (2H, m, H_2-12), 1.27 (2H, overlapped, H_2-13), 1.29 (2H, m, H_2-14), 1.27 (2H, overlapped, H_2-15), 1.25 (2H, overlapped, H_2-16), 0.88 (3H, t, *J* = 7.1 Hz, H_3-17); ^{13}C NMR values in ppm ($CDCl_3$, 75 MHz): δ_C 136.2 (CH, C-2), 133.0 (CH, C-10), 116.9 (CH_2, C-1), 122.1 (CH, C-9), 80.0 (C, C-7), 74.5 (C, C-4), 71.1 (C, C-5), 64.3 (C, C-6), 63.3 (CH, C-3), 31.9 (CH_2, C-15), 29.3 (CH_2,C-13) 29.3 (CH_2, C-14), 27.2 (CH_2, C-11), 29.2 (CH_2, C-12), 22.7 (CH_2, C-16), 17.7 (CH_2, C-8), 14.1 (CH_3, C-17).

Preparation of MTPA esters of compound 2

(*R*)- and (*S*)-MTPA-Cl (10 µL) and a catalytic amount of DMAP were separately added to two different aliquots of falcarinol (**2**) (1.0 mg each) in dry CH_2Cl_2 (0.5 mL). The resulting mixtures were allowed to stand at rt for 12 h. After evaporation of the solvent, the mixtures were purified on a SiO_2 pipette *Pasteur* (CH_2Cl_2/MeOH, 99 : 1) affording pure (*S*)- and (*R*)-MTPA esters of **2**, respectively.

*(S)-MTPA ester of **2***: selected ^{1}H NMR values ($CDCl_3$, 400 MHz): δ_H 5.516 (1H, d, *J* = 17.0 Hz, H-1a), 5.332 (1H, d, *J* = 10.2 Hz, H-1b), 5.821 (1H, ddd, *J* = 5.5, 10.2, 17.0 Hz, H-2), 6.104 (1H, d, *J* = 5.7 Hz, H-3), 3.0443 (1H, br d, *J* = 6.8 Hz, H-8), 5.534 (3H, m, H-9), 5.375 (1H, m, H-10); ESIMS *m/z* 483 $[M + Na]^+$.

*(R)-MTPA ester of **2***: selected ^{1}H NMR values ($CDCl_3$, 400 MHz): δ_H 5.595 (1H, d, *J* = 17.0 Hz, H-1a), 5.394 (1H, d, *J* = 10.2 Hz, H-1b), 5.926 (1H, ddd, *J* = 5.5, 10.2, 17.0 Hz, H-2), 6.073 (1H, d, *J* = 5.7 Hz, H-3), 3.0374 (1H, br d, *J* = 6.8 Hz, H-8), 5.525 (3H, m, H-9), 5.365 (1H, m, H-10); ESIMS *m/z* 483 $[M + Na]^+$.

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Conflict of Interest

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The authors have no conflict of interest to declare.

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