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New neo-clerodane diterpenes from *Teucrium polium* subsp. *capitatum*

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ABSTRACT

Phytochemical investigation of a methanolic extract from the aerial parts of *Teucrium polium* subsp. *capitatum* afforded six acetoxylated neo-clerodane diterpenes, including 20-acetylauropolin and 6-acetylteucjaponin A, along with four previously undescribed congeners. The compounds were isolated by a combination of silica gel column chromatography and preparative HPLC-ESIMS. Their structures were determined by extensive NMR analysis, HRESIMS, and by comparison with literature data of related compounds. The absolute configuration of 20-acetylauropolin was confirmed by X-ray crystallographic diffraction analysis. Some of the isolated diterpenes possess structural features unusual in the class of neo-clerodane diterpenes, such as a rare C-20 hemiacetal function which forms an oxepane ring to C-7 of the *trans*-decalin core structure.

1. Introduction

Teucrium polium subsp. *capitatum* (L.) Arcang (syn. *Teucrium capitatum* L.; Lamiaceae), also known as felty or golden germander, is a perennial subshrub that natively grows in Mediterranean regions of Europe, Northern Africa, and Southwestern Asia [1]. The aerial parts of the plant are traditionally used in Algeria as a decoction or ointment in the treatment of hypertension, diabetes, and wounds [2,3]. *T. polium* including subspecies and varieties is known to contain a wide variety of polyphenolic compounds including flavonoids and phenylethanoid glycosides, as well as various terpenoids, such as triterpenes, sterols, sesquiterpenoids, iridoids, and diterpenoids [2,4,5]. In particular, these plants are a source of neo-clerodane diterpenes [4–10], which are known for their antifeedant effects on insects and are thought to be responsible for the hepatotoxicity occasionally observed after the consumption of *Teucrium*-containing herbal products [11–16]. Recently, neo-clerodane diterpenes from *T. yemense* were shown to increase glucose-triggered release of insulin from murine pancreatic islets [17].

In a previous study we reported the wound healing properties of a methanolic extract from the aerial parts in a wound excision model in rabbits. A comprehensive analysis of the polyphenolic profile of this extract led to the isolation and identification of 12 flavonoids and two phenylethanoid glycosides [2]. Herein, we report on the further investigation of the methanolic extract leading to the isolation and structure elucidation of six furanoid neo-clerodane diterpenes including four new congeners.

2. Experimental

2.1. General procedures

Normal phase flash chromatography was performed on a Premium Flash-Prep LC system PuriFlash® 4100 equipped with an UV detector and a fraction collector (Interchim, Montluçon, France). Preparative HPLC was performed on a Preparative LC/MSD System (Agilent Technologies, Santa Clara, CA, USA) consisting of a 1260 binary pump, a 1100 diode array detector, and a 6120 single quadrupole MS detector. A SunFire Prep C18 OBD column (5 µm, 150 × 30 mm i.d., Waters, Milford, MA, USA), equipped with a C18 Prep Guard Cartridge (10 × 30 mm i.d.) was used. Water (A) and MeCN (B), both containing 0.1% formic acid, were used as mobile phase. A flow rate of 20 mL/min was applied. A 1290 Infinity II Valve Drive manual injection system (Agilent Technologies) was used for injection. Detection was with ESIMS in positive scan mode in combination with UV at 210 nm. For ESIMS detection a Quicksplit™ adjustable nano flow splitter (Analytical Scientific In-

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struments Inc., Richmond, CA, USA) was used with a 1:100 flow split. A make-up flow of 0.4 mL/min (50% aqueous MeCN with 0.1% formic acid) generated by an Agilent 1290 Infinity II quaternary pump was added post-split. Data acquisition and processing was done by ChemStation software (Agilent Technologies).

Semi-preparative separations were carried out on an HP1100 Series HPLC instrument (Agilent Technologies) equipped with a binary pump, auto sampler, column oven, and a diode array detector. Chromatography was performed on a SunFire C18 column (5 µm, 150 × 10 mm i.d., Waters) equipped with a guard column (10 × 10 mm i.d.). The mobile phase consisted of A and B as described above at a flow rate of 4 mL/min. Data acquisition and processing was performed using ChemStation software (Agilent Technologies).

HPLC-PDA-ELSD-ESIMS was conducted on a LC-MS 8030 chromatographic system (Shimadzu, Kyoto, Japan) consisting of a degasser, auto-sampler, quaternary pump, a column oven, a diode array detector, a triple quadrupole MS, and an ELSD 3300 detector (Alltech, Deerfield, IL, USA). Analytical investigations were carried out on a SunFire C18 column (3.5 µm, 150 × 3 mm i.d., Waters) equipped with a guard column (10 × 3 mm i.d.). Solvents A and B (see above) were used as mobile phase. The gradient was 5–80% B in 30 min followed by 80–100% B from 30 to 35 min, and a final hold for 5 min at 100% B. The flow rate was 0.4 mL/min. The LabSolutions software (Shimadzu) was used for data acquisition and processing.

TLC was performed on silica gel 60 F254-coated aluminium plates (Macherey-Nagel, Düren, Germany). Detection was at UV 254 nm, and after spraying with 1% ethanolic vanillin and 10% sulfuric acid in EtOH followed by heating at 100 °C.

HRESIMS spectra were recorded on a LTQ Orbitrap XL hybrid ion trap–Orbitrap mass spectrometer (Thermo-Fisher Scientific, Massachusetts, MA, USA). Optical rotations were measured at 25 °C on a JASCO P-2000 polarimeter (Brechbühler, Schlieren, Switzerland) equipped with a 10 cm temperature-controlled microcell. UV and ECD spectra were recorded in MeOH (333 or 400 µg/mL) on a Chirascan CD spectrometer using 1 mm path precision cells (110 QS, Hellma Analytics, Müllheim, Germany). NMR spectra were recorded on a Bruker Avance III spectrometer (Bruker BioSpin, Rheinstetten, Germany) operating at 500.13 MHz for ^{1}H and 125.77 MHz for ^{13}C nuclei. ^{1}H NMR, ^{1}H,^{1}H-COSY, HSQC, HMBC, and ROESY spectra were recorded in $CDCl_3$ (ARMAR Chemicals, Döttingen, Switzerland) in a 1 mm TXI or a 5 mm BBO probe at 23 °C. Data were analysed using Bruker Topspin 3.5 and ACD/Labs NMR Workbook suites (Advanced Chemistry Development, Toronto, Canada) software. Chemical shifts are reported as δ values (ppm) using the solvent signal (δ_H 7.27; δ_C 77.00, $CDCl_3$) as internal reference; coupling constants (J) are given in Hz.

Silica gel 60 (0.040–0.063 mm) used for flash chromatography was from Merck (Darmstadt, Germany). Methanol for extraction was from Honeywell (Offenbach, Germany). HPLC-grade solvents MeCN, MeOH (Avantor, Radnor, PA, USA), and water from a Milli-Q water purification system (Merck Millipore, Billerica, USA) were used for HPLC separations. HPLC-grade *n*-BuOH, formic acid, and DMSO were obtained from Scharlau (Scharlab S.L., Barcelona, Spain). HPLC-grade isopropanol and EtOAc used for recrystallization were from Avantor and Scharlau, respectively. Technical grade EtOAc, MeOH, and *n*-hexane (Rheuss Chemie, Tägerig, Switzerland) were redistilled before use for preparative isolation.

