PERGAMON

Phytochemistry 60 (2002) 847–852

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Alkaloids from *Narcissus angustifolius* subsp. *transcarpathicus* (Amaryllidaceae)☆

Josep Labraña[a], Alex King'ori Machocho[a], Vladimir Kricsfalusy[b], Reto Brun[c], Carles Codina[a], Francesc Viladomat[a], Jaume Bastida[a,*]

[a]*Departament de Productes Naturals, Facultat de Farmàcia, Universitat de Barcelona, Avda. Diagonal 643, E-08028 Barcelona, Spain*
[b]*Department of Botany, Laboratory of Plant Population, Uzhgorod State University, Voloshyma 54, Uzhgorod, Ukraine*
[c]*Swiss Tropical Institute, Protozoology Laboratory, Socinstraβe 57, CH-4002 Basel, Switzerland*

Received 19 December 2001; received in revised form 15 April 2002

Abstract

Seven alkaloids have been isolated from fresh bulbs of *Narcissus angustifolius* subsp. *transcarpathicus* (Amaryllidaceae). Nangustine, reported here for the first time, is the first 5,11-methanomorphanthridine alkaloid with a C-3/C-4 substitution. The structure and stereochemistry of this new alkaloid, as well as those previously known, have been determined by physical and spectroscopic methods. Spectroscopic data of pancracine have been completed. The in vitro assay activity against the parasitic protozoa *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani* and *Plasmodium falciparum* was carried out with the compounds nangustine and pancracine.

Keywords: *Narcissus angustifolius* subsp. *transcarpathicus*; Amaryllidaceae; Bulbs; Alkaloids; Pseudolycorine; 8-*O*-Demethylhomolycorine; Cherylline; Ungeremine; Vasconine; Pancracine; Nangustine; Antiprotozoal activity

1. Introduction

Narcissus angustifolius Curt. subsp. *transcarpathicus* Kricsfalusy (*N. poeticus* L. subsp. *stellaris* (Haw.) Aschers. et Graebn.), is distributed throughout the Transcarpathian region of Ukraine. The present investigation deals with the isolation and characterisation of seven alkaloids from the bulbs of this hitherto unstudied species. The minor alkaloids cherylline and ungeremine are reported for the first time in the *Narcissus* genus. Cherylline is a 4-arylisoquinoline derivative, a group with several potential medicinal properties (Jacob et al., 1981; Zára-Kaczián et al., 1986; Maryanoff et al., 1990; Kihara et al., 1997), and has recently been the subject of many synthetic studies (Couture et al., 1999; Honda et al., 2001; Ruchirawat et al., 2001). Ungeremine has been shown to be active against leukemia (Zee-Cheng et al., 1978) and responsible, at least in part, for the growth-inhibitory and cytotoxic effects of lycorine (Ghosal et al., 1988). Significant efficiency against human ovarian and stomach cancers has been claimed for semisynthetic ungeremine acetate in human clinical trials conducted in China (Chen et al., 1997; Xu and Wang, 1997). Nangustine (**1**) as well as the known alkaloid pancracine (**2**) are representatives of the rare 5,11-methanomorphanthridine ring system.

