ALKALOIDS OF *Gloriosa superba* L.

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Dedicated to Professor A. Brossi on the occasion of his 60th birthday.

From the corms and seeds of Indian *Gloriosa superba* L., 19 tropolone and 3 non-tropolone alkaloids were isolated. Of these, cornigerine, (*S*)-(+)-floramultine (bechuanine), 1,12-dihydroxy-2,10,11-trimethoxyhomoaporphine, colchicamide, 2-demethylcolchifoline, 3-demethylcolchifoline, colchicoside, and isoperlolyrine are reported for the first time from this plant. The new alkaloid isoperlolyrine has been assigned the structure *III*. This is the first alkaloid of carboline type found in the subfamily *Wurmbaeoideae*.

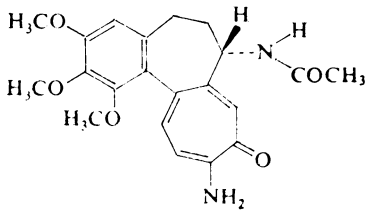
Previous studies on the chemotaxonomy of the plant *Gloriosa superba* L. showed that it mainly contained tropolone alkaloids of neutral type: colchicine[1-9], N-formyl-N-deacetylcolchicine[1-9], 3-demethylcolchicine[1,5-7], β-lumicolchicine[1,5-7], γ-lumicolchicine[8], N-formyl-N-deacetyl-β-lumicolchicine[8], N-formyl-N-deacetyl-γ-lumicolchicine[8], 3-demethyl-β-lumicolchicine[8], 3-demethyl-N-formyl-N-deacetylcolchicine[8,9], 3-demethyl-N-formyl-N-deacetyl-β-lumicolchicine[8,9], 3-demethyl-γ-lumicolchicine[9], 2,3-demethyl-N-deacetylcolchicine[9], 2,3-demethylcolchicine[9]; compound G-1 (corms)[6,7] recently identified as colchifoline[10], and compound X-1 (leaves)[9] identified now as 10,11-oxy-10,12a-cyclo-10,11-secocolchicine[10]. Only the African *G. superba* L. gave the unidentified non-tropolone alkaloids G-2 (ref.[6,7]), G-3 (ref.[7]) and G-4 (ref.[7]).

In this paper, we present the results of the reinvestigation of alkaloids from corms of *G. superba* L. of Indian origin. The seeds of this plant have not yet been studied for their alkaloidal content.

From the seeds and corms, 19 tropolone and 3 non-tropolone alkaloids were isolated by column and preparative thin-layer chromatography. Of these, 13 tropolone alkaloids had been isolated previously[1-9], *i.e.* colchicine, N-formyl-N-deacetylcolchicine, 3-demethylcolchicine, β- and γ-lumicolchicine, N-formyl-N-deacetyl-β-lumicolchicine, N-formyl-N-deacetyl-γ-lumicolchicine, 3-demethyl-β-lumicolchicine, 3-demethyl-N-formyl-N-deacetylcolchicine, 3-demethyl-N-formyl-N-deacetyl-β-lumicolchicine, 3-demethyl-γ-lumicolchicine, 2,3-demethyl-N-deacetylcolchicine, 2,3-demethylcolchicine. The tropolone alkaloid cornigerine (corms), the non-tropolone alkaloids

(*S*)-(+)-floramultine (bechuanine) (corms and seeds) and 1,12-dihydroxy-2,10,11--trimethoxyhomoaporphine (corms and seeds) were isolated from *G. superba* L. for the first time. Only the seeds gave the tropolone alkaloids 2-demethylcolchifoline, 3-demethylcolchifoline, and colchicoside. All these alkaloids had been isolated earlier from other plant species of the subfamily *Wurmbaeoideae*[11–15]. The neutral extract of the seeds gave a crystalline compound of m.p. 264°C, and the basic extract a crystalline compound of m.p. 186°C.

