

Colchicinoids from *Colchicum Crocifolium* Boiss.: A Case Study in Dereplication Strategies for (-)-Colchicine and Related Analogs Using LC-MS and LC-PDA Techniques

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Abstract:

As a part of a project designed to investigate *Colchicum* species in Jordan, the chemical constituents of *Colchicum crocifolium* Boiss. (Colchicaceae) were investigated using LC-MS and LC–UV/Vis PDA. A decision tree for working with colchicinoids has been developed by incorporating data from LC-UV/PDA and LC-MS. This dereplication strategy draws upon the UV/PDA spectra to classify compounds into one of four structural groups and combines this with retention time and mass spectra/molecular weight to identify the compounds. This strategy was applied on a small amount of extract (2 mg) of *Colchicum crocifolium* to dereplicate 10 known compounds from four different structural groups, namely (-)-demecolcine, 2-demethyl(-)-colchicine or 3-demethyl(-)-colchicine, *N*-deacetyl(-)-colchicine, (-)-colchicine, (-)-colchicine, β -lumidemecolcine, 2-demethyl- β -lumicolchicine or 3-demethyl- β -lumicolchicine, *N,N*-dimethyl-*N*-deacetyl- β -lumi-cornigerine, (-)-isoandrocymbine and (-)-autumnaline. Furthermore, a new compound was identified as *N,N*-dimethyl-*N*-deacetyl(-)-cornigerine. Three compounds, which had molecular ions at m/z 325, 340 and 374, could not be dereplicated into any obvious structural classes that have been isolated in our laboratories previously or reported in the literature.

Keywords: bioactive natural products | hyphenated techniques | Jordanian medicinal plants | biodiversity | colchicaceae

Article:

INTRODUCTION

To expedite discovery of new bioactive natural products from plant sources, it is of great importance to be able to discriminate between previously isolated, known compounds and new compounds, particularly at the level of the crude extract. This process, which is termed dereplication, is critical for the efficient use of valuable, and often scant, human and financial resources; science is not served by the reinvention of the proverbial wheel. By doing so, one can avoid the tedious isolation of known constituents, and thus focus on the targeted isolation of constituents presenting novel or unusual spectroscopic features (Hostettmann *et al.*, 2001; Wolfender *et al.*, 2006).

Several hyphenated techniques are finding growing use in dereplication strategies. Traditionally, UV, MS and NMR spectroscopy are used separately for structure elucidation of pure natural compounds. However, the integration of all these techniques in their hyphenated forms, liquid chromatography–ultraviolet photodiode array detection (LC-UV/PDA), liquid chromatography–mass spectrometry (LC-MS), liquid chromatography–nuclear magnetic resonance (LC-NMR) and liquid chromatography–nuclear magnetic resonance–mass spectrometry (LC-NMR-MS), may enable the complete characterisation of discrete compounds in mixtures in a single linear run of coupled analyses. In essence, hyphenated techniques can be defined as the coupling of HPLC separation technologies with on-line spectroscopic/spectrometric detection technologies (Wolfender *et al.*, 2003, 2006). The extraordinary improvements in hyphenated analytical methods over the past two decades have broadened their applications in the analyses of biomaterials significantly, especially natural products. Several hyphenated techniques are used complementarily as powerful and economical tools for dereplication of natural products in crude plant extracts. They are able to give sufficient information to identify known components or, in the case of LC–NMR, full structural elucidation of unknown compounds. The combined use of these hyphenated techniques is also very useful for the on-line structure determination of compounds that are not separable at the preparative level and for the study of labile constituents. Moreover, they are very efficient for the recording of spectra in crude reaction mixtures at the microgram level, often enabling the determination, using Mosher's esters, of absolute configurations on-line (Queiroz *et al.*, 2003). Hyphenated techniques are also being used in related fields, such as chemotaxonomic studies, chemical fingerprinting, quality control of herbal products and metabolic studies (Wolfender *et al.*, 2003; Yang, 2006). In short, the impact of hyphenated techniques in many forms of analytical chemistry is tremendous and cannot be overlooked, especially for the natural product chemist (Stobiecki, 2001).