2. Results and discussion

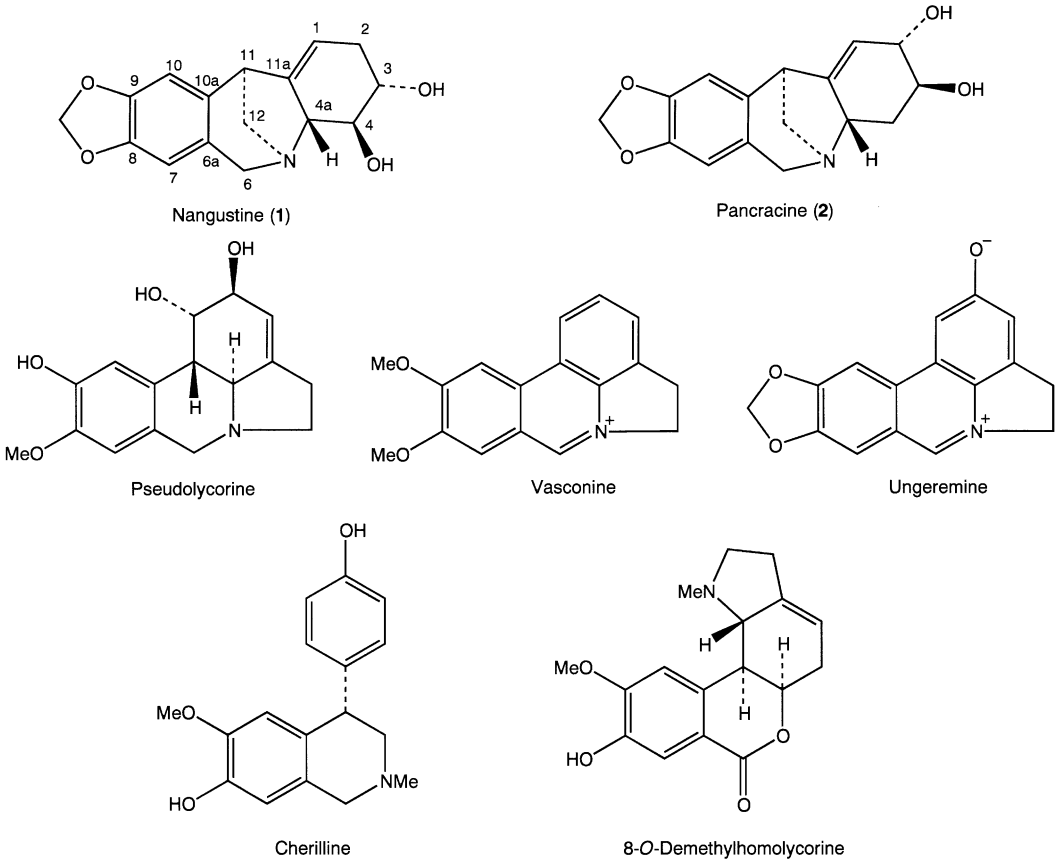
Compound **1**, $C_{16}H_{17}NO_4$ by HR–EIMS, was isolated from fraction III (see Experimental) and the name nangustine was proposed for this alkaloid. Its IR spectrum displayed a broad absorption band centred at 3192 cm^{-1} as well as a band at 930 cm^{-1} associated to hydroxyl and methylenedioxy groups, respectively. Its MS showed the parent base peak at m/z 287 $[M]^+$ and several ions of considerable abundance (m/z 185, 199, 214), which were also observed for compound **2** but not for other montanine type alkaloids like montanine and coccinine

☆ Part 26 in the series "*Narcissus* alkaloids". For part 25 see Labraña et al. (1999).

* Corresponding author. Tel.: +34-93-402-44-93; fax: +34-93-402-90-43.

E-mail address: bastida@farmacia.far.ub.es (J. Bastida).

0031-9422/02/$ - see front matter
PII: S0031-9422(02)00154-1



(Wildman and Brown, 1968). The nature of the substituents and their particular configuration have a very significant effect on the electron-induced fragmentation of these molecules.

The ^{1}H NMR spectrum (500 MHz, CD_3OD) (Table 1), exhibited two singlets at δ 6.56 and 6.51 for the aromatic protons H-10 and H-7, respectively. The assignment was made according to the benzylic couplings with H-11 and H-6, observed in the COSY experiment (Table 2). The two H-6 protons were clearly differentiated as an AB-system with geminal coupling of 16.5 Hz. The H-6α was assigned at lower fields as a consequence of the NOE contour correlation with H-12axial. Furthermore, H-6β showed a long range W-coupling with H-12equatorial, all of which confirmed the proposed assignments. The ring C bore an equatorial 4-OH and a pseudoequatorial 3-OH groups and existed in the C_4 half-chair conformation. The large vicinal coupling constants between H-4 and H-4a and between H-4 and H-3 denoted their *trans*-diaxial relationship, which was consistent with the NOE contour correlations between H-4a and H-3 and between H-4 and H-12 equatorial. The signal centred at δ 2.05 was assigned to the H-2α from ROESY experiments and because of the $J_{2\alpha,3}$ value of 9.0 Hz. Additional long range allylic couplings of H-1 with H-11 as well as H-1 with H-4a were observed by 2D COSY experiments (Table 2), causing line-broadening of these protons.