For the compound of m.p. 264°C, the molecular formula $C_{21}H_{24}N_2O_5$ has been derived from high resolution spectroscopic measurements M^+ and $M^+ - 1$ ions. The fragmentation pattern in the mass spectrum is indistinct, and the typical characteristics of colchicine alkaloids are missing[16]. A comparison of the NMR data of the compound with those of colchicine shows that one methoxyl is absent, instead of it the ^{1}H NMR spectrum exhibits a two proton exchangeable singlet at 6·08 ppm (Table I). The spectrum is in good agreement with values published for the colchicamide[18,19] (*I*) where the methoxyl at $C_{(10)}$ of the tropolone ring is replaced by an amino group. The ^{13}C NMR chemical shifts of the carbon atoms of the tropolone ring differ from those of colchicine, mainly those of $C_{(10)}$ (8·3 ppm upfield,) which indicates its changed nature; a substituent at $C_{(10)}$ with a lower electronnegativity than CH_3O, a different distribution of the electron mass similar to that of colchiceine[20]. The chemical shifts of the atoms of the rings A and B and of the side chains differ only slightly. The m.p., TLC, UV/Vis, IR and mass spectra are identical with those of colchicamide prepared from colchicine[19]. The isolation of colchicamide (*I*) is the first evidence of its occurrence in the subfamily *Wurmbaeoideae*.



I

For the compound of m.p. 186°C, the molecular formula $C_{16}H_{12}N_2O_2$ was established by high resolution mass spectrometry. The presence of a carboline nucleus was suggested by the UV spectrum (Fig. 1). Of the known compounds of this type, perlolyrine (*II*) has similar UV and mass spectra[21,22]. A comparison of the ^{1}H NMR spectra of these two compounds (Table II) shows that both of them consist of four spin systems of the same type (ABCD system of four vicinal aromatic protons, two AB systems of heteroaromatic protons, and the A_2-system of the primary alcohol group) but the differences in the chemical shifts of the corresponding protons

are well above the concentration effect. The largest difference is between the $\Delta\delta_{AB}$ values of the two AB systems (0·690 against 1·009 and 0·390 *versus* 0·239) of the alkaloid *II* and the examined compound. The coupling between the protons of the furane ring is sensitive to the type of substitution[23]: 1·8–2·1 Hz for the substitution 2,3-; 0·8–0·9 Hz for 2,4-; 3·3–3·7 Hz for 2,5-. The found values 4 and 3·8 Hz

TABLE I

NMR Spectral data of colchicine and colchicamide (*I*)

Group	atom	^{13}C NMR			^{1}H NMR	
		colchicine	colchicamide	$\Delta\delta$[a]	colchicine	colchicamide[b]
Ring A	1a	125·6	126·7	1·1	—	—
	1	151·1	151·1	0·0	—	—
	2	141·6	141·6	0·0	—	—
	3	153·5	153·1	−0·4	—	—
	4	107·3	107·2	−0·1	6·56	6·53
	4a	134·2	134·5	0·3	—	—
Ring B	5	29·9	30·0	0·1	—	—
	6	36·2	37·2	1·0	—	—
	7	52·9	52·6	−0·3	4·62	4·70
Ring C	7a	152·9	150·9	−2·0	—	—
	8	130·3	125·3	−5·0	7·67	7·59
	9	179·4	175·2	−4·2	—	—
	10	163·9	155·6	−8·3	—	—
	11	113·0	112·3	−0·7	6·93[c]	6·89[c]
	12	135·5	138·9	3·4	7·40[c]	7·34[c]
	12a	137·1	132·3	−4·8	—	—
Methoxyls		61·5	61·4	−0·1	3·67	3·63
		61·3	61·3	0·0	3·92	3·90
		56·4	—	—	4·03	—
		56·1	56·2	−0·1	3·95	3·94
$C_{(7)}$ Substituent					8·69[d]	8·12[e]
		170·1	169·8	−0·3	—	—
		22·6	22·9	0·3	1·98	1·98
NH_2		—	—	—	—	6·08

[a] $\delta_I - \delta_{colch.}$; [b] proton chemical shifts for colchicamide (*I*) from ref.[17]: 6·56 ($C_{(4)}$—H), 7·59 ($C_{(8)}$—H), 6·92 ($C_{(11)}$—H), 7·38 ($C_{(12)}$—H), 3·65, 3·93 and 3·97 (OCH_3 groups), 6·05 (NH_2); [c] $J_{11,12} = 11$ Hz; [d] $_{7,NH} = 6·4$ Hz; [e] $J_{7,NH} = 7·3$ Hz.

are consistent with 2,5-disubstitution. A further possibility of isomerism is only an other location of the nitrogen atom in ring C. Of the isomeric α-, γ- and δ-carbolines, the γ-carboline skeleton comes into consideration because the UV spectra

TABLE II

Comparison of the ^{1}H NMR parameters of perlolyrine (*II*) and isoperlolyrine (*III*) (200 MHz, $C^2HCl_3 + C^2H_3O^2H$ 4 : 1, 25°C)

