Structure and Stereochemistry of Nardostachysin, a New Terpenoid Ester Constituent of the Rhizomes of *Nardostachys jatamansi*

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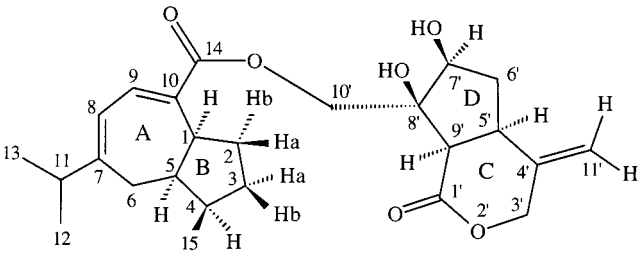
The structure and stereochemistry of a new terpenoid ester, nardostachysin (**1**), isolated from the rhizomes of *Nardostachys jatamansi*, were established as the 7′,8′-dihydroxy-4′-methylene hexahydrocyclopenta-[*c*]pyran-1′-one-8′-methyl ester of 7,9-guaiadien-14-oic acid, by spectral and chemical studies.

Nardostachys jatamansi DC. (Valerianaceae), popularly known as "Jatamansi", is native to the Alpine Himalayas. The rhizomes and roots are used in the Indian system of medicine as a sedative and an antistress remedy.[1] The rhizomes also constitute an important ingredient in Ayurvedic formulations as an anticonvulsant.[2]

The occurrence of a number of sesquiterpenes, lignans, and neolignans in the roots of this species has been observed,[1] but a detailed chemical investigation on the rhizomes of *N. jatamansi* has not yet been reported. This stimulated the authors to undertake a detailed chemical examination of the rhizomes from which a new terpenoid ester, nardostachysin (**1**), was isolated. The structure and stereochemistry were established from its chemical and spectral analyses.



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The methylene chloride fraction of an ethanolic extract of the powdered rhizomes of *N. jatamansi* was chromatographed over Si gel to yield nardostachysin (**1**) as white crystals, which analyzed for $C_{25}H_{34}O_6$. The molecular ion peak was observed at *m/z* 430 [M^+] from EIMS. HRMS was also obtained, and the presence of ion fragments at *m/z* 234 ($M^+ - C_{10}H_{12}O_4$), 216 ($M^+ - C_{10}H_{12}O_4 - H_2O$), 173 ($M^+ - C_{10}H_{12}O_4 - H_2O - C_3H_7$), and 145 ($M^+ - C_{10}H_{12}O_4 - H_2O - C_3H_7 - CO$), suggested the occurrence of C_{15} and C_{10} units in nardostachysin (**1**). The structure of these moieties was determined from ^{1}H, ^{13}C, and 2D NMR spectra (Table 1).