The ^{13}C NMR spectrum of **1** (Table 1), confirmed by HMQC (Bax and Subramanian, 1986), was consistent with the proposed structure. The multiplicities of the 16 carbons accounted for were determined by DEPT experiments. The location of one of the hydroxyl groups at C-4 was confirmed by the pronounced deshielding effect at this position as well as at the β-carbon C-4a, which differs from the ^{13}C NMR data of compound **2**. The quaternary carbons of the aromatic ring and the olefinic bond were confirmed by HMBC correlations (Bax and Summers, 1986). The absolute configuration of this alkaloid was determined from the CD spectrum, where the curve was qualitatively similar to that of the known 5,11-methanomorphanthridine alkaloids with an α-configuration for the methane-bridge (Ali et al., 1984; De Angelis and Wildman, 1969).

Pancracine ($C_{16}H_{17}NO_4$) (**2**) had been fully characterized (Ali et al., 1984; Wildman and Brown, 1968). Both the physical and spectral data of the compound we isolated were in close agreement with those that have been reported. However, we report here the unequivocal assignment of the ^{13}C NMR spectrum using 2D NMR techniques, providing additional information with respect to previously published data (Ali et al., 1984).

Antiparasitic activities of the 5,11-methanomorphanthridine alkaloids **1** and **2** are summarized in Table 3. Compound **2** showed a higher activity than compound **1** against all the 4 protozoan parasites tested. While

Table 1
^{1}H NMR, HMQC and HMBC data for nangustine (**1**)[a]

Proton	δ_H	Correlated C-atom	
		HMQC	HMBC
1	5.52 *dt* (3.5, 2.5)	114.7 *d*	
2α	2.05 *ddt* (18.0, 9.0, 3.5)	35.5 *t*	
2β	2.57 *dddd* (18.0, 7.0, 3.5, 2.0)	35.5 *t*	C-1
3	3.62 *ddd* (9.0, 9.0, 7.0)	72.3 *d*	C-4
4	3.31 *t* (9.0)	75.5 *d*	C-3, C-4a
4a	3.16 *brd* (9.0)	70.0 *d*	C-4
6α	4.32 *d* (16.5)	62.0 *t*	C-4a, C-6a, C-10a
6β	3.83 *d* (16.5)	62.0 *t*	C-4a, C-6a, C-7, C-10a, C12
		125.2 *s* (C-6a)	
7	6.51 *s*	107.8 *d*	C-6, C-9, C-10a
		148.3 *s* (C-8)	
		147.6 *s* (C-9)	
10	6.56 *s*	108.3 *d*	C-6a, C-8, C-11
		133.6 *s* (C-10a)	
11	3.33 *brd* (2.5)	46.4 *d*	C-4a, C-10, C-10a
		147.5 *s* (C-11a)	
12*ax*	3.03 *d* (11.0)	56.9 *t*	C-4a, C-6, C-10a, C-11a
12*eq*	2.94 *dd* (11.0, 2.0)	56.9 *t*	C-6, C-10a
OCH_2O	5.86 *d* – 5.85 *d* (1.5)	102.1 *t*	C-9

[a] Chemical shifts in ppm. Coupling constants (*J*) in Hz. C-multiplicities were determined by DEPT data.

Table 2
COSY and ROESY data for nangustine (**1**)

Proton	COSY	ROESY
1	H-2α, H-2β, H-4a, H-11	H-2α, H-11
2α	H-1, H-2β, H-3, H-4a	H-1, H-2β, H-4
2β	H-1, H-2α, H-3, H-4a	H-2α, H-3
3	H-2α, H-2β, H-4	H-2β, H-4, H-4a
4	H-3, H-4a	H-2α, H-3, H-4a, H-12eq
4a	H-1, H-2α, H-2β, H-4	H-3, H-4, H-6β
6α	H-6β, H-7	H-6β, H-7, H-12ax
6β	H-6α, H-7, H-12eq	H-4a, H-6α, H-7
7	H-6α, H-6β,	H-6α, H-6β
10	H-11	H-11
11	H-1, H-10, H-12ax, H-12eq	H-1, H-10, H-12ax, H-12eq
12axial	H-11, H-12eq	H-6α, H-11, H-12eq
12equatorial	H-6β, H-11, H-12ax	H-4, H-11, H-12ax
OCH_2O		

compound **1** has to be classified as inactive against the 4 parasite species, compound **2** showed weak activity against *Trypanosoma brucei rhodesiense* and *T. cruzi*, but no activity against *Leishmania donovani*. The IC_{50} values of 0.75 and 0.70 μg/ml, respectively, for two strains of *Plasmodium falciparum* also represent an activity which has to be classified as weak. On the other hand, no cytotoxic activity for L-6 cells (rat skeletal myoblasts) was found which confirms the selective activity of compound **2** for *T. brucei rhodesiense* and *P. falciparum*.

