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Absolute configuration assignment to anticancer Amaryllidaceae alkaloid jonquailine

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ABSTRACT

Jonquailine, a new alkaloid recently isolated from *Narcissus jonquilla quail*, an Amaryllidaceae species cultivated for its flowers fragrance in Europe and USA, shows very significant anti-proliferative activity against several malignant cancer cell types. Although it was reported that this activity is related to the functionalities and to its stereochemistry at C-8 of B ring, the absolute configuration at this stereocenter was not known. Density functional theory (DFT) calculations of chiroptical properties, namely electronic circular dichroism (ECD), vibrational circular dichroism (VCD), and optical rotatory dispersion (ORD) are employed here to complete assignment of absolute configuration of jonquailine, and then, by extension, to its analogues pretazettine and 8-*O*-methylpretazettine. While ECD is not discriminating and ORD is of limited use, VCD reveals decisive in the task of absolute configuration assignment.

1. Introduction

Plants belonging to the Amaryllidaceae genus are spread worldwide, mainly in Andean South America, Mediterranean basin, and South Africa. They consist of 75 genera and 1100 species commonly referred to as "Amaryllis". Lycorine was the first alkaloid isolated from this plant family and up to now, nearly 500 alkaloids have been isolated [22, 36]. Amaryllidacea alkaloids are grouped into twelve distinct ring types [26] and exhibit a wide range of bioactivities, such as anticancer, antiviral, antibacterial, antifungal, antimalarial, analgesic, cytotoxic, and acetylcholinesterase inihibitor [22]. Recent efforts focused on the anticancer activity of these alkaloids and of the closely related isocarbostyrils in view of their potential application in medicine [14, 28, 29]. As for most natural products, the bioactivity of Amaryllidacea alkaloids is strictly dependent on their relative and absolute stereochemistry [9]. This aspect is particularly evident for alkaloids of the [2]benzopyrano[3,4-*c*]indole subgroup reported in Fig. 1. In fact, while tazettine (**1a**), the most abundant alkaloid of this subgroup, is devoid of anticancer activity [49–51], the closely related pretazettine (**2a**) [53] has shown promising anticancer effects and has been studied in numerous models of murine cancer [16–21]. However, an important impediment that apparently halted further studies of **2a** in human cancer models is its instability in aqueous solution, where **2a** rearranges to **1a** (*vide infra*) [54, 55]. Also the structurally very similar alkaloid jonquailine (**2c**), recently isolated from dried bulbs of *Narcissus jonquilla quail* [31], revealed significant antiproliferative effects against glioblastoma, melanoma, uterine sarcoma and non-small-cell lung cancer cells displaying various forms of drug resistance, including resistance to apoptosis and multi-drug resistance [31]. **2c** was also found to synergize with paclitaxel in its antiproliferative action against drug-resistant lung cancer cells [31].

Notably, methylation of the acetal functionality makes **2c** more stable than **2a**, and less prone to rearrangement to **1a**, then improving its potential application as anticancer.

As far as the absolute configuration of these compounds is concerned, the one of **1a** was assigned by X-ray analysis of its methyl iodide [44] and later confirmed by chemical correlation [24] and total synthesis [4, 38]. Notably, Wildman and Bailey [54] demonstrated that in mild basic conditions **2a** and its C-3 epimer precriwelline smoothly rearranges to **1a** and to its C-3 epimer criwelline, respectively. This allowed one to assign the absolute configuration at C-3, C-4a and C-6a in **2a** (*i.e.* the junction of the B, C, D tricyclic system), later confirmed by total synthesis [4, 38]. The relative and absolute stereochemistry at the junction between rings B and D, and C and D, as well as that at C-3 in **2c** and in its epimer 8-*O*-methylpretazettine (**2b**), isolated from *Eucharis amazonica* [8], were then assigned by comparing their NMR and

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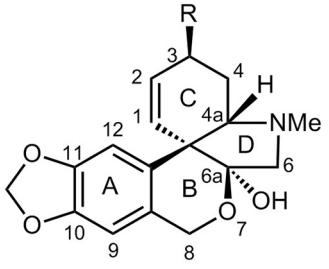
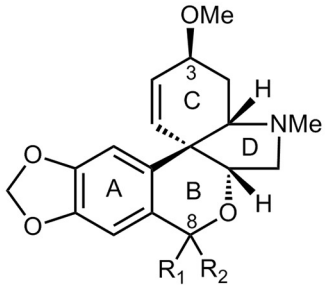


1a Tazettine; R = OMe
1b 3-*O*-Demethyltazettine; R =OH



2a Pretazettine; R_1= H, R_2 = OH
2b 8-*O*-Methylpretazettine; R_1= H, R_2 = OMe
2c Jonquailine; R_1= OMe, R_2 = H

Fig. 1. Structure of Amaryllidaceae alkaloids of [2]benzopyrano[3,4-*c*]indole subgroup. The previously defined stereochemistry of four carbon atoms is shown.

Electronic Circular Dichroism (ECD) spectra with those of **2a**. However, for all the three compounds **2a**, **2b**, and **2c** the absolute configuration at C-8 remained still unassigned. Notably, even NMR NOE analysis resulted unsuitable to ascertain the relative configuration at C-8. In fact, preliminary inspection of jonquailine (**2c**) Dreiding models for both epimers at C-8 shows that, while in the 8*R* epimer a NOE effect could be observed between H-8 and H-1, in the 8*S* epimer the NOE correlation between H-8 and H-6a appears uncertain.

