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ALKALOIDS AND PHENOLICS OF THREE *MERENDERA* SPECIES*

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Key Word Index—*Merendera* spp.; Liliaceae; tropolone alkaloids; baytopine; homoaporphine alkaloids; CD, UV; luteolin; aromatic acids.

Abstract—The alkaloid content (colchicine, 2-demethydemecolcine, 3-demethyldemecolcine, *N*-formyl-*N*-deacetylcolchicine, cornigerine, demecolcine) of corms, leaves and flowers of *Merendera kurdica* and of corms of *M. manissadjianii* and *M. sobolifera* was determined by HPLC. In addition to these compounds, all corms contained the flavone luteolin, and benzoic, 2-hydroxy-6-methoxybenzoic and vanillic acids. A new homoaporphine alkaloid baytopine was isolated from the leaves and flowers of *M. kurdica*. The UV and CD spectra of five homoaporphine alkaloids, baytopine, bechuanine, CC-24, kreysigine, and *O*-methylkreysigine, measured in ethanol and sodium ethoxide show marked differences which are discussed.

INTRODUCTION

Plants of the genus *Merendera* (tribe Colchiceae) grow wild in northwest Africa, Asia Minor, and India [2]. The seeds and corms of *Merendera* plants have, like *Colchicum*, been used in folk medicine as a specific treatment of gout. Phytochemical analysis within the genus *Merendera* has revealed that tropolone alkaloids constitute the major secondary metabolites [3]. Four *Merendera* species grow wild in Turkey [4]. Phytochemical studies have been reported only for *M. caucasica* (syn. *M. manissadjianii*) [5, 6] which has been found to produce colchicine, *N*-formyl-*N*-deacetylcolchicine, 2-demethylcolchicine, β-lumicolchicine, two polypeptides, some amino acids, and saccharides.

Here we report the results of a chemical investigation of *M. kurdica* Bornm., *M. manissadjianii* Aznav. (syn. *M. caucasica* or *M. trigyna*) and *M. sobolifera* C. A. Meyer collected in Turkey.

RESULTS AND DISCUSSION

Plants of the genus *Merendera* belong to the oldest evolutionary group within the tribe Colchiceae [2]. This is supported by the presence of homoaporphine alkaloids that are absent in more recent genera. The phylogenetically oldest genera *Merendera*, *Iphigenia*, and *Kreysigia* within the family Liliaceae represent the single natural source of homoaporphines. These alkaloids originate via a metabolic pathway from phenethyltetrahydroisoquinoline precursors [7].

As part of our continuing phytochemical study of the family Liliaceae, we have investigated three *Merendera* species, namely *M. kurdica*, *M. manissadjianii*, and *M. sobolifera*. In the corms of all three plants the major alkaloid is colchicine, while demecolcine was the only basic tropolone present (Table 1). In addition, we analysed leaves and flowers of *M. kurdica*. In the aboveground parts of this plant the major alkaloid was the homoaporphine baytopine (**1**), the structure of which has recently been described by us [1], and the 2- and 3-*O*-demethylated derivatives of colchicine predominated over colchicine, which is similar to findings for other plants of the tribe Colchiceae [2] (Table 2). Ether extracts of the three species were examined by TLC for aromatic acid content. Benzoic, 2-hydroxy-6-methoxybenzoic and vanillic acids were detected with test reagents [8] and identified by direct comparison with authentic samples. They were present in quantities insufficient for isolation. The flavone luteolin was present in corms of all studied species.

The UV and CD spectra of five homoaporphines were measured in ethanol and sodium ethoxide solution (Table 3). Except for O-methylkreysigine (**5**), the UV and CD spectra of all studied homoaporphines **1**–**4** showed changes due to ionization of phenolic groups, particularly in baytopine (**1**) and bechuanine (**2**). The changes in the UV and CD spectra of **1** and **2** after alkalization are attributed to ionization of the phenolic hydroxyl in position 11 ($pK_a = 10.00 \pm 0.04$ and 10.46 ± 0.06, respectively). The pK_a values of a phenolic hydroxyl in position 13 or 1 are over 12.

EXPERIMENTAL

General. UV: EtOH, 0.001 M NaOEt. CD: EtOH, 0.001 M NaOEt. IR: KBr discs. ^{1}H NMR: $CDCl_3$, at 400 MHz; ^{13}C NMR: $CDCl_3$, at 100 MHz. MS were done at 70 eV. Prep. TLC

*Dedicated to Professor Tadeus Reichstein on the occasion of his 90th birthday.

Part 103 in the series 'Substances from the Plant of the Subfamily Wurmbaeoideae and their Derivatives'. For part 102 see ref. [1].