2.2. Plant material

The flowering aerial parts of *Teucrium polium* subsp. *capitatum* L. were collected in May 2018, in M'Sila Algeria, at 35° 12′ 36.97″ N latitude and 4° 10′ 46.08″ E longitude. The taxonomic identity of the plant material was confirmed by Dr. Sarri Dj., botanist at the Department SNV/M'Sila, Mohamed Boudiaf University. A voucher specimen (AB-92) has been deposited at the department's herbarium of the University of M'Sila. The plant material was cleaned, dried at room temperature, and subsequently powdered.

2.3. Extraction and isolation

The powdered plant material was extracted in 50 g portions with MeOH (500 mL, each) by Soxhlet extraction for six hours to afford after filtration and evaporation under reduced pressure an oily residue (yield 17.5%). A portion of 32 g of the MeOH extract was suspended in water and partitioned with EtOAc (3 × 500 mL) to yield an EtOAc-soluble fraction (8.2 g). The EtOAc-soluble fraction was prepared as a dry load adsorbed on 25 g silica gel 60 for flash chromatography. The sample was loaded onto a self-packed silica gel 60 glass column (47 × 5 cm i.d.) and fractionated by applying a gradient of EtOAc in *n*-hexane [2% (0–10 min), 2–70% (10–420 min), 70–100% (420–480 min], followed by MeOH in EtOAc [0–20% (480–540 min), 20–100% (540–600 min)] at a flow rate of 20 mL/min. A total of 540 fractions were collected and combined based on TLC analysis into 22 fractions (F1-F22).

Further preparative purification of F15 (363 mg) by HPLC-PDA-ESIMS with a gradient of 35–70% B (see General procedures) in 30 min (ESIMS detection in positive mode at *m/z* 505–507) yielded **1** (19.0 mg, t_R 18.5 min) and **2** (4.7 mg, t_R 21.3 min). Compound **3** (9.1 mg, t_R 23.2 min) was obtained from F12 (120 mg) by preparative HPLC-PDA-ESIMS with a gradient of 15–90% B in 30 min, using ESIMS detection in positive mode at *m/z* 378–380. Compound **4** (1.8 mg, t_R 24.8 min) was isolated from fraction F13 (49.4 mg) by preparative HPLC-PDA-ESIMS using a gradient of 5–80% B in 30 min (ESIMS detection in positive mode at *m/z* 474–476). Compound **5** (9.6 mg, t_R 22.5 min) was isolated from F10 (192 mg) using preparative HPLC-PDA-ESIMS with a gradient of 5–80% B in 30 min, with ESIMS detection in positive mode at *m/z* 415–417. A second, later eluting peak (**5a**, t_R 27.6 min) was collected which yielded, however, after evaporation to dryness also **5** (10 mg). Preparative HPLC-PDA-ESIMS of F17 (79.4 mg) with a gradient from 5 to 80% B in 30 min and ESIMS detection in positive scan mode at *m/z* 402–405 afforded crude **6** (4.7 mg, t_R 20.6 min). Final purification by semi-preparative HPLC using a gradient of 5–80% B in 30 min (UV detection at 210 nm) yielded pure **6** (2.1 mg, t_R 17.7 min).

*((1′S,2R,2′R,4′S,5a'S,9a'R,10′S)–2′-Acetoxy-1′-((S)–2-acetoxy-2-(furan-3-yl)ethyl)–10′-methyl-5′-oxooctahydro-5a'H-spiro[oxirane-2,6′-[1,4]methanobenzo[d]oxepin]–5a'-yl)methyl acetate (20-Acetylauropolin, **1**).* White amorphous solid; $[\alpha]_D^{25}$ +55.0 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 195 (3.8), 270 (3.0) nm; ECD (MeOH, c 0.79 mM, 0.1 cm) $\Delta\varepsilon$ +3.13 (197 nm), −0.98 (214 nm), +0.54 (317 nm); ^{1}H and ^{13}C NMR data: Table 1 and Supplementary Materials Figs. S7-S12; HRESIMS *m/z* 527.1889 $[M+Na]^+$ (calcd for $C_{26}H_{32}NaO_{10}^+$, 527.1888).

*((2′R,3R,4′R,4a'R,5S,5′R,8a'S)–4′-Acetoxy-5-(furan-3-yl)–2′-methyl-2-oxooctahydro-2H-dispiro[furan-3,1′-naphthalene-5′,2′'-oxiran]–4a'(2′H)-yl)methyl acetate (6-Acetylteucjaponin A, **2**).* White amorphous solid; $[\alpha]_D^{25}$ −8.6 (c 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 195 (3.9), 217 (3.6, sh), 270 (2.9) nm; ECD (MeOH, c 0.90 mM, 0.1 cm) $\Delta\varepsilon$ +2.21 (195 nm), −0.24 (254 nm); ^{1}H and ^{13}C NMR: Table 1 and Supplementary Materials Figs. S13-S18; HRESIMS *m/z* 469.1832 $[M+Na]^+$ (calcd for $C_{24}H_{30}NaO_8^+$, 469.1833).

*((1S,2R,4S,5aS,6R,9aR,10S)–2-Acetoxy-1-(2-acetoxy-2-(furan-3-yl)ethyl)–6-(chloromethyl)–6–hydroxy-10-methyl-5-oxooctahydro-1,4-methanobenzo[d]oxepin-5a(2H)-yl)methyl acetate (20-Acetylauropolin chlorohydrin, **3**).* White amorphous solid; $[\alpha]_D^{25}$ +20.0 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 195 (3.9), 208 (3.8, sh), 272 (2.9) nm; ECD (MeOH, c 0.74 mM, 0.1 cm) $\Delta\varepsilon$ +2.56

Table 1
^{1}H and ^{13}C NMR spectroscopic data of **1–3** ($CDCl_3$; 500 MHz for ^{1}H and 126 MHz for ^{13}C NMR; δ in ppm).

