Chiroptical study and absolute configuration of securinine oxidation products

Egor Chirkin a, William Atkatlian a, Quyên Do b, Thomas Gaslonde a, Thi-Hanh Dufat a, Sylvie Michel a, Pascale Lemoine c, Grégory Genta-Jouve a* and François-Hugues Porée a*

aLaboratoire de Pharmacognosie UMR CNRS 8638 COMETE, Faculté de Pharmacie, Université Paris Descartes, 4 Avenue de l’Observatoire, F-75006 Paris, France; bHanoi University of Pharmacy, No. 15, Le Thanh Tong str., Hanoi, Viet Nam; cLaboratoire de Cristallographie et RMN biologiques UMR CNRS 8015, Faculté de Pharmacie, Université Paris Descartes, 4 Avenue de l’Observatoire, F-75006 Paris, France

(Received 13 January 2015; final version received 20 February 2015)

Time-dependant density functional theory–electronic circular dichroism spectra prediction was carried out to study the absolute configuration of phyllanthidine-type derivatives 5 and 6, derived from securinine (1) and its enantiomer virosecurinine (2), respectively. This method demonstrated to be very reliable in this alkaloid series. Thus, 5 and 6 shared the same stereochemistry as their parent precursors, confirming the retentive nature of the oxidation sequence. In addition, this study highlighted the key role of the methylene bridge (BC ring) in the chiroptical activity of these compounds. These results fully clarified the stereochemical relationships between the phyllanthidine and the securinine subgroups.

Keywords: securinine; phyllanthidine; TDDFT-ECD; X-ray crystallography; absolute configuration

1. Introduction

Securinine (1) and its enantiomer virosecurinine (2) belong to the Securinega alkaloids family, a group comprising more than 60 compounds (Snieckus 1973; Chirkin et al. 2015). They are characterised by an original strained tetracyclic backbone featuring a butenolide moiety (ring D) and an azabicyclo [3.2.1] octane ring system (rings B and C) (Figure 1). This structural complexity has stimulated many research groups culminating in several total syntheses (Weinreb 2009; Chirkin et al. 2015). In addition, they exhibit interesting pharmacological...
activities such as central nervous system, anti-infectious and also anti-tumour agents (Gupta et al. 2011; Holmes et al. 2011; Zhang et al. 2011; Klochkov et al. 2014). For the latter, an American patent was recently published (Wald 2014). Interestingly, securinine (1) and its enantiomer virosecurinine (2) are present in different plants and have never been reported as a racemic mixture. Thus, (−)-securinine (1) was first isolated from Securinega suffruticosa (Pall.) Rehd (Murav’eva & Ban’kovskii 1956), and virosecurinine (2) was first isolated from Flueggea virosa (Roxb. ex Willd.) Royle (Nakano et al. 1962). In addition, co-occurring C-2 epimers are also usually found together with 1 and 2, namely allosecurinine (3) and allovirosecurinine (4) (Satoda et al. 1962; Saito et al. 1964). Compounds 5 and 6, the ‘oxidation’ products of securinine, were reported by Nakano et al. (1966) and Li et al. (2012), respectively, and should be the C-2 epimer of the known natural products phyllanthidine (7) (Parello 1965; Horii et al. 1972) or its enantiomer (8) (Lajis et al. 1992).

It could be assumed that 5–8 shared the same biochemical transformations, but until now 5 and 6 have never been isolated from any Securinega alkaloids containing plants. While a deep investigation of the absolute configuration of phyllanthidine (7) was conducted by Nakano et al. (1966), the more recent study did not treat the question of the absolute stereochemistry of derivatives 5 or 6 (Nakano et al. 1966; Li et al. 2012). Several questions can be asked such as the influence of the proton at C-2 on the stereoselectivity of the oxidation or its influence on the signs of the Cotton effects (CEs) observed on the electronic circular dichroism (ECD) spectra.

Herein we report the in-depth characterisation of the chiroptical properties of both enantiomers 5 and 6 resulting from the oxidation of compounds 1 and 2, respectively, using theoretical calculation of the ECD spectra. In addition, the stereochemistry in the phyllanthidine series was fully addressed.

