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ISOLATION AND STRUCTURE (X-RAY ANALYSIS) OF *ENT*-NORSECURININE, AN ALKALOID FROM *PHYLLANTHUS NIRURI*

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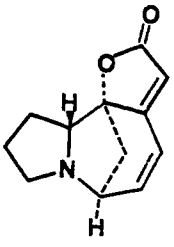
ABSTRACT.—The isolation and structure determination of the alkaloid *ent*-norsecurinine (**4**) from *Phyllanthus niruri* L. is described. The structure and absolute stereochemistry have been confirmed by an X-ray analysis of *ent*-norsecurinine hydrochloride.

Phyllanthus niruri L. (Syn. *Phyllanthus asperulata*) (Euphorbiaceae) is a small plant that grows throughout the hotter parts of India during the monsoon season. The root of the plant is reputed in folklore to be a remedy for jaundice, although no clinical reports appear in the literature (1). A number of flavonoids, quercetin, quercitrin, isoquercitrin, astragalin, rutin (2), kaempferol-4′-rhamnopyranoside, eridictyol-7-rhamnopyranoside (3), fisetin-4′-*O*-glucoside (4), 5,6,7,4′-tetrahydroxy-8-(3-methylbut-2-enyl)flavanone-5-*O*-rutinoside (nirurin) (5); the lignans, phyllanthin and hypophyllanthin (6); and the triterpene lup-20(29)-en-3-β-ol (7) have been isolated from *P. niruri*. An unnamed alkaloid was isolated earlier from this plant, and solely on the basis of spectral data, it was assigned the antipodal structure of norsecurinine (**1**) (8), the lower A ring homologue of securinine (**2**) (9). The opposite rotation alone does not establish with certainty the absolute stereochemistry of the molecule, especially because four theoretical isomers are possible for the alkaloid.



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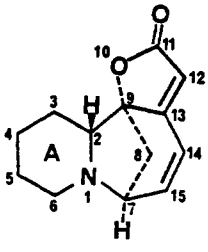

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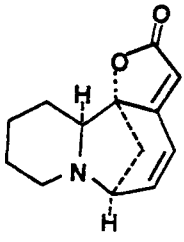

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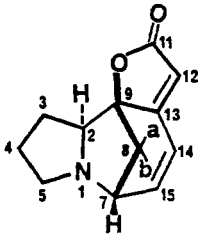
We report here the isolation and structure determination of *ent*-norsecurinine from *P. niruri*. The whole plant was extracted with MeOH and the crude alkaloidal fraction was isolated as an oil. Chromatographic separation on silica gel afforded an alkaloid as pale yellow oil that was rather unstable on storage, as in the case of norsecurinine (9,10). Securinine alkaloids, however, are crystalline and are stable. For purification,

[1]Deceased.



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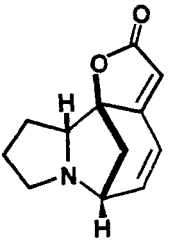

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the oil was converted to its crystalline hydrochloride, mp 275-276°, and the free base was regenerated after basification and extraction with CH_2Cl_2. The analysis of the hydrochloride agreed with the formula $C_{12}H_{13}NO_2 \cdot HCl$. The alkaloid showed the presence of an extended α,β-unsaturated-γ-lactone moiety: ir (nujol) 1805, 1760 cm^{-1} and uv λ max 256 nm (log ε, 4.13). Catalytic hydrogenation afforded the tetrahydro derivative. The mass spectrum of the alkaloid showed major peaks at *m/z* 203 (M^+), 134, 106, 78, and 70. These fragments strongly suggested that the alkaloid belongs to the securinega type and the ring A possesses a pyrrolidine ring as in **1** (9). The ^{1}H-nmr spectrum of the base is in agreement with the gross formulation **1** and double resonance studies confirmed the assignments (Table 1).

TABLE 1. ^{1}H-nmr Spectrum (360 MHz) of **4** in $CDCl_3$.