In the ^{13}C NMR spectrum of nardostachysin (**1**) signals for an ester and a lactone carbonyl (discernible in the IR spectrum at 1760 cm^{-1}) appeared at δ 170.1 (s) and 170.3 (s) and four olefinic carbons appeared at δ 133.4 (s), δ 134.9 (d), δ 116.9 (d), and δ 160.9 (s) (Table 1), thereby accounting for two olefinic groups in the molecule. In the ^{13}C NMR spectrum the chemical shift for one exocyclic methylene group at C-11′ was observed at δ 114.4 (t) with the quaternary carbon at C-4′ associated with the methylene group seen at δ 140.3 (s). The proton scalar-coupled spin network and the association with ^{1}H NMR with directly bonded carbons via HMQC are presented in Table 1. In this context it needs special mention that the ^{1}H NMR spectrum exhibited two methyl signals at δ 1.05 (6H, d, J = 7.5 Hz) and one methyl at δ 1.07 (3H, d, J = 7.0 Hz). Signals for two hydroxyl groups were discernible at δ 2.98 (br) and δ 3.37 (br) and were exchangeable with deuterium oxide (Table 1). The HMBC spectrum (Table 1) afforded several important pieces of information regarding the structure of nardostachysin (**1**). The analysis of this spectrum revealed long-range coupling of H-6 to C-4 and C-1; H-8 to C-6, C-10 and C-11; H-9 to C-1 and C-14; H-2 to C-5; H-3 to C-5, C-1, and C-15; and H-15 to C-3. All these correlations established a seven/five (A/B) fused ring system in nardostachysin (**1**). Initially, it was revealed that part of the ^{1}H and ^{13}C NMR data were identical with those of jatamansic acid,[4] thereby confirming the structure of the sesquiterpene unit as a 7,9-guaiadien-14-oic acid derivative. The remaining part of the molecule was recognized as a bicyclic monoterpene alcohol derivative with a six/five-membered fused ring (C/D) system from ^{1}H and ^{13}C NMR, HMBC, HMQC, and ^{1}H−^{1}H COSY experiments. HMBC NMR data (Table 1) show long-range coupling of H-3′ to C-1′, C-5′, and C-11′; H-4′ to C-1′; H-11′ to C-3′ and C-5′; H-6′ to C-8′ and C-9′; H-9′ to C-7′ and C-10′; and H-10′ to C-9′. From the long-range coupling between H-9′ and H-10′ to C-14, it could be established that the 7,9-guaiadien-14-oic acid unit is involved in ester formation with C-10′ of the monoterpene unit.

Two hydroxyl groups in the monoterpene unit of **1**, already ascertained from the ^{1}H NMR spectrum, could be located at C-7′ and C-8′ from 2D NMR spectral analysis. NOE experiments (Table 1) were studied to determine the stereochemistry of both sesquiterpene (A/B ring) and monoterpene (C/D ring) units. It was possible to assess their relative configuration from NOE difference experiments. It was revealed that H-1 (δ 3.04) was α oriented due to its NOE correlation with H-5 (δ 1.68) and Hb-2 (δ 2.34). The H_3-15 signal (δ 1.07) exhibited a strong NOE correlation with Hb-3 (δ 1.76) and Ha-2 (δ 1.49), indicating β orientation in the sesquiterpene unit. From NOE experi-

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Table 1. ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR Data for Nardostachysin (**1**) ($CDCl_3$)[a]