2. Results and discussion

Securinine (1) and its enantiomer virosecurinine (2) were isolated from the leaves of S. suffruticosa (Pall.) Rehd. and F. virosa (Roxb. ex Willd.) Royle, respectively, and were further converted into phyllanthidine-type derivatives 5 and 6 according to a known procedure (Wei et al. 2013). As anticipated, compounds 5 and 6 presented the same NMR and MS data, and differed only by the sign of their optical rotation ([α]D20 = −268 (c = 0.04, CHCl3) and +254 (c = 0.05, CHCl3), respectively). The previous observation made for virosecurinine (2) and its
oxidation product 6 was confirmed as the sign of the optical rotation was conserved during the transformation (Nakano et al. 1966). But in order to fully understand the contribution of all stereogenic centres to the optical rotation, a circular dichroism study was undertaken.

Theoretical calculations of ECD have demonstrated to be very efficient for the determination of the absolute configuration of numerous natural products (Cachet et al. 2009; Bondu et al. 2012; Genta-Jouve & Thomas 2013; Genta-Jouve et al. 2013), but was never applied to this series. In order to confirm that time-dependant density functional theory (TDDFT) could be applied to this family of compounds, a first simulation was performed using the couple of enantiomers 1 and 2. For this purpose, geometry of the conformer resulting from the X-ray diffraction of 2 was first optimised using the B3LYP method at the 6-311G level (Figure 2).

The conformer of lower energy was then subjected to ECD calculation using the same method at the same level of theory. As depicted in Figure 3(a), three CEs are observable at $\lambda = 210 (+10)$, 255 ($-5.5$) and 355 ($-7$) nm for the experimental spectra (plain lines). The predicted spectra (dashed line) were rather consistent with both in term of CE’s wavelengths and signs. These calculations demonstrated that the TDDFT approach can be applied to this Securinea series with confidence and this methodology was then employed on the oxidation products of both 1 and 2.

Compounds 5 and 6 were obtained using the conditions described by Wei et al. (2013) from 1 and 2, respectively. Acquisition of ECD spectra for these compounds furnished interesting results as the oxidation step gave rise to important changes on the observed CE. As shown in Figure 3(b), while the CE at 207 nm was conserved, only one additional CE was observed at 265 nm instead of the two initial ones at 255 and 345 nm. This change could be attributed to an influence of the nitrogen (N1) lone pair to the $\alpha, \beta$ unsaturated $\gamma$-lactone through the additional oxygen (O7) lone pair (phyllanthidine numbering). Indeed, contribution of the nitrogen (N1) lone pair to the butenolide chromophoric unit was already proposed for securinine (1) during its
conformational analysis (Snieckus 1973). In order to confirm the conjugation through the oxygen (O7) lone pair, ECD calculations were undertaken using two conformers as input for the geometry optimisation. The first conformer described in Figure 4(a) corresponded to the X-ray structure of 6, while the second one (Figure 4(b)) was the one that prevented the direct nitrogen lone pair conjugation to the butenolide moiety.

Figure 4. (a) ORTEP representation of compound 6; (b) conformation of 6 preventing direct nitrogen lone pair; (c) predicted ECD spectrum of compound 6 (X-ray tridimensional structure); (d) predicted ECD spectrum of compound 6 (conformation of 6 preventing direct nitrogen lone pair influence).
As depicted in Figure 4(c),(d), the predicted ECD spectra of both conformations were similarly shaped with one major CE at ca. 250 nm relevant for the influence of oxygen (O7). These results confirmed the key assistance of the oxygen atom (O7) lone pairs in this chromophoric system.

As pointed out in the introduction, three stereogenic centres can be identified on securinine and phyllanthidine-type compounds, two of them being involved in the BC rings bridge and the latter at C-2. Contribution of H-2 in the global ECD spectrum was investigated using phyllanthidine (7), the C-2 epimer of compound 5. Experimental ECD spectra are presented in Figure 5. The results clearly showed that both epimers 5 and 7 exhibited the same broad CE at 250 nm, implying that the major contribution to the CE was produced by the bridged BC ring system.

