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1,2-DIDEMETHYLCOLCHICINE: A NEW ALKALOID FROM *GLORIOSA SUPERBA*[1]

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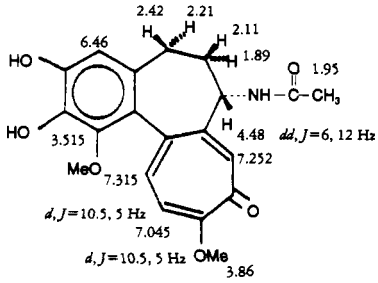
ABSTRACT.—From the seeds of *Gloriosa superba*, the new alkaloid 1,2-didemethylcolchicine [**2**], was isolated, accompanied by known naturally occurring alkaloids colchicine, 2,3-didemethylcolchicine [**1**], 3-demethylcolchicine, *N*-formyl-*N*-deacetylcolchicine, and colchicoside. The structure of 1,2-didemethylcolchicine was secured by ^{1}H nmr with the help of decoupling and nOe difference spectra, ms, and [α]D.

Gloriosa superba L. (Liliaceae) is a branched herbaceous climber common in low jungles almost throughout India up to an altitude of 6000 ft and is commonly grown in gardens as an ornamental plant (1). The Ayurvedic drug, consisting of the roots and rhizomes, is reported to be used for a variety of medicinal purposes (1). There are previous reports on chemical investigations of the tubers, corms, and flowers (2–6). Recently, Dvorackova *et al.* reported the isolation of a new carboline alkaloid from the seeds (7). In continuation of our work on the Indian medicinal plants (8) used in the Ayurvedic system of medicine, we report here the isolation of alkaloids related to colchicine from the seeds of *G. superba*.

The crude MeOH extract of the defatted seeds was subjected to extraction at different pH's (2.5–8.5). After $CHCl_3$ extraction at different pH's, the aqueous solution was extracted with *n*-BuOH resulting in the isolation of brown gummy materials. After purification by XAD-2 and Si gel chromatography, compounds **1** and **2** were obtained. Compound **2**, mp 238°, an amorphous yellow solid, gave positive Dragendorff's and Ziesel's tests, indicating a tropolonic alkaloid (9). The uv spectrum of **2**, with λ max 240 and 355 nm, was also characteristic of a tropolonic alkaloid (10). The ir spectrum showed the presence of OH, C=O, and amide bands at 3455, 3320, 1655, 1640, 1610, 1595, 1490, and 1355 cm^{-1}. The 400 MHz ^{1}H-nmr spectrum (CD_3OD) showed two aromatic singlets at δ 6.425 and 7.152, each integrating for one proton, and two doublets at δ 6.98 (J=10.5 Hz) and 7.225 (J=10.5 Hz), respectively, indicating the presence of the tropolonic ring C of the colchicum alkaloids (11). Moreover, two 3H singlets were present at δ 3.91 and 3.86 for two MeO groups. A sharp singlet at δ 1.88 was due to an amide Me group of the acetyl side chain.

In order to confirm the positions of all the signals, a series of nOe experi-



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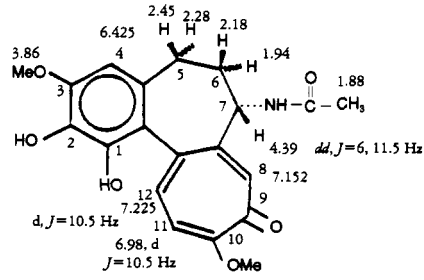

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ments were performed. Irradiation of the aromatic singlet at δ 6.425 (H-4) produced an nOe enhancement of the singlet at δ 3.86 and for the multiplet at δ 2.45 (H-5β). The signal at δ 3.86 was assigned to the MeO at C-3 in the A ring. The MeO singlet at δ 3.91 showed nOe interaction with the aromatic doublet at δ 6.98 (J=10.5 Hz, H-11) assigning the MeO to the tropolone ring, and the signal at δ 7.225 (d, J=10.5 Hz) was therefore assigned to H-12. H-8 appeared at δ 7.152 as a singlet. A doublet of doublet at δ 4.39 was assigned to H-7 based on the observed coupling constant of the H-6 protons (J=11.5 Hz and 6 Hz) (11). Further irradiation of the H-7 proton at δ 4.39 simplified the multiplet at δ 2.18 and 1.94, thus revealing the presence of two H-6 protons. The large coupling constant between H-7 and one of the H-6 protons (d, J=11.5 Hz) suggested a trans diaxial relationship, and, consequently, the acetamido group at C-7 is in the equatorial position. Compound **2** has, therefore, an absolute configuration similar to that of (−)-colchicine: this conclusion was supported by its [α]D (−148°, DMF) (11,13). Mp and [α]D of **2** are in good agreement with the reported data (10,13). Irradiation at δ 7.152 showed nOe enhancement of the double doublet centered at δ 4.39. The observed nOe, together with the large diaxial coupling of H-7 with one of the H-6 protons, indicated the boat-like configuration of the B ring (11,13). Thus, the structure of **2** as 1,2-didemethylcolchicine was established. This is the first report of its natural occurrence.

