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FIVE NEW ALKALOIDS FROM *COLCHICUM RITCHII*

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ABSTRACT.—*Colchicum ritchii* of Jordanian origin has yielded the diphenolic phenethyltetrahydroisoquinoline (−)-isoautumnaline [**2**], which is the first naturally occurring analog of the known (−)-autumnaline [**1**]. The absolute configuration of both alkaloids was established through a study of their cd spectra. Two new colchicine-type alkaloids are (−)-specioritchine [**5**] and its structural isomer (−)-speciocolchine [**6**]. Androcymbine, which was previously known only in the levorotatory form, has now been found as the dextrorotatory enantiomer **7**. It is accompanied in the plant by the related base (+)-colchiritchine [**8**].

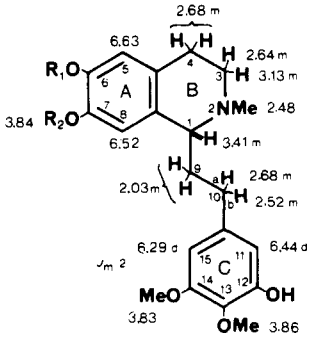
Initial investigations on *Colchicum ritchii* R. Br. (Liliaceae) had revealed the presence of (−)-colchicine [**3**], (−)-*N*-formyldeacetylcolchicine, (−)-demecolcine, (−)-colchiceine, and a compound believed to be either (−)-2- or (−)-3-demethylcolchicine (1-3).

Presently, we were able to collect this plant in southern Jordan, and the study of its alkaloidal content yielded a variety of alkaloids belonging to the autumnaline [**1**], colchicine [**3**], and androcymbine [**4**] series.

With the exception of (−)-autumnaline [**1**] and (−)-isoautumnaline [**2**], Tables 1 and 2 list the alkaloids isolated and characterized by us, together with their ^{1}H-nmr chemical shifts obtained at either 200 or 360 MHz in $CDCl_3$ solution. Chemical shift assignments were confirmed through appropriate decoupling and nOe experiments.

Because the present work represents our initial effort in the realm of *Colchicum* alkaloids, and because we did not possess in our alkaloidal collection samples of such compounds for comparison purposes, with the single exception of the commercially available (−)-colchicine [**3**], we endeavored first to obtain complete high resolution nmr data for the known alkaloids we had isolated. Subsequently, this information was used in the characterization of five new alkaloids found in the plant.

Our first new alkaloid was the diphenolic phenethyltetrahydroisoquinoline (−)-isoautumnaline [**2**], $C_{21}H_{27}NO_5$, which was obtained mixed with the known structur-



1 R_1=Me, R_2=H
2 R_1=H, R_2=Me (^{1}H nmr)

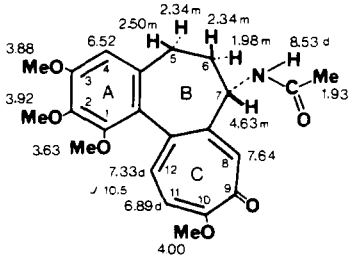


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TABLE 1. Nmr Chemical Shifts (δ Values) in $CDCl_3$ for Compounds of the Colchicine Series Tabulated According to Substitution on Nitrogen

