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A Novel Alkaloid from *Stapelia hirsuta*

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A novel alkaloid (1,8,8-trimethyl-5,8-dihydro-1H-pyrano[3,4-b]pyridine-4,6-dione) was isolated from the chloroformic traction obtained from the total alcoholic extract of the aerial parts of *Stapelia hirsuta* L. In addition, apigenin, luteolin and β-sitosterol-3-O-β-D-glucopyranoside were also isolated.

Introduction

The genus *Stapelia* belongs to the family Asclepiadaceae, which comprises a group of some 50 species, which have been used in medicine for their anti-inflammatory and antiarithritic properties[1]. The presence of alkaloids in the genus *Stapelia* was first reported by Keller[2], where hordenine was isolated from the aerial part of *S. gigantea*. In addition, Meyer et al.[3] reported the detection of *N*-methyl tyramine, choline and candicine in the aerial parts of *S. gigantea*. Several pigments have been identified in the flowers of different *Stapelia* species[4]. Cyanidin-3-monoglucoside, cyanidin-3-rutinoside and cyanidin-3-sophoroside were identified in *S. vareigata, S. comparabilis* and *S. ambigua*, while cyanidin-3-rustinoside was the only pigment detected in *S. nobilis*.

In a continuation of our interest in members of the family Asclepiadaceae [5−9], we investigated the chloroformic fraction of the aerial parts *S. hirsuta* and we report our findings herein.

In this paper, we describe the isolation and identification of a new pyrano pyridine-dione alkaloid, in addition to the isolation of apigenin, luteolin and β-sitosterol-3-*O*-glucoside from the chloroformic fraction of the aerial parts of the plant.

Experimental

General. Melting points were determined on an electrothermal 9100 apparatus (U.K.). UV spectra were measured using Beckman Du-7 and Shimadzu-265 spectrophotometers. IR spectra were measured on a

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Shimadzu-IR-435 infrared spectrophotometer (Japan). NMR spectra were measured with a JEOL JNA-LA 400 WB-FT (^{1}H-NMR, 395.75 MHZ, ^{13}C, 100 MHZ (Japan), using TMS as an internal standard, and chemical shifts were given in δ values.

Plant Material. The aerial parts of *S. hirsuta* L. were collected in 1999 from plants growing in the Orman garden, Giza governorate, Egypt. The plant material was identified by Dr. Nabil El-Hadidi, Professor of Plant Taxonomy, Faculty of Science, Cairo University, Egypt. A voucher specimen was deposited in the herbarium of the College of Pharmacy, Cairo University.

Extraction and Isolation. The air-dried powder of S. hirsuta (750 g) was extracted with 70% ethyl alcohol by percolation. The alcohol extract was concentrated under reduced pressure to give 15 g of dark green residue. This residue was suspended in water (300 mL) and partitioned successively with n-hexane (5 x 150 mL), chloroform (5 x 150 mL) and finally with n-butanol (5 x 150 mL) to yield after evaporation dry residues of 5.3, 4.7 and 4.5 g, respectively.

The chloroform-soluble fraction (4 g) was fractionated on a Si gel column (8 x12 cm). Elution was carried out using $CHCl_3$-MeOH mixtures of increasing polarity. Fractions of 50 m**L** each were collected and monitored by TLC on Si gel F_{254} plates using Benz-EtOAc (8:2) and $CHCl_3$-MeOH (8.2:1.8) as solvent systems and the plates were inspected in UV light and/or after spraying with *p*-anisadehyde reagent followed by heating. Similar fractions were pooled together to give 3 main fractions (**A**, **B** and **C**). Rechromatography of fr **A** on a Si gel column using $CHCl_3$-MeOH (9.5:0.5) as eluting solvent afforded compound **1** (23 mg). Fraction **B** was subjected to rechromatography on a Si gel column eluted with $CHCl_3$-MeOH (9:1) followed by Sephadex LH-20 (eluted with MeOH) to afford compounds **2** (8 mg) and **3** (14 mg) as faint yellow powder. Compound **4** (40 mg) was isolated as an off-white powder from fr **C** after rechromatography on a column of Si gel using $CHCl_3$-MeOH (9:1) as eluting solvent.