3. Experimental

3.1. General

Mps: uncorr. Optical rotations: Perkin-Elmer 241 Polarimeter. CD: Jasco J-700 Spectropolarimeter. IR spectra: Perkin-Elmer 1600 FTIR series Spectrometer in dry film. EIMS: Hewlett Packard 5989A Mass Spectrometer at 70 eV. ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC, HMBC and ROESY spectra: Varian VXR 500 or Gemini 200, in $CDCl_3$ or CD_3OD. Chemical shifts are reported in units of δ (ppm) relative to the TMS signal and coupling constants (*J*) in Hz. Silica gel SDS Chromagel 60 G Merk (mean particle size 15 μm) and silica gel Merck (70–230 mesh) were used for VLC and CC, respectively. Sephadex LH-20 Pharmacia was used for gel filtration, and silica gel SIL G/UV_{254} (Macherey-Nagel) for analyt. (0.25 mm) and SIL G-25 UV_{254} for prep. (0.25 mm) TLC. Spots on chromatograms were detected under UV light (254 nm) and by Dragendorff's reagent.

3.2. Plant material

Bulbs of *N. angustifolius* subsp. *transcarpathicus* (*N. poeticus* subsp. *stellaris*) were collected in May 1999, during the flowering period, in Kireshi, Khust district, Ukraine. A voucher specimen (N 269) has been deposited in the Herbarium of the Uzhgorod State University, Ukraine.

Table 3
In vitro assays for nangustine (**1**) and pancracine (**2**)

Parasite	*T. b. rhodesiense*	*T. cruzi*	*L. donovani*	*P. falciparum*		Cytotoxicity
Stage	Trypomastigote	Trypomastigote	Amastigotes	Erythrocytic form		
Strain	STIB 900	Tulahuen C4	MHOM-ET-67/L82	K1	NF54	L-6
Standard/compound	IC_{50}[a]	IC_{50}[a]	IC_{50}[a]	IC_{50}[a]	IC_{50}[a]	MIC[a]
Melarsoprol	0.0014					
Benznidazole		0.4				
Pentostam			33			
Chloroquine				0.057	0.004	
Artemisinine				0.003	0.004	
Compound **1**	9.6	54.6	>30	2.14	2.93	>90
Compound **2**	0.7	7.1	>30	0.75	0.70	>90

[a] All values as: μg ml^{-1}.

3.3. Extraction and isolation of alkaloids

Fresh bulbs of *N. angustifolius* subsp. *transcarpathicus* (4.7 kg) were crushed and macerated with EtOH for 48 h and the process repeated twice. The crude alcoholic extracts were evaporated under reduced pressure, the residue dissolved in H_2O and acidified with 5% H_2SO_4 to pH 3–4. After removing the neutral material with Et_2O, the acidic soln. was extracted with CH_2Cl_2 to provide extract A (1.85 g). Basifying the aq. soln. up to pH 8–9 with 10% NH_4OH and extracting it again with CH_2Cl_2 several times gave extract B (7.75 g). Finally, a CH_2Cl_2–MeOH (3:2) extraction of the basic soln. gave extract C (1.60 g). Extract A was absent of alkaloids. After combining extracts B and C, the brown gummy residue was dissolved in MeOH from which pseudolycorine (850 mg) crystallized directly. The soln. was dried again and subjected to VLC on silica gel eluting initially with *n*-hexane, increasing the polarity with CH_2Cl_2, and later up to CH_2Cl_2–MeOH (4:1). Four major fractions (I–IV) containing alkaloids were obtained. Fraction I (688 mg), eluded with CH_2Cl_2-MeOH (99.5:0.5→97.8:2.2) from the initial column, was rechromatographed by VLC on silica gel using Me_2CO as solvent, giving 3 fractions. Fraction I-2 (122 mg), after final purification on Sephadex LH-20 using MeOH as eluent, afforded 8-*O*-demethylhomolycorine (20 mg) and more pseudolycorine (45 mg). Fractions II (358 mg) and III (443 mg) were eluted with CH_2Cl_2–MeOH (97.7:2.3→92:8) from the initial column. 8-*O*-Demethylhomolycorine (73 mg) was obtained directly from fraction II by recrystallization with MeOH. The rest of fraction II was combined with fraction III due to their overlapping TLC pattern. This combined fraction was first subjected to a silica gel VLC using EtOAc–MeOH (gradient, 100:0→90:10) mixtures for elution, giving 5 fractions. Fractions III-2 (135 mg), III-3 (114 mg) and III-4 (143 mg) from this column, which eluted with EtOAc–MeOH (97.2:2.8→92.5:7.5), were subjected directly to preparative TLC, eluting twice with MeOH and Me_2CO as solvents. Cherylline (26 mg) was obtained from fraction III-2, vasconine (20 mg) from fraction III-3, and ungeremine (16 mg) and **1** (15 mg) from fraction III-4. Fraction IV (865 mg), eluded with CH_2Cl_2–MeOH (91:11→86:14), was treated as fraction II and III above, giving 3 new fractions. Separation of the alkaloids from fraction IV-2 (106 mg), which eluted with EtOAc–MeOH (93.8:6.2→91.5:8.5), was again done by preparative TLC (twice with MeOH and Me_2CO) giving **2** (15 mg).

