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Isolation of optically active nevirapine, a dipyridodiazepinone metabolite from the seeds of *Cleome viscosa*

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ABSTRACT

The optically active nevirapine, a natural analogue of the previously designed and synthesized optically inactive nevirapine, a non-nucleoside inhibitor of HIV-1 reverse transcriptase, has been isolated and fully characterized from the seeds of *Cleome viscosa*. The structure of natural nevirapine has been confirmed by X-ray crystallography. It is an interesting discovery to find out that optically inactive nevirapine was designed and synthesized before the isolation of optically active nevirapine from the seeds of *C. viscosa*.

1. Introduction

Cleome viscosa (Syn. *Cleome icosandra*) (Capparidaceae) is an annual wasteland weed with yellow flowers and strong penetrating odour. This weed is very common in India. The leaves of the plant are rubefacient, vesicant and sudorific. The seeds are small, dark brown or black and granular. They are reported to have rubefacient, vesicant and anthelminthic properties. The seeds are occasionally used as a condiment in curries.[1]

Chemical investigation of the plant has disclosed the presence of several interesting classes of chemical compounds, macrocyclic diterpene cleomaldic acid,[1a] glycoflavanones,[2] lipoflavanones,[3] glucosinolates,[4] etc. Previous work on the aerial parts of *C.viscosa* has resulted in the isolation of a diterpene lactone cleomeolide,[5] which has anticancer properties. Phytochemical investigation of the seeds of the plant has also resulted in the isolation of a new class of chemical entities known as coumarinolignoids in which a C_6–C_3 unit is linked with a coumarin moiety through a dioxane bridge. Four coumarinolignoids cleomiscosins A, B, C and D have been isolated and identified from the seeds of *C.viscosa*.[6,7] Cleomiscosins A, B and C possess liver protective activity.[8] The mixture of the three coumarinolignoids cleomiscosins A, B and C is termed as Cliv-92, which has hepatoprotective activity comparable to that of silymarin the antihepatotoxic drug currently in use throughout the world.

2. Results and discussion

As a part of our ongoing studies regarding optimization of batch process parameters for the large scale isolation of Cliv-92, we have undertaken an up scaling process from the dried seeds of this plant at a level of 100 kg/batch.[9] The crude MeOH extract (12 kg on dry weight) obtained after the fixed oil expulsion and hexane defatting was adsorbed in Celite and the adsorbed material was partitioned with toluene, EtOAc and MeOH, respectively. The EtOAc extract obtained was subjected to column chromatography and was eluted with mixtures of hexane and EtOAc in the ratio of 1:1, 1:3 and finally with EtOAc. From the fractions obtained from hexane/EtOAc (1:1) eluate, we have isolated a new optically active natural product (**1**), mp 241–243 °C; $[\alpha]_D^{28}$ +7.04 (*c* 0.0011, $CHCl_3$). The molecular structure of this metabolite was proposed initially on the basis of comprehensive analysis of the ^1H and ^{13}C NMR, IR, UV and DART-HRMS spectra. A single crystal X-ray structure analysis was further carried out to confirm the proposed structure.

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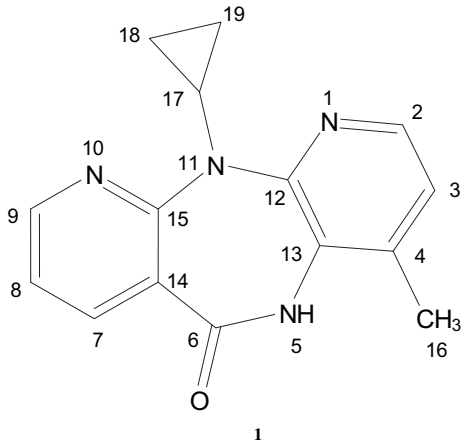

1

Structure of the isolated optically active (+) nevirapine (**1**) from the seeds of *C. viscosa*.

The metabolite **1** obtained as transparent needles was assigned the molecular formula $C_{15}H_{14}N_4O$ from its DART-HRMS spectrum *m*/*z* 267.1231, $[M+H]^+$. The UV [λ_{max} 325, 275 (sh) and 240 nm] and IR spectra of the molecule indicated that it possessed α, β unsaturated lactam system. The nature of 15C atoms present in the molecule was revealed from ^{13}C NMR spectrum of the compound, which showed aromatic methyl (δ 18), a cyclopropyl ring (δ 9.4, 9.2, 29.9) and five CH groups (δ 119.3, 120.7, 140.6, 144.7, and 152.4), one lactam carbonyl (δ 169.4) and five quaternary carbons (δ 122.4, 125.3, 139.9, 154.5 and 161). In the HMBC spectrum H-2 (δ 8.16) showed cross peaks with C-3 (δ 120.7), C-4 (δ 139.9) and C-12 (δ 154.5) and H-3 (δ 6.93) to C-2 (δ 140.6), C-4 (δ 139.9), C-16 (δ 18.1) and to the quaternary carbon C-13 (δ 125.3). Also, the HMBC correlation of H-7 (δ 8.11) to C-8 (δ 119.3), C-9 (δ 152.4), C-14 (δ 122.4), C-15 (δ 161.0) and the characteristic correlation of H-7 to the carbonyl C (δ 169.0) indicated the presence of a dipyridodiazepinone system in the molecule. The key correlation diagram for $^1H-^{13}C$ HMBC has been shown in Fig. 1.