3. Experimental

*General:* Solvents were purchased from Carlo Erba (Val de Reuil, France). Flash chromatography was performed with silica gel Carlo Erba 60 A (particle size 35–70 μm). Yield refers to chromatography and spectroscopically pure compounds unless otherwise noted. Rotary values were recorded on a Perkin Elmer Model 341 polarimeter (Perkin Elmer, Courtaboeuf, France) at 20°C. ECD spectra were recorded on a JASCO J-810 spectropolarimeter (Jasco, Bourguenais, France).

*Plant material:* The aerial parts (twigs and leaves) of *F. virosa* (Roxb. ex Willd.) Royle (= *Securinega virosa*) were collected by Dr D. Quyên and identified in Viet Nam (Herbarium of Hanoi University of Pharmacy, Voucher specimen number HNIP/17849/13). *S. suffruticosa* (Pall.) Rehd. was purchased at the Conservatoire National des Plantes à Parfums, Médicinales, Aromatiques et Industrielles at Milly-la-Forêt (France) and cultivated in the botanical garden of the Faculty (Voucher specimen number AWEC/022013/598).

*Extraction conditions and isolation:* Virosecurinine (2) was extracted from the aerial parts of *F. virosa* (Roxb. ex Willd.) Royle (= *S. virosa*) and securinine (1) was extracted from the aerial parts of *S. suffruticosa* (Pall.) Rehd. Extraction was carried out under pressure at room temperature with a Speed Extractor Apparatus (Büchi). For this purpose, the pulverised dried material (20 g) was first treated with an aqueous ammoniac solution (30 mL), then transferred into a stainless steel cartridge and extracted with CH₂Cl₂ at 100 bar for 15 min. Two successive cycles were performed to assure complete alkaloids extraction (Dragendorff reagent control). Then the organic layers were combined and reduced to half of their volume. A typical alkaloid extraction was then performed with first an acidic treatment and after elimination of the organic

![Figure 5. Experimental ECD spectra of compounds 5 and 7.](image-url)
layer, alkalinisation (NH₄OH) and extraction with CH₂Cl₂. The organic layers were dried over MgSO₄ and concentrated in vacuo. The crude residue was purified by flash chromatography (cyclohexane/ethyl acetate, 80:20) to yield virosecurinine (2) in 0.3% (leaves) and 0.08% (stems), respectively, in the case of F. virosa (Roxb. ex Willd.) Royle, and securinine (1) in 0.43% (leaves) and 0.1% (stems), respectively, from S. suffruticosa (Pall.) Rehd. Their ¹H NMR spectral data and rotary values were identical to the reported data (Snieckus 1973; Livant & Beutler 1987). In addition, virosecurinine (2) was crystallised from n-butanol. Allosecurinine (3) was generously given by Pr Y. Li (Shanghai University of TCM, China).

General procedure for oxidation of the securinane skeleton with m-CPBA: conditions described by Wei et al. (2013) were applied. At 0°C, to a solution of allosecurinine (3) (10 mg, 0.046 mmol), or securinine (1) (76 mg, 0.35 mmol) or virosecurinine (2) (114 mg, 0.52 mmol) in methanol was added m-CPBA (70% m/m, 1.5 equiv.). The mixtures were stirred at this temperature for 10 min and an excess amount of Ca(OH)₂ was added. The resulting suspensions were filtered off through a pad of Perlite and the filtrates were concentrated under vacuum to give the N-oxide derivatives, which were used in the next step without further purification. The N-oxide compounds made as described above were separately dissolved in xylene (40 mL), and heated at reflux for 30 min. After solvent removal under vacuum, the residues were dissolved in CH₂Cl₂ and washed with an aqueous NaHCO₃ solution. The organic layers were dried over MgSO₄, filtered and concentrated in vacuo. The residues were purified by flash chromatography on silica gel (pentane/ethyl acetate, 1:1) to yield the corresponding rearranged compounds 7 (3.5 mg, 0.015 mmol, from allosecurinine), 5 (43 mg, 0.18 mmol, from securinine) and 6 (66 mg, 0.28 mmol, from virosecurinine) in 32%, 51% and 54%, respectively, as white solids. Compound 6 derived from virosecurinine was crystallised from n-butanol, which allowed X-ray diffraction analysis (CCDC number 997650).

