Colchicinoids from Colchicum Crocifolium Boiss. (Colchicaceae)

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Abstract:

A new colchicinoid from *Colchicum crocifolium* Boiss. (Colchicaceae) was isolated and identified as *N*,*N*-dimethyl-*N*-deacetyl-(−)-cornigerine (**5**), along with four known compounds, but new to the species: (−)-colchicine (**1**), (−)-demecolcine (**2**), (−)-*N*-methyl-(−)-demecolcine (**3**) and 3-demethyl-*N*-methyl-(−)-demecolcine (**4**). All isolated compounds showed potent cytotoxicity against a human cancer cell panel.

Keywords: Colchicum crocifolium | colchicinoids | Jordan medicinal plants | colchicine

Article:

1. Introduction

As part of our studies on the unique and under explored biodiversity of the Hashemite Kingdom of Jordan (Jordan) (Alali et al., 2005, 2006a, 2006b; Alali, Gharaibeh, Ghawanmeh, Tawaha, & Oberlies, 2008; Al-Mahmoud, Alali, Tawaha, & Qasaymeh, 2006), the colchicinoids of *Colchicum crocifolium* Boiss. (Colchicaceae) were pursued. Alkaloids of the colchicinoid structural class are well known from this genus, particularly (−)-colchicine, and these compounds have been investigated extensively for both toxicological and potential medical properties. For example, (−)-colchicine is a drug of choice to relieve acute gout attack (Terkeltaub, 2003) and is used to combat a variety of pro-inflammatory disorders, such as familial Mediterranean fever (Drenth & van der Meer, 2001) and Behçet's disease (Sakane & Takeno, 2000). *Colchicum crocifolium* grows wildly in Jordan in clayey sandy desert, is a delicate, perennial herb, with corms covered by thick, dark-brown to reddish scales and is found flowering from March to April (Al-Eisawi, 1998). In preliminary studies where *C. crocifolium* was used as a model

organism to demonstrate the feasibility and value of an LC-MS and LC-PDA system to dereplicate colchicinoids (Alali et al., 2008), 10 known alkaloids were dereplicated in addition to a new compound, which was proposed to be *N*,*N*-dimethyl-*N*-deacetyl-(−)-cornigerine (Alali et al., 2008). Those promising results stimulated our research team to conduct a full phytochemical characterisation of the active components in *C. crocifolium*, which to the best of our knowledge had not been studied previously.

From extracts of the whole plant (leaves, stems, corms and seeds), five colchicinoids were isolated and characterised. One of these, *N*,*N*-dimethyl-*N*-deacetyl-(−)-cornigerine (**5**), is a new colchicinoid analogue, while the other four are known compounds that are new to the species: (−)-colchicine (**1**), (−)-demecolcine (**2**), (−)-*N*-methyl-(−)-demecolcine (**3**) and 3-demethyl-*N*-methyl-(−)-demecolcine (**4**). The structures of all compounds were elucidated using a series of spectrometric and spectroscopic techniques. All compounds (**1**–**5**) were evaluated for their cytotoxicity against a human cancer cell panel.