	NH	O ‖ C-CH_3	H-7	H-4	H-8	H-11[a]	H-12[a]	H-5β	H-5α	H-6β	H-6α	1-OCH_3	2-OCH_3	3-OCH_3	10-OCH_3
Type A:															
(−)-Colchicine [3]	8.53[b]	1.93	4.63	6.52	7.64	6.89	7.33	2.50	2.34	2.34	1.98	3.63	3.92	3.88	4.00
(−)-3-Demethyl-colchicine	7.78[b]	1.99	4.65	6.59	7.56	6.90	7.34	2.49	2.30	2.30	1.93	3.64	4.00	OH	4.01
(−)-Cornigerine	7.38[b]	1.99	4.66	6.47	7.53	6.87	7.27	2.49	2.32	2.38	1.86	3.79	6.01 (OCH_2O)		4.01
(−)-Colchiceine	7.10[b]	2.01	4.70	6.53	7.48	6.89	7.34	2.49	2.38	2.26	1.90	3.62	3.94	3.90	5.96(OH)
(−)-Cornigereine	7.59[b]	2.03	4.72	6.47	7.49	6.91	7.31	2.45	2.30	2.20	1.80	3.79	5.99 (OCH_2O)		5.99(OH)
Type B:															
(−)-Colchifoline	7.54[c]	4.03[d] 4.18[d] (CH_2OH)	4.72	6.56	7.56	6.88	7.35	2.56	2.43	2.30	1.96	3.65	3.95	3.92	4.00
Type C:															
(−)-*N*-Formyldeacetyl-colchicine	6.25[e]	8.18 (CHO)	4.74	6.55	7.39	6.84	7.36	2.52	2.32	2.04	1.87	3.66	3.95	3.91	4.01
Type D:															
(−)-Demecolcine	2.18 (NCH_3)		3.23	6.51	7.66	6.78	7.20	2.40	2.34	2.12	1.62	3.57	3.89	3.87	3.97
(−)-3-Demethyl-demecolcine	2.30 (NCH_3)		3.36	6.59	7.70	6.82	7.24	2.43	2.26	2.26	1.75	3.59	3.99	OH	4.01
(−)-2-Demethyl-demecolcine	2.22 (NCH_3)		3.29	6.53	7.69	6.80	7.25	2.41	2.36	2.20	1.63	3.56	OH	3.94	4.01
(−)-Demecolceine	2.29 (NCH_3)		3.35	6.54	7.68	6.89	7.29	2.45	2.35	2.24	1.74	3.58	3.93	3.90	6.01(OH)
Type E:															
(−)-Specioritchine [5]	2.24 (NCH_3)		3.16	6.59	7.62	6.77	7.25	2.51	2.35	2.35	1.96	3.52	3.99	OH	3.99
(−)-Speciocolchine [6]	2.23 (NCH_3)		3.15	6.52	7.62	6.77	7.28	2.52	2.37	2.37	1.99	3.54	OH	3.96	3.99

[a]d, $J \simeq 10.5$ Hz.
[b]d, $J_{vic} \simeq 6.3$ Hz.
[c]d, $J_{vic} \simeq 7.5$ Hz.
[d]d, $J_{gem} = 16.3$ Hz.
[e]d, $J_{vic} = 5.8$ Hz.

TABLE 2. Nmr Chemical Shifts (δ Values) in $CDCl_3$ for Compounds of the Androcymbine Series

	NCH_3	H-7	H-4	H-8	H-11	H-5β	H-5α	H-6β	H-6α
(+)-Androcymbine [7]	2.39	3.91	6.42	6.30	6.80	2.36	2.88	1.79	2.14
(−)-*O*-Methylandrocymbine	2.40	3.90	6.33	6.30	6.82	2.36	2.94	1.80	2.15
(+)-CC-20	2.38	3.88	6.29	6.30	6.81	2.35	2.87	1.76	2.14
(+)-Colchiritchine [8]	NH	4.11	6.27	6.31	6.81	2.34	2.82	1.70	2.42

	H-12β	H-12α	H-13β	H-13α	1-OCH_3	2-OCH_3	3-OCH_3	10-OCH_3
(+)-Androcymbine [7]	1.70	2.92	2.58	2.78	4.00	3.85	OH	3.64
(−)-*O*-Methylandrocymbine	1.69	2.96	2.58	2.78	4.03	3.83	3.83	3.64
(+)-CC-20	1.64	3.05	2.55	2.74	4.08	5.92	5.96 (OCH_2O)	3.66
(+)-Colchiritchine [8]	1.40	3.27	2.85	2.85	4.08	5.93	5.97 (OCH_2O)	3.67

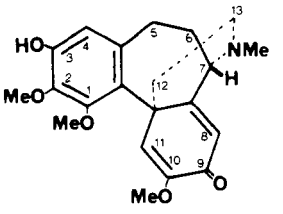
ally isomeric base (−)-autumnaline [**1**] (4,5). Subsequently, repeated tlc on Si gel precoated glass plates using the system C_6H_6-diethylamine (4:1) led to a clean separation of the two isomers. (−)-Isoautumnaline [**2**] presented the same mass spectrum as (−)-autumnaline [**1**] with a weak molecular ion *m/z* 373 and base peak *m/z* 192 due to rings A and B of the molecule.

The ^{1}H-nmr spectrum of (−)-isoautumnaline [**2**] differed from that of **1** only by a slight shift of the H-5 and H-8 absorptions. This indicated that the isomerism resided in ring A, more specifically in the relative positions of the methoxyl and hydroxyl substituents. Complete nmr nOe studies on (−)-autumnaline [**1**] and (−)-isoautumnaline [**2**] (see Experimental) conclusively established the substitution pattern on rings A and C in each case. (−)-Autumnaline [**1**] is, thus, the 6-methoxy-7-hydroxy isomer, while (−)-isoautumnaline [**2**] corresponds to the 6-hydroxy-7-methoxy analog.

