SHORT COMMUNICATION

Chemical constituents of *Fumaria densiflora* and the effects of some isolated spirobenzylisoquinoline alkaloids on murine isolated ileum and perfused heart

Adel M. Al-Ghazzawi\(^a\), Musa H. Abu Zarga\(^a\) and Shtaywy S. Abdalla\(^b\)

\(^a\)Department of Chemistry, The University of Jordan, Amman, Jordan; \(^b\)Department of Biological Sciences, The University of Jordan, Amman, Jordan

**ABSTRACT**

Twenty-two alkaloids, were isolated from *Fumaria densiflora*. Two of these alkaloids, N-methyl-5-hydroxystylopine chloride and fumari-cine N-oxide, were isolated for the first time from natural sources. Parfumine and fumaritine, in concentrations ranging from \(3 \times 10^{-7}\) to \(9 \times 10^{-4}\) M, caused concentration-dependent relaxation of ileum longitudinal segment. Also, parfumine and fumaritine in concentrations ranging from \(3 \times 10^{-4}\) to \(9 \times 10^{-2}\) M, caused concentration-dependent decrease in heart rate of the isolated perfused heart. A concentration of parfumine of \(3 \times 10^{-2}\) M increased but a higher concentration (\(9 \times 10^{-2}\) M) decreased the amplitude of contraction of the isolated perfused heart. On the other hand, fumaritine, in concentrations ranging from \(3 \times 10^{-4}\) to \(3 \times 10^{-2}\) M, caused concentration-dependent increase, but a higher concentration (\(9 \times 10^{-2}\) M) caused a decrease in the amplitude of contraction of the isolated perfused heart.

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CONTACT Adel M. AL-Ghazzawi \(\text{algawazy@kku.edu.sa}\); Shtaywy S. Abdalla \(\text{shtaywy@ju.edu.jo}\)

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1. Introduction

*Fumaria densiflora* DC. (Papaveraceae) is one of 55 species of the genus *Fumaria* which are distributed in the Mediterranean region, Central Europe and South Africa. *Fumaria* species are usually diffuse and branching annual herbs, and occasionally climbing. Leaves are compound and dissected into narrow, oblong–filiform segments (Ghakravarty 1976). Plants of the genus *Fumaria* have been used in traditional medicine as anti-hypertensive, diuretic, hepatoprotectant and laxative, as well as in the treatment of some skin diseases (rashes and conjunctivitis) (Gulshan et al. 2012; Altundag and Ozturk 2011; Orhan et al. 2012). *F. densiflora* has been found to have significant antiplasmodial and antitrypanosomal activity (Orhan et al. 2015) and to possess acetylcholinestase and butyrylcholinesterase inhibitory activity (Adewusi et al. 2010). *F. densiflora* is one of five species of *Fumaria* in Jordan (AL-Eisawi 1998). Seventeen benzylisoquinoline alkaloids have been isolated from *F. densiflora* of Jordanian origin (Aboudi et al. 1986; Abu Zarga et al. 1987; Taborska et al. 1997). Also, many isoquinoline alkaloids such as protopine, cryptopine, coptisine, palmatine, adlumidine, (±)-sinactine, fumafalorine, and densiflorine, were found in *F. densiflora* as well as phenolic acids with antiprotozoal activity (Orhan et al. 2015). No work has been done on the effect of spirobenzylisoquinolines alkaloids on isolated ileum and perfused heart. This work investigates the alkaloid content of *F. densiflora* of Jordanian origin and the effect of two spirobenzylisoquinoline alkaloids on mouse isolated ileum and rat isolated perfused heart.

2. Results and discussion

2.1. Chemical analysis of *Fumaria densiflora*

Chemical analysis of *F. densiflora* afforded twenty-two alkaloids, two of which were isolated for the first time from natural sources. The new alkaloids were N-methyl-5-hydroxystylopine chloride (1) and fumaricine N-oxide (2) (Figure 1). Norfumaritine was isolated for the second time from natural sources (Colton et al. 1985) and for the first time from natural sources (Colton et al. 1985) and for the first time from natural sources.
time from *F. densiflora*. The $^{13}$C-NMR spectrum for norfumaritine is reported here for the first time. Also, a simple benzylisoquinoline alkaloid, norjuziphine, is isolated for the first time from *F. densiflora*.