In our ongoing research on plants native to the Hashemite Kingdom of Jordan (Jordan), we have isolated and identified colchicine and more than 30 related analogues from different species of *Colchicum*. (–)-Colchicine (**I**) is one of the most well studied natural compounds, as it was first isolated in 1820 by Pelletier and Caventou (Pelletier and Caventou, 1820). It is still used as a pharmaceutical for the treatment of gout (Terkeltaub, 2003) and in a number of proinflammatory disorders, such as familial Mediterranean fever (Drenth and Meer, 2001) and Behcet's disease (Sakane and Takeno, 2000). (–)-Colchicine (**I**) was found to possess potent antitumour activity in clinical investigations. However, its use as an antineoplastic agent is limited due to lack of tumour selectivity and high toxicity (Eigsti and Dustin, 1955). However, its promising potency encouraged medicinal and organic chemists to synthesise hundreds of colchicine analogues in an attempt to reduce toxicity and to refine the structure–activity relationships (Bergemann *et al.*, 2003; Cifuentes *et al.*, 2006; Nakagawa-Goto *et al.*, 2005). Among (–)-colchicine analogues,

isoandrocymbine (**1**); and phenethyltetrahydroisoquinoline-type alkaloids—(–)-autumnaline (**2**). This has led to the development of a decision tree that can be implemented even at the level of the crude extract to dereplicate known compounds and at least classify the major structural features of new compounds.

EXPERIMENTAL

Plant material. *Colchicum crocifolium* flowers from March to April in the northeastern desert of Jordan, usually in clayey/sandy desert soils, and it is characterized as perennial herb with corms covered by thick, dark brown to reddish scales (Al-Eisawi, 1998). 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 (elev. 691 m, lat. 32°33.465'N, long. 38°16.854'E). The collected materials were identified by one of the authors (K.T.). A voucher specimen (PHC# 110) was deposited in the herbarium of the Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan. The plant raw material was cleaned and air-dried at room temperature with the corms sliced into small pieces to speed up the process. The plant material (corms, leaves, stems and seeds) were mixed together and then ground to a fine powder using a laboratory mill, RetschMühle (RETSCH GmbH, Haan, Germany), passed through a 24 mesh sieve to generate a homogeneous powder, stored at room temperature (22–23°C), and protected from light until required for analyses.

Extraction and isolation. 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 method of Simánek and coworkers (Santavy *et al.*, 1981; Sutlupinar *et al.*, 1988). Briefly, the MeOH-extract was dissolved in 5% acetic acid and 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; 1.15 g). The acidic aqueous residues were made alkaline (pH 9) with 10% NH₄OH followed by extraction three times with CH₂Cl₂ (fraction C; 1.94 g). The aqueous residues were then adjusted to pH 12 with 10% NaOH, and then extracted three times with diethyl ether (fraction D; 0.05 g), and finally three times with CH₂Cl₂ (fraction E; 0.1 g). All fractions were dried *in vacuo*. For the dereplication studies, an aliquot of fraction C (2 mg) was dissolved in mobile phase, filtered through a 0.45 µm Teflon filter, and then transferred to 2 mL amber HPLC vials. A 100 µL aliquot was injected onto the LC-UV/PDA and LC-MS systems. (–)-Colchicine standard (Fluka Chemie AG, Buchs) was used for method development and retention time matching.

Liquid chromatography–mass spectrometry. LC-MS utilised an Agilent® (Palo Alto, CA, USA) ion-trap LC/MS coupled with APCI positive ionisation mode and an Agilent® 100 series HPLC equipped with a Hypersil BDS (125 × 4 mm; 5 µm) column (Thermo Electron, Auchtermuchty, UK). The mobile phase used H₂O acidified with 0.1% formic acid (A) and MeOH (B) in the following gradient combinations: 0–2 min, 90% A; 2–27 min, 10% A; 27–30 min 90% A.

The mass detector conditions were as follows: APCI positive-ionisation mode, full-scan mode from 50 to 800 *m/z*, capillary voltage set at –4500 V, APCI temperature 400°C, gas flow-rate 4 L/min. The mobile phase flow-rate was 1 mL/min with an injection volume of 100 µL.

LC-UV/PDA. UV-vis PDA spectra were obtained on a Lachrom[®] Merck-Hitachi (Tokyo, Japan) HPLC, equipped with quaternary gradient L-7150 pump, L-7455 Diode-Array Detector, L-7200 auto-sampler and D-7000 Interface in the range between 200 and 400 nm. Mobile phase, flow rate, analytical column, injection volume and run times were the same as those used for LC-MS.

NMR. For the new compound (**12**), ¹H and ¹³C-NMR spectra were recorded using a 400 MHz Bruker spectrometer (Switzerland), at the Faculty of Pharmacy, JUST, Jordan.

RESULTS AND DISCUSSION

The general dereplication strategy used a three-step approach. First, LC-MS was used to acquire the molecular ion and hence the molecular weight of the compound. These data were searched across natural product databases, particularly the *Dictionary of Natural Products* (Chapman & Hall, version 15:2), typically narrowing the search as much as possible by using the molecular weight to the first decimal place. This information was cross referenced against our compiled library, which has been constructed by gathering all spectral data reported in the literature for compounds isolated and characterised from *Colchicum* and related genera. Next, LC-UV/PDA was used to acquire the UV-vis spectra of a compound, and this fingerprint was used to group each compound into one of four structural classes that are typical for colchicine analogues (Rosso and Zuccaro, 1998; Shamma, 1972). Finally, the mass fragmentation data and the chromatographic retention times (and hence, relative polarity) were used to identify the structures of the compounds. Where possible, authentic reference standards were used to verify these assignments. By using this integrated approach, 10 known compounds were dereplicated rapidly, one new structure was identified and three compounds were grouped into complete unknowns (although structurally related to each other).