II[a]	*III*[a]
4·746 s[b]	4·712 s[b]
6·526 d (4)	6·614 d (3·8)
7·216 d (4)	7·623 d (3·8)
7·300 ddd (7·4, 7·2, 1·2)	7·385 td (8, 1·2)
7·576 td (7·4, 1·2)	7·696 td (8, 1·2)
7·690 td (7·4, 1·2)	7·793 td (8, 1·2)
7·930 d (5·6)	8·104 d (6)
8·148 dd (7·2, 1·2)	8·215 d (8)
8·320 d (5·6)	8·343 d (6)

[a] Chemical shift in ppm, multiplicity, coupling constant *J* in Hz (in parentheses); [b] 2 H.

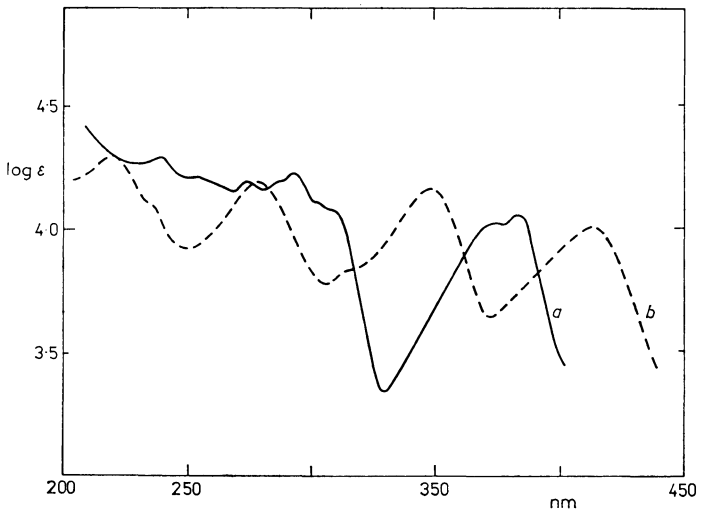
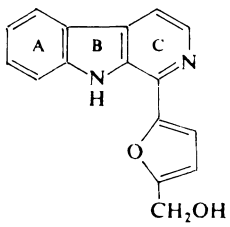
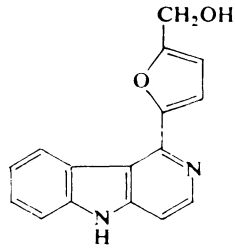


FIG. 1

Ultraviolet spectrum of the alkaloid isoperlolyrine (*III*) in ethanol (*a*) and ethanol + hydrochloric acid (*b*)



II



III

of β- and γ-carbolines are similar to each other, whereas those of the α- and δ-carbolines markedly differ[24]. Since there is an AB system (J_{AB} = 6 Hz) of two vicinal protons in the molecule, the hydroxymethyl substituted furyl group must be in the δ-position. On the basis of the spectral data, the structure of the new alkaloid can be represented by *III*. We have named this alkaloid isoperlolyrine. This is the first case of the isolation of γ-carboline from plant material.

EXPERIMENTAL

The corms and seeds of *G. superba* L. were of Indian orlgin, and were procured as commercial material.

Melting points have been determined on the Kofler block. For spectral measurements, the compounds were dried at 60°/130 Pa to a constant weight. The UV spectra were measured on a Unicam SP 700 spectrophotometer in 95% ethanol, in ethanolic sodium hydroxide (0·1 mol . $.l^{-1}$) or in ethanolic HCl (0·1 mol . l^{-1}). The IR spectra were measured on a Perkin–Elmer, Model 567 or on an Infragraf H 1 200 in KBr pellets. The mass spectra were registered on the instrument Varian MAT-311 (70 eV, direct inlet 190—220°C). The ^{1}H NMR spectra were measured on a Varian T-60, XL-200 and Tesla BS-487 (80 HMz) spectrometers. The chemical shifts are given in δ (ppm) values with respect to TMS as internal standard. ^{13}C NMR spectra (FT) were measured on Jeol FX-60 at 15·036 MHz. The optical rotations were measured on a polarimeter Polamat (Zeiss, Jena, GDR). Preparative column chromatography was carried out on Al_2O_3 (Reanal, activity II, Hungary, and Woelm neutral, activity I, Germany)[10] and TLC on silica gel G (Merck, GFR) or Silufol (Kavalier, ČSSR) in solvent systems S_1 (benzene–ethyl acetate–diethylamine–methanol 50 : 40 : 10 : 8) for tropolone compounds; S_2 (benzene–ethyl acetate–diethylamine 50 : 40 : 10) for non-tropolone compounds; S_3 (chloroform–ethanol 70 : 30) for colchicoside. Detection in UV light, and by spraying with Dragendorff and iodoplatinate reagents.