Assignment of the proton	No. of protons	Chemical shift (δ)	Multiplicity	Coupling	*J* Hz	Decoupling results	
						Irr.	Change
H-15	1	6.75	d,d	H_{15}, H_{14}	9.5		↑
				H_{15}, H_7	6.0		↑
H-14	1	6.49	d	H_{14}, H_{15}	9.5		
H-12	1	5.7	s				
H-7	1	3.67	d,d	H_7, H_{15}	6.0	↓	
				H_7, H_{8a}	5.0	↓	
H-5	1	3.31				↓	↑
			m				↑
	1	2.56					
H-2	1	3.24	d,d	H_2, H_{3a}	14.0	↓	
H-8 a,b	1	2.54		H_{8a}, H_{8b}	10.7		↑
			m				↑
	1	1.76					↑
H-3	2	2.0	m				
H-4	2	1.81	m			↓	↑

The ^{13}C-nmr spectrum showed 12 lines for the twelve carbon atoms of the molecule. The proton decoupled and SFORD spectra are in conformity with the gross formulation as in structure **1** (Table 2). The ^{13}C-assignments have been made as in the case of other securinega alkaloids (11), securinine (**2**) and allosecurinine (**3**).

The alkaloid showed a molecular rotation $[\alpha]^{20}D$ +255.8° (c 4.2, MeOH) and norsecurinine (**1**) is known to have the rotation $[\alpha]^{23}D$ −272° (11-13); the low optical rotation of $[\alpha]D$ − 19.5° reported for norsecurinine by Iketubosin and Mathieson is probably recorded on an impure sample (14). The optical rotatory dispersion (ord) curve of the alkaloid hydrochloride showed a single strong positive Cotton efffect and as shown

TABLE 2. Carbon-13 Chemical Shifts and Assignments for *Ent*-Norsecurinine Hydrochloride (**4**), Securinine (**2**) (11) and Allosecurinine (**3**) (11)

Carbon	Compounds		
	4[a]	**2**[b]	**3**[b]
2	69.0 d	62.7	60.5
3	30.3 t[c]	27.4[c]	20.8
4	28.3 t[c]	24.6[c]	21.9
5	58.1 t	26.0	18.3
6	—	48.8	43.4
7	63.3 d	58.9	58.5
8	37.8 t	42.4	42.4
9	90.2 s	89.5	91.0
11	176.0 s	173.4	172.4
12	113.6 d	105.0	110.6
13	167.8 s	170.2	167.3
14	139.5 d	140.3	148.4
15	128.2 d	121.4	122.4

[a]Solvent D_2O.
[b]Solvent $CDCl_3$.
[c]Assignments tentative.

in Figure 1, was opposite to the ord of norsecurinine hydrochloride (12,13). As the chemical and ord studies have established the absolute configuration of norsecurinine is as in **1** (10,12,13), the alkaloid isolated from *P. niruri* can be represented by the structure **4** indicating N(1)*R*, C(2)*S*, C(9)*R*, C(7)*R* or **5** having N(1)*S*, C(2)*R*, C(9)*R*, C(7)*R* configuration. The stereochemistry at the centers C(7) and C(9) was established by the ord determination. However, the streochemistry at C(2) cannot be defined with certainty. Securinine (**2**) and allosecurinine (**3**), which differ in their