3.4. Nangustine (*1*)

White solid, m.p. 261 °C. $[\alpha]_D^{20}$ −69.6 ° (MeOH; *c* 0.3533). CD $[\theta]_{255}$ −6698, $[\theta]_{270}$ −1263, $[\theta]_{307}$ −1018. IR ν_{max} (film on NaCl) cm^{-1}: 3192 (–OH), 2899, 2354, 1607, 1502, 1481, 1369, 1332, 1279, 1232, 1197, 1176, 1134, 1078, 1061, 1037, 991, 971, 930 (–OCH_2O–), 901, 857, 823, 755. ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (50 MHz, CD_3OD), see Table 1. EIMS 70 eV, *m*/*z* (rel. int.): 287 $[M]^+$ (100), 270(7), 243 (1), 226 (19), 223 $[C_{15}H_{11}O_2]^+$ (22), 215 (22), 214 (16), 212 (22), 199 $[C_{13}H_{11}O_2]^+$ (35), 197 (24), 186 (20), 185 $[C_{12}H_9O_2]^+$ (87), 173 (19), 153 (20), 141 (43), 129 (29), 128 (50), 127 (30), 116 (19), 115 (72), 103 (19), 102 (20), 91 (31), 89 (32), 83 (22), 77 (62), 76 (32), 73 (19), 72 (37), 71 (26), 69 (24), 65 (32), 64 (21), 63 (40), 60 (27), 57 (48), 55 (60), 53 (30), 51 (46). HR–EIMS *m*/*z*: 287.1161 (calc. for $C_{16}H_{17}NO_4$, 287.1158).

3.5. Pancracine (*2*)

White solid, m.p., $[\alpha]_D$, CD, IR, EIMS and ^1H NMR data are in agreement with those reported (Ali et al., 1984; Wildman and Brown, 1968). ^{13}C NMR (50 MHz, CD_3OD): δ 29.3 (*t*, C-4), 44.7 (*d*, C-11), 54.5 (*t*, C-12), 58.5 (*d*, C-4a), 59.2 (*t*, C-6), 68.2 (*d*, C-2), 70.4 (*d*, C-3), 100.7 (*t*, OCH_2O), 106.2 (*d*, C-7), 106.8 (*d*, C-10), 115.6

(*d*, C-1), 122.2 (*s*, C-6a), 131.1 (*s*, C-10a), 146.3 (*s*, C-9), 146.8 (*s*, C-8), 150.0 (*s*, C-11a). HR–EIMS *m*/*z*: 287.1155 (calc. for $C_{16}H_{17}NO_4$, 287.1158).

Pseudolycorine, 8-*O*-demethylhomolycorine and vasconine (Bastida et al., 1998), cherylline (Kobayashi et al., 1984) and ungeremine (Bastida et al., 1996) were identified by comparison of their chromatographic and spectroscopic properties (TLC, $[\alpha]_D$, CD, IR, MS, ^1H and ^{13}C NMR) with those of authentic samples obtained from other plant sources.