Extraction

Corms: The finely ground dry corms (19 kg) were extracted with methanol (130 l). The methanolic extract was concentrated *in vacuo*, diluted with water and slightly acidified to pH 4 with citric acid, extracted with ether, then with chloroform (neutral phenolic portions), yield 101·55 g (0·55%). The aqueous residue was made alkaline with ammonia (pH 11—12), and again extracted with chloroform (basic portion), yield 3·8 g (0·02%). The two chloroform extracts were separated

by column chromatography on Al_2O_3. The mother liquors after crystallization were separated by thin-layer preparative chromatography on silica gel.

Seeds: The seeds (6 kg) were worked up in the same manner as the corms. The yield of the neutral phenolic portion was 59·5 g (0·99%) and that of the basic portion 1·02 g (0·02%). On working up the seeds, the aqueous residue of pH 11 was extracted with chloroform, neutralized with sulfuric acid (0·5 mol . l^{-1}) to pH 7, concentrated *in vacuo* to a viscous mass, and extracted with a mixture ethanol–chloroform 1 : 4 to give colchicoside (0·59 g; 0·01%).

Identification of Alkaloids

All the known alkaloids were identified on the basis of their UV, IR, NMR, and mass spectra. From the neutral phenolic portion of the corms and seeds there were isolated or demonstrated the alkaloids colchicine, N-formyl-N-deacetylcolchicine, 3-demethylcolchicine, β- and γ-lumicolchicine, N-formyl-N-deacetyl-β- and γ-lumicolchicine, 3- demethyl-β- and γ-lumicolchicine, 3-demethyl-N-formyl-N-deacetyl-β-lumicolchicine, 2,3-demethylcolchicine, 3-demethyl-N-formyl--N-deacetylcolchicine; from the corms cornigerine (8 mg), m.p. 270°C, $[\alpha]_D^{22}$ −150° (*c* 0·8, $CHCl_3$), and from the seeds 2-demethylcolchifoline (6 mg, amorphous) and 3-demethylcolchifoline (8 mg, amorphous).

The basic portion of the corms and seeds gave the alkaloids 2,3-demethyl-N-deacetylcolchicine; *S*-(+)-floramultine, m.p. 230°C, $[\alpha]_D^{22}$ +78 (*c* 0·7, $CHCl_3$), 1,12-dihydroxy-2,10,11-trimethoxyhomoaporphine, m.p. 246°C. Only in the seeds there was present colchicamide (98 mg), m.p. 264°C, $[\alpha]_D^{22}$ −124° (*c* 0·7, $CHCl_3$); R_F 0·44 (S_1). Mass spectrum (70 eV, 180/200°C): 384 (100), 356 (19), 342 (13), 341 (15), 325 (17), 313 (16), 299 (12), 297 (15), 282 (15), 85 (44), 83 (65), 47 (20), 43 (14).

In the seeds there was also found isoperlolyrine (43 mg), m.p. 186°C (decomposition), $[\alpha]_D^{22}$ ±0° (*c* 0·6, $CHCl_3$), R_F 0·43 (S_2). Mass spectrum: M^+ 264·0897 ($C_{16}H_{12}N_2O_2$), unsaturation 12·0, fragmentation $C_{16}H_{11}N_2O$ (247·0871), $C_{16}H_{10}N_2O$ (246·0796), $C_{15}H_{11}N_2$ (219·0919), $C_{14}H_9N$ (205·0766), $C_{11}H_7N_2$ (167·0610), $C_{12}H_7$ (151·0548), and $C_{10}H_6N$ (140·0503). UV spectrum ethanol: λ_{max} 239 nm (log ε 4·29), 254 (4·22), 265 sh (4·17), 274 (4·19), 289 sh (4·20), 293 (4·23), 301 sh (4·12), 309 sh (4·08), 372 (4·03), 385 (4·06); (ethanol + HCl): 220 nm (log ε 4·30), 234 sh (4·11), 278 (4·20), 316 sh (3·84), 349 (4·17), 413 (4·01). From the seeds there was also isolated colchicoside (0·59 g), m.p. 195/218°C, $[\alpha]_D^{22}$ −360° (*c* 0·573, H_2O).

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