	1		**2**		**3**	
position	δ_C, type	δ_H, (J in Hz)	δ_C, type	δ_H, (J in Hz)	δ_C, type	δ_H, (J in Hz)
1	21.0, CH_2	1.70, qd (13.0, 13.0, 13.0, 4.1), (H_α) 2.17, m, (H_β)	23.0, CH_2	1.66, m, (H_α) 1.89[a], (H_β)	22.0, CH_2	1.46, qd (12.7, 12.7, 12.7, 3.6), (H_α) 2.04[a], (H_β)
2	24.5, CH_2	2.08, m, (H_α) 1.48[a], (H_β)	24.2, CH_2	2.05, m, (H_α) 1.58, m, (H_β)	22.6, CH_2	1.87, m, (H_α) 1.30, m, (H_β)
3	31.3, CH_2	2.48, m, (H_α) 1.09, dd (14.2, 4.7), (H_β)	32.7, CH_2	2.39, m, (H_α) 1.13, dt (14.1, 3.7), (H_β)	29.8, CH_2	1.69, td (15.0, 15.0, 5.0), (H_α) 2.24, dd (15.0, 4.0), (H_β)
4	62.3, C	–	61.4, C	–	76.8, C	–
4-OH	–	–	–	–	–	3.12, s
5	52.8, C	–	44.9, C	–	57.4, C	–
6	203.0, C	–	69.7, CH	5.14, t (2.1, 2.1)	208.1, C	–
7	91.0, CH	4.19, s	30.7, CH_2	2.21, m, (H_α) 1.85[a], (H_β)	90.1, CH	4.18, s
8	46.4, CH	2.02[a]	33.3, CH	1.87[a]	46.7, CH	2.03[a]
9	53.1, C	–	51.7, C	–	54.0, C	–
10	51.0, CH	2.02[a]	47.3, CH	2.25, m	46.8, CH	1.80, dd (12.7, 1.2)
11	31.1, CH_2	2.09[a] 2.28, dd (15.6, 7.6)	45.3, CH_2	2.43, dd (14.0, 8.8) 2.52, dd (14.0, 8.8)	31.2, CH_2	2.06[a] 2.32, dd (15.9, 8.2)
12	65.5, CH	5.85, dd (7.6, 4.9)	71.8, CH	5.40, t (8.8, 8.8)	65.8, CH	5.86, dd (8.2, 4.0)
13	125.1, C	–	125.1, C	–	125.6, C	–
14	108.5, CH	6.43, d (1.5)	108.0, CH	6.40, dd (1.6, 0.6)	108.7, CH	6.42, dd (1.7, 0.9)
15	143.8, CH	7.42, t (1.5, 1.5)	144.2, CH	7.45, t (1.6, 1.6)	144.3, CH	7.42, t (1.7, 1.7)
16	140.1, CH	7.44, s	139.5, CH	7.47, d (0.6)	140.3, CH	7.45, t (0.9, 0.9)
17	14.7. CH_3	1.16, d (7.0)	16.2, CH_3	1.00, d (6.7)	15.2, CH_3	1.22, d (7.0)
18	48.8, CH_2	2.38, d (5.2) 3.04, dd (5.2, 1.8)	51.6, CH_2	2.28, m 2.98, dd (5.0, 2.0)	48.3, CH_2	3.80, d (11.6) 3.92, dd (11.6, 1.8)
19	62.5, CH_2	4.82, d (11.3) 5.24, d (11.3)	62.4, CH_2	4.97, d (13.1) 5.04, d (13.1)	64.8, CH_2	4.42, d (11.3) 4.85, d (11.3)
20	97.7, CH	6.11, s	176.9, C	–	98.3	6.31, s
6-OAc-1′	–	–	169.1, C	–	–	–
6-OAc-2′	–	–	21.6, CH_3	2.10, s[a]	–	–
12-OAc-1′	170.0, C	–	–	–	170.6, C	–
12-OAc-2′	21.5, CH_3	2.05, s	–	–	21.9, CH_3	2.07,s[a]
19-OAc-1′	170.8, C	–	170.9, C	–	170.2, C	–
19-OAc-2′	20.9, CH_3	2.09, s[a]	21.1, CH_3	2.10, s[a]	21.3, CH_3	1.98, s
20-OAc-1′	169.5, C	–	–	–	169.7, C	–
20-OAc-2′	21.2, CH_3	2.10, s[a]	–	–	21.6, CH_3	2.09, s

[a] Overlapping signals.

(196 nm), −0.77 (217 nm), +0.38 (311 nm); ^{1}H and ^{13}C NMR: Table 1 and Supplementary Materials Figs. S19-S24; HRESIMS *m/z* 563.1653 $[M+Na]^+$ (calcd for $C_{26}H_{33}ClNaO_{10}^+$, 563.1654).

((1S,2R,4S,5aR,6R,9aS,10S)−2-Acetoxy-1-(2-acetoxy-2-(furan-3-yl)ethyl)−6–hydroxy-10-methyl-5-oxodecahydro-1,4-methanobenzo[d]oxepin-6-yl)methyl acetate (Teupocapin A, ***4****).* white amorphous solid; $[\alpha]_D^{25}$ +2.8 (c 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 195 (3.9), 268 (3.1, sh) nm; ECD (MeOH, c 0.81 mM, 0.1 cm) $\Delta\varepsilon$ +0.08 (195 nm), −0.85 (207 nm), +0.42 (311 nm); ^{1}H and ^{13}C NMR: Table 2 and Supplementary Materials Figs. S25-S30; HRESIMS *m/z* 515.1887 $[M+Na]^+$ (calcd for $C_{25}H_{32}NaO_{10}^+$, 515.1888).

(5aR,6S,7R,9S,10S)−6-(2-Acetoxy-2-(furan-3-yl)ethyl)−9a–hydroxy-10-methyl-3,4,5,5a,6,7,9,9a-octahydro-2H-6,9-methanooxepino[3,4,5-cd]isobenzofuran-7-yl acetate (Teupocapin B, ***5****).* Slightly yellow amorphous solid; $[\alpha]_D^{25}$ +58.0 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 195 (4.0), 240 (3.4, sh), nm; ECD (MeOH, c 0.77 mM, 0.1 cm) $\Delta\varepsilon$ +9.46 (201 nm); ^{1}H and ^{13}C NMR: Table 2 and Supplementary Materials Figs. S31-S36; HRESIMS *m/z* 455.1676 $[M+Na]^+$ (calcd for $C_{23}H_{28}NaO_8^+$, 455.1676).

2-((3R,5S,5aS,7aR,11R,11aS,12S,13S)−5a,7a-Dihydroxy-13-methyloctahydro-1H-3,11,5-(epiethane[1,1,2]triyl)[1,3]dioxepino[5,6-c]isobenzofuran-12-yl)−1-(furan-3-yl)ethyl acetate (Teupocapin C, ***6****).* White amorphous solid; $[\alpha]_D^{25}$ −22.0 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 195 (3.8), 215 (3.5, sh) nm; ECD (MeOH, c 0.95 mM, 0.1 cm) $\Delta\varepsilon$ −0.97 (198 nm), +0.01 (218 nm), −0.33 (241 nm); ^{1}H and ^{13}C NMR: Table 2 and Supplementary Materials Figs. S37-S42; HRESIMS *m/z* 443.1675 $[M+Na]^+$ (weak, calcd for $C_{22}H_{28}NaO_8^+$, 443.1676), 403.1758 $[M+H-H_2O]^+$ (calcd for $C_{22}H_{27}O_7^+$, 403.1757).

2.4. Single-crystal X-ray crystallographic analysis of ***1***

X-ray diffraction data were collected on a Stoe StadiVari diffractometer (STOE & Cie GmbH, Darmstadt, Germany) equipped with an Oxford Cryosystems low-temperature device operating at T = 150 K (OxfordCryosystems, Oxford, United Kingdom). Data were measured using rotation method, ω scans using a metaljet source with GaK_α radiation (λ = 1.34143 Å).

Single colorless block-shaped crystals of **1** were recrystallized from a mixture of EtOAc and *i*-PrOH by solvent layering. A suitable crystal 0.13 × 0.09 × 0.06 mm was selected and the crystal was mounted on a mylar loop in perfluoroether oil. The crystal was kept at a steady T = 150 K during data collection. The structure was solved with the ShelXT [18,19] structure solution program using the Intrinsic Phasing solution method and by using Olex2 [20] as the graphical interface. The model was refined with version 2018/3 of ShelXL [18,19] using Least Squares minimization.

Crystallographic data for the structure of 20-acetylauropolin (**1**) were deposited at the Cambridge Crystallographic Data center (CCDC) as supplementary crystallographic data with number CCDC 2,236,028.