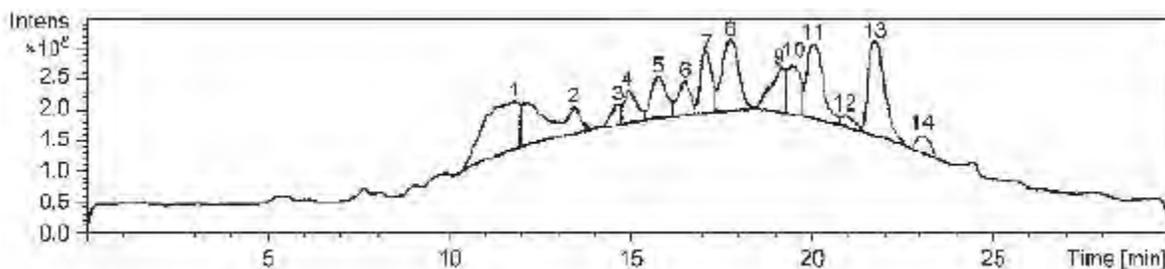


Figure 1. (+)-APCI TIC chromatogram of the alkaloid rich fraction of *C. crocifolium*.

For LC-MS and LC-UV/PDA analyses, the optimum separation conditions for the alkaloid-rich fraction (fraction C) of *C. crocifolium* were determined as outlined in the Experimental. Figure 1 shows a typical LC-MS total ion chromatogram (TIC) of *C. crocifolium*, and this gave enough resolution that data from distinct compounds could be acquired. The mass fragmentation patterns for each peak are shown in Fig. 2, and Table 1 summarizes the retention times, UV_{max} (nm), and mass spectral data of each peak as well. In general, data obtained from LC-MS, using atmospheric pressure chemical ionisation (+)-APCI as the ionisation interface, were complementary to those obtained using LC-UV/PDA, hence enabling dereplication of colchicine and 10 related compounds in *C. crocifolium*.