2. Materials and methods

2.1. General

Optical rotation, IR and UV data were obtained on a Rudolph Research Autopol III polarimeter, a Nicolet Avatar 360 FT-IR, and a MultiSpec-1501, SHIMADZU® photodiode diode array spectrophotometer (Kyoto, Japan), respectively. All NMR experiments were performed in $CDCl_3$ with TMS as an internal standard; ^{1}H-, ^{13}C-NMR, DEPT-135, gs-COSY and gs-HSQC NMR spectra were acquired on a Bruker 400 MHz NMR spectrometer (Industriestr., Fällanden, Switzerland), while a Varian Unity Inova-500 instrument with a 5 mm broad-band inverse probe with z-gradient (Varian Inc., Palo Alto, CA) was utilised for ROESY and gs-HMBC NMR spectra. Low-resolution ESIMS and APCIMS were determined on an Agilent® (Palo Alto, CA, USA) ion-trap LC/MS system; HRMS were acquired using an Applied Biosystems (Framingham, MA) TOF/TOF mass spectrometer, equipped with a Nd : YAG laser operating at 355 nm and 200 Hz. This instrument was operated in the reflectron mode, and the matrix employed was 2,5-dihydroxybenzoic acid prepared at a concentration of 9 mg mL^{-1} in 70 : 30 (v : v) acetonitrile–0.1% trifluoroacetic acid. HPLC was performed on a Lachrom® MERCK-HITACHI (Tokyo, Japan), equipped with quaternary gradient L-7150 pump, L-7455 diode-array detector, L-7200 auto-sampler and D-7000 interface. The preparative HPLC column was a Hibar® MERCK, pre-packed column RT 250-25, Lichrosorb® RP-18 (7 μm). PTLC was carried out on 20 × 20 cm plates with silica gel F_{254} (Merck KGaA, Germany). Column chromatography was carried out using silica gel 60 (0.06–0.2 mm; 70–230 mesh), and TLC utilised silica gel 60 with gypsum and pigment addition for UV visualisation (both from Scharlau Chemie S.A., Barcelona, Spain). TLC spots were visualised by UV (VILBER LOURMAT, 4 W-254 nm tube) or by spraying the developed plates with 5% phosphomolybdic acid in EtOH. (−)-Colchicine standard was obtained from Fluka Chemie AG, Buchs.

2.2. Plant material

Corms, leaves, stems and seeds of *C. crocifolium* were collected during the seeding stage in April of 2005 and 2006 in the northeastern part of Jordan, from Ar-Rwaished (elevation 691 m, latitude 32°33′465″N, longitude 38°16′854″E), and identified by one of the authors (Khaled Tawaha). A voucher specimen (PHC# 110) was deposited in the Herbarium of the Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan.

2.3. Extraction and isolation

Air-dried plant raw material was ground to a fine powder using a laboratory mill, RetschMühle (RETSCH GmbH, Haan, Germany), and then it was passed through a 24 mesh sieve. Dried plant material (537.7 g) was extracted with MeOH in a Soxhlet apparatus for 3 h. The solvent was evaporated under reduced pressure to yield a MeOH-extract (148.1 g), which was fractionated based on the methods of Santavy et al. (1981) and Sutlupinar et al. (1988). Briefly, the MeOH-extract was dissolved in 5% acetic acid, extracted with light petroleum (Fraction A) (12.0 g), after which the aqueous acid residue was re-extracted three times with diethyl ether (Fraction B) (1.2 g). The acidic aqueous residues were made alkaline (pH 9) with 10% NH_4OH followed by triple extraction with CH_2Cl_2 (Fraction C) (1.9 g). All fractions were dried *in vacuo*. Fraction C (1.9 g) was subjected to chromatography over silica gel using a gradient of 100% hexane to 100% CH_2Cl_2 to 2.4% MeOH in CH_2Cl_2, to yield 13 pools. Pure compounds were isolated via prep-HPLC from alkaloid-rich pools (3–10) using a gradient solvent system of CH_3CN and 3% acetic acid in water (10 : 90 to 60 : 40 over 40 min) with a 10 mL min^{-1} flow rate, monitoring at 245 nm, and injecting between 50 and 400 mg of material dissolved in 2 mL of MeOH and mobile phase in a 1 : 1 ratio. The purity of the isolated compounds was verified by TLC developed with either $CHCl_3$: MeOH [9 : 1] or CH_2Cl_2 : acetone : diethylamine [12 : 6 : 2].

2.3.1. *N*,*N*-Dimethyl-*N*-deacetyl-(−)-cornigerine (***5***)

Yellowish powder (95.9 mg); UV (MeOH) λ_{max}(log ε) 242 (4.64) nm; α_D^{23} −106 (*c* 0.0035, MeOH); IR ν_{max} 3686, 3017 (base peak), 2436, 2396, 2362, 1557, 1520, 1474, 1419, 1217, 1029 and 930 cm^{-1}; for ^1H (400 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) NMR data, see Table 1; ESI MS (pos. ion mode) *m*/*z* 370.3 [M + H^+], 338.1 [MH–$MeOH^+$], 327.0 MH–N(Me) = CH_2^+, 299.1 [MH–N(Me) = CH_2–CO^+]; HR TOF/TOF MS *m*/*z* 370.16374 [M + H^+] (Calcd for [$C_{21}H_{23}NO_5$ + H]$^+$, 370.16490).