The presence of a cyclopropyl group, two pyridine rings, lactam carbonyl and the diazepinone framework accounted for 11° of unsaturation for the molecule. The molecular formula and ^{13}C NMR of the compound **1** showed a close resemblance with nevirapine **1**,[10] except the optical rotation.[11] Compound **1**, was crystallized from ethyl acetate as needles. The detailed structure and stereochemistry of compound **1** was established unambiguously by single crystal X-ray crystallography. An ORTEP diagram of molecule **1** is given in Fig. 2.

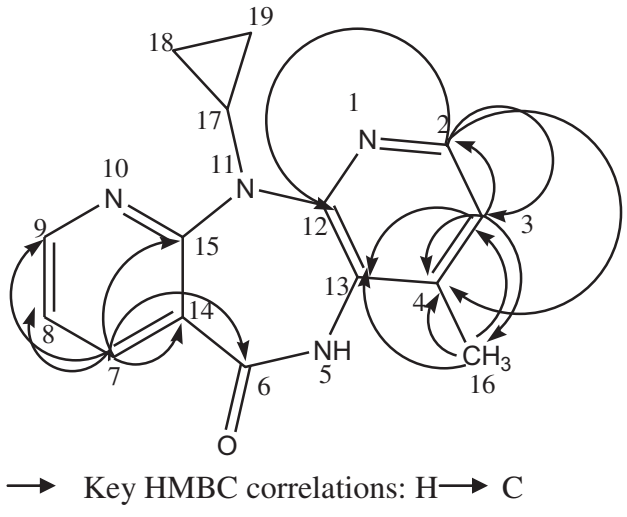

Fig. 1. Key HMBC correlations.

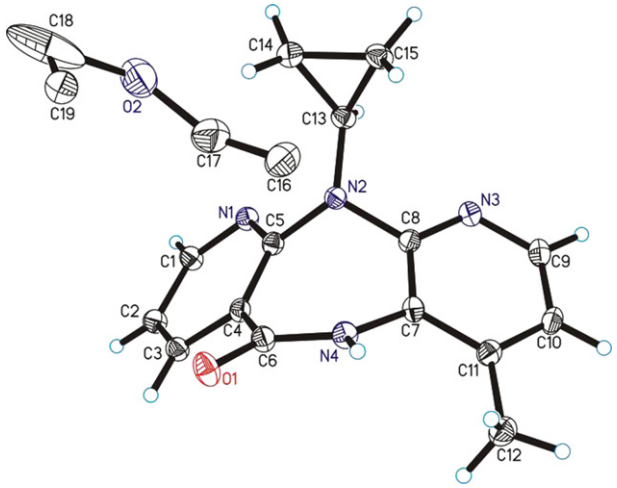

Fig. 2. ORTEP-Diagram: stereoscopic view of the molecule (**1**) in atomic numbering scheme at 30% probability with disordered solvent molecule.

3. Conclusion

From the X-ray and NMR analysis of the molecule,[12–14] the structure of **1** was established as nevirapine (**1**). The optically inactive nevirapine was designed as a selective non-nucleoside inhibitor of HIV-1 reverse transcriptase and is in clinical use.[10] This is a very interesting discovery that naturally occurring optically active nevirapine has been biosynthesized in the seeds of *C.viscosa* and the optically inactive nevirapine was designed as a selective non-nucleoside inhibitor of HIV-1 reverse transcriptase. It is also a remarkable finding that the seed of *C.viscosa* is the source of optically active nevirapine, which was also designed and synthesized[10] before its isolation from natural source. It is worthwhile to mention here that nevirapine was isolated in small quantities when we were isolating the coumarinolignoids from 10 kg seeds of *C.viscosa*. However, its structure could not be determined due to paucity of the sample.

The discovery of optically active nevirapine from natural source clearly demonstrates that nature still holds the key to the discovery of unique biologically active molecules.[15]

4. Experimental

4.1. General

Melting points are uncorrected and were recorded on a Buchi—Melting point Apparatus. U.V. spectra were recorded on a Spectronic® GENESYS™ with a 10 mm quartz cell and IR spectra were recorded on a Perkin—Elmer Spectrum BX FT-IR spectrometer. Sample pellets were prepared in KBr using hydraulic pellet press of Kimaya manufacturers. Optical rotation was measured on Horiba SEPA-300 high sensitive digital polarimeter. NMR spectra were recorded on a Bruker—Avance 300 MHz FT-NMR using $CDCl_3$, a deuterated solvent, the chemical shift of which was used as an internal standard. HR-ESI-MS data were obtained with a JEOL ACCU TOF DART JMS-T100LC mass spectrometer.

4.2. Plant material

The seeds of *C.viscosa* required for the up scaling process were collected from the outskirts of Lucknow during the month of October. The seed samples have been deposited in the 'National Gene Bank of Medicinal and Aromatic Plants' CIMAP, Lucknow (accession number CIMAP 3426). The harvested plants were dried in open sun and seeds were removed by thrashing them.