The stereochemistry of the B:D ring junction in alkaloids of the [2] benzopyrano[3,4-*c*]indole subgroup, and then the absolute configuration at the carbon stereocenters joining B, C, D rings has been correlated [52] to the sign of the Cotton effects at around 240 nm and 290 nm in their ECD spectrum, allied to the dominant methylenedioxybenzenechromophore (Table 1). Wagner et al. [52], analyzing the ECD spectra of **1a**,**b** and **2a** concluded that a positive/negative sequence of the two Cotton effects is allied to a *cis* B:D ring junction, while a negative/positive sequence indicates a tricyclic system having a *trans* B:D fusion [52]. As inferred from Table 1, such empirical correlation fails in the case of **2c** which, although having a *trans* B:D ring junction, exhibits two positive Cotton effects at 242 nm and 291 nm, thus raising a doubt on the rule reliability. These data instead reveal that jonquailine (**2c**) should be indeed a C-8 epimer of 8-*O*-methylpretazettine (**2b**), while the latter and pretazettine (**2a**), sharing the same ECD profile and having essentially the same chromophoric system, must have the same absolute configuration at C-8 [31]. This highlights that assignment of the absolute configuration at C-8 in **2c** could allow one to assign, at the same time, the absolute configuration at the same stereocenter also in **2a** and **2b**.

As reported above, absolute stereochemistry has great importance on the bioactivity of Amaryllidaceae alkaloids [9] and, in particular, it seems that the presence of an oxygenated stereogenic center at C-8 has a role on the anticancer activity of **2a** and **2c** when compared to inactive **1a**. Therefore, we consider the assignment of the absolute configuration to this stereocenter in jonquailine (**2c**) of utmost importance, and we attempt here to reach this goal by computational analysis of its chiroptical spectra, *i.e.* ECD, Optical Rotatory Dispersion (ORD), and Vibrational Circular Dichroism (VCD). In this case the latter technique is revealed to be crucial in the AC assignment. In the literature it is also well documented that the use of several independent chiroptical methods leads to more reliable AC assignment [23, 32, 35, 40, 41]. Also Raman optical activity (ROA), not used in this work, could be very useful [5, 7, 37] especially in the case of aqueous solutions. As reported above this leads, as a consequence, to the assignment of the absolute configuration at C-8 also in 8-*O*-methylpretazettine (**2b**) and pretazettine (**2a**).

Table 1
Long wavelength ECD Cotton effects sign and Δε maximum value in ECD spectra of alkaloid molecules discussed here.

Compound	Ring fusion	λ(nm)[Δε]	λ(nm)[Δε]
Tazettine (**1a**)[a]	B:D *cis*	240.4 [+9.88][e]	289.2 [−0.94]
3-*O*-demethyltazettine (**1b**)[a]	B:D *cis*	240.0 [+4.80][e]	295.0 [−0.53]
Pretazettine (**2a**)[b]	B:D *trans*	249.6 [−2.98][e]	291.0 [+3.98]
8-*O*-Methylpretazettine (**2b**)[c]	B:D *trans*	244.0 [−1.33][e]	296.0 [+1.37]
Jonquailine (**2c**)[d]	B:D *trans*	240.0 [+10.82]	289.0 [+3.23]

[a] Data from [39].
[b] Data from [52].
[c] Data from [8].
[d] Data from [31] and this work.
[e] Δε values obtained from literature values of molar ellipticity by applying the formula [θ] = 3300 Δε.

2. Results and discussion

2.1. Absolute configuration of jonquailine

The assignment of the absolute configuration at C-8 of **2c** was first attempted by *ab initio* computational analyses of its ORD and ECD spectra [2, 3]. This approach was demonstrated to be particularly straightforward and reliable for the absolute configuration assignment of natural products [32, 42, 43, 47], allowing analysis in solution and sometimes on the microscale. Taking into account the known absolute configuration at C-3, C-4a and C-6a of **2c** the ECD spectra for the two epimers at C-8 were computed. Therefore, conformational analysis was carried out on the two (12b*S*,3*S*,4a*S*,6a*R*,8*S*)-**2c** and (12b*S*,3*S*,4a*S*,6a*R*,8*R*)-**2c** stereoisomers. The geometry optimization at DFT level (B3LYP/TZVP//B3LYP/6-31G(d) gas phase) of the conformers, defined by MM conformational search, provided five and three appreciably populated conformers for (8*S*)-**2c** and (8*R*)-**2c**, respectively (Figs. 2 and 3 and Tables S1 and S2 in Supporting Information). In the different conformers the polycyclic scaffold has, as expected, essentially the same conformation and only rotation of the two methoxy moieties at C-3 and C-8 occurs.

UV and ECD spectra of naturally occurring **2c** were then recorded in methanol in the 180–350 nm range (Fig. 4). The UV spectrum exhibits a maximum at 206 nm (ε 5060), followed by two broad bands centered at 240 nm (ε 877) and 290 (ε 525), respectively. The ECD spectrum shows a couplet-like feature at short wavelengths, with an intense negative Cotton effect at 197.0 nm (Δε − 32.3), followed by a positive band at 211.8 nm (Δε + 72.8), while at lower energy two weaker positive Cotton effects are visible at 240.0 (Δε + 10.8) and 289.0 nm (Δε + 3.23), respectively.

The UV and ECD spectra of both C-8 epimers were then calculated in gas phase at TDDFT/CAM-B3LYP/aug-cc-pVDZ level on the conformers optimized at the DFT/B3LYP/TZVP level of theory, and averaged over conformers' Boltzmann populations. Computed ECD spectra for (8*S*)-**2c** and (8*R*)-**2c** were then compared with experimental one for **2c** (Fig. 4).