2.4. Human cancer cell panel

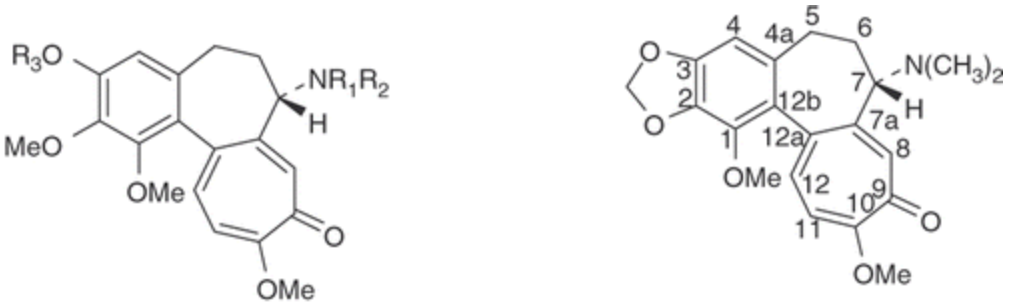
The cytotoxicity measurements against the MCF-7 human breast carcinoma (Barbara A. Karmanos Cancer Center, Detroit, MI), NCI-H460 human large cell lung carcinoma (American Type Culture Collection, Manassas, VA) and SF-268 human astrocytoma (NCI Developmental Therapeutics Program, Frederick, MD) cell lines were performed as described previously (Alali, 2005).

Table 1. ^{1}H, ^{13}C, DEPT-135 and HMBC NMR data (in $CDCl_3$) for compound **5.**

Position	δ_C	DEPT	δ_H, multiplicity (*J* in Hz)	HMBC (H → C)
1	140.1	C		
2	137.3	C		
3	148.7	C		
4	103.2	CH	6.43 s	1, 2, 3, 5, 12b
4a	134.0	C		
5	30.3	CH_2	2.21 m, 2.40 m	4, 4a, 6, 7, 12b
6	36.4	CH_2	1.70 m, 2.19 m	4a, 5, 7, 7a, 12b
7	68.2	CH	2.68 dd (6, 6)	6, 7a, 8, 12, 12a
7a	152.3	C		
8	133.5	CH	8.07 s	7, 7a, 12, 12a, 9, 10
9	180.0	C		
10	163.9	C		
11	111.6	CH	6.80 d (10.5)	9, 10, 12, 12a
12	134.1	CH	7.17 d (10.5)	7a, 10, 12a, 12b
12a	136.5	C		
12b	125.1	C		
O–CH_2–O	101.2	CH_2	5.99, 6.02 d (13)	2, 3
1-OCH_3	59.9	CH_3	3.84 s	1
10-OCH_3	56.1	CH_3	3.98 s	10
$N(CH_3)_2$	43.5	$(CH_3)_2$	2.09 s	