Although (−)-autumnaline [**1**] was first characterized almost 20 years ago, (−)-isoautumnaline [**2**] is only the second pentaoxygenated phenethyltetrahydroisoquinoline to be identified. Originally, a specific rotation of $-5°\pm3°$ in $CHCl_3$ was reported for autumnaline [**1**], while the alkaloid appeared to be optically inactive in EtOH (5). Thus, it was not completely clear whether the natural compound was optically active or racemic. In our hands, (−)-autumnaline [**1**] showed a specific rotation of $-8°$ in either $CHCl_3$ or MeOH. (−)-Isoautumnaline exhibited specific rotations of $-6°$ in $CHCl_3$ and $-1°$ in MeOH. More significant were the cd curves for the two isomers in MeOH, both of which displayed positive maxima at 270 nm with strong positive tails at 220 nm, indicating the 1*R* configuration (6). Thus, in spite of their small specific rotations, both bases are optically active. The small magnitude of the specific rotations could be accounted for by the conformations of the two molecules. As suggested by the nOe results, the phenethyl residue prefers to lie approximately midway between ring A and the *N*-methyl group.

Our second new alkaloid was the diphenolic (−)-specioritchine [**5**], $C_{27}H_{29}NO_6$, which incorporates a colchicine nucleus. The mass spectral molecular ion, *m/z* 463, was very weak (0.6%) due to facile loss of the hydroxylated benzylic side chain, OH-C_6H_4-CH_2, with concurrent formation of the fairly strong (26%) *m/z* 357 ion.

The ^{1}H-nmr spectrum in $CDCl_3$, summarized around expression **5**, immediately suggested a colchicine nucleus (see Table 1). Aside from the clearly evident *N*-methyl function, the nature of the substitution on nitrogen was indicated by the presence of four adjacent aromatic protons as multiplets between δ 6.73 and 7.14. Significantly, H-7 appeared relatively upfield as a multiplet at δ 3.16.



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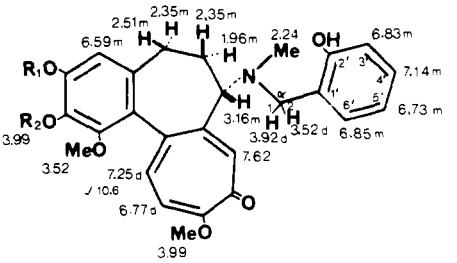
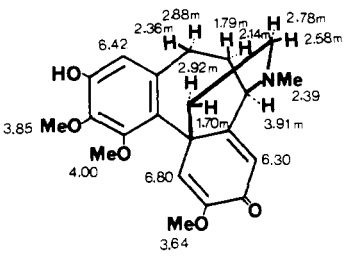


5 R_1=H, R_2=Me (^{1}H nmr)
6 R_1=Me, R_2=H



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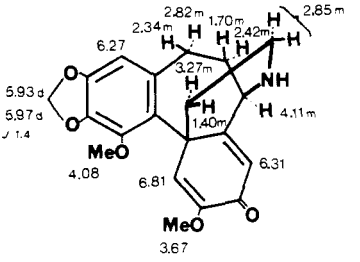

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Substantial difficulty was initially encountered in locating the nmr absorptions for the benzylic methylene protons of the side chain of species **5**. A complete nmr nOe study (see Experimental section) revealed that they overlap with the methoxyl protons at δ 3.52 and 3.99. Interestingly, when the ^{1}H-nmr spectrum was rerun under somewhat different conditions, i.e., in CD_2Cl_2 at −80°, two conformations for the benzylic side chain could be observed, resulting in two pairs of doublets for the methylene group in question at δ 3.13 and 4.41 and at δ 2.89 and 4.03.

A C-2 methoxyl in the colchicine series usually absorbs between δ 3.85 and 3.95. The fact that in the (−)-specioritchine [**5**] case this methoxyl appears at δ 3.99 suggested the presence of an *o*-phenolic function. Indeed, diazomethane *O*-methylation of (−)-specioritchine [**5**] provided (−)-*O,O*-dimethylspecioritchine, $C_{29}H_{33}NO_6$, for which the C-2 methoxyl absorption falls at δ 3.95.

The negative specific rotation of (−)-specioritchine [**5**] was indicative of the fact that the alkaloid possesses the same absolute configuration as (−)-colchicine [**3**].

