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Piperazirum, a novel bioactive alkaloid from *Arum palaestinum* Boiss.

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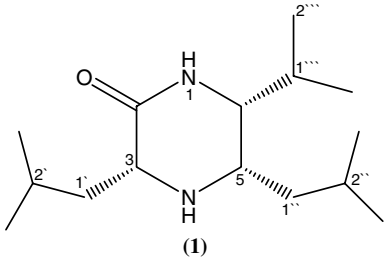
Abstract—A novel alkylated piperazine (3α,5α-diisobutyryl-6α-isopropyl-piperazine-2-one, **1**) has been isolated from the *n*-butanol soluble part of the leaves extract of *Arum palaestinum* Boiss., the chemical structure and the relative stereochemistry of **1** were elucidated on the basis of 1D and 2D NMR spectroscopic data. Piperazirum showed a significant cytotoxicity against cultured tumor cell lines in vitro.

Arum is a genus of about 26 species of flowering plants in the family Araceae, native to Europe, Northern Africa and Western Asia, with the highest species diversity in the Mediterranean region.

Little is known about the phytochemical studies on this plant species, one of which showed the occurrence of flavonoid C-glycosides.[1]

Piperazine and substituted piperazines are important pharmacophores that can be found in many marketed drugs, such as the Merck HIV protease inhibitor, Crixivan and drugs under developments.[2]

The present Letter describes the isolation and the structure elucidation of a new sesquiterpenic alkaloid, piperazirum (**1**), from *n*-butanol fraction of *Arum palaestinum*.[3]



(1)

The water extract of the air-dried leaves of *A. palaestinum* was suspended in H_2O and partitioned successively with ethyl acetate and *n*-butanol. The *n*-butanol layer was subjected to repeated silica gel column chromatography to afford **1**.[4]

Piperazirum (**1**) was isolated as an amorphous white powder, $[\alpha]_D$ +50.0 (*c* 0.024, MeOH), and gave a positive reaction to Dragendorff reagent, its ESIMS displayed the molecular ion peak at *m*/*z* 253 $[M-1]^+$. Its molecular formula $C_{15}H_{30}N_2O$ was established by HRESIMS (*m*/*z* 253.2289 $[M-H]^+$; calcd for $C_{15}H_{29}N_2O$, 253.2280).

The IR spectrum of **1** contained characteristic absorption bands at λ_{max} 3250–3170 (broad NH-stretching), 1680 (C=O) and at 1578 (NH-bending).

In the ^1H NMR spectrum (DMSO-d_6) of **1**, most of the proton signals appeared in the extremely upfield chemical shifts region (0.8–2.1 ppm), except for three proton signals which appeared in the range 3.4–3.6 ppm and two broad signals at δ 8.45 and δ 9.75 exchangeable with D_2O.

These two broad signals were determined as two NH groups since they had no HSQC correlations. In addition, the ^{13}C NMR spectrum of **1** revealed the presence of 15 carbon resonances. All the above spectral

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evidences together with chemical shifts and coupling constants suggested **1** to be a sesquiterpenic alkaloid.

The structure and the relative stereochemistry of such a novel alkaloid were deduced from the careful analysis of ^{1}H and ^{13}C NMR spectra, including a DEPT measurement.

The assignment of the spectra was accomplished through the carbon–proton (HSQC, HMBC) chemical shifts correlation techniques carried out at 600 MHz, whereas the relative configuration of the molecule was deduced from the NOESY spectrum.

The detailed analysis of ^{1}H and ^{13}C NMR spectra of **1** (Table 1) indicated the presence of six methyl groups, six methine groups, two methylene groups and one quaternary carbonyl group (imide or amide).

All protons were assigned unambiguously from the HSQC experiment in which all protons were correlated with those of corresponding carbons.

The presence of the carbonyl group at position 2 was deduced from the long range correlations in the HMBC spectrum of **1** (Fig. 1) where it showed correlations with H-3 (δ 3.59), H-1′a (δ 1.54), and H-6 (δ 3.47).

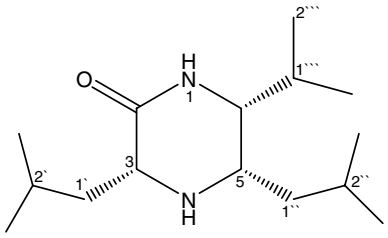
The fragment ion peaks at *m/z* 43 $[C_3H_7]^+$ and at *m/z* 57 $[C_4H_9]^+$ observed in the EIMS spectrum of **1** supported the assumption of the presence of isopropyl and isobutyryl side chains, respectively. Furthermore, the fragment peaks at *m/z* 85 $[C_5H_{11}N]^+$ and at *m/z* 72 $[C_4H_{10}N]^+$ confirmed that each of these residues was attached to a nitrogen atom through one methine group.