position	δ_C	δ H (*J* Hz)	HMBC	^{1}H-^{1}H COSY	NOESY
1	45.9 d	3.04 m	Hb-3,Ha-6,Hb-6,H-9	Ha-2, Hb-2, H-5	Hb-2,Ha-3[b],H-4[b],H-5
2	32.5 t	Ha 1.49 m Hb 2.34 m	Ha-3	H-1,Hb-2,Ha-3,Hb-3 H-1,Hb-2,Ha-3,Hb-3	Hb-3, H_3-15[b] H-1,Ha-3,H_3-15[b]
3	27.9 t	Ha 1.12 m Hb 1.76 m	H_3-15	Ha-2,Hb-2,Hb-3,H-4 Ha-2,Hb-2,Ha-3,H-4	H-1[b],Hb-2,H-4,H-5[b] Ha-2, H_3-15
4	38.6 d	2.22 m	Ha-3, Hb-6	Ha-3, Hb-3, H-5	H-1[b],Hb-2[b],Ha-3,H-5[b]
5	43.5 d	1.68 dt (11.0, 4.5)	Ha-2,Hb-3,Ha-6,Hb-6 H_3-15	H-1, Ha-6	
6	26.1 t	Ha 1.78 m Hb 2.23 m	H-8, H-11	H-5, Hb-6, H-8, H-5, Ha-6	H-8, H_3-15[b,c] Ha-3[b,c],H-4[b],H-5, H-9[b,c]
7	160.9 s		Ha-6,Hb-6,H-8,H-11 H_3-12,H_3-13		
8	116.9 d	5.75 d (8.0)	H-11	Ha-6, Hb-6, H-9	Ha-6, H-11[b]
9	134.9 d	7.10 dd (8.0, 1.0)	H-8	H-8	H-1[b,c], Hb-6[b,c]
10	133.4 s		H-8, H-9		
11	38.3 d	2.43 seventh (7.5)	H-8, H_3-12, H_3-13	Hb-6, H_3-12,H_3-13	
12	20.9 q	1.05 d (7.5)	H-11	H-11	
13	20.7 q	1.05 d (7.5)	H-11	H-11	
14	170.1 s		H-1,H-9,Ha-10′, Hb-10′		
15	16.0 q	1.07 d (7.0)	Ha-3	H-4	Ha-2[b], Hb-3
1′	170.3 s		Ha-3′,Hb-3′,Ha-5′, Hb-9′		
3′	70.0 t	Ha 4.50 d (12.0) Hb 5.05 d (12.0)	H-11′	Hb-3′ Ha-3′	H-4[-b], H-9[-b,c] Ha-6[-b,c]
4′	140.3 s		Ha-3′,Hb-3′,Ha-11′, Hb-11′		
5′	36.5 d	3.06 m	H-3′,Ha-6′,H-9′, Ha-11′,Hb-11′	Ha-6′,Hb-6′,H-9′	Ha-3[-b], Hb-6′, H-9′
6′	36.0 t	Ha 1.94 dt (13.0, 12.0) Hb 2.26 m		H-5′,Hb-6′,H-7′ H-5′,Hb-6′,H-7′	Hb-3[-b,c] Ha-3[-b,c],H-5′,H-7′, H-9[-b,c]
7′	72.6 d	3.85 dd (8.0,12.0)	Ha-6′,OH-8′,H-9′, Ha-10′,Hb-10′	Ha-6′, Hb-6′, OH-7′	Hb-6′
8′	80.7 s		Hb-6′,OH-8′,H-9′, Ha-10′,Hb-10′		
9′	47.0 d	3.13 d (11.0)	H-5′,Hb-6′,OH-8′, Ha-10′,Hb-10′	H-5′	
10′	64.2 t	Ha 4.20 d (11.0) Hb 4.43 d (11.0)	H-9′	Hb-10′ Ha-10′	H-5[-c], H-7′, H-9′
11′	114.4 t	Ha 5.07 s Hb 5.12 s	Ha-3′, Hb-3′	Hb-11′ Ha-11′	
7′		OH 2.98 br		H-7′	
8′		OH 3.37 br			

[a] Assignments based on 2D experiments (COSY, HMQC, and HMBC). [b] Weak. [c]Negative.

ments, it was revealed that H-5′, H-7′, and H-9′ in the monoterpene unit are spatially correlated, having the α configuration, and the hydroxyl groups occurring therein at 7′ and 8′ were established as cis, having the *β* orientation. In this context it is worthwhile to mention that the *β* isomer is more stable than α by 2 kcal/mol from conformational energy calculations.[5,6] Accordingly, from detailed spectral and chemical studies the structure and stereochemistry of nardostachysin (**1**) were established as the 7′,8′-dihydroxy-4′-methylene hexahydrocyclopenta[*c*]-pyran-1′-one-8′-methyl ester of 7,9-guaiadien-14-oic acid.

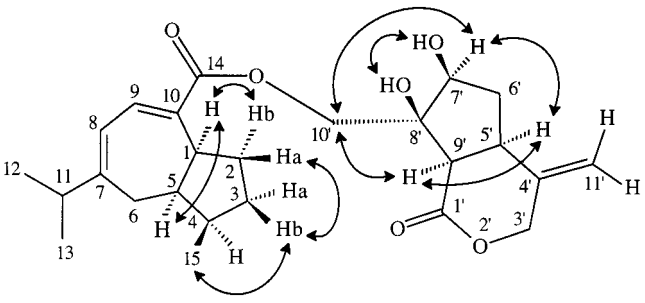

Figure 1. NOESY correlations for nardostachysin (**1**). (Arrows indicate positive NOEs.)