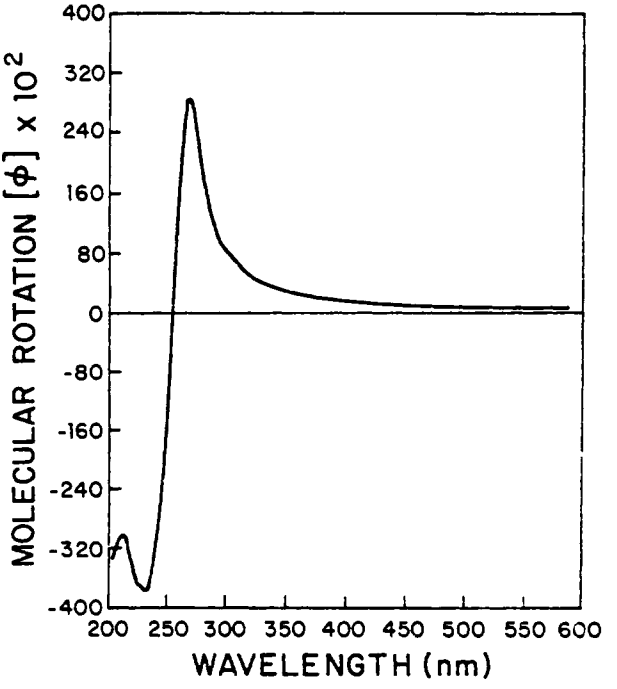

FIGURE 1. Ord curve of *ent*-norsecurinine hydrochloride.

stereochemistry only at C(2), showed a large negative rotation and the antipodal alkaloids virosecurinine and viroallosecurinine, differing in their stereochemistry at C(2) only, exhibited an opposite positive rotation.

In order to confirm the structure and absolute sterochemistry of the alkaloid, we decided to obtain an X-ray crystal structure determination of the hydrochloride. Suitable crystals were grown from MeOH.

A crystal of dimensions $0.18\times0.25\times0.35$ mm was used for determination of cell dimensions and data collection. The hydrochloride [$C_{12}H_{13}NO_2HCl\cdot H_2O$ (257.72)] crystallized in the monoclinic space group $P2_1$ with cell dimensions a=12.898 (3)Å, b=6.766(1)Å, c=13,286(2)Å and β=147.53(2)Å, z=2, volume=622.5Å and Dc=1.375 g cm^{-3}. The cell constants were determined by the least squares fit of 25 centered reflections. Intensity data were collected on an ENRAF-NONIUS CAD-4 diffractometer by the ω-2θ scan technique using μ(CuKα)=27.3 cm^{-1} radiation. Of the 1443 unique reflections collected with $2\theta<154$, 1346 had intensities greater than 2σ (I) and hence were considered observed. During the intensity data collection three reflections monitored every 2 h showed no crystal deterioration. The intensities were corrected for Lorentz polarization effects and an empirical absorption correction was also applied.

The position of chlorine atom was determined from a Patterson map and the structure was developed from sucessive Fourier maps. The asymmetric unit contained one alkaloid molecule, one chlorine atom, and one water molecule for a total of 17 non-hydrogen atoms. A few cycles of full-matrix refinement with isotropic temperature factor for all the atoms dropped the conventional R-factor to 0.13. Changing of isotropic thermal parameters to anisotropic thermal parameters and a few more cycles of refinement brought the R-factor to 0.06. At this stage all the hydrogen atoms were located from difference Fourier maps and were included in the refinement. The final R-factor for 1347 reflections is 0.031 and Rw=0.031.

To determine the absolute configuration of the molecule both enantiomorphs were refined with the unmerged data set. The R-factor for configuration **4** was 0.0319 and Rw=0.0321, while for the enantiomorph R=0.0367 and Rw=0.0366. According to the Hamilton test (15) the Rw ratio of 1.140 implies that the enantiomorph **4** is correct with a probability exceeding 99.5%.

The final atomic parameters are given in Table 3.[2] The average estimated standard deviation in bond lengths and angles involving the non-hydrogen atoms are 0.003Å and 0.1° respectively.