Compound **1** was established as 2,3-didemethylcolchicine by extensive nOe studies, and all of its proton assignments are indicated on structure **1**. The mp and [α]D of **1** are in good agreement with the reported data (10,13). The other alkaloids isolated were colchicine, *N*-formyl-*N*-deacetylcolchicine, 3-demethylcolchicine, and colchicoside: all were identified by direct comparisons with the authentic samples.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a Toshniwal mp apparatus and are uncorrected. Uv spectra were measured with a Pye-unican SPP-100, and the ir spectra were determined with a Perkin-Elmer 1710 Ft-ir. The ^{1}H nmr were recorded with a Bruker AM-400 spectrometer (400 MHz) in CD_3OD. Optical rotations were measured with a JASCO DIP-181 digital polarimeter. Eims was obtained on a JEOL JMS DX-303 spectrometer. Al_2O_3 and Si gel (BDH, India) was used for cc and analytical tlc. Amberlite XAD-2 polymeric resin was used for purification. The spots were visualized with Dragendorff's reagent and I_2 staining.

PLANT MATERIAL.—*G. superba* seeds were collected in 1990 from the Delhi (India) market as a commercial material, and they were of Indian origin. The seeds were identified in our botany division where the voucher specimens are kept.

EXTRACTION.—The finely ground seeds (1 kg) were defatted with *n*-hexane and extracted with MeOH (7×2 liters). The MeOH extract was concentrated in a Buchi evaporator at 30°. The concentrate was treated with 3% HCl and filtered to remove the insoluble materials. The aqueous solution was further defatted with *n*-hexane and extracted with $CHCl_3$ at pH's 2.5, 4, and 6 to yield the neutral portion (11.25 g). The aqueous solution was made alkaline with 25% aqueous NH_3 to pH 8.5 and extracted wth *n*-BuOH to yield the basic alkaloids (5 g).

ISOLATION OF COMPOUNDS **1** AND **2**.—The basic part (5 g) was purified by passing through Amberlite XAD-2 (20 g) using H_2O and H_2O-MeOH (4:1, 7:3, and 2:3). The residues from the MeOH-H_2O (4:1) were further purified over Al_2O_3, using $CHCl_3$/MeOH mixtures of increasing MeOH content as eluents. The earlier fractions from a 10% MeOH/$CHCl_3$ eluent afforded **1**, and the later fractions yielded a mixture of **1** and **2**. The mixture of **1** and **2** was further purified by preparative tlc on Si gel [$CHCl_3$-MeOH (4:1)].

2,3-Didemethylcolchicine [**1**].—Yield 0.01 g; mp 205°; amorphous; pale yellow color; R_f 0.45 [Si gel, $CHCl_3$-MeOH (17:3)]; [α]D −230° (c=0.23, MeOH); uv (MeOH) λ max 243, 355 nm; ir (KBr) ν max 3520, 3330, 1660, 1643, 1620, 1610, 1485, 1410, 1350, 1070, 620; ^{1}H nmr (400 MHz, CD_3OD) see **1**. Principal nOe's were H-4 to H-5β, H-11 to 10-OMe; H-12 to 1-OMe; H-8 to H-6α; decoupling at δ 4.39 simplified the signals at δ 2.11 and 1.81. Eims *m/z* (% rel. int.) $[M]^+$ 371 (100), 343 (11.5), 328 (13), 312 (14), 284 (10.5), 191 (4.3), 157 (2.1), 105 (5.21). Identical by mp and [α]D with published data (10,13).

1,2-Didemethylcolchicine [**2**].—Yield 8.008 g; R_f 0.42 [Si gel, $CHCl_3$-MeOH (17:3)]; $[\alpha]_D$ −148° (*c*=0.167, DMF); uv (MeOH) λ max 240, 355 (log ε 4.23, 3.01); ir (KBr) ν max 3455, 3320, 1655, 1640, 1610, 1595, 1490, 1355; ^{1}H nmr (400 MHz, CD_3OD) see **2**; eims *m/z* (% rel. int.) $[M]^+$ 371 (100), 356 (2.3), 328 (3.1), 313 (5.4). Identical by $[\alpha]_D$ and uv with published data (10,12).

Colchicoside (3-demethylcolchicine-3-O-β-D-glucoside).—The aqueous residue after the extraction of the basic portion was neutralized with 4 N H_2SO_4 and evaporated at 40° to a syrup. The syrup was extracted with $CHCl_3$-MeOH (4:1), and the extract was washed with H_2O. The aqueous extract was cleared with charcoal and evaporated to dryness to afford colchicoside (0.15 g) as an amorphous powder. It was identical in all respects (ir, $[\alpha]_D$, ^{1}H nmr, tlc and hplc) to an authentic sample by direct comparison.

Colchicine and N-formyl-N-deacetylcolchicine.—On cc over neutral Al_2O_3, the neutral portion gave colchicine (9.9 g) and *N*-formyl-*N*-deacetylcolchicine (0.1 g) both of which were identified by comparison to the authentic samples.

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