20-Acetylauropolin (**1**): $C_{26}H_{32}O_{10}$, M_r = 504.51, orthorhombic, $P2_12_12$ (No. 18), a = 17.3203(7) Å, b = 17.4805(9) Å, c = 8.3553(3) Å, $\alpha = \beta = \gamma$ = 90°, V = 2529.71(19) Å^3, T = 150 K, Z = 4, Z' = 1, $\mu(GaK_\alpha)$ = 0.547, 52,085 reflections measured, 5209 unique (R_{int} = 0.1141) which were used in all calculations. The final wR_2 was 0.1418 (all data) and R_1 was 0.0557 ($I > 2(I)$). The Flack parameter was refined to 0.02(12). Additional information is available in Supplementary Materials.

Table 2
^{1}H and ^{13}C NMR spectroscopic data of **4–6** ($CDCl_3$; 500 MHz for ^{1}H and 126 MHz for ^{13}C NMR; δ in ppm).

	4		5		6	
position	δ_C, type	δ_H, (J in Hz)	δ_C, type	δ_H, (J in Hz)	δ_C, type	δ_H, (J in Hz)
1	22.4, CH_2	1.37[a] (H_α) 1.89[a], (H_β)	24.0, CH_2	1.31, m, (H_α) 2.28, m, (H_β)	22.8, CH_2	1.44[a], (H_α) 1.98[a], (H_β)
2	28.1, CH_2	2.25[a], (H_α) 1.36[a], (H_β)	22.1, CH_2	2.00[a], (H_α) 1.55, m, (H_β)	24.7, CH_2	1.95[a], (H_α) 1.22, m, (H_β)
3	35.0, CH_2	1.94, m, (H_α) 1.39[a], (H_β)	22.2, CH_2	2.09[a], (H_α, H_β)	29.5, CH_2	1.63, m, (H_α) 1.91[a], (H_β)
4	73.4, C	–	140.4, C	–	80.3, C	–
4-OH	–	2.63, s	–	–	–	3.69, s
5	55.3, CH	2.79, d (11.9)	132.2, C	–	51.4, C	–
6	208.8, C	–	108.7, C	–	109.1, C	–
6-OH	–	–	–	n.d.	–	3.95, s
7	89.2, CH	4.08, s	86.8, CH	4.05, s	86.5, CH	3.81, s
8	44.7, CH	2.13[a]	41.4, CH	2.57[a]	46.4, CH	2.26, q (7.0, 7.0, 7.0)
9	52.9, C	–	53.2, C	–	49.4, C	–
10	43.4, CH	1.86[a]	37.9, CH	2.54[a]	45.5, CH	1.44[a]
11	31.0, CH_2	2.05, dd (15.9, 3.4) 2.29, dd (15.9, 8.9)	30.9, CH_2	1.94, dd (15.4, 3.1) 2.10[a]	34.7, CH_2	1.82, dd (15.5, 3.1) 2.46, dd (15.5, 10.1)
12	66.2, CH	5.91, dd (8.9, 3.4)	66.4, CH	5.93, dd (8.5, 3.1)	66.3, CH	6.01, dd (10.1, 3.1)
13	125.2, C	–	125.8, C	–	125.9, C	–
14	108.8, CH	6.43, dd (1.5, 0.9)	108.6, CH	6.42, dd (1.5, 0.9)	108.9, CH	6.41, dd (1.8, 0.9)
15	144.0, CH	7.41, t (1.5, 1.5)	143.6, CH	7.39, t (1.5, 1.5)	143.8, CH	7.39, t (1.8, 1.8)
16	140.4, CH	7.49, t (0.9, 0.9)	140.1, CH	7.47, t (0.9, 0.9)	140.5, CH	7.46, t (0.9, 0.9)
17	14.8, CH_3	1.18, d (7.0)	14.6, CH_3	1.10, d (7.0)	15.0, CH_3	1.01, d (7.0)
18	64.5, CH_2	4.25, d (11.9) 4.50, d (11.9)	75.4, CH_2	4.49, dd (13.1, 3.3) 4.73, dd (13.1, 3.3)	79.2, CH_2	4.14, d (10,1) 4.31, d (10,1)
19	–	– –	–	– –	59.0, CH_2	3.91, d (11.3) 4.11, d (11.3)
20	98.2, CH	6.21, s	98.9, CH	5.87, s	106.8, CH	5.18, s
12-OAc-1′	170.4, C	–	170.1, C	–	170.2, C	–
12-OAc-2′	21.6, CH_3[a]	2.09, s[a]	21.3, CH_3	2.03, s[a]	21.7, CH_3	2.03, s
18-OAc-1′	171.6, C	–	–	–	–	–
18-OAc-2′	21.3, CH_3	2.09, s[a]	–	–	–	–
20-OAc-1′	170.1, C	–	169.9, C	–	–	–
20-OAc-2′	21.6, CH_3[a]	2.11, s[a]	21.4, CH_3	2.04, s[a]	–	–

[a] Overlapping signals, n.d. not detected.

3. Results and discussion

HPLC-PDA-ESIMS analysis of the MeOH extract of *T. polium* subsp. *capitatum* L. revealed, besides the previously reported flavonoids and caffeic acid derivatives [2], the presence of a series of compounds that strongly ionized in the positive ESIMS mode but did not show any signals in the UV (254 nm) trace (Fig. 1). Their MS data in conjunction with chemotaxonomic information suggested that these compounds were diterpenes.

Targeted isolation of the corresponding peaks afforded six neo-clerodane type diterpenes (Fig. 2), including four previously undescribed compounds (**3**–**6**). Compound **1** was identified as 20-acetylauropolin. This compound has been reported as an acetylation product of auropolin, which has previously been isolated from several *T. polium* subspecies and *T. asiaticum*, but has never been directly isolated from plant material [12,21,22]. Optical rotation and NMR data of **1** were identical to those described in literature [21,22]. The absolute configuration of **1** was determined by X ray diffraction analysis (Fig. 3 and Supplementary Materials).

Compound **2** was identified as 6-acetylteucjaponin A based on NMR analysis and comparison with literature data (Supporting Material Fig. S19). It was originally obtained by acetylation of teucjaponin A and later isolated from *T. polium* [23–25]. The structures of the previously undescribed neo-clerodanes **3**–**6** were established by extensive 1D and 2D NMR analysis and by comparison with the NMR data recorded for **1**.

Compound **3** had a molecular formula of $C_{26}H_{33}ClO_{10}$ deduced from the sodium adduct ion peak in HRESIMS at *m/z* 563.1653 (calcd for $C_{26}H_{33}ClNaO_{10}^{+}$, 563.1654). The presence of a chlorine atom was confirmed by the isotopic pattern. Comparison of the NMR data of compounds **1** and **3** revealed that ^{1}H and ^{13}C NMR chemical shifts and coupling constants of both compounds were very similar. The main difference was observed for C-4, which was more downfield shifted in **3** (δ_C 76.8) compared to **1** (δ_C 62.3). Additionally, while the ^{13}C NMR chemical shifts for C-18 were very similar in both compounds (δ_C 48.8 in **1** *vs* δ_C 48.3 in **3**) a clear difference was observed in the H-18 chemical shifts [δ_H 2.38 (*d*, J = 5.2 Hz, H_a-18) and δ_H 3.04 (*dd*, J = 5.2, 1.8 Hz, H_b-18) in **1**, and δ_H 3.80 (*d*, J = 11.6 Hz, H_a-18) and δ_H 3.92 (*dd*, J = 11.6, 1.8 Hz, H_b-18) in **3**]. The H-18 proton signals in **1** displayed the typical chemical shifts and coupling constants for a α,α-disubstituted oxirane ring system [21,26]. This pattern was not found in **3**, thereby indicating a different substitution at C-4. ^{1}H and ^{13}C NMR shifts were in full agreement with the replacement of the oxirane ring in **1** by a chlorohydrin in **3** (Table 1 and Supplementary Materials). The presence of the hydroxyl group at C-4 of the chlorohydrin could be clearly confirmed by ^{2}J, ^{3}J, and ^{4}J HMBC correlations between the hydroxyl proton (δ_H 3.12, *s*) and C-2 (δ_C 22.6), C-3 (δ_C 29.8), C-4 (δ_C 76.8), and C-5 (δ_C 57.4) (Fig. 4). Chlorinated neo-clerodane diterpenes have already been reported several times and were either described as artifacts resulting from the use of chlorinated solvents in the isolation procedure, or as naturally occurring compounds [26–33]. Indeed, the chlorohydrin **3** was detected by HPLC-ESIMS analysis in the crude methanolic extract, which was obtained by Soxhlet extraction. However, in a freshly prepared methanolic extract obtained by maceration of the same dried plant material the chlorohydrin was only detected in minute amounts (data not shown). Although no chlorinated chemicals were used in the isolation procedure, we assume that the chlorohydrin **3** is likely to be an artifact formed by the opening of the oxirane in **1**,