Experimental Section

General Experimental Procedure. Melting points were determined using an electrical melting point apparatus, and the optical rotation was measured on a Perkin-Elmer 241 polarimeter. UV spectra were recorded using a Hitachi U-2000 spectrophotometer and IR spectra using KBr pellets on a Perkin-Elmer 782 spectrophotometer. ^{1}H and ^{13}C NMR studies were performed on a 400 MHz Varian XL-400 spectrometer. EIMS (EI, 70 eV) were obtained on a AEI MS 3074 spectrometer and HRMS on a Finnigan MAT-H-SQ-30 mass spectrometer.

For energy calculations the DISCOVER module of the Insight II package (Molecular Simulation Inc.) running on a Silicon Graphics 02 workstation was operated. Si gel (60−120 mesh, S.D. Fine-Chemicals Ltd., Mumbai, India) was used for

column chromatography. Si gel G (Spectrochem Pvt. Ltd., Mumbai, India) was used for TLC.

Plant Material. The rhizomes of *N. jatamansi* DC. were collected in April, 1999, and were identified by Dr. Nanda Dulal Paria, Department of Botany, Ballygunge Science College, Calcutta University. A voucher specimen (NH-6) has been deposited at the Centre of Advanced Studies on Natural Products, Department of Chemistry, Calcutta University.

Extraction and Isolation. The dried rhizomes (5 kg) of *N. jatamansi* were cut into small pieces, powdered, and extracted with ethanol at room temperature. The alcohol extract on removal of the solvent under reduced pressure afforded a reddish brown residue (125 g, 2.5%). A portion of this residue (37 g) was partitioned between aqueous ethanol and methylene chloride, 2:3 (500 mL). The methylene chloride layer on evaporation yielded a pale brown residue (3.7 g). It was purified by column chromatography over Si gel. The column was eluted with hexane and subsequently with mixtures of benzene and ethyl acetate in various proportions. Nardostachysin (**1**) migrated from the column on elution with benzene and ethyl acetate (9:1) and crystallized from ethyl acetate as white crystals (1.0 g). The homogeneity of nardostachysin (**1**) was confirmed by TLC over Si gel G using benzene and ethyl acetate (1:1) as developing solvent (R_f 0.63).

Nardostachysin (1): white crystals; mp 192 °C; $[\alpha]_D^{23}$ −147.9° (*c* 113.6 × 10^{-3} g/100 mL, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 215 (3.2) nm; IR (KBr) 3500, 2980, 1760, 1680 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 1; EIMS *m*/*z* 430 [M^+]; HRMS *m*/*z* 234 ($M^+ - C_{10}H_{12}O_4$), 216 ($M^+ - C_{10}H_{12}O_4 - H_2O$), 173 ($M^+ - C_{10}H_{12}O_4 - H_2O - C_3H_7$), 145 ($M^+ - C_{10}H_{112}O_4 - H_2O - C_3H_7 - CO$).

Hydrolysis of Nardostachysin (1) with 5% Aqueous Sodium Carbonate Solution. Nardostachysin (**1**) (0.25 g) was covered with methylene chloride (50 mL) and stirred with 5% aqueous sodium carbonate (25 mL) solution for 5 h and kept overnight. The alkaline solution was extracted with methylene chloride to remove neutral materials, and the alkaline solution was subsequently acidified at 0 °C with 2 N hydrochloric acid (30 mL). The acidified solution was extracted with methylene chloride (2 × 50 mL), washed with water (2 × 25 mL), and dried over anhydrous sodium sulfate. The residue (0.11 g) from methylene chloride crystallized from petroleum ether as colorless needles, mp 124 °C (0.08 g). It was identified as jatamansic acid from its mp, mixed mp, and IR and NMR spectra and by comparison with an authentic sample.[3,4] All attempts to isolate the monoterpene alcohol derivative from the neutral portion in methylene chloride were unsuccessful.

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