3.6. In vitro assays

Trypanosoma brucei rhodesiense. Serial drug dilutions in Minimum Essential Medium supplemented according to Baltz et al. (1985) were added to microtiter plates. Trypomastigotes of *T. brucei rhodesiense* STIB 900 were added to each well and the plates incubated for 72 h. Viability was assessed by Alamar Blue leading to a colour reaction which was read in a fluorescence scanner (Millipore Cytofluor 2300) (Räz et al., 1997). Fluorescence development was expressed as percentage of the control, and IC_{50} values determined.

Trypanosoma cruzi. Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells/well/100 μl in RPMI 1640 medium with 10% FBS and 2 mM L-glutamine. After 24 h 5000 trypomastigotes of *T. cruzi* (Tulahuen strain C2C4 containing the β-galactosidase (Lac Z) gene) were added in 100 μl per well with 2x of a serial drug dilution. The plates were incubated at 37 °C in 5% CO_2 for 4 days. For measurement of the IC_{50} the substrate CPRG/Nonidet was added to the wells. The colour reaction which developed during the following 2–4 h was read photometrically at 540 nm. From the sigmoidal inhibition curve IC_{50} values were calculated.

Leishmania donovani. Mouse peritoneal macrophages were seeded in RPMI 1640 medium with 10% heat-inactivated FBS into Lab-tek 16-chamber slides. After 24 h, *L. donovani* amastigote were added at a ratio of 3:1 (amastigotes to macrophages). The medium containing free amastigotes was replaced by fresh medium 4 h later. Next day the medium was replaced by fresh medium containing different drug concentrations. The slides were incubated at 37 °C under a 5% CO_2 atmosphere for 96 h. Then the medium was removed, the slides fixed with methanol and stained with Giemsa. The ratio of infected to non-infected macrophages was determined microscopically, expressed as percentage of the control and the IC_{50} value calculated by linear regression.

Plasmodium falciparum. Antiplasmodial activity was determined using two strains of *P. falciparum:* NF54 (sensitive to all known drugs) and K1 (resistant to chloroquine and pyrimethamine). A modification of the [^3H]-hypoxanthine incorporation assay was used (Ridley et al., 1996). Briefly, infected human red blood cells were exposed to serial drug dilutions in microtiter plates for 48 h. Viability was assessed by measuring the incorporation of [3H]-hypoxanthine by liquid scintillation counting. From the sigmoidal inhibition curves IC_{50} values were calculated.

Acknowledgements

Part of this work was financially supported by CIRIT-CICYT and Commissionat per a Universitat i Recerca, Generalitat de Catalunya (1999SGR-00070) and by the Spanish Ministerio de Educación y Ciencia (PB98–1211). AKM also thanks the Agencia Española de Cooperación Internacional for provision of a doctoral fellowship. This investigation also received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (RB). The technical assistance for the assessment of biological activities was kindly provided by Elke Gobright and Christian Scheurer.

References

Ali, A.A., Mesbah, M.K., Frahm, A.W., 1984. Phytochemical investigation of *Hippeastrum vittatum*. Part IV. Stereochemistry of pancracine, the first 5,11-methanomorphanthridine alkaloid from *Hippeastrum*—structure of hippagine. Planta Medica 50, 188–189.

Baltz, T., Baltz, D., Giroud, C., Crockett, J., 1985. Cultivation in a semi-defined medium of animal infective forms of *Trypanosoma brucei, T. equiperdum, T. evansi, T. rhodesiense* and *T. gambiense*. European Molecular Biology Organization Journal 4, 1273–1277.

Bastida, J., Codina, C., Porras, C.L., Paiz, L., 1996. Alkaloids from *Hippeastrum solandriflorum*. Planta Medica 62, 74–75.

Bastida, J., Viladomat, F., Codina, C., 1998. *Narcissus* alkaloids. In: Atta-ur-Rahman (Ed.), Studies in Natural Product Chemistry, Vol. 20. Elsevier Science, Amsterdam, pp. 323–405.

Bax, A., Subramanian, S., 1986. Sensitivity-enhanced two-dimentional heteronuclear shift correlation NMR spectroscopy. Journal of Magnetic Resonance 67, 565–569.

Bax, A., Summers, M.F., 1986. ^1H and ^{13}C assignments from sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by multiple quantum NMR. Journal of the American Chemical Society 108, 2093–2094.

Chen, J., Chen, K., Jiang, H., Lin, M., Ji, R., 1997. Theoretical investigation on interaction binding of analogs of AT-1840 to double-stranded polynucleotide. Progress in Natural Science 7, 329–335.