3. Results, discussion and conclusions

Air-dried ground plant material of *C. crocifolium* Boiss. (Colchicaceae) was processed into three fractions (A–C) according to the schemes of Santavy, Preininger, Simanek, and Potesilova (1981) and Sutlupinar et al. (1988). A high concentration of colchicinoids was noted in the TLC of fraction C by distinctive yellow spots after spraying with 5% phosphomolybdic acid in EtOH. Thus, fraction C was subjected to further purification using a series of chromatographic techniques: open columns, preparative TLC and semi-preparative HPLC. Five pure compounds, four known but new to the species, along with a new one, were isolated and their structures were elucidated using a suite of spectroscopic techniques, principally: 1D-NMR (^{1}H and ^{13}C), 2D-NMR (COSY, ROESY, HMBC and HSQC), mass spectra analyses (low-resolution ESI-MS and high-resolution TOF/TOF MS) and by comparisons with data in the literature (Alali, 2005, 2006a). These compounds were all of (−)-colchicine-type: (−)-colchicine (**1**) (23.6 mg, 0.004% w/w), (−)-demecolcine (**2**) (50.3 mg, 0.009% w/w), (−)-*N*-methyl-(−)-demecolcine (**3**) (12.6 mg, 0.002% w/w), 3-demethyl-*N*-methyl-(−)-demecolcine (**4**) (6.02 mg, 0.001 w/w) and *N*,*N*-dimethyl-*N*-deacetyl-(−)-cornigerine (**5**) (95.9 mg, 0.018% w/w) (Figure 1). The spectroscopic and spectrometric data of the four known compounds: (−)-colchicine (**1**) (Alali, 2005), (−)-demecolcine (**2**) (Alali, 2005), (−)-*N*-methyl-(−)-demecolcine (**3**) (Alali, 2006a) and 3-demethyl-*N*-methyl-(−)-demecolcine (**4**) (Alali, 2006a), were in full agreement with the literature.



1: R_1=H, R_2=$COCH_3$, R_3=CH_3

2: R_1=H, R_2=CH_3, R_3=CH_3

3: R_1=CH_3, R_2=CH_3, R_3=CH_3

4: R_1=CH_3, R_2=CH_3, R_3=H

5

Figure 1. Chemical structures of (−)-colchicine (**1**), (−)-demecolcine (**2**), (−)-*N*-methyl-(−)-demecolcine (**3**), 3-demethyl-*N*-methyl-(−)-demecolcine (**4**) and *N*,*N*-dimethyl-*N*-deacetyl-(−)-cornigerine (**5**).

Compound **5** (95.9 mg) was obtained as a yellowish powder. The HR TOF/TOF MS data (observed *m/z* 370.16374 for $[M + H]^+$) revealed the molecular formula to be $C_{21}H_{23}NO_5$. The ^1H NMR spectrum (Table 1) showed signals assignable to five methine protons at δ_H 6.43 (H-4), 2.68 (H-7), 8.07 (H-8), 6.80 (H-11) and 7.17 (H-12); three pairs of methylene protons 2.21 (H-5a), 2.40 (H-5b), 1.70 (H-6a), 2.19 (H-6b), and 5.99 and 6.02 for (O–CH_2–O); and four sets of methyl protons 3.84 (1-OCH_3), 3.98 (10-OCH_3) and 2.09 ($N(CH_3)_2$). In the ^{13}C and DEPT-135 NMR spectra, signals were observed for: eight quaternary carbons at δ_C 140.1 (C-1), 137.3 (C-2), 148.7 (C-3), 134.0 (C-4a), 152.3 (C-7a), 163.9 (C-10), 136.5 (C-12a) and 125.1 (C-12b); four tertiary carbons at 59.9 (1-OCH_3), 56.1 (10-OCH_3) and 43.5 ($N(CH_3)_2$); three secondary carbons at 30.3 (C-5), 36.4 (C-6) and 101.2 (O–CH_2–O); five primary carbons at 103.2 (C-4), 68.2 (C-7), 133.5 (C-8), 111.6 (C-11) and 134.1 (C-12); and a ketone carbonyl carbon at 180.0 (C-9). The complete ^1H, ^{13}C, DEPT-135 and HMBC data sets are shown in Table 1. The 1D-NMR data revealed the presence of a methylenedioxy ring, which was characteristic of (−)-cornigerine-related compounds (Alali, 2005). In particular, compound **5** differed from (−)-cornigerine by having an *N*,*N*-dimethyl group at C-7 instead of the *N*-acetyl moiety. A broad singlet at δ_H8.07 (H-8), an AB pattern at δ_H 7.17 and 6.80 (d, J = 10.5 Hz; H-12 and H-11, respectively), and an upfield-shifted ketone carbonyl at δ_C 180.0 (C-9) were characteristic for a tropolone C-ring (Alali, 2005), whose presence was confirmed by corresponding HMBC data (Table 1, Figure 2). One of the methoxy moieties (δ_H 3.98) was placed at C-10 due to an HMBC correlation. The other methoxy group (δ_H/δ_C 3.84/59.9) was placed at C-1 due to an HMBC correlation, as is typical in colchicinoids (Figure 2) (Alali, 2005). The presence of the characteristic methylenedioxy ring (δ_H/δ_H/δ_C 5.99/6.02/101.3) was confirmed by the HMBC correlations of its two protons with C-3 and C-2 (Figure 2), and by ROESY correlations with (H-4) and (1-OCH_3) (Figure 3). A broad peak at δ_H/δ_C 2.09/43.5 was assigned to the *N,N*-dimethyl residues; this unusual NMR pattern of the *N,N*-dimethyl at C-7 was observed previously by our research team in (−)-*N*-methyl-(−)-demecolcine and 3-demethyl-*N*-methyl-(−)-demecolcine (Alali, 2006a). The structure of **5** was thus established as *N,N*-dimethyl-*N*-deacetyl-(−)-