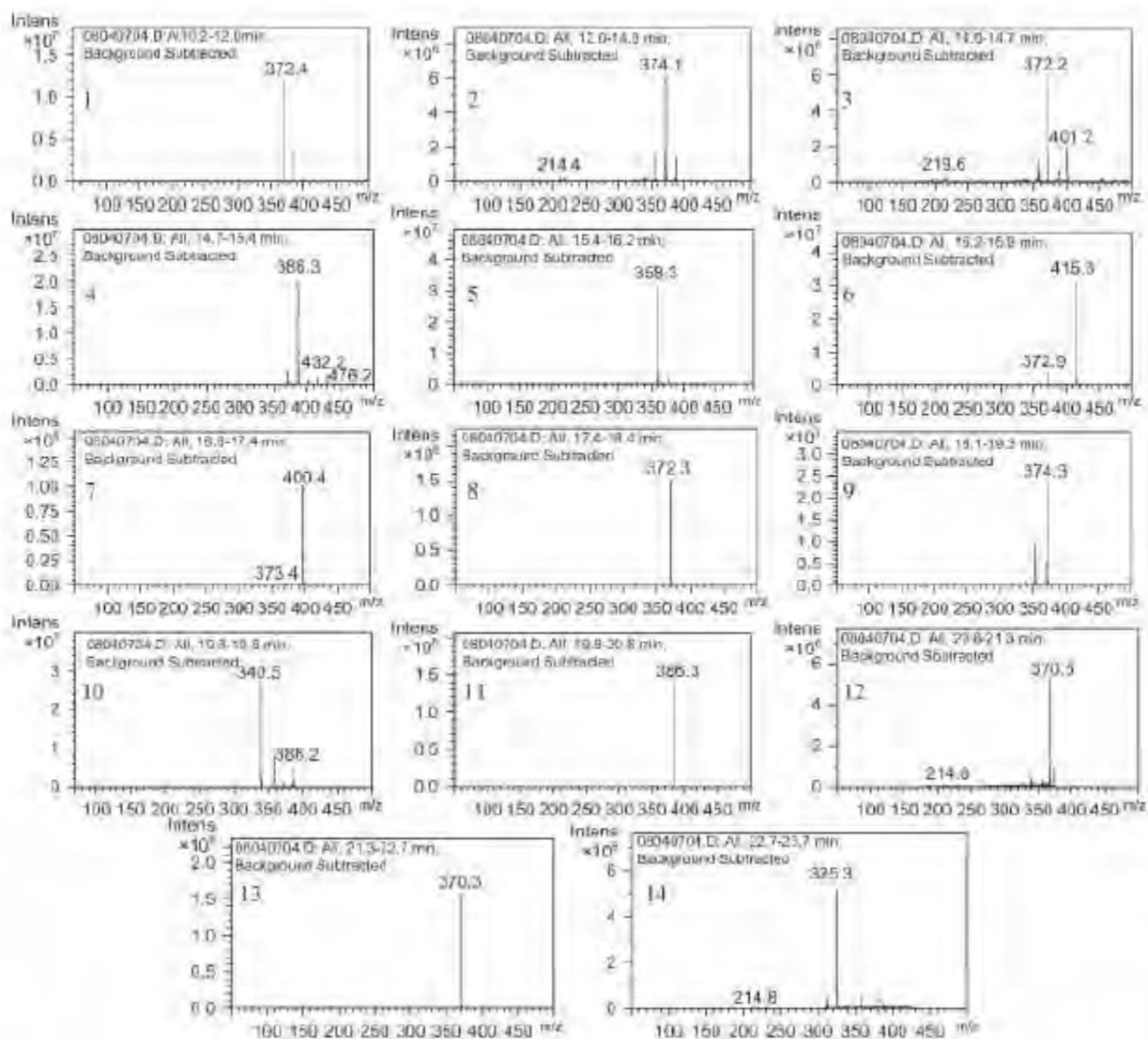


Figure 2. (+)-APCI Mass fragmentation patterns of the TIC chromatographic peaks of *C. crocifolium*.

The strategy used in identifying these compounds was integrative in nature. Based on retention times (polarity), UV max (nm) pattern, mass spectral data (base peak and fragments), authentic standards of key colchicinoids, our long experience in *Colchicum* chemical constituents and general background in natural products biosynthesis, 14 compounds were tentatively identified in the (+)-APCI TIC chromatogram (1–14; Fig. 1). The chemical structures of one new (12) and 10 known (1–8, 11 and 13) compounds were identified. Of these, six (3–7 and 12) were colchicine or colchicine-like compounds, three were lumiderivatives (photoisomers) (8, 11 and 13), one was an androcymbine-type (1) alkaloid, and one was a phenethyltetrahydroisoquinoline-type (2) alkaloid. Figure 3 shows typical UV/PDA spectra of the general classes of *Colchicum* alkaloids that were detected in the investigated species (Rosso and Zuccaro, 1998; Shamma, 1972). UV/PDA spectra were very informative in determining the general structural class for each compound; even just by eye, the general patterns are quite characteristic. In the TIC from LCMS

the following molecular ions m/z were observed: 371 (11.8, 14.7 and 17.7 min), 373 (13.5 and 19.2 min), 385 (15.0 and 20.0 min), 358 (15.8 min), 416 (16.5 min), 400 (17.1 min), 340 (19.5 min), 370 (20.9 and 21.7 min) and 325 (23.0 min); (Figs 1 and 2, and Table 1).