This was further confirmed by the presence of three methine carbon signals bearing nitrogen atoms at δ_C 53.6, 59.7 and 60.6 for C-3, C-5 and C-6, respectively, in the ^{13}C NMR spectrum of **1**.

The positions of attachment of the 5-isobutyryl and 6-isopropyl residues to the piperazine ring were inferred

Table 1. NMR spectral data for **1** in D_2O

	^{13}C	DEPT	^{1}H
2	175.7	C	
3	53.6	CH	3.59 (dd, $J = 3.6/4.8$ Hz)
5	59.7	CH	3.53 (m)
6	60.6	CH	3.47 (dd, $J = 4.2/5.9$ Hz)
1′	40.0	CH_2	1.55 (m, H-1′a)
			1.61 (m, H-1′b)
2′	24.4	CH	1.56 (m)
3′	21.2	CH_3	0.87 (d, $J = 6.0$ Hz)
4′	22.2	CH_3	0.91 (d, $J = 7.2$ Hz)
1″	24.6	CH_2	1.13 (m, H1″a)
			1.33 (m, H1″b)
2″	36.5	CH	1.85 (m)
3″	11.2	CH_3	0.80 (d, $J = 7.2$ Hz)
4″	14.9	CH_3	0.82 (d, $J = 6.0$ Hz)
1‴	29.2	CH	2.14 (m)
2‴	16.3	CH_3	0.84 (d, $J = 6.0$ Hz)
3‴	18.1	CH_3	0.85 (d, $J = 7.2$ Hz)



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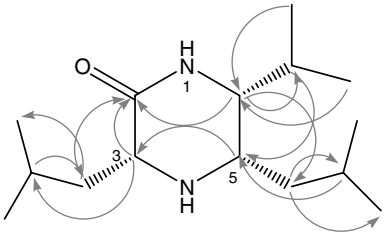

Figure 1. Most significant HMBC correlations for **1**.

through the HMBC correlations of **1**, where the correlations between H-6 (δ 3.47)/C-1″(δ 24.6) and H-1‴(δ 2.14)/C-5 (δ 59.7) supported the 5,6-positions of both the alkyl residues.

Similarly, the location of the second isobutyryl residue was confirmed to be at C-3 from the HMBC correlation of H-1′a (δ 1.55) to C-2 (δ 175.7).

The relative stereochemistry of **1** was determined by NOESY experiments. The important NOE cross-peaks observed between H-3 and H-5 and between H-5 and H-6 demonstrated that H-3, H-5 and H-6 exist on the same β-face of the piperazine ring and therefore the substituents at C-3, C-5 and C-6 have α-orientations. Further correlations between H-1″a and H_3-2‴ and between H_3-2‴ and H_3-3″ support the relative configuration of **1** (Fig. 2).

Piperazirum (**1**) was found to exhibit a significant inhibition for all examined cultured human cell lines using the SRB method,[5] such as A549 (non-small cell lung, $ED_{50} = 4.26 \pm 0.2$ μM), SK-OV-3 (ovary, $ED_{50} = 1.38 \pm 0.1$ μM), SK-MEL-2 (melanoma, $ED_{50} = 0.51 \pm 0.1$ μM) and HCT-15 (colon, $ED_{50} = 2.47 \pm 0.3$ μM). Detailed experimental procedures were described in a previous paper.[6]

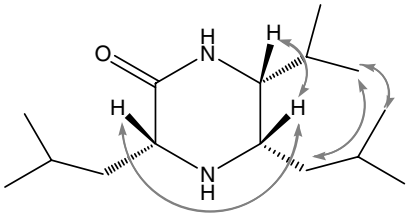

Figure 2. Selected NOESY correlations and relative stereochemistry for **1**.

Acknowledgements

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References and notes

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3. *Arum palaestinum* leaves were collected from Wadi Mujeb, Jordan, in March 2006 and were identified by Professor S. A. Kawashty, Department of Phytochemistry and Plant Systematic, National Research Center, Egypt. A voucher specimen (YKP04-624) was deposited at the herbarium of the National Research Center, Cairo, Egypt.
4. The *n*-butanol fraction of *A. palaestinum* (5 g) was fractionated using vacuum liquid chromatography with the gradient system CH_2Cl_2/MeOH, in which a fraction eluted with CH_2Cl_2/MeOH (1:1) was purified using silica gel column chromatography ($CH_2Cl_2/CH_3OH/H_2O$, 4.5:1:0.5) to afford an amorphous white powder of **1** (80 mg).
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