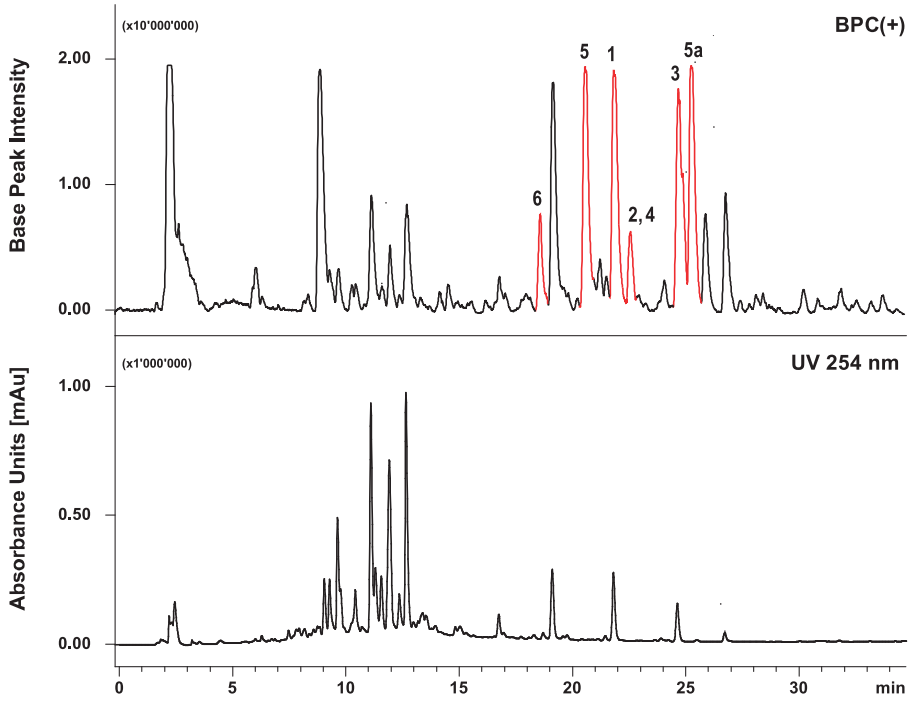

Fig. 1. HPLC profile of the methanolic *T. polium* subsp. *capitatum* extract. The profile was obtained on a SunFire C18 column (3.5 µm, 150 × 3 mm i.d) applying a gradient of 5–80% MeCN in water containing 0.1% formic acid in 30 min at a flow rate of 0.4 mL/min. The upper trace shows the base peak chromatogram (BPC) recorded with ESIMS in positive mode. The lower trace shows the PDA chromatogram at 254 nm. Peak numbers refer to neo-clerodane diterpenes **1–6**. The black peaks correspond to polyphenolic compounds [2].

probably due to the presence of traces of chlorinated contaminants in the solvent used for Soxhlet extraction. Such oxirane opening reactions in the presence of traces of chloroform and/or hydrochloric acid have been reported in 4α,18-epoxy-neo-clerodanes from *Teucrium* species and in epoxy-sesquiterpene lactones from *Centaurea solstitialis* [34,35].

Compound **4** had a molecular formula of $C_{25}H_{32}O_{10}$ (HRESIMS *m/z* 515.1887 $[M+Na]^+$; calcd for $C_{25}H_{32}NaO_{10}^+$, 515.1888). As in **1**–**3**, the NMR data of **4** showed ^1H and ^{13}C NMR resonances of a β-substituted furan ring [(δ_H 6.43, *dd*, J = 1.5, 0.9 Hz, H-14; δ_H 7.41, *t*, J = 1.5, 1.5 Hz, H-15; δ_H 6.49, *t*, J = 0.9, 0.9 Hz H-16), (δ_C 125.2 (C-13), 108.8 (C-14), 144.0 (C-15), 140.4 (C-16))]. The furan ring was linked to an acetoxylated methine (δ_H 5.91 *dd*, J = 8.9, 3.4 Hz, H-12; δ_C 66.2, C-12) further connected via a methylene carbon to C-9 (δ_C 52.9) of the decalin core. The one-proton singlet at δ_H 6.21 (H-20) could be assigned to an acetal function containing an acetoxy group (δ_C 98.2) without vicinally connected protons. The ring closure of this acetal function was corroborated by a ^2J HMBC correlation between H-20 and C-9, and a ^3J HMBC correlation from H-20 to C-7 (δ_C 89.2). Compared to the spectra of compounds **1**–**3** differences were observed at positions C-4 (δ_C 73.4) and C-5 (δ_C 55.3). The ^{13}C NMR chemical shift of C-4 (δ_C 73.4) indicated the presence of a hydroxyl group at this position. Furthermore, ^2J HMBC correlations between the methylene H-18 protons (δ_H 4.25, *d*, J = 11.9 Hz, H_a-18; δ_H 4.50, *d*, J = 11.9 Hz, H_b-18) and C-4, in conjunction with ^3J HMBC correlations from these protons to the acetyl carbonyl at δ_C 171.6, suggested an acetoxymethylene group attached to C-4. The relative configuration at C-4 was indicated by a ROESY correlation between the methylene protons H_2–18 and H_β–10 (δ_H 1.86). HSQC correlations from C-5 (δ_C 55.3) to H-5 (δ_H 2.79, *d*, J = 11.9 Hz), and ^1H,^1H-COSY correlations of H-5 to H-10 revealed C-5 as a sp^3 methine group with the proton in axial orientation. Compound **4** was thus a 19-nor-neo-clerodane diterpene (Fig. 5). The presence of a hydroxyl group together with an acetoxymethylene group at C-4 is quite rare in neo-clerodane diterpenes and has been described so far only in picropolinol from *T. polium* subsp. *capitatum,* in alysin B and its 3-deacetyl derivative identified in *T. alyssifolium*, and in a neo-clerodane diterpene found in *Ajuga decumbens* [33,36,37]. Compound **4** was named teupocapin A in reference to the name of the species and subspecies.