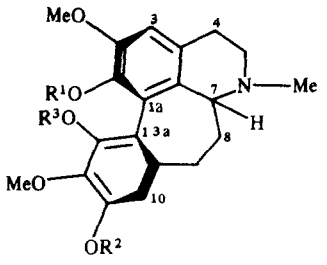
Table 1. Content of some alkaloids in corms of *Merendera* species

Alkaloid	mg/g dry wt (CV, %) *M. kurdica*	*M. manissadjianii*	*M. sobolifera*
Colchicine	0.42 (0.5)	0.76 (2.1)	2.14 (0.5)
2-Demethylcolchicine	0.02 (2.5)	0.01 (3.0)	—
3-Demethylcolchicine	0.08 (1.5)	0.11 (3.5)	0.53 (0.9)
N-Formyl-*N*-deacetylcolchicine	trace	trace	—
Cornigerine	0.002 (11.5)	—	0.22 (0.5)
Demecolcine	—	0.003 (4.3)	0.03 (0.4)

Table 2. Contents of some alkaloids in leaves and flowers of *M. kurdica*

Alkaloid	R_t (min)	mg/g dry wt (CV, %) Leaves	Flowers
Colchicine	7.17	0.29 (3.0)	0.16 (3.3)
2-Demethylcolchicine	4.03	n.q.	0.50 (1.5)
3-Demethylcolchicine	2.30	0.29 (6.5)	0.28 (39.7)
N-Formyl-*N*-deacetylcolchicine	6.63	0.14 (13.2)	0.04 (6.1)
Cornigerine	9.93	trace	trace
Demecolcine	9.02	—	—
Baytopine (1)	6.57	0.68 (2.8)	0.79 (1.9)

n.q.: Not quantified.



1 R^1 = Me, R^2 = R^3 = H (7*S*)
2 R^1 = R^2 = H, R^3 = Me (7*S*)
3 R^1 = R^3 = H, R^2 = Me (7*R*)
4 R^1 = H, R^2 = R^3 = Me (7*R*)
5 R^1 = R^2 = R^3 = Me (7*R*)

was carried out on Merck silica gel 60 glass plates (0.5 mm); analytical TLC on Merck silica gel 60 F_{254} glass plates (0.25 mm). Colchicine and its derivatives were obtained in pure form by prep. TLC of neutral $CHCl_3$ extracts in C_6H_6–EtOAc–Me_2NH–MeOH (27:5:2), demecolcine was detected by TLC of basic $CHCl_3$ extracts of *M. manissadjianii* and *M. sobolifera* in C_6H_6–EtOAc–Me_2NH (7:2:1), luteolin was obtained from all Et_2O extracts by prep. TLC in toluene–$CHCl_3$–Me_2CO (8:5:7), and aromatic acids were detected by TLC of the Et_2O extracts in C_6H_6–$CHCl_3$–MeOH (3:2:1). Full details of the detection of alkaloids and aromatic acids by TLC are described in refs [8, 9]. The identity of the known alkaloids and luteolin was established by comparison of their physical data with those of authentic samples. These include mps and UV, IR, ^{1}H NMR, ^{13}C NMR and EIMS spectra.

Plant material and extraction. M. kurdica was collected in Van-Bahcesaray in June, 1981. Air-dried material: corms 50 g, leaves 230 g, flowers 20 g. *M. manissadjianii* in Amasya in June, 1982; corms 295 g. *M. sobolifera* in Eskisehir in March, 1983; corms 20 g. Voucher specimens are deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul. Powdered plant material was extracted exhaustively with MeOH. The dried methanolic extract was taken up in 0.1% H_2SO_4. After extraction with Et_2O, the aq. soln was treated as described in ref. [9]. The yields of extracts (mg/g dry wt) were as follows: *M. kurdica*, corms 7.32 (Et_2O extract = EE), 2.20 (neutral $CHCl_3$ extract = NCE), 1.00 (basic $CHCl_3$ extract = BCE), leaves 9.15 (EE), 5.00 (NCE), 1.17 (BCE), flowers 9.62 (EE), 6.82 (NCE), 1.59 (BCE); *M. manissadjianii*, corms 8.80 (EE), 2.67 (NCE), 0.31 (BCE); *M. sobolifera*, corms 6.72 (EE), 9.90 (NCE), 1.50 (BCE).

HPLC quantification. Neutral and basic $CHCl_3$ extracts of *Merendera* corms and of leaves and flowers of *M. kurdica* were analysed by HPLC using a Spectra-Physics SP 8700 liquid chromatograph equipped with non-thermostated steel column (250 × 4.6 mm, filled with Separon SGX C18 7 μm (Lachema)). The solvent system was MeOH–H_2O–Me_3N (54:46:0.015) with a flow rate of 1 ml/min. The detection by UV was at 353 nm for tropolones and at 294 nm for their lumiderivatives and for baytopine (1). For R_ts see Table 2. Detailed procedures of samples preparation and of data evaluation are available in ref. [9].

pK_a value determination. The ionization constants were measured spectrophotometrically. The pH values of the sample were adjusted with citrate, triethanolamine, glycine buffers, different concentrations of perchloric acid, and NaOH, resp. All absorbance and pH measurements were made at 25° and ionic strength 0.1. The pH values were measured with a glass and Ag/AgCl electrode filled with 0.1 M NaCl in H_2O–EtOH (1:1). The ionization constants and molar absorptivities of alkaloids were refined with least-squares program SQUAD (84) [10]. The values are the mean of seven determinations.