### 2.2. Structural elucidation

N-methyl-5-hydroxystylopine chloride (1) was obtained as white solid with melting point of 282 °C (decomposition). The HR-ESI-MS spectrum of N-methyl-5-hydroxystylopine chloride gave a molecular ion peak at $m/z = 390.59173$, which corresponds to the ion [C$_{20}$H$_{20}$O$_5$NCl + H]$^+$. The $^1$H-NMR spectrum (DMSO-d$_6$) (experimental part) of (1) is similar to stylopine except for the downfield shift of signals, the disappearance of a methylene group signal at $\delta = 2.62$ and 3.15 ppm and the appearance instead of signal for a hydroxymethine at $\delta = 5.04$ ppm and also the appearance of a singlet at $\delta = 2.91$ ppm integrating for three protons assigned to N–Me group. The doublet of doublet at $\delta = 4.18$ ppm ($J = 12.2, 6.4$ Hz) is assigned to H-6a. The second coupling is equatorial-axial interaction with H-5 proton indicating that the OH group is equatorial while H-6b appears as multiplet at $\delta = 3.67$ ppm. The multiplet at $\delta = 5.04$ ppm is assigned to H-5a. The OH proton appears as a broad singlet at $\delta = 6.66$ ppm. Again, the $^{13}$C-NMR spectrum (DMSO-d$_6$) (experimental part) is similar to stylopine except for the presence of extra signals at $\delta = 40.2$ ppm for N–CH$_3$ and the downfield shift of C-5 signal which appears in stylopine at $\delta = 29.6$ ppm but is shifted in this compound to $\delta = 60.1$ ppm, indicating that C-5 is attached to OH group. This was further approved by the DEPT experiments which confirmed the presence of one methyl signal for N–CH$_3$ and 5 methylene groups. The sixth methylene group of stylopine was replaced by a hydroxymethine proton in compound 1. The structure of N-methyl-5-hydroxystylopine chloride (1) was confirmed by 2D NMR experiments (COSY, HMQC and HMBC) (S. 1). The COSY experiment showed correlations between H-5a, H-6a and H-6b. The chemical shifts of the different carbons of compound 1 were assigned with the help of HMQC and HMBC experiments. The position of 5-OH group was confirmed through the HMBC correlation between H-4 ($\delta = 7.09$ ppm) and C-5 ($\delta = 60.1$ ppm).

Fumaricine N-oxide (2) was obtained as amorphous yellowish solid. The HR-ESI-MS spectrum of compound (2) gave a molecular ion peak at $m/z = 386.15936$, which is 16 units higher than that of fumaricine, suggesting that compound (2) contained an extra oxygen atom which is located in the only place available, the N atom. The $^1$H-NMR and $^{13}$C-NMR spectra (CDCl$_3$) of fumaricine N-oxide (2) (experimental part) are comparable to those of fumaricine except for the downfield shift of H-6a ($\delta = 4.00$, m) and the N-Methyl protons ($\delta = 3.25$, s). This was confirmed by the downfield shifts of C-6, C-14 and the N-methyl carbon signals which appear in fumaricine at $\delta = 47.6$, 74.5 and 38.2 ppm, respectively, but are shifted to $\delta = 62.5$, 89.2 and 52.4 respectively, in the N-oxide.

### 2.3. Effect of parfumine and fumaritine on mice isolated ileum

Parfumine and fumaritine, in concentrations ranging from $3 \times 10^{-7}$ to $9 \times 10^{-4}$ M caused concentration – dependent relaxation of ileum longitudinal segments (S. 2).
The median effective concentration (EC50) and the maximum relaxant effect of parfumine and fumaritine are shown in Table 1. The two compounds which belong to the same benzylisoquinoline alkaloid sub-group caused similar relaxant effects on mouse isolated ileum. Since, there are no studies dealing with the effects of the spirobenzylisoquinoline alkaloids on mouse isolated ileum, we compared our observations with those from other studies that showed the effect of other benzylisoquinoline sub-groups on mouse isolated ileum. The effects of spirobenzylisoquinoline alkaloids on mouse isolated ileum are similar to those reported for other benzylisoquinoline alkaloids on isolated smooth muscles. The relaxant effect of benzylisoquinoline alkaloids was documented in many preparations, including rat aorta, rat pulmonary artery, rat ileum and rat trachea (Huddart and Saad 1980; Lee et al. 1999; Huai-Liang et al. 2002).