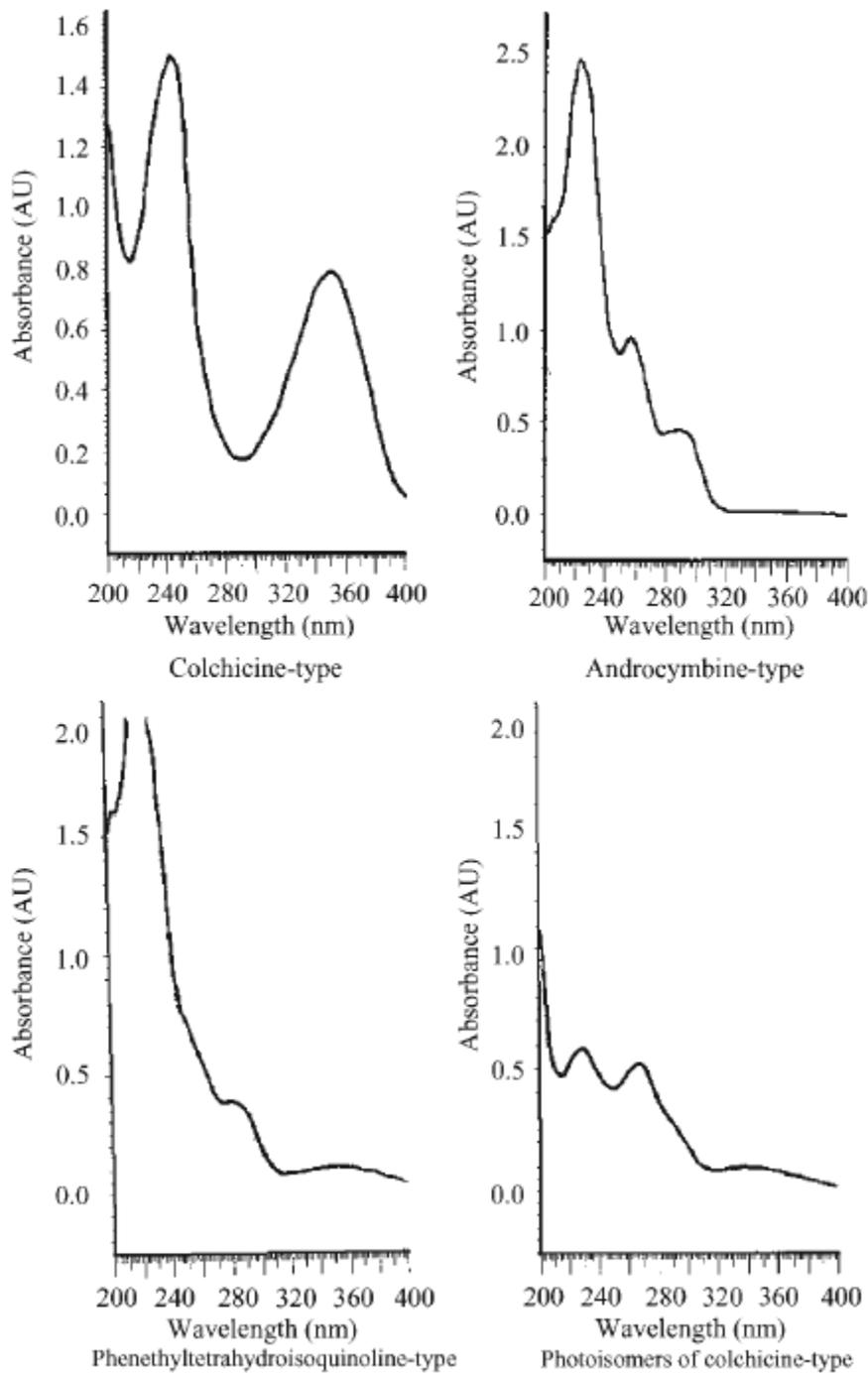
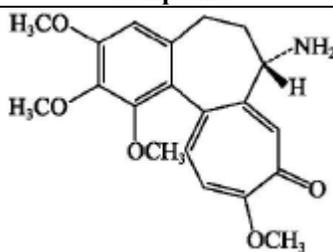
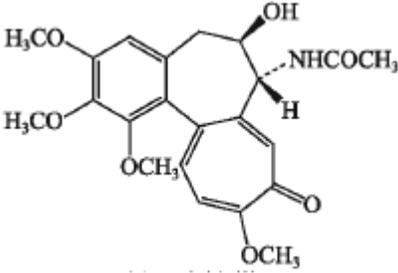
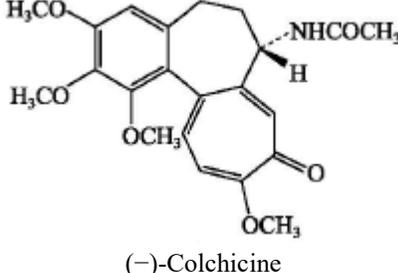
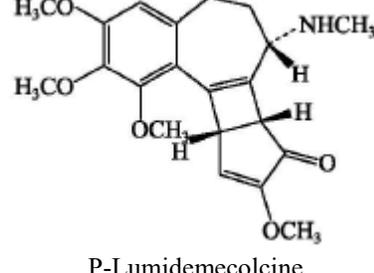
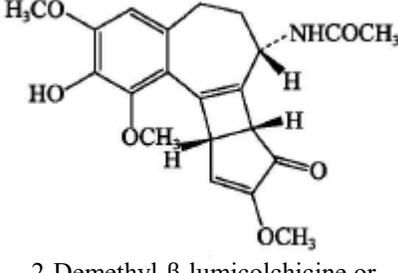
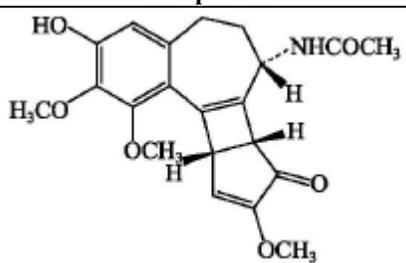
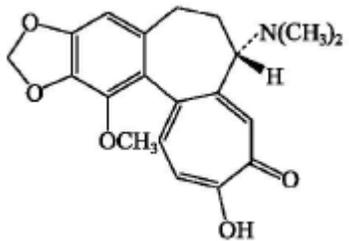
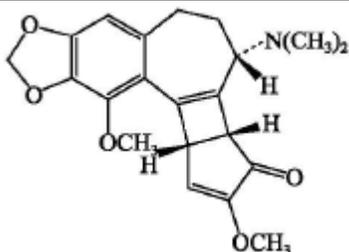


Figure 3. Typical UV/PDA spectra of general classes of *Colchicum* alkaloids.