cornigerine, a new colchicine analogue. Figure 4 shows the fragmentation pattern of *N,N*-dimethyl-*N*-deacetyl-(−)-cornigerine (**5**); this is the first attempt to interpret (+)-ESI-MS/MS fragments of colchicinoids. Based on the well-established biosynthetic pathway of colchicinoids (Herbert, 2001; Maier, 1997; McDonald et al., 1998; Nasreen, Rueffer, & Zenk, 1996), the stereochemistry at position C-7 was presumed to be *S*.

Compounds **1**–**5** were tested for cytotoxicity against a human cancer cell panel, where **1** and **2** were the most potent (Table 2). The new compound **5** exhibited an unusual activity profile; while it had modest activity against two of the cell lines (H460 and SF268), its growth inhibitory potency against MCF-7 breast cancer cells was nearly equal to that of the positive control, camptothecin.

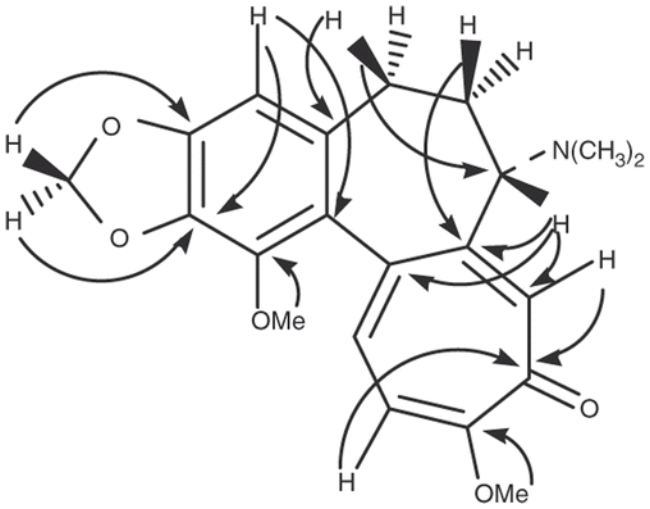


Figure 2. Key HMBC correlations for *N,N*-dimethyl-*N*-deacetyl-(−)-cornigerine (**5**).

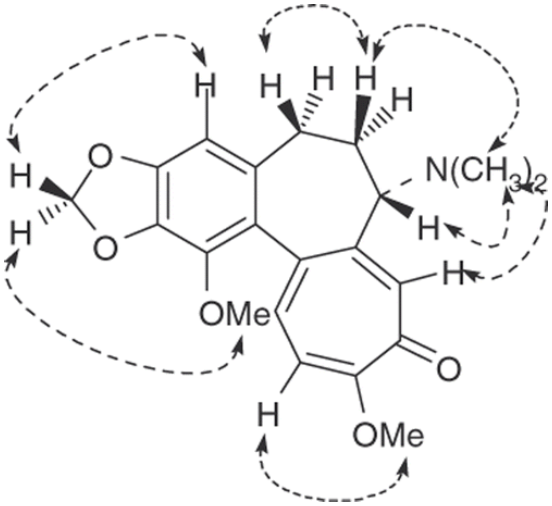


Figure 3. Key ROESY correlations for *N,N*-dimethyl-*N*-deacetyl-(−)-cornigerine (**5**).