2.4. Effect of parfumine and fumaritine on rat isolated perfused heart

Parfumine and fumaritine, in concentrations ranging from $3 \times 10^{-4}$ to $9 \times 10^{-2}$ M, caused concentration–dependent decrease in heart rate of the isolated perfused heart (S. 3). The median effective concentration (EC50) of parfumine and fumaritine and the maximum decrease in heart rate are shown in Table 2. Also, parfumine, in a concentration of $3 \times 10^{-2}$ M increased, but a higher concentration $9 \times 10^{-2}$ M decreased the amplitude of contraction of the isolated perfused heart (Figure 4). On the other hand, fumaritine, in concentration ranging from $3 \times 10^{-4}$ to $3 \times 10^{-2}$ M caused a slight increase, but a higher concentration $9 \times 10^{-2}$ M caused a decrease in the amplitude of contraction of the isolated perfused heart (S. 4).

The two compounds parfumine and fumaritine, which belong to the same alkaloid class, caused similar effect on the isolated perfused heart. When we compared our observations with those from other benzylisoquinoline alkaloids subgroups, we found that benzylisoquinoline subgroups have similar effects as spirobenzylisoquinoline alkaloids subgroups.

Table 1. EC50 and the maximum response induced by parfumine and fumaritine on mouse isolated ileum\textsuperscript{a}.

| Compound | N\textsuperscript{b} | EC50\textsuperscript{c} (M) | Maximum relaxation (% of papaverine maximum) |
|----------|----------------|-----------------|---------------------------------|
| Parfumine | 6 | $(2.2 \pm 0.3) \times 10^{-4}$ | $55.8 \pm 5.8$ |
| Fumaritine | 6 | $(1.3 \pm 0.2) \times 10^{-5}$ | $54.0 \pm 7.5$ |

\textsuperscript{a}Values are expressed as means ± SEM.

\textsuperscript{b}Number of experiments.

\textsuperscript{c}EC50 = Median effective concentration (the concentration causing 50% of the maximum response).

Table 2. EC50 and the maximum response induced by parfumine and fumaritine on rat isolated perfused heart\textsuperscript{a}.

| Compound | N\textsuperscript{b} | EC50\textsuperscript{c} (M) | Maximum heart rate (% of control maximum) |
|----------|----------------|-----------------|---------------------------------|
| Parfumine | 6 | $(2.4 \pm 0.2) \times 10^{-2}$ | $44.0 \pm 11.4$ |
| Fumaritine | 6 | $(0.5 \pm 0.1) \times 10^{-2}$ | $43.0 \pm 5.0$ |

\textsuperscript{a}Values are expressed as means ± SEM.

\textsuperscript{b}Number of experiments.

\textsuperscript{c}EC50 = Median effective concentration (the concentration causing 50% of the maximum response).
3. Conclusion

Chemical analysis of the alkaloid part of Fumaria densiflora afforded twenty-two alkaloids that belong to four known and two new alkaloid classes in the plant. We also report here the isolation of two new natural compounds: N-methyl-5-hydroxystylopine chloride (1) and fumaricine N oxide (2).

Parfumine and fumaritine, which belong to spirobenzylisoquinoline alkaloids subgroup, caused a relaxant effect on mouse isolated ileum longitudinal segments, and reduced heart rate of the isolated perfused heart. These two compounds have moderate positive inotropic effect but at higher concentrations \((9 \times 10^{-2} \text{ M})\) have negative inotropic effect.

We can conclude that different benzylisoquinoline alkaloids subgroups have the same effect on isolated smooth muscles and isolated perfused heart but with different activity and specificity.

These differences are due to differences in geometry and in the basicity of the nitrogen.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Adel M. Al-Ghazzawi http://orcid.org/0000-0001-7273-0131

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