4.3. Extraction and isolation of nevirapine

The process was primarily aimed at the extraction of coumarinolignoids, which involved the fixed oil removal from 100 kg dried seeds of the plant by subjecting them to an oil expeller. The miscella (89 kg) was charged into pilot scale extractors of 100 kg/batch of a multiutility solid–liquid extraction plant where the initial defatting of the crude material was done in hexane (200 L×6). The marc left was next extracted with MeOH (210 L×6). The solvent free dark green coloured crude methanolic extract (12 kg) was then adsorbed with Celite (6.4 kg), which served as a base for the solid-matrix partition process. A nutsche type filtration unit of 50 kg capacity was packed with Celite (6.0 kg) in toluene and the adsorbed extract was then partitioned sequentially by toluene (25 L×4), EtOAc (25 L×4) and MeOH (25 L×4), respectively. The vacuum concentrated toluene, EtOAc and MeOH extracts were in 1.98%, 5.69%, 0.55% yields, respectively. The semi solid concentrated EtOAc extract was adsorbed with 11.0 kg silica gel and was loaded onto pilot scale stainless steel columns of 50 kg gel holding capacity. Silica gel (60–120 mesh) (23 kg) was packed with hexane along with the adsorbed slurry and the column was eluted with a mixture of hexane–EtOAc in the ratio of 1:1, 1:3 and finally with EtOAc. TLC analysis of the fractions of hexane/EtOAc (1:1) eluate in $CHCl_3$/acetone (93:7) showed almost a major spot in U.V. at a R_f of 0.46 and it was stained with Dragendorff's reagent. The fractions containing the above spot were pooled and concentrated. Recrystallisation of the concentrate from EtOAc yielded colourless needles of nevirapine (1) (3.54 gm, 0.00397%); mp 241–243 °C; R_f 0.46 (7% $CHCl_3/CH_3COCH_3$); $[\alpha]_D^{28}$ +7.04 (*c* 0.0011, $CHCl_3$); IR ν_{max} (KBr) 3047, 2921, 2868, 1744, 1656, 1586, 1463, 1417 cm^{-1}; UV($CHCl_3$) λ_{max} (log ε) 325 nm (3.5), 275 nm (sh,3.5) 240.4 nm (4.0); ^1H NMR δ_H (300 MHz, $CDCl_3$) 8.54 (1H, dd, *J* 2.1, 7.8 Hz, 9-H), 8.16 (1H, d, *J* 4.8 Hz,2-H), 8.11 (1H, dd, *J* 2.1, 7.8 Hz, 7-H), 7.06 (1H, dd, *J* 4.8, 7.5 Hz, 8-H), 6.93 (1H, d, *J* 4.8 Hz,3-H), 3.75 (1H, m, 17-H), 2.38 (3H, s, CH_3), 0.98 (2H, m, CH_2), 0.45 (2H, m, CH_2); ^{13}C NMR δ_C (75 MHz, $CDCl_3$) 169.4, 161.0, 154.5, 152.4, 144.7, 140.6, 139.9, 125.3, 122.4, 120.7, 119.3, 29.9, 18.1, 9.4, 9.2; DART-HRMS: $[M+H]^+$, found 267.1231. $C_{15}H_{15}N_4O$ requires 267.1246 (Δ −1.5 mmu).

4.4. Single crystal X-ray structure determination of nevirapine (1) at 293 (2) K

The X-ray data were collected at 293 K with a Bruker Smart Apex CCD diffractometer with graphite monochromator and Mo Kα radiation (λ=0.71073 Å), SMART32 (Bruker) and SAINT (Bruker) softwares. The structure was solved by direct methods and refinements by full-matrix least-squares methods on F^2 using SHELXTL-NT [Bruker AXS Inc.: Madison, Wisconsin, USA 1997]. Crystal data: $C_{15}H_{14}N_4O$, Empirical formula $C_{17}H_{14}N_4O_2$, M_r=306.32, triclinic, space group *P*(−1), *a*=7.767(3) Å, *b*=8.420 (4) Å, *c*=12.466(5) Å, α (°)=84.70, β (°)=89.37, γ (°)=68.39, *V* (Å^3)= 754.5(5), *Z*=2, ρ_{calcd}=1.348 Mg/m^3, λ (Mo Kα)=0.71073 Å, μ=0.092 (mm^{-1}).Data collection and reduction: crystal size, 0.225×0.20×0.275 mm^3, θ range=2.61–28.31, 4942 reflections collected, 3553 independent reflections (R_{int}=0.0229), *R* indices (all data)=R_1=0.1219 and wR_2=0.3301 final *R* indices [$I > 2\sigma(I)$] R_1=0.0931 and wR_2=0.2450 for 233 variable parameters, GOF=1.12. The X-ray crystallographic file of the synthetic nevirapine has already been deposited in Cambridge Crystallographic Data Centre, CCDC Nos. 649751–649756.[13]

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Supplementary data

Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2010.11.020.

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