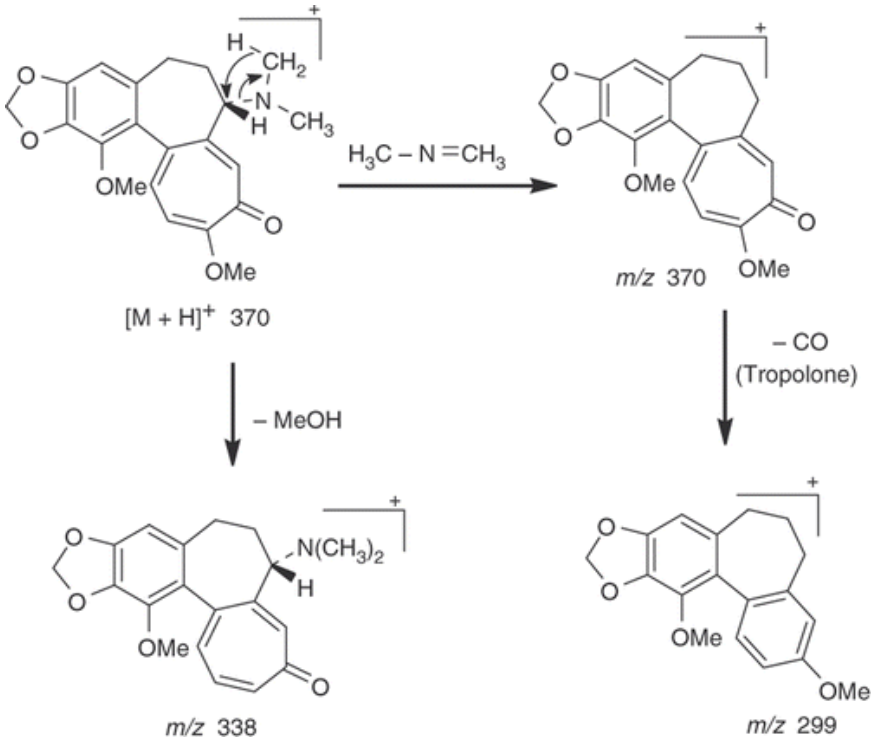


Figure 4. Mass fragmentation pattern of *N,N*-dimethyl-*N*-deacetyl-(−)-cornigerine (**5**).

Table 2. Human cancer cell panel results for compounds **1–5.**

Compound	MCF-7	H460	SF268
1	0.062	0.032	0.025
2	0.052	0.044	0.039
3	0.151	0.165	0.354
4	2.44	3.20	9.81
5	0.515	1.00	1.77
Camptothecin[a]	0.309	0.024	0.043

Note: Cytotoxicity results are expressed as IC_{50} values (μM; concentration to inhibit growth by 50%) and are derived from single experiments using 12 data points, each run in triplicate. IC_{50} values were determined as the concentration required to reduce cellular staining with sulforhodamine B by 50% relative to untreated controls following 72 h of continuous exposure (Alali, 2005); the percent standard deviation for each IC_{50} determination averaged 11% of each respective mean value, with none exceeding 35%.
[a]Positive control.

In summary, via our preliminary studies, a 2 mg aliquot of *C. crocifolium* extract was analysed using an LC-MS and LC-PDA systems to dereplicate 10 known alkaloids; constituents from each of the four major structural groups of colchicinoids were identified readily using that system. Moreover, the presence of new compounds was also suggested, particularly one that was proposed to be *N*,*N*-dimethyl-*N*-deacetyl-(−)-cornigerine (Alali et al., 2008). Those findings stimulated the present investigation, which was aimed at isolating cytotoxic components in *C. crocifolium*, both to have a larger amount of material to verify the dereplication data

spectroscopically and to evaluate the compounds against a human cancer cell panel. To the best of our knowledge, this represents the first phytochemical evaluation of *C. crocifolium*, a species native to Jordan.

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