1, 8, 8-trimethyl-5,8-dihydro-1H-pyrano[3,4-b] pyridine-4,6-dione (**1**; $C_{11}H_{13}$ NO_3)

Needle crystals, m.p. 178-180 ^{o}C; IR (KBr): υ_{max}= 3446, 1749, 1671, 1594, 1546, 1151, 1083 cm^{-1}; UV (MeOH): λ_{max}= 322 and 234 nm;^{1}H-NMR (400 MHz, $CDCl_3$): $\delta_=$ 7.72 (1H, *d*, *J*= 7 Hz, H-2), 6.29 (1H, *d*, *J*= 7 Hz, H-3), 3.63 (3H, *s*, H-12), 1.60 (6H, *s*, H-11$_{a,b}$), 1.20 (2H, *s*, H-5). **^{13}C NMR** (100 MHz, $CDCl_3$): $\delta_=$ 171.8 (C-4), 167.1 (C-6), 157.8 (C-9), 145.6 (C-2), 111.1 (C-10), 98.3 (C-3), 82.5 (C-8), 37.6 (C-12), 29.6 (C-5), 2 x 25.8 (C-11$_{a,b}$); EI-MS: *m/z* (%) = 207 [M$^+$] (0.35), 167 (1.24), 149 (4.8),129 (17), 111 (37), 99 (35), 86 (100), 83 (33.8), 82 (11.9), 81 (13.7), 71 (11.7), 57 (68.6), 55 (14).

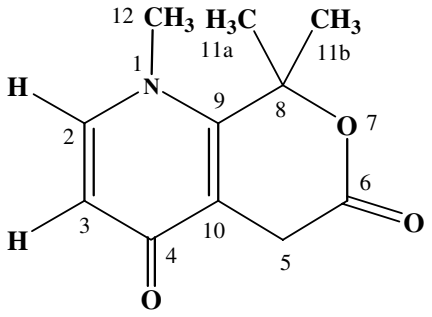
Results and Discussion

Compound **1** was isolated as white crystalline needles (23 mg), m.p. 178-180 °C and had the molecular formula $C_{11}H_{13}$ NO_3 as deduced from the EI/MS and ^{13}C-NMR (DEPT). Compound **1** gave a purple fluorescence in UV light (366 nm), which was intensified on exposure to ammonia vapors. It gave a positive reaction with Dragendorff's and ninhydrine reagents, indicating its possible alkaloidal or nitrogenous nature. It showed 2 maxima in the UV spectrum at $\lambda_{\max}$ (MeOH) 322 and 234 nm.

^{13}C NMR and DEPT spectra of compound **1** showed the presence of 10 signals corresponding to 11 carbons; 2 of them are aromatic methines, 1 aliphatic methylene, 3 methyl groups and 5 quaternary carbons. The ^{1}H-NMR revealed the presence of 2 ortho aromatic protons as deduced from the presence of 2 doublet signals at δ_H7.72 (1H, *d*, *J*= 7 Hz, H-2) and 6.29 (1H, *d*, *J* = 7 Hz, H-3), which were directly correlated

to carbon signals at δ_c145.6 and 98.3 (C-2 and C-3), respectively. The singlet at δ_H3.63 (3H, *s*, N-Me) was assigned to the N-methyl group positioned at N-1 ortho to the proton at δ_H7.72 (H-2). This was confirmed by the long-range correlation observed between H-12 (δ_H 3.63) and both of C-2 (δ_c145.6) and C-9 (δ_c 157.8) in the HMBC spectrum (Figure). The presence and the position of a carbonyl group at C-4 (δ_C 171.8) were verified from the upfield shift of C-3 (δ_C 98.3) and further confirmed by the long-range correlation between C-4 and H-2 (δ_H7.72). The appearance of a proton singlet signal at δ_H1.20 (2H, *s*, H-5) revealed the presence of a methylene group attached to C-10 of a pyridine-4-one ring, which is directly correlated to the carbon signal at δ_c29.8 (HMQC). The position of H-5 was confirmed by the long correlation observed between H-5 (δ_H 1.20.) and C-4 (δ_C 171.8) in the HMBC spectrum (Figure). The singlet at δ_H1.60 (6H, *s*, H-11$_{a,b}$) was assigned for 2 methyls positioned at C-11. The presence of a quaternary carbon (C-8) attached to the pyridine-4-one ring at C-9 and to an oxygen atom of the COO group and bearing 2 methyl groups (C-11$_{a,b}$) was deduced from its appearance at a relatively downfield value (δ_c82.5). The carbon signals at δ_c157.8 and 111.1 were assigned to C-9 and C-10, respectively.