Couture, A., Deniau, E., Lebrun, S., Grandclaudon, P., 1999. Total syntheses of (±)-cherylline and (±)-latifine. Journal of the Chemical Society Perkin Transactions I, 789–794.

De, Angelis, G.G., Wildman, W.C., 1969. Circular dichroism studies I. A quadrant rule for the optically active aromatic chromophore in rigid polycyclic systems. Tetrahedron 25, 5099–5112.

Ghosal, S., Singh, S.K., Kumar, Y., Unnikrishnan, S., Chattopadhyay, S., 1988. The role of ungeremine in the growth-inhibiting and cytotoxic effects of lycorine: evidence and speculation. Planta Medica 54, 114–116.

Honda, T., Namiki, H., Satoh, F., 2001. Palladium-catalyzed intramolecular δ-lactam formation of aryl halides and amide-enolates: syntheses of cherylline and latifine. Organic Letters 3, 631–633.

Jacob, J.N., Nichols, D.E., Kohli, D., Glock, D., 1981. Dopamine agonist properties of *N*-alkyl-4-(3,4-dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinolines. Journal of Medicinal Chemistry 24, 1013–1015.

Kihara, M., Ikeuchi, M., Yamauchi, A., Nukatsuka, M., Matsumoto, H., Toko, T., 1997. Synthesis and biological evaluation of 7-hydroxy-3,4-diphenyl-1,2-dihydroisoquinolines as new 4-hydroxytamoxifen analogues. Chemical and Pharmaceutical Bulletin 45, 939–943.

Kobayashi, S., Tokumoto, T., Kihara, M., Imakura, Y., Shingu, T., Taira, Z., 1984. Alkaloidal constituents of *Crinum latifolium* and *Crinum bulbispermum* (Amaryllidaceae). Chemical and Pharmaceutical Bulletin 32, 3015–3022.

Labraña, J., Choy, G., Solans, X., Font-Bardia, M., de la Fuente, G., Viladomat, F., Codina, C., Bastida, J., 1999. Alkaloids from *Narcissus bujei* (Amaryllidaceae). Phytochemistry 50, 183–188.

Maryanoff, B.E., Vaught, J.L., Shank, R.P., McComsey, D.F., Costanzo, M.J., Nortey, S.O., 1990. Pyrroloisoquinoline antidepressants. 3. A focus on serotonin. Journal of Medicinal Chemistry 33, 2793–2797.

Räz, B., Iten, M., Grether-Bühler, Y., Kaminsky, R., Brun, R., 1997. The Alamar Blue assay to determine drug sensitivity of African trypanosomes (*T. b. rhodesiense* and *T. b. gambiense*) in vitro. Acta Tropica 68, 139–147.

Ridley, R.G., Hofheinz, W., Matile, H., Jacquet, C., Dorn, A., Masciadri, R., Jolidon, S., Richter, W.F., Guenzi, A., Girometta, M.A., Urwyler, H., Huber, W., Thaitong, S., Peters, W., 1996. 4-Aminoquinolone analogs of chloroquine with shortened side chains retain activity against chloroquine-resistant *Plasmodium falciparum*. Antimicrobial Agents and Chemotherapy 40, 1846–1854.

Ruchirawat, S., Tontoolarug, S., Sahakitpichan, P., 2001. Synthesis of 4-aryl-tetrahydroisoquinolines: application to the synthesis of cherylline. Heterocycles 55, 635–640.

Wildman, W.C., Brown, C.L., 1968. Mass Spectra of 5,11b-methanomorphanthridine alkaloids. The structure of pancracine. Journal of the American Chemical Society 90, 6439–6446.

Xu, B., Wang, X.W., 1997. Lycobetaine acetate. Antineoplastic alkaloid. Drugs of the Future 22, 123–129.

Zára-Kaczián, E., György, L., Deák, G., Seregi, A., Dóda, M., 1986. Synthesis and pharmacological evaluation of some new tetrahydroisoquinoline derivatives inhibiting dopamine uptake and/or possessing a dopaminomimetic property. Journal of Medicinal Chemistry 29, 1189–1195.

Zee-Cheng, R.K.Y., Yan, S.J., Cheng, C.C., 1978. Antileukemic activity of ungeremine and related compounds. Preparation of analogues of ungeremine by a practical photochemical reaction. Journal of Medicinal Chemistry 21, 199–203.