(−)-Speciocolchine [**6**], $C_{27}H_{29}NO_6$, our third new alkaloid, is a structural isomer of (−)-specioritchine [**5**]. Its mass, ir, and uv spectra were very close to those of **5**. The only significant difference resided in the ^{1}H-nmr spectrum in which H-4 appeared at δ 6.52 instead of δ 6.59; while the 3-methoxyl singlet was at δ 3.96, as compared to the 2-methoxyl singlet of (−)-specioritchine [**5**], which was evident at δ 3.99. The 2-hydroxy-3-methoxy substitution pattern for (−)-speciocolchine [**6**] was then confirmed by a partial nmr nOe that showed an enhancement of the methoxyl three-proton singlet at δ 3.96 upon irradiation of the H-4 aromatic singlet located at δ 6.52.

The fourth new alkaloid is (+)-androcymbine [**7**], $C_{21}H_{25}NO_5$. This is the first report of the occurrence of dextrorotatory androcymbine, inasmuch as only the levo form was previously known (2). ^{1}H-nmr chemical shifts for our (+)-androcymbine are quoted around expression **7**. The shift assignments, as well as the boat-like conformation of ring B, were confirmed through nOe experiments (Experimental).

Our last new alkaloid, (+)-colchiritchine [**8**], $C_{20}H_{21}NO_5$, displayed a ^{1}H-nmr

spectrum related to that of (+)-androcymbine [**7**]. Significant divergences involved (a) the presence of two close doublets at δ 5.93 and 5.97, reflecting a 2,3-methylenedioxy group, and (b) the absence of an *N*-methyl singlet. Consonant with the presence of a secondary amine function was the downfield shift of H-7 that was found at δ 4.11 instead of within the customary range of δ 3.88-3.92.

As with (+)-androcymbine [**7**], the positive rotation of (+)-colchiritchine [**8**] gave a clear indication of the absolute configuration.

The present findings are illustrative of an interesting facet of *Colchicum* phytochemistry. Within one and the same plant, androcymbine type alkaloids of opposite absolute configurations may be found. In the case of *C. ritchii*, the bases in question would be, on the one hand, (+)-androcymbine [**7**], (+)-colchiritchine [**8**], and (+)-CC-20; and the known (−)-*O*-methylandrocymbine, on the other (1-3). Turning now to the colchicine type alkaloids, these have been shown conclusively to be formed through the intermediacy of (−)-*O*-methylandrocymbine (3). So far, however, only colchicine alkaloids of the *S* configuration are known, all of which are levorotatory and derived from (−)-*O*-methylandrocymbine (3). It could, thus, be argued that the enzymes necessary to transform dextrorotatory androcymbine analogs into dextrorotatory colchicine species of the *R* configuration are not normally present in *Colchicum* species.

EXPERIMENTAL

PLANT MATERIAL.—*C. ritchii* (whole plant, 19 kg) was collected near the Petra archaeological site. The plant was identified by Prof. D. el-Isawi of the Department of Botany, University of Jordan, and a specimen was deposited in the university herbarium.

EXTRACTION AND ISOLATION.—The powdered plant material was first defatted with petroleum ether and then extracted with cold EtOH. The concentrated extracts were placed on a Si gel chromatographic column. Elution was with $CHCl_3$ gradually enriched with MeOH. Final purification was by tlc on Si gel, using the systems $CHCl_3$-Me_2CO-$(Et)_2NH$ (80:10:10), EtOAc-EtOH (75:25), and C_6H_6-$CHCl_3$-$(Et)_2NH$ (80:10:10). The following amounts of pure compounds were obtained:(−)-autumnaline [**1**], 30 mg; (−)-isoautumnaline [**2**], 6 mg; (−)-colchicine [**3**], 33 mg; (−)-3-demethylcolchicine, 66 mg; (−)-cornigerine, 10 mg; (−)-colchiceine, 14 mg; (−)-cornigereine, 10 mg; (−)-colchifoline, 5 mg; (−)-*N*-formyldeacetylcolchicine, 2 mg; (−)-demecolcine, 147 mg; (−)-3-demethyldemecolcine, 22 mg; (−)-2-demethyldemecolcine, 16 mg; (−)-demecolceine, 12 mg; (−)-specioritchine [**5**], 7 mg; (−)-speciocolchine [**6**], 1.5 mg; (+)-androcymbine [**7**], 2 mg; (−)-*O*-methylandrocymbine, 11 mg; (+)-CC-20, 5 mg; and (+)-colchiritchine [**8**], 4 mg. All compounds were amorphous.

