NEW NITROGEN-CONTAINING COMPOUNDS IN *Lilium candidum* L.

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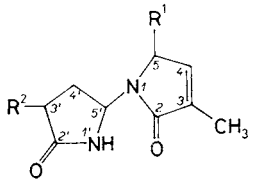
1-(3'-Methyl-2'-oxo-5'-pyrrolidinyl)-3-methyl-3-pyrrolin-2-one, 1-(2'-oxo-5'-pyrrolidinyl)-3-methyl-3-pyrrolin-2-one and 1-(3'-methyl-2'-oxo-5'-pyrrolidinyl)-5-hydroxy-3-methyl-3-pyrrolin-2-one were isolated from petals of *Lilium candidum* L. (*Liliaceae*). Presence of these substances, obtained by column chromatography of the extract on silica gel, has not been reported in any plant material as yet; their structures were adduced from spectral data.

Our preceding papers[1,2] concerned the isolation and structures of two nitrogen-containing compounds in petals of *Lilium candidum* L.: jatropham $C_5H_7NO_2$, separated for the first time from *Jatropha macrorrhiza* (*Euphorbiaceae*) by Wiedhopf and coworkers[3], and a new flavone alkaloid lilaline[4] $C_{20}H_{17}NO_2$. This paper presents the isolation and structure elucidation of further three new compounds from this plant.



I, $R^1 = H$; $R^2 = CH_3$
II, $R^1 = R^2 = H$
III, $R^1 = OH$; $R^2 = CH_3$

The mass spectrum of compound *I* showed the formula $C_{10}H_{14}N_2O_2$; its IR spectrum revealed absorptions due to a double bond in conjugation with a carbonyl group and another carbonyl group. The structure of compound *I* was unambiguously determined by X-ray analysis[5] as 1-(3'-methyl-2'-oxo-5'-pyrrolidinyl)-3-methyl-3-pyrrolin-2-one. A complex analysis of the ^1H NMR spectrum of *I*, in which signals

belonging to pyrrolidine and pyrroline moieties could be separated, served for structure elucidation of compounds *II* and *III*. Worthfull were especially signals of methyl groups C(3)—CH_3 and C(3′)—CH_3 at δ 1·78 and 1·14, respectively.

The peak of high resolution mass spectral measurement of molecular radical ion of compound *II* appearing at *m/z* 180·0912 corresponded to formula $C_9H_{12}N_2O_2$. The 14 mass units difference between composition of compounds *II* and *I* indicated that *II* can be the demethyl compound *I*. Absorption bands in the IR spectrum of *II* disclosed the presence of a conjugated carbonyl group, a double bond and a carbonyl group similarly as with *I*. The ^{1}H NMR spectrum of the pyrroline moiety of *II* also coincided with that of *I* the difference being in the pyrrolidine moiety lacking the signal of C(3′)—CH_3 at δ 1·14. Accordingly, structure 1-(2′-oxo-5′-pyrrolidinyl)-3-methyl-3-pyrrolin-2-one was assigned to compound *II*.

Compound *III*, $C_{10}H_{14}N_2O_3$ showed in its IR spectrum absorption bands associated with vibrations of the respective conjugated carbonyl, C=C double bond, and hydroxyl group. The ^{1}H NMR spectrum revealed signals of two methyl groups analogously as with compound *I*, but a little upfield shifted; signals of protons of the pyrrolidine moiety were found at the same positions. Signals in the pyrroline moiety appeared at δ 5·54 (H-5) and δ 4·83 (OH, J(H-5, OH) = 10·1 Hz) and therefore, the hydroxyl group can be at C-5. Consequently, structure 1-(3′-methyl-2′-oxo-5′-pyrrolidinyl)-5-hydroxy-3-methyl-3-pyrrolin-2-one can be ascribed to compound *III*. As seen, the hydroxyl group is located in the same position as with jatropham[2,3] (5-hydroxy-3-methyl-3-pyrrolin-2-one). All nitrogen-containing compounds hitherto isolated from *Lilium candidum* L. contain variously substituted 5-membered lactam ring and it is, therefore probable that this drug also includes further derivatives of this type.