Table 3. UV and CD spectral data of compounds **1–5**

Alkaloid		λ_{max} nm (log ε or Δε)
1	UV	215 (4.55), 257 (4.02), 287sh (3.67), 296sh (3.62)
		[a] 217 (4.39), 287 (4.00), 296sh (3.97)
	CD	209 (20.09), 258 (−11.2)
		[a] 222 (8.00), 279 (−6.25), 298 (−5.54)
2	UV	216 (4.61), 258 (4.05), 285sh (3.72)
		[a] 210 (4.58), 288 (4.16)
	CD	200 (14.9), 257 (−19.2)
		[a] 229 (15.75), 290 (−10.3), 305 (−10.8)
3	UV	217 (4.50), 256 (4.02), 285 (3.73)
		[a] 225 (4.43), 263sh (3.90), 305 (3.83)
	CD	200 (−30.8), 239sh (4.93), 258 (16.84), 291 (6.58)
		[a] 210 (−41.5), 224sh (−28.78), 250sh (9.87), 265 (13.32), 303 (12.56)
4	UV	217 (4.59), 256 (4.07), 290 (3.68)
		[a] 217 (4.57), 253 (4.06), 290 (3.60), 320 (3.20)
	CD	206 (−32.6), 232sh (−1.80), 258 (19.28), 294 (2.84)
		[a] 211 (−44.9), 226 (15.91), 241 (−3.22), 260 (9.06), 295 (0.75)
5	UV	218 (4.47), 257 (3.98)
		[a] 216 (4.46), 255 (3.96)
	CD	200 (−17.3), 258 (14.98), 290 (2.35)
		[a] 210 (−16.3), 226 (−0.97), 259 (12.26), 291 (1.97)

[a] In 0.001 M NaOEt.

Baytopine (**1**). Isolated by prep. TLC (C_6H_6–EtOAc–Et_2NH, 7:2:1) from BCE of leaves and flowers of *M. kurdica* in yields of 17.5 and 1.8 mg respectively (yellowish solid). On spot test on paper, it displayed a grey-blue colour with $FeCl_3$. $[\alpha]_D^{20} = +74°$ ($CHCl_3$; *c* 0.28); IR ν_{max} cm^{-1}: 3400–3500 (OH, *s*); ^1H NMR: δ2.07 (1H, *m*, H-8a), 2.26 (1H, *m*, H-9a), 2.39 (1H, *m*, H-8b), 2.48 (1H, *m*, H-9b), 2.59 (3H, *s*, N-Me), 2.83 (1H, *m*, H-5a), 3.11 (1H, *m*, H-5b), 3.15 (1H, *m*, H-4a), 3.35 (1H, *m*, H-4b), 3.63 (1H, *dd*, *J* = 11.7 Hz and 6.3 Hz, H-7), 3.64 (3H, *s*, 1-OMe), 3.92 (3H, *s*, 2-OMe), 3.95 (3H, *s*, 12-OMe), 6.65 (1H, *s*, H-10), 6.68 (1H, *s*, H-3); ^{13}C NMR: δ24.6 (*t*, C-8), 30.0 (*t*, C-4), 33.7 (*t*, C-9), 40.8 (*q*, N-Me), 44.8 (*t*, C-5), 55.1 (*q*, 2-OMe), 58.6 (*d*, C-7), 61.2 (*q*, 1-Me or 12-Me), 61.2 (*q*, 12-OMe or 1-OMe), 110.6 (*d*, C-3), 111.0 (*d*, C-10), 118.9 (*s*, C-13a), 121.8 (*s*, C-9a)[a], 122.4 (*s*, C-7a)[a], 124.2 (*s*, C-3a)[a], 135.6 (*s*, C-1a)[a], 138.6 (*s*, C-13 or C-11), 141.3 (*s*, C-11 or C-13), 147.6 (*s*, C-12)[b], 149.7 (*s*, C-2)[b], 149.8 (*s*, C-1)[b]; MS *m/z* (rel. int.): 371 $[M]^+$ (53), 354 ($C_{21}H_{24}NO_4$, 100), 340 ($C_{20}H_{22}NO_4$, 97), 60 ($C_2H_4O_2$, 17), 58 (C_3H_8N, 8) ([a, b] assignments may be reversed).

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