(−)-*Autumnaline* [**1**].—$[\alpha]_D$ −8° (*c* 0.26, $CHCl_3$), −8° (*c* 0.29, MeOH); cd in MeOH Δε (nm) 0 (312), +0.8 (270), +0.4 (240), positive tail near 220 nm; nmr $CDCl_3$ (360 MHz) δ 6.67 (s, H-8), 6.56 (s, H-5), 6.45 (d, J=2 Hz, H-11), 6.32 (d, J=2 Hz, H-15), 3.87 (s, MeO-6), 3.86 (s, MeO-13), 3.84 (s, MeO-14), 3.45 (m, H-1), 3.17 (m, H-3a), 2.70 (m, 4H, H-3b, H-4a, H-4b, H-10b), 2.49 (s, NMe), 2.43 (m, H-10a), 2.04 (m, 2H, H-9a, H-9b). Principal nOe's were MeO-6 to H-5 (20%), H-5 to MeO-6 (11%), H-5 to H-4 (4%), H-4 to H-5 (21%), H-8 to H-1 (5%), H-1 to H-8 (7%), H-8 to H-9 (7%), H-9 to H-8 (22%), NMe to H-1 (10%), MeO-14 to H-15 (17%), H-15 to MeO-14 (15%), H-10b to H-15 (7%), H-15 to H-10b (5%).

(−)-*Isoautumnaline* [**2**].—Ms *m/z* 373 (M^+, 0.5), 3.72 (0.5), 358 (0.3), 192 (100), 177 (11), 167 (2); $[\alpha]_D$ −6° (*c* 0.4, $CHCl_3$), −1° (*c* 0.4, MeOH); cd in MeOH Δε (nm) 0 (310), +2.4 (272), +9.5 (213). Principal nOe's were H-8 to MeO-7 (11%), MeO-7 to H-8 (9%), H-8 to H-1 (6%), H-1 to H-8 (5%), H-9 to H-8 (8%), H-8 to H-9 (3%), H-1 to H-9 (5%), H-9 to H-1 (8%), H-1 to NMe (5%), NMe to H-1 (8%), H-4 to H-5 (12%), MeO-14 to H-15 (14%), H-15 to MeO-14 (13%), H-10a to H-11 (4%). The separation of **2** from **1** may be achieved using precoated preparative Si gel glass plates, and the system C_6H_6-$(Et)_2NH$ (4:1). The glass plates were developed once, dried, and then redeveloped in the same solvent system.

(−)-*Specioritchine* [**5**].—Ms *m/z* 463 (M^+, 0.6), 448 (0.9), 357 (26), 342 (9), 328 (18), 314 (13), 298 (31), 193 (100), 107 (10), 106 (35); ν max ($CHCl_3$) 1585, 1613 cm^{-1}; $[\alpha]_D$ −57° (*c* 0.11, MeOH). Principal nOe's were H-4 to H-5β (2%), H-5β to H-4 (18%), MeO-1 to H-12 (1%), H-12 to MeO-1 (3%), H-11 to MeO-10 (14%), MeO-10 to H-11 (21%), H-7 to H-8 (5%), H-6α to H-8 (3%), H-6β to H-7 (4%), H-α_1 to H-7 (2%), H-α_1 to H-8 (2%), H-α_2 to H-6′ (1%).

(−)-*Speciocolchine* [**6**].—Ms *m/z* 463 (M^+, 0.6), 448 (1), 357 (29), 342 (8), 328 (19), 314 (13), 298 (33), 193 (100); ν max ($CHCl_3$) 1565, 1585, 1610, 3530 cm^{-1}; λ max (MeOH) 243, 359 nm (log ε 4.26, 3.95); $[\alpha]_D$ −37° (*c* 0.13, MeOH).

(+)-*Androcymbine* [**7**].—$[\alpha]_D$ +178° (*c* 0.08, MeOH), +130° (*c* 0.09, $CHCl_3$). Principal nOe's were H-5β to H-4 (24%), H-4 to H-5β (13%), MeO-1 to H-11 (8%), H-11 to MeO-1 (3%), H-11 to MeO-10 (7%), MeO-10 to H-11 (20%), H-8 to H-7 (17%), H-7 to H-8 (26%), H-7 to NMe (4%), NMe to H-7 (13%), H-12α to MeO-1 (3%), MeO-1 to H-12α (3%), H-12α to H-11 (5%), H-6α to H-7 (9%), H-6β to H-13α (3%).

(+)-*Colchiritchine* [**8**].—Ms *m/z* 355 (M^+, 100), 354 (39), 340 (23), 326 (21), 312 (17), 269 (20), 194 (44); λ max (MeOH) 213, 241, 279 (log ε 4.41, 4.08, 3.70); ν max ($CDCl_3$) 1610, 1630, 1660 cm^{-1}; $[\alpha]_D$ +207° (*c* 0.15, MeOH).

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