Compound **5** had a molecular formula of $C_{23}H_{28}O_8$ (HRESIMS *m/z* 455.1676 $[M+Na]^+$; calcd for $C_{23}H_{28}NaO_8^+$, 455.1676). The ^1H and ^{13}C NMR spectra of **5** showed also the typical pattern of a β-substituted furan ring [(δ_H 6.42, *dd*, J = 1.5, 0.9 Hz, H-14; δ_H 7.39, *t*, J = 1.5, 1.5 Hz, H-15; δ_H 7.47, *t*, J = 0.9, 0.9 Hz, H-16), (δ_C 125.8 (C-13), 108.6 (C-14), 143.6 (C-15), 140.1 (C-16))]. As for **1, 3**, and **4,** ^3J HMBC correlations from H-12 (δ_H 5.93 (*dd*, J = 8.5, 3.1 Hz) to C-14 and C-16 revealed that the furan ring was linked to an acetoxylated methine group at C-12 (δ_C 66.4) further connected to C-9 (δ_C 53.2) of the decalin moiety via a methylene group. Furthermore, the cyclic acetoxylated acetal function at C-20 was also present in **5**, as indicated by a ^2J HMBC correlation between H-20 (δ_H 5.87, *s*) and C-9, and a ^3J HMBC correlation from H-7 (δ_H 4.05, *s*) to C-20 (δ_C 98.9). Compared to the neo-clerodane diterpenes **1**–**4**, the resonances of C-4 (δ_C 140.4) and C-5 (δ_C 132.2) were observed at lower field. Diterpene **5** thus had a double bond at this position and consequently a 19-nor-neo-clerodane diterpene scaffold. Furthermore, the NMR data revealed the presence of an allylic hemiketal function ranging from C-4 (δ_C 140.4) to C-6 (δ_C 108.7) with cyclization via the C-18 methylene group [(δ_H 4.49 (*dd*, J = 13.1, 3.3 Hz, H_a-18); δ_H 4.73 (*dd*, J = 13.1, 3.3 Hz, H_b-18); (δ_C 75.4)]. The partial structure was supported by ^2J and ^3J HMBC correlations (Fig. 7). Such a hemiketal has so far only been reported from the chemical transformation of eriocephalin into a series of 19-nor-neo-clerodane derivatives [38]. Initially, two well separated peaks (**5** and **5a**) were collected after preparative HPLC separation of fraction F10 from the initial fractionation on a silica gel column. However, after evaporation to dryness, both peaks afforded the same compound **5** as confirmed by HPLC and NMR analysis. We hypothesize that the acidic mobile phase used during isolation

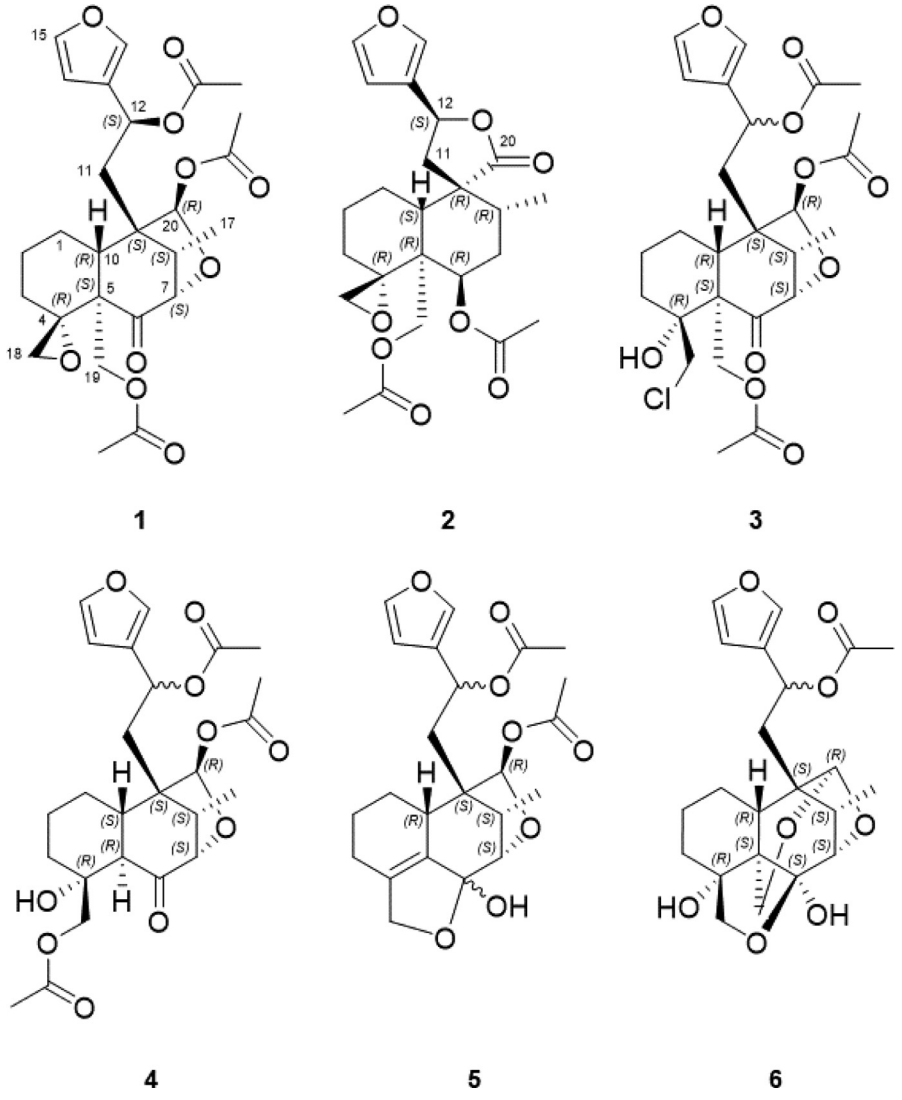

Fig. 2. Structures of neo-clerodane diterpenes **1–6**.

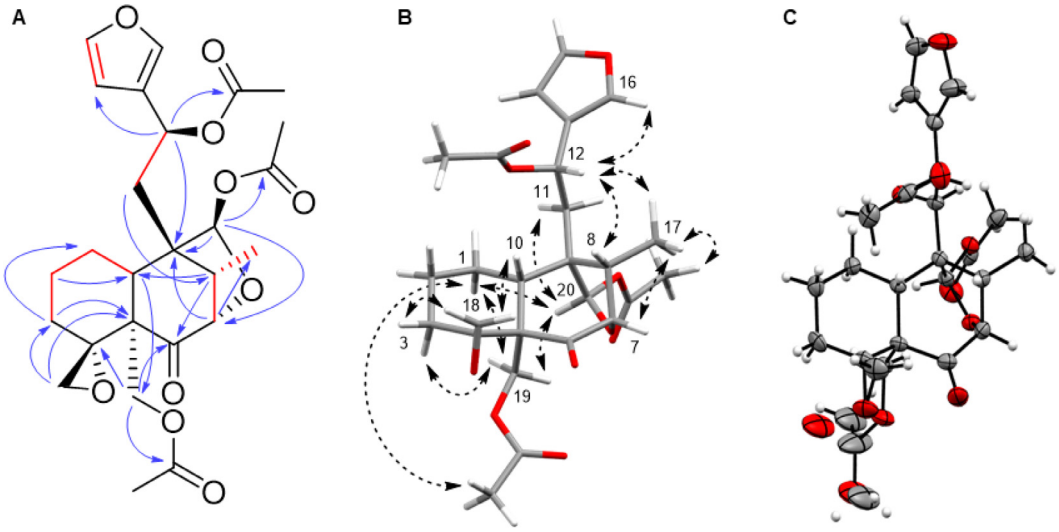

Fig. 3. (A) COSY (red) and key HMBC correlations (blue arrows), (B) key ROESY correlations (black dotted arrows), and (C) ORTEP drawing obtained by single crystal X-ray diffraction (ellipsoids at 50% probability) of **1**.