Peak no.	<i>t_R</i> (min)	UV _{max} (nm)	LC-(+)-APCI mass fragments (<i>m/z</i>)	Compound
1	11.8	230, 260, 290	372 (M + H) ⁺ , 341, 214	<p>(-)-Isoandrocybine</p>
2	13.5	225, 285	374 (M + H) ⁺ , 358, 341, 214	<p>(-)-Autumnaline</p>
3	14.7	247, 350	372 (M + H) ⁺ , 360, 358	<p>(-)-Dernecolcine</p>
4	15.0	243, 353	386 (M + H) ⁺ , 373, 373, 372	<p>2-Dernethyl(-)-colchicine or</p> <p>3-Dernethyl(-)-colchicine</p>

Peak no.	t_R (min)	UV_{max} (nm)	LC-(+)-APCI mass fragments (m/z)	Compound
5	15.8	248, 350	358 (M + H) ⁺ , 357, 345, 327	 <p><i>N</i>-deacetyl(-)-colchicine</p>
6	16.5	244, 350	416 (M + H) ⁺ , 387, 386, 358	 <p>(-)-Colchicine</p>
7	17.1	248, 348	400 (M + H) ⁺ , 383, 373, 358	 <p>(-)-Colchicine</p>
8	17.7	228, 266	372 (M + H) ⁺ , 358, 341	 <p>P-Lumidemecolcine</p>
9	19.2	230, 270	374, 372, 358, 341	Unknown
10	19.2	244, 280	340, 372, 356, 340	Unknown
11	20.0	230, 266	386 (M + H) ⁺ , 372, 355, 192	 <p>2-Demethyl-β-lumicolchicine or</p>

Peak no.	t_R (min)	UV _{max} (nm)	LC-(+)-APCI mass fragments (m/z)	Compound
				 <p>3-Demethyl-β-lumicolchicine</p>
12	20.9	244, 358	370 ($M + H$) ⁺ , 369, 358, 343	 <p><i>N,N</i>-Dimethyl-<i>N</i>-deacetyl(-)-comigerine</p>
13				 <p><i>N,N</i>-Dimethyl-<i>N</i>-deacetyl-β-Lumi(-)-cornigerine</p>
14	23.0	240	325, 314, 313	Unknown

The UV spectra of compounds **3**, **4**, **5**, **6**, **7** and **12** revealed the presence of absorption maxima at ~ 350 nm, suggesting the presence of the *O*-methyl tropolone system characteristic of (-)-colchicine and structurally related colchicinoids (Rosso and Zuccaro, 1998; Shamma, 1972). In general the (+)-APCI mass fragments, being few and with low intensity, were not always structurally informative as one might expect from this ‘soft’ ionisation technique. However, the molecular ion could be identified, which facilitated the literature cross referencing component.

The (+)-APCI mass spectrum of (-)-colchicine (peak 7, $t_R = 17.1$ min) showed a parent molecular ion at m/z 400 $[M + H]^+$, and $[M + H - COCH_2]^+$ gave a peak at m/z 358. The UV/PDA spectrum of (-)-colchicine showed two absorption maxima at 248 and 348 nm, typical values for (-)-colchicine, supporting the identity of this compound (O’Neil *et al.*, 2001; Rosso and Zuccaro, 1998). This was verified by comparison of the APCI mass spectrum, UV/PDA spectrum and HPLC retention time of an authentic standard of (-)-colchicine, where complete matching was observed (Alali *et al.*, 2005).

The (+)-APCI mass spectrum of peak 3 ($t_R = 14.7$ min) showed a parent molecular ion at m/z 372 for $[M + H]^+$, 28 Da less than the analogous peak in (-)-colchicine; it was also at an earlier retention time (i.e. more polar) than (-)-colchicine. The few and low-intensity mass fragments of this compound were not diagnostic. The UV/PDA spectrum of the compound showed two absorption maxima at 247 and 350 nm, implying structural similarities to (-)-colchicine. These

data suggested that this compound was (-)-demecolcine (Freyer *et al.*, 1987), and this was verified by comparison of the retention time and UV/PDA spectrum with that of (-)-demecolcine, which was isolated previously in our laboratory (Alali *et al.*, 2005).