From the above-mentioned data and from the physico-chemical properties, and by comparison with the available literature[10,11], the structure of compound **1** was elucidated as depicted in the Figure.



1,8,8-Trimethyl-5,8-dihydro-1H-pyrano[3,4-b]pyridine-4,6-dione

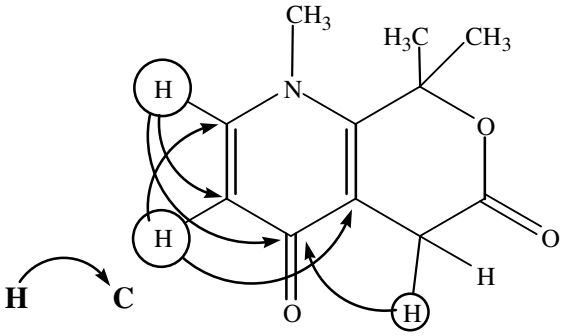


Figure. The significant long range correlation detected in the HMBC spectrum of compound **1**.

Compounds **2** and **3** were identified as apigenin and luteolin, respectively, using m.p., TLC comparison to authentic samples, UV analysis[12] and ^{1}H-NMR. In addition, compound **4** was identified as β-sitosterol 3-*O*-glucoside using m.p. and TLC comparison to an authentic sample and NMR analysis (^{1}H and ^{13}C-NMR)[13,14].

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References

1. B.M. Reddy, V.V. Byahatti, A.N.V.A. Rao, M. Ramesh and R.A.N. Appa, **Fitoterapia 67**, 545-547 (1996).
2. W.J. Keller, **Lloydia 44**, 366-367 (1981).
3. B.N. Meyer, J.L. McLaughlin and W.J. Keller, **Planta Medica 43**, 304-306 (1981).
4. L.E. Knaak, R.P. Collins and N.C. Chang, **Lloydia 32**, 148-152 (1969).
5. A. Gohar, M.M. El-Olemy, E. Abdel-Sattar, M. El-Said and M. Niwa, **J. Nat. Prod. Sci. 63**, 142-146 (2000).
6. M. A. Al-Yahya, E. Abdel-Sattar and E. Guittet, **J. Nat. Prod. 63**, 1451-1453 (2000).
7. E. Abdel-Sattar, M.A. Al-Yahya, N. Nakamura and M. Hatori, **Phytochemistry 57**, 1213-1217 (2001).
8. E. Abdel-Sattar, M.R. Meselhy and M.A-A. Al-Yahya, **Planta medica 68**, 430-434 (2002).
9. S.M. El-Zalabani, E. Abdel-Sattar, F.I. Fathy and N.G. Shehab, Egypt. **J. Biomed. Sci. 11**, 233 (2003).
10. M. Shamma and D.M. Hindenlang, "**Carbon-13 NMR Shift Assignments of Amines and Alkaloids**", Plenum Press, New York, p 111 (1979).
11. A.U. Rahman and V.U. Ahmed, "^{13}**C-NMR of Natural Products**", Plenum Press, New York and London (1992).
12. T.J. Mabry, K.R. Markham and M.B. Thomas, "**The Systematic Identification of Flavonoids**", vol. 1, 2, Springer Verlag, New York (1970).
13. J.L. Good and T. Akisha, "**Analysis of Sterols**", Blackie Academic & Professional Press, Chapman & Hall (1997).
14. M.A.C. Leitão, M.A. Kaplon, F.D. Monache, T. Akihisa and T. Tamura, **Phytochemistry 31,** 2813-2817 (1992).