Electronic circular dichroism: ECD spectra of compounds 1, 2, 5, 6 and 7 were recorded on a JASCO J-810 spectropolarimeter at Imagif – Laboratoire d’enzymologie et biochimie structurales (ICSN, Gif sur Yvette, France). The compounds were dissolved in ethanol (0.1 mg/mL) and their spectra were recorded between 200 and 400 nm (interval 0.5 nm). For each sample, three successive measurements were done.

X-ray crystallography: X-ray analyses of virosecurinine (2) and compound 6 were performed by Dr P. Lemoine (Université Paris Descartes). All the detailed data are available in the supplementary material.

Theoretical calculations of the electronic dichroism spectra: the Gaussian09 package was used for the ECD calculations on the most stable conformer of each compound. Density functional theory with B3LYP functional and Pople’s 6.311G basis set was used on the lowest energy conformers. TDDFT was employed to calculate excitation energy (in eV) and rotatory strength R in dipole velocity (Rvel) and dipole length (Rlen) forms. The calculated rotatory strengths were simulated in the ECD curve by using SpecDis v1.61 (Bruhn et al. 2013).

4. Conclusions
The use of TDDFT ECD spectra prediction demonstrated to be very efficient for the study of the absolute configuration phyllanthidine-type alkaloids 5 and 6. Thus, these compounds shared the same stereochemistry as their parent precursors, securinine (1) and virosecurinine (2), respectively. This point clearly confirmed the retentive nature of the oxidation sequence in this series and also demonstrated that each securinine stereoisomer could give rise to its own phyllanthidine derivative. In addition, this study highlighted the key role of the methylene bridge (BC ring) in the chiroptical activity of these series of compounds.

Supplementary material
Supplementary material relating to this paper is available online.
Acknowledgements
We express our deep thanks to Dr Christophe Velours (ICSN Gif/Yvette) and Dr Serge Bouaziz (CNRS/Paris Descartes University) for their technical assistance (ECD measurement). We also thank Pr Yiming Li (Shanghai University of TCM, China) for the generous gift of allosecurinine. This work was granted access to the HPC resources of IDRIS under the allocation 2014-100483 OUMOLPO made by GENCI.

Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
We gratefully acknowledged the financial support from Paris Descartes University and CNRS.

References
Bondu S, Genta-Jouve G, Leiros M, Vale C, Guigonis J-M, Botana LM, Thomas OP. 2012. Additional bioactive guanidine alkaloids from the Mediterranean sponge Crambe crambe. RSC Adv. 2:2828–2835. doi:10.1039/c2ra00045h.

Bruhn T, Schaumloffel A, Hemberger Y, Bringmann G. 2013. SpecDis: quantifying the comparison of calculated and experimental electronic circular dichroism spectra. Chirality. 25:243–249. doi:10.1002/chir.22138.

Cachet N, Genta-Jouve G, Regalado EL, Mokrini R, Amade P, Culioi G, Thomas OP. 2009. Parazoanthines A–E, hydantoin alkaloids from the Mediterranean sea anemone Parazoanthus axinellae. J Nat Prod. 72:1612–1615. doi:10.1021/nl900437y.

Chirk E, Atkatlian W, Porée F-H. 2015. The securinega alkaloids. In: Knölker HJ, editor. The alkaloids. New York: Academic Press; Vol 74, pp. 1–120. doi:10.1016/bs.alkal.2014.11.001.

Genta-Jouve G, Thomas OP. 2013. Absolute configuration of the new 3-epi-cladocroic acid from the Mediterranean sponge Haliclanus fulva. Metabolites. 3:24–32. doi:10.3390/metabo3010024.

Genta-Jouve G, Weinberg L, Cocandeau V, Maestro Y, Thomas OP, Holderith S. 2013. Revising the absolute configurations of coatlines via density functional theory calculations of electronic circular dichroism spectra. Chirality. 25:180–184. doi:10.1002/chir.22129.