The (+)-APCI mass spectrum of peak 4 ($t_R = 15.0$ min) showed a parent molecular ion at m/z 386 for $[M + H]^+$, 14 Da less than the analogous peak in (-)-colchicine; it was also at an earlier retention time (i.e. more polar) than (-)-colchicine. The UV/PDA spectrum of the compound showed two absorption maxima at 243 and 353 nm, similar to colchicine-type alkaloids. These data suggested that this compound was either (-)-2-demethyl(-)-colchicine or (-)-3-demethyl(-)-colchicine (Chommadov *et al.*, 1990). Both (-)-2-demethyl(-)-colchicine and (-)-3-demethyl(-)-colchicine were isolated previously in our laboratory (Alali *et al.*, 2005; Alali *et al.*, 2006a, d; Al-Mahmoud *et al.*, 2006). The retention time and UV/PDA spectrum of this compound matched well with both of the above compounds, and due to their close structural similarities, 2D-NMR data are required to confirm its identity completely. Nevertheless, this dereplication strategy narrowed it to two possibilities.

The (+)-APCI mass spectrum of peak 5 ($t_R = 15.8$ min) showed a parent molecular ion at m/z 358 for $[M + H]^+$, 42 Da less than (-)-colchicine and 14 Da less than (-)-demecolcine. It is more polar than (-)-colchicine and less polar than (-)-demecolcine. A peak at m/z 326 suggests $[M + H - CH_3OH]^+$. The UV/PDA spectrum of the compound showed two absorption maxima at 248 and 350 nm, similar to colchicine-type alkaloids. These data suggested that this compound was *N*-deacetyl(-)-colchicine (Cordell *et al.*, 1989).

The (+)-APCI mass spectrum of peak 6 ($t_R = 16.5$ min) showed a parent molecular ion at m/z 416 for $[M + H]^+$, 16 Da more than the analogous peak of (-)-colchicine, and it was more polar than (-)-colchicine based on the retention time. A peak at m/z 358 suggests $[M + H - NHCOCH_3]^+$. The UV/PDA spectrum of the compound showed two absorption maxima at 244 and 350 nm, similar to colchicine-type alkaloids. These data suggested that this compound was (-)-colchicine (Potesilova *et al.*, 1977).

The (+)-APCI mass spectrum of peak 1 ($t_R = 11.8$ min) showed a parent molecular ion at m/z 372 for $[M + H]^+$. The UV/PDA spectrum of the compound differs significantly from that of colchicine-type alkaloids. It showed three absorption maxima at 230, 260 and 290 nm, similar to androcymbine-type alkaloids (Freyer *et al.*, 1987). The early retention time of this compound is also characteristic for this class of compounds. These data suggested that this compound was isoandrocymbine, a compound that was isolated previously in our laboratory (Al-Mahmoud *et al.*, 2006).

The (+)-APCI mass spectrum of peak 2 ($t_R = 13.5$ min) showed a parent molecular ion at m/z 374 for $[M + H]^+$. Peaks at m/z 341 and 359 suggested $[M - CH_3OH]^+$ and $[M + H - CH_3]^+$, respectively. The UV/PDA spectrum of the compound differs from that of colchicine-type alkaloids, as it showed two absorption maxima at 225 and 285 nm, indicating the presence of a non-conjugated benzene chromophore, which are typical of phenethyltetrahydroisoquinoline-type alkaloids (Shamma, 1972). The early retention time of this compound is also characteristic of this class of compounds. These data suggested that this compound was (-)-autumnaline (Freyer *et al.*, 1987).

The (+)-APCI mass spectrum of peak **8** ($t_R = 17.7$ min) showed a parent molecular ion at m/z 372 for $[M + H]^+$, which was the same molecular weight as (-)-demecolcine. Peaks at m/z 341 and 357 suggested $[M + H - OCH_3]^+$ and $[M + H - CH_3]^+$, respectively. The UV/PDA spectrum of the compound showed two absorption maxima at 228 and 266 nm, indicating that this compound was a photoisomer of (-)-demecolcine. The extended conjugation of (-)-demecolcine was interrupted upon isomerization to lumidemecolcine, and thus a shift of the absorption maxima to shorter wavelengths was observed at 228 and 266 nm compared with 247 and 350 nm in (-)-demecolcine. These data suggested that this compound is β -lumidemecolcine (Chommadov *et al.*, 1990).

The (+)-APCI mass spectrum of peak **11** ($t_R = 20.0$ min) showed a parent molecular ion at m/z 386 for $[M + H]^+$, which was the same molecular weight as 2-demethyl- or 3-demethyl(-)-colchicine. The few and small-intensity fragments of this compound were not very informative. However, the UV/PDA spectrum of the compound showed two absorption maxima at 230 and 266 nm, indicating that this compound was a photoisomer of 2-demethyl- or 3-demethyl(-)-colchicine. These data suggested that this compound was either 2-demethyl- β -lumicholchicine or 3-demethyl- β -lumicholchicine (Chommadov *et al.*, 1990). As with peak **4** described above, this dereplication strategy quickly narrowed the structure to two possibilities.