EXPERIMENTAL

Melting points were determined on a Kofler micro hot-stage, optical rotations of methanolic solutions were measured with the respective Polamat A (Zeiss, Jena), UV spectra of methanolic solutions with a UV-VIS (Zeiss, Jena), IR spectra of KBr pellets with a Perkin–Elmer, model 477, mass spectra with an AEI MS 902, and the ^{1}H NMR spectra of acetone solutions containing tetramethylsilane as an internal reference with AM 300 (Bruker) apparatuses. Silica gel No. 4 (Silpearl), modified according to Pitra et al.[6], and silica gel G (according to Stahl, Merck) and Silufol UF_{254} and UF_{366} sheets were employed for column, and thin-layer chromatographies, respectively.

Extraction and Isolation of Nitrogen-Containing Compounds

Dried flowers (3 500 g) were repeatedly macerated with 95% and 70% ethanols at room temperature. The combined macerate was evaporated under diminished pressure, the residue (1 370 g) was dissolved in 5% hydrochloric acid and the solution was stepwise extracted with light petroleum, ether, and chloroform. The aqueous phase was made alkaline to pH = 11 and extracted

with chloroform and chloroform–ethanol (2 : 1). Compounds present in the chloroform–ethanolic extract (8·6 g) were separated by column chromatography on silica gel (500 g) using benzene–acetone (8·5 : 1·5, 1 : 1), acetone, and methanol as eluents. The 150 ml-fractions were checked by thin-layer chromatography in the solvent systems benzene–acetone (8·5 : 1·5, 8 : 2) and chloroform–methanol (9 : 1, 8 : 2, 7 : 3). The spots were detected with sulfuric acid in ether and UV 254 and 366 nm light. Fractions with compounds of the same R_F values were combined, totally 128 fractions were collected.

Fraction 33 (benzene–acetone 1 : 1) afforded compound *I* (12 mg), $C_{10}H_{14}N_2O_2$, m.p. 172–174°C, $[\alpha]_{546}^{20}$ +248° (*c* 0·25, methanol). UV spectrum (methanol): λ_{max} 241 nm (sh). IR spectrum (cm^{-1}): 1 710 (CO), 1 670 (conj. CO), 1 640 (C=C). Mass spectrum (*m/z*): M^+ 194·1012, for $C_{10}H_{14}N_2O_2$ calculated 194·1055, 166, 151, 136, 125, 124, 98 (C_5H_8NO, b.p.), 82, 70, 69. ^{1}H NMR spectrum: 7·12 (br. s, 1 H, *J*(1′, 5′) = 1·3 Hz, *J*(1′, 4a′) = 0·7 Hz, *J*(1′, 3′) = = 0·3 Hz, H-1′); 6·85 (ddqd, 1 H, *J*(4, 5a) = 2·0 Hz, *J*(4, 5b) = 2·0 Hz, *J*(4, C(3)—CH_3) = = 1·8 Hz, *J*(4, 5′) = 0·6 Hz, H-4); 5·70 (dddddd, 1 H, *J*(5′, 4b′) = 8·1 Hz, *J*(5′, 4a′) = 1·9 Hz, *J*(5′, 5a) = 0·5 Hz, *J*(5′, 5b) = 0·5 Hz, H-5′); 3·96, 3·90 (m, 2 H, *J*(5a, 5b) = 19·3 Hz, *J*(5a, C(3)—CH_3) = 2·0 Hz, *J*(5b, C(3)—CH_3) = 2·0 Hz, H-5a, H-5b); 2·63 (ddqd, 1 H, *J*(3′, 4b′) = = 9·5 Hz, *J*(3′, 4a′) = 8·7 Hz, *J*(3′, C(3′)—CH_3) = 7·1 Hz, H-3′); 2·32 (dddd, 1 H, *J*(4a′, 4b′) = = 13·7 Hz, H-4a′); 2·13 (ddd, 1 H, H-4b′); 1·78 (ddd, 3 H, C(3)—CH_3); 1·14 (d, 3 H, C(3′)—CH_3).