Gupta K, Chakrabarti A, Rana S, Ramdeo R, Roth LBL, Agarwal LML, Tse W, Agarwal KMK, Wald NDN, Minna D. 2011. Securinine, a myeloid differentiation agent with therapeutic potential for AML. PLOS ONE. 6:e21203. doi:10.1371/journal.pone.0021203.

Holmes M, Crater AK, Dhudshia B, Thadani AN, Ananvaranich S. 2011. Toxoplasma gondii: inhibitory activity and encystation effect of securinine and pyrrolidine derivatives on Toxoplasma growth. Exp Parasitol. 127:370–375. doi:10.1016/j.exppara.2010.09.002.

Hori Z, Imanishi T, Yamauchi M, Hanaoka M, Parello J, Munavalli S. 1972. Structure of phyllantine and phyllantidine. Tetrahedron. 13:1877–1880. doi:10.1016/S0040-4020(01)86830-7.

Klochkov SG, Neganova ME, Afanas’eva SV, Shevtsova EF. 2014. Synthesis and antioxidant activity of securine derivatives. Pharm Chem J. 48:15–17. doi:10.1007/s11094-014-1035-5.

Lajis NH, Guan OB, Sargent MV, Skelton BW, White AH. 1992. Viroallosecurinine and ent-phyllantidine from the leaves of Bresnia coronata (Euphorbiaceae). Aust J Chem. 45:1893–1897.

Li J-Y, Zhao B-X, Zhang W, Li C, Huang X-J, Wang Y, Sun P-H, Ye W-C, Chen W-M. 2012. Unexpected ring contraction and oxidation rearrangement reactions of securinine. Tetrahedron. 68:3972–3979. doi:10.1016/j.tet.2012.03.084.

Livant PD, Beutler JA. 1987. Conformations of the securinine alkaloids as studied by high field 1H and 2-D NMR, and molecular mechanics calculation. Tetrahedron. 43:2915–2924. doi:10.1016/S0040-4020(01)86830-7.

Murav’eva VI, Ban’kovskii AI. 1956. Securinine a new alkaloid of strychnine-like action. Med Prom-st SSSR. 10:27–28.

Nakano T, Terao S, Lee KH, Saeki Y, Durham LJ. 1966. Some observations on the oxidation of virosecurinine with monoperphthalic acid 1,2. J Org Chem. 31:2274–2279. doi:10.1021/jo01345a044.

Nakano T, Yang TH, Terao S. 1962. Structure of virosecurinine. Chem Ind (London, UK). 1651–1652.

Parello J. 1965. Phyllantine and Phyllantidine, alcaloids du Phyllantus discoides Mull. Arg. (Euphorbiaceae) [Phyllantine and Phyllantidine, alkaloids from Phyllantus discoides Mull. Arg. (Euphorbiaceae)]. C.R. Acad Sci. 260:337–340.

Saito S, Iwamoto T, Tanaka T, Matsumura C, Sugimoto N, Hori Z, Tamura Y. 1964. Two new alkaloids, viroallosecurinine and virosine, isolated from Securinega virosa. Chem Ind (London, UK). 1263–1264.
Satoda I, Murayama M, Tsuji J, Yoshii E. 1962. Studies on securinine and allosecurinine. Tetrahedron Lett. 3:1199–1206. doi:10.1016/S0040-4039(00)70585-5.

Snieckus V. 1973. The Securinega alkaloids. In: Manske RH, editor. The alkaloids. New York: Academic Press; Vol 14, p. 425–506.

Wald D. 2014. US Patent 2014/0018383 A1.

Wei H, Qiao C, Liu G, Yang Z, Li C-C. 2013. Stereoselective total syntheses of (−)-flueggine a and (+)-virosaine b. Angew Chem Int Ed. 52:620–624. doi:10.1002/anie.201208261.

Weinreb SM. 2009. Total synthesis of the Securinega alkaloids. Nat Prod Rep. 26:758–775. doi:10.1039/b902265a.

Zhang W, Li J-Y, Lan P, Sun P-H, Wang L, Ye W-C, Chen WM. 2011. Chemical synthesis and biological activities of Securinega alkaloids. J Chin Pharm Sci. 20:203–212.