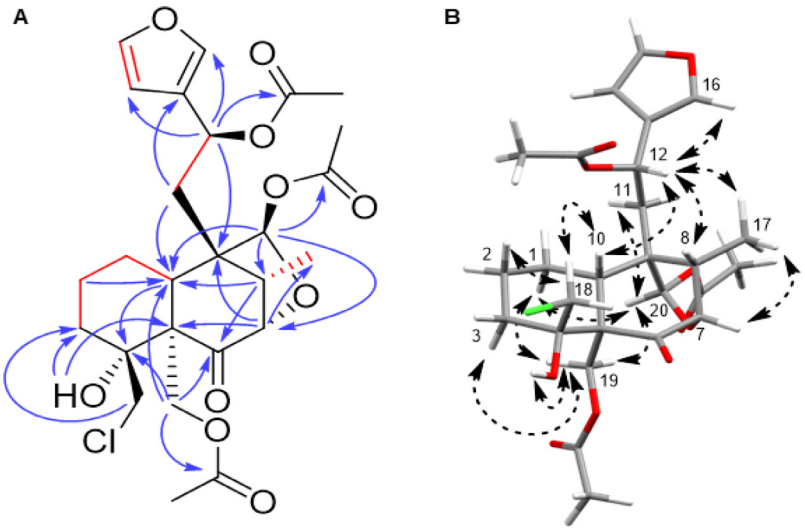

Fig. 4. (A) COSY (red) and key HMBC correlations (blue arrows), (B) key ROESY correlations of **3** (structure depicted as (12*S*) isomer).

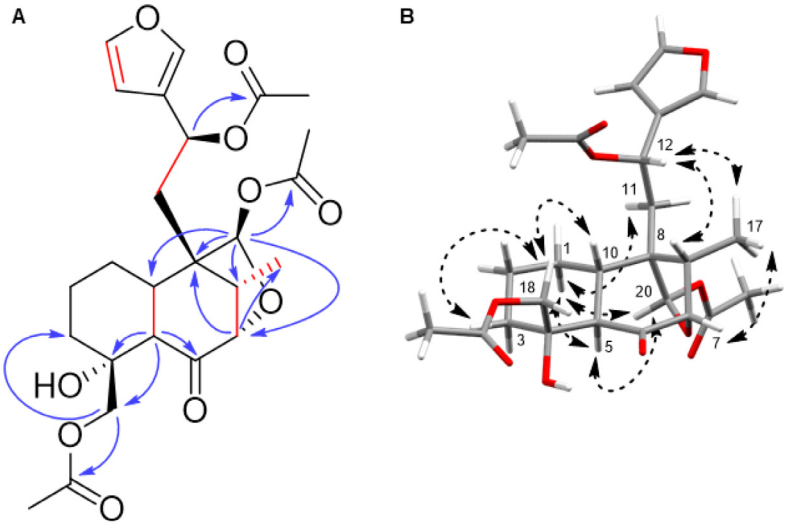

Fig. 5. (A) COSY (red) and key HMBC correlations (blue arrows), (B) key ROESY correlations of **4** (structure depicted as (12*S*) isomer).

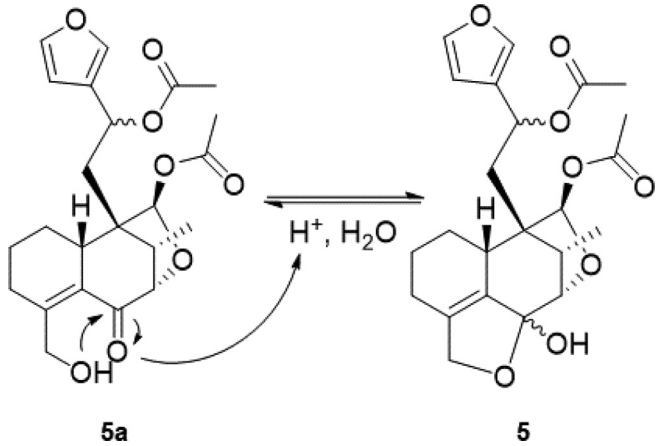

Fig. 6. Putative formation of **5** from **5a** under the acidic conditions used during isolation.

catalyzed an intramolecular hemiketal formation by reaction of the hydroxyl group at position C-18 with the carbonyl group at C-6 in **5a** to form **5** (Fig. 6). The structure of **5** was named as teupocapin B.

Compound **6** had a molecular formula of $C_{22}H_{28}O_8$ as revealed by a minor HRESIMS peak at *m/z* 443.1675 $[M+Na]^+$ (calcd for $C_{22}H_{28}NaO_8^+$, 443.1676) and further supported by an intense fragment ion peak at *m/z* 403.1758 ($[M+H-H_2O]^+$; calcd for $C_{22}H_{27}O_7^+$, 403.1757). Compound **6** also showed the chemical shifts for a β-substituted furan ring attached at the C-12 acetoxylated methine group [(δ_H 6.01, *dd*, *J* = 10.1, 3.1 Hz, H-12); (δ_C 66.3 (C-12)] and the C-11 methylene group [(δ_H 1.82, *dd*, *J* = 15.5, 3.1 Hz, H_a-11 and δ_H 2.46, *dd*, *J* = 15.5, 10.1 Hz, H_b-11); (δ_C 34.7 (C-11))] connected to C-9 (δ_C 49.4) of the decalin core structure. In addition, **6** also contained an acetal function at C-20 (δ_C 106.8), with a ring closure to C-7, as revealed by the ^3J HMBC correlation from H-20 (δ_H 5.18, *s*) to C-7 (δ_C 86.5) (Fig. 8). However, in contrast to the neo-clerodane diterpenes **1** and **3**–**5**, the C-20 acetal function did not include an acetoxyl group, but showed a further ring closure to C-19 (δ_C 59.0). The linkage was corroborated by ^3J HMBC correlations from the C-19 oxo-methylene protons (δ_H 3.91, *d*, *J* = 11.3 Hz, H_a-19 and δ_H 4.11, *d*, *J* = 11.3 Hz, H_b-11) to C-20 (δ_C 106.8), C-4 (δ_C 80.3), C-5 (δ_C 51.4), C-6 (δ_C 109.1), and C-10 (δ_C 45.5). Such a C-20/C-19 and C-20/C-7 bridged acetal function

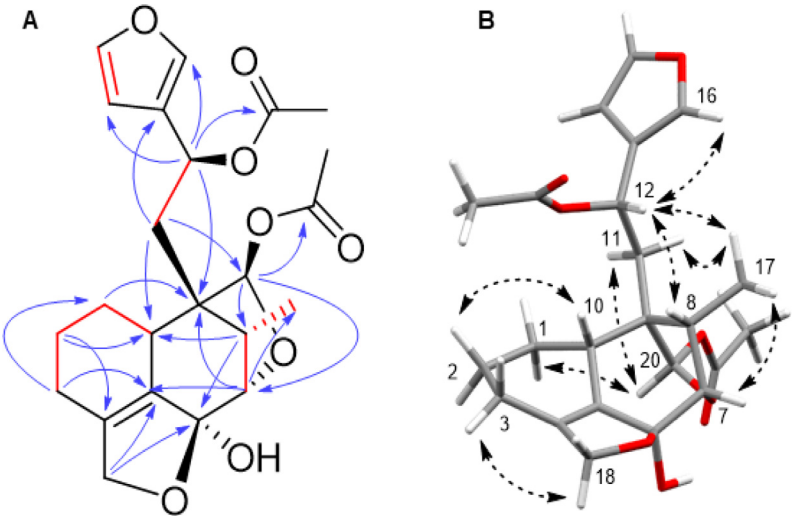

Fig. 7. (A) COSY (red) and key HMBC correlations (blue arrows), (B) key ROESY correlations of **5** (structure depicted as (6*S*,12*S*) isomer).