Rechromatography of fractions 46–66 (3·4 g, obtained with acetone on silica gel No. 4 (350 g)) with chloroform–methanol (9 : 1, 8 : 2, 1 : 1) and methanol yielded 80 fractions. Fraction 3 (chloroform–methanol 9 : 1) furnished compound *II* ($C_9H_{12}N_2O_2$ (15 mg), m.p. 167–169°C. UV spectrum (methanol): λ_{max} 241 nm (sh). IR spectrum (cm^{-1}): 1 705 (CO), 1 675 (conj. CO), 1 640 (C=C). Mass spectrum (*m/z*): M^+ 180·0912, for $C_9H_{12}N_2O_2$ calculated 180·0898, 152 ($C_8H_{12}N_2O$), 98, 97 (C_5H_7NO), 84. ^{1}H NMR spectrum 7·07 (br. s, 1 H, *J*(1′, 5′) = 1·3 Hz, H-1′); 6·86 (ddqd,1 H, *J*(4, 5a) = 2·0 Hz, *J*(4, 5b) = 2·0 Hz, *J*(4, C(3)—CH_3) = 1·8 Hz, *J*(4, 5′) = 0·6 Hz, H-4); 5·79 (m, 1 H, *J*(5′, 4b′) = 7·8 Hz*, *J*(5′, 4a′) = 3·3 Hz*, *J*(5, 5a′) = = 0·5 Hz, *J*(5′, 5b′) = 0·5 Hz, H-5′); 3·96 and 3·90 (m, 2 H, *J*(5a, 5b) = 19·3 Hz, *J*(5a, C(3)—CH_3) = 2·0 Hz, *J*(5b, C(3)—CH_3) = 2·0 Hz, H-5a, H-5b), 2·02–2·56 (4 H, H-3a′, H-3b′ H-4a′, H-4b′), 1·78 (ddd, 3 H, C(3)—CH_3).

Fraction 6 (chloroform–methanol 9 : 1) gave compound *III* $C_{10}H_{14}N_2O_3$, m.p. 169–171°C, $[\alpha]_{546}^{20}$ +206° (*c* 0·15 methanol). UV spectrum (methanol): λ_{max} 246 nm (sh). IR spectrum (cm^{-1}): 1 700 (CO), 1 680 (conj. CO), 1 660 (C=C). Mass spectrum (*m/z*): M^+ 210·0996, for $C_{10}H_{14}N_2O_3$ calculated 210·1004, 167, 141, 113, 98, 97, 96. ^{1}H NMR spectrum: 6·86 (br. s., 1 H, *J*(1′, 5′) = 1·4 Hz, *J*(1′, 4a′) = 0·8 Hz, H-1′); 6·20 (qd, 1 H, *J*(4, C(3)—CH_3) = 1·8 Hz, *J*(4, 5) = 1·8 Hz, H-4); 5·54 (ddq, 1 H, *J*(5, OH) = 10·1 Hz, *J*(5, C(3)—CH_3) = 1·3 Hz, H-5); 5·40 (ddd, 1 H, *J*(5′, 4b′) = 8·5 Hz, *J*(5′, 4a′) = 1·4 Hz, H-5′; 4·83 (d, 1 H, OH-5); 2·85 (ddq, 1 H, *J*(3′, 4b′) = 9·5 Hz, *J*(3′, 4a′) = 8·7 Hz, *J*(4′, C(3′)—CH_3) = 7·2 Hz, H-3′); 2·54 (dddd, 1 H, *J*(4a′, 4b′) = 13·7 Hz, H-4a′); 2·10 (ddd, 1 H, H-4b′), 1·77 (dd, 3 H, C(3)—CH_3); 1·12 (d, 3 H, C(3′)—CH_3).

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* Coupling constants referred to H-5′ in the first order approximation.

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