The (+)-APCI mass spectrum of peak **12** ($t_R = 20.9$ min) and peak **13** ($t_R = 21.7$ min) showed parent molecular ions at m/z 370 and 370, respectively for $[M + H]^+$. The UV/PDA spectra of the two compounds were different. The former showed two absorption maxima at 244 and 358 nm, characteristic of colchicine-type alkaloids, while the later showed two absorption maxima at 238 and 270 nm, characteristic of colchicine-type photoisomers. Judging from their close retention times on the relatively low polar side of the chromatogram, and from their mass fragments and PDA patterns, compound **13** was presumed to be a photoisomer of **12**. Thus, compound **12** was determined to be a structurally new compound *N,N*-dimethyl-*N*-deacetyl(-)-cornigerine and compound **13** was the known photoisomer of it, *N,N*-dimethyl-*N*-deacetyl- β -lumicornigerine (Potesilova *et al.*, 1985). Indeed, as a continuation of this work, *N,N*-dimethyl-*N*-deacetyl(-)-cornigerine was isolated and purified for full spectral characterisation and biological evaluation, which will be the subject of a forthcoming publication. The 1H and ^{13}C -NMR data for compound (**12**) are as follows: 1H NMR (400 MHz, $CDCl_3$)—1.70 (1H, m, H-6), 2.09 [6H, s, $N(CH_3)_2$], 2.19 (1H, m, H-6), 2.21 (1H, m, H-5), 2.40 (1H, m, H-5), 2.68 (1H, dd, $J = 6, 6$ Hz, H-7), 3.84 (3H, s, 1-OMe), 3.98 (3H, s, 10-OMe), 6.01 (2H, d, $J = 13$ Hz, O- CH_2 -O), 6.43 (1H, s, H-4), 6.80 (1H, d, $J = 10.5$ Hz, H-11), 7.17 (1H, d, $J = 10.5$ Hz, H-12), 8.07 (1H, s, H-8). ^{13}C NMR (125 MHz, $CDCl_3$)—30.3 (CH_2 , C-5), 36.4 (CH_2 , C-6), 43.5 $N(CH_3)_2$, 56.1 (CH_3 , 10- OCH_3), 59.9 (CH_3 , 1- OCH_3), 68.2 (CH, C-7), 101.2 (CH_2 , O- CH_2 -O), 103.2 (CH, C-4), 111.6 (CH, C-11), 125.1 (C, C-12b), 133.5 (CH, C-8), 134.03 (C, C-4a), 134.08 (CH, C-12), 136.5 (C, C-12a), 137.3 (C, C-2), 140.1 (C, C-1), 148.7 (C, C-7a), 152.3 (C, C-3), 163.9 (C, C-10), 180.0 (C, C-9). In our previous studies on *Colchicum* species, we have not isolated previously compounds **1**, **2**, **5**, **6**, **8**, **11** and **13**.

We were unable to suggest chemical structures for compounds **9**, **10** and **14**, which had molecular ions at m/z at 374, 340 and 325, respectively for $[M + H]^+$. Their UV/PDA spectra did

not fit to any of the four major classes studied, and a search of the *Dictionary of Natural Products* did not reveal any obvious candidates.

In short, a decision tree for working with colchicinoids has been developed by incorporating data from LC-UV/PDA and LC-MS. This dereplication strategy draws upon the UV/PDA spectra to classify compounds into one of four structural groups and combines this with retention time and mass spectra/molecular weight to identify the compounds, at least tentatively. This decision tree was used on a small amount of extract (2 mg) of *Colchicum crocifolium* to dereplicate 10 known compounds from four different structural groups. Furthermore, we identified one compound as having a new structure that may be *N,N*-dimethyl-*N*-deacetyl-(-)-cornigerine (**12**) and which is related to the known photoisomer, *N,N*-dimethyl-*N*-deacetyl- β -lumicornigerine (**13**).

Importantly, three compounds could not be dereplicated into any obvious structural class that has been isolated in our laboratories previously or reported in the literature. The decision tree has thus focused our ongoing studies, both to verify the structure of the presumed new compound (**12**) and to further classify and identify the three unknowns (**9**, **10** and **14**).

From a broader scientific perspective, we have developed a strategy to speed the discovery of new compounds from crude alkaloid extracts. The described methods can be applied to the isolation/dereplication of other related classes of alkaloids of similar basicity, and the analytical settings are general and can be used for the analysis of a large group of diverse structures. Thus, we hope that many scientists may benefit by applying similar tools and strategies to other classes of alkaloids. While the described use of PDA spectra was specific for distinct classes of colchicinoids, other PDA spectra may serve as fingerprints for different alkaloid classes, and the LC-MS methods are quite general and should be applicable to structurally diverse groups of compounds.

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