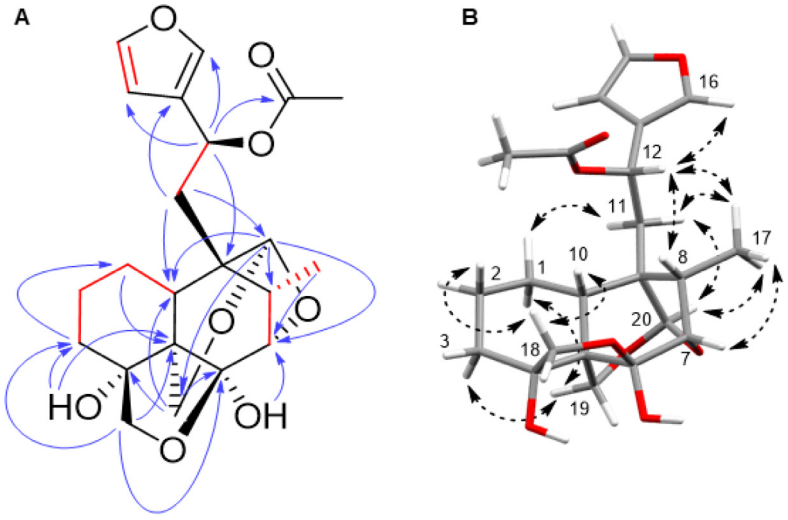

Fig. 8. (A) COSY (red) and key HMBC correlations (blue arrows), (B) key ROESY correlations of **6** (structure depicted as (12*S*) isomer).

is a rare structural feature in neo-clerodane diterpenes and has so far only been described in a diterpene isolated from *Salvia minata* [39]. As in compound **5**, ^{2}J and ^{3}J HMBC correlations in **6** revealed a hemiketalic ring closure from C-6 (δ_C 109.1) to C-4 (δ_C 80.3) via the C-18 methylene group [(δ_H 4.14, *d*, J = 10.1 Hz, H_a-18 and δ_H 4.31, *d*, J = 10.1, H_b-18); (δ_C 79.2 (C-18))]. The relative configuration at C-4 was established by a ROESY cross-peak between the methylene proton H_b-18 and $H_\beta-10$ (δ_H 1.44). The same structural feature with an oxolane ring and a tertiary hydroxy group at C-4 has been found in teulanigerin, teupolin V, teugnaphalodin and its derivatives, and teulepicephin [40–44]. Compound **6** was named as teupocapin C.

Except compound **2**, all isolated neo-clerodanes contain a rarely occurring axial oxy-function at C-7 that forms a hemiacetalic bridge to C-20 [12,45]. Compound **2** possesses instead a five-membered lactone ring connecting C-20 to C-12, a structural feature that is very often found in neo-clerodane diterpenes from *Teucrium* species [7–9,45].

The absolute configuration of compounds **2**–**6** could not be ascertained from the data above. Attempts at obtaining crystals suitable for single X-ray diffraction analysis were not successful. However, clerodane diterpenes found in the genus *Teucrium* so far all belonged to the neo-clerodane series [6,7,45]. Additionally, given that the absolute configuration of **1** could be established by X-ray crystallographic analysis it can be reasonably assumed that diterpenes **2**–**6** are also neo-clerodanes.

The vast majority of neo-clerodane diterpenes from *Teucrium* has been shown to possess the *S* configuration at C-12 [7,46]. Since the crystal structure of **1** revealed the *S*-configuration at C-12, it is likely that diterpenes **2**–**6** possess the same configuration at this position. Moreover, a method based on NOESY spectroscopy in combination with molecular mechanics calculations of different conformers has been developed by Jiménez-Barbero for establishing the absolute configuration at C-12 in neo-clerodanes bearing a furanyl ethyl side chain with a hydroxyl or acetoxyl substituent at C-12, as found in **1** and in **3**–**6** [47]. Thus, an *S* configuration at C-12 leads to conformers in which H-12 shows strong NOE signals with H-8 and H-17, while NOE cross-peaks with $H_\beta-1$ and H-10 are weak or not detectable. An *R* configuration at C-12, in contrast, leads to strong NOESY cross-peaks of H-12 with $H_\beta-1$ and H-10, while signals with H-8 and H-17 are rather weak or absent. In the ROESY spectrum of **1**, a strong cross-peak was detected between H-12 and H-17, and between H-12 and H-8, while the signal with $H_\beta-1$ was much weaker, in accord with the *S* configuration established by X-ray diffraction analysis. ROESY cross-peak intensities observed in **3**–**6** were similar to those detected in **1,** thereby

supporting the *S* configuration at C-12 in these compounds. However, confirmation by X-ray diffraction analysis will be needed for a definitive configurational assignment. As for neo-clerodanes containing a lactone ring, the absence of an NOE cross-peak between H-12 and H-17 has been found indicative of the *S* configuration at C-12, since H-12 is located on the other face of the plane which is spanned by the C-12/C-20 lactone [7,48,49]. This was also the case for compound **2**, confirming the *S* configuration at C-12.

4. Conclusion

The genus *Teucrium* is a rich source of neo-clerodane diterpenoids, and the occurrence of this type of compounds is considered as a chemotaxonomic marker [8]. Even though the phytochemistry of *Teucrium* species has been extensively investigated it was shown in several studies that the composition in neo-clerodane diterpenes can significantly vary depending on geographic origin and environmental conditions [6–10]. This is confirmed by the present study given that most diterpenes detected in the methanolic extract of *T. polium* subsp. *capitatum* collected in Algeria were previously undescribed compounds, while 20-acetylauropolin was known up to now only as a natural product derivative. X ray crystallographic data of 20-acetylauropolin support the validity of a previously reported method based on molecular mechanic calculations and NOESY correlations for the determination of the absolute configuration at C-12 in neo-clerodanes bearing a furanyl ethyl side chain. Taken together, the compounds described here extend the structural diversity of neo-clerodane diterpenoids, as they combine common structural characteristics with features that are rare in naturally occurring neo-clerodanes.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Matthias Hamburger reports financial support, administrative support, article publishing charges, and equipment, drugs, or supplies were provided by University of Basel, Department of Pharmaceutical Sciences. Amel Boudjelal reports financial support, administrative support, equipment, drugs, or supplies, and travel were provided by Algerian Ministry of Higher Education and Scientific Research.

CRediT authorship contribution statement

Morris Keller: Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Sarra Chabane:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – review & editing. **Amel Boudjelal:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Project administration. **Ombeline Danton:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Alessandro Prescimone:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Matthias Hamburger:** Resources, Writing – review & editing, Funding acquisition. **Olivier Potterat:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – review & editing, Supervision, Project administration.

Data availability statement

The datasets generated for this study are available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2023.135447.

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