The conformation of the molecule in the correct absolute configuration is shown in Figure 2. The nitrogen atom is protonated with a N-H distance of 0.93Å and there is a tetrahedral arrangement around nitrogen. The average $C(sp^3)$-$N(sp^3)$ distance of 1.516Å is slightly longer than the accepted value of 1.479Å reported by Sutton (16) for the C-N bonds at 4-covalent nitrogen, but agrees well with the value of 1.52Å deduced from a survey of alkaloids (17). The chloride ion forms a good hydrogen bond with the nitrogen atom with the N....Cl distance of 3.050(2)Å and the NH....Cl distance of 2.51Å. The N-H....Cl angle is 162°. It also has a contact distance of 3.283(3)Å with the water molecule with H20W....Cl distance being 2.44Å and the OW-H2....Cl angle of 156°. The crystal packing viewed down the b-axis is shown in Figure 3. The alkaloid molecules are linked through NH....Cl....H-O-H....Cl....H-N hydrogen bonds.

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[2]Atomic coordinates have been deposited with the Cambridge Crystallographic Centre and can be obtained, on request, from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

TABLE 3. Table of Positional Parameters and their Estimated Standard Deviations[a]

Atom	x	y	z	B(A2)
C1	0.91728(5)	−0.250	0.75699(5)	4.44(2)
N1	0.6784(2)	0.0982(4)	0.4855(1)	3.04(5)
C2	0.5856(2)	0.0417(4)	0.3098(2)	2.84(5)
C3	0.3773(2)	−0.0058(5)	0.1648(2)	3.85(7)
C4	0.3837(2)	−0.0615(5)	0.2806(2)	4.26(7)
C5	0.5221(2)	0.0875(5)	0.4364(2)	3.79(6)
C7	0.7673(2)	0.3007(4)	0.5423(2)	3.75(7)
C8	0.6235(2)	0.3941(4)	0.3512(2)	3.52(6)
C9	0.6171(2)	0.2272(5)	0.2701(2)	2.92(5)
O10	0.4710(1)	0.2380(4)	0.0746(1)	3.34(4)
C11	0.5567(2)	0.2277(5)	0.0519(2)	3.55(5)
O11	0.4593(1)	0.2282(4)	−0.1029(1)	4.66(5)
C12	0.7649(2)	0.2185(5)	0.2363(2)	3.80(6)
C13	0.8029(2)	0.2217(5)	0.3649(2)	3.28(6)
C14	0.9775(2)	0.2364(6)	0.5681(2)	4.24(7)
C15	0.9612(2)	0.2746(5)	0.6522(2)	4.44(8)
OW	0.8301(2)	−0.0643(5)	0.9093(2)	8.35(8)
HN1	0.774(2)	0.009(5)	0.584(2)	4.5(7)*
HC2	0.664(2)	−0.074(4)	0.343(2)	3.1(5)*
H1C3	0.306(2)	0.116(4)	0.101(2)	3.2(6)*
H2C3	0.324(2)	−0.100(4)	0.080(2)	4.8(7)*
H1C4	0.245(2)	−0.056(6)	0.192(2)	7.9(9)*
H2C4	0.439(2)	−0.198(5)	0.333(2)	6.5(8)*
H1C5	0.462(2)	0.222(5)	0.388(2)	4.9(6)*
H2C5	0.591(2)	0.048(5)	0.555(2)	4.8(7)*
HC7	0.774(2)	0.368(4)	0.609(2)	4.5(7)*
H1C8	0.662(2)	0.509(4)	0.355(2)	4.1(6)*
H2C8	0.497(2)	0.418(4)	0.271(2)	4.5(7)*
HC12	0.856(2)	0.215(5)	0.265(2)	4.7(6)*
HC14	1.098(2)	0.208(5)	0.630(2)	4.8(7)*
HC15	1.072(2)	0.269(6)	0.785(2)	6.5(7)*
H10W	0.921(2)	0.017(6)	1.018(2)	8(1)*
H20W	0.892(3)	−0.111(7)	0.904(2)	9(1)*

[a]Starred atoms were refined isotropically. Anisotropically refined atoms are given in the form of the isotropic equivalent thermal parameter defined as: (4/3)*[A2*B(1,1)+B2*B(2,2)+C2*B(3,3)+AB(COS Gamma)*B(1,2)+AC(COS Beta)*B(1,3)+BC(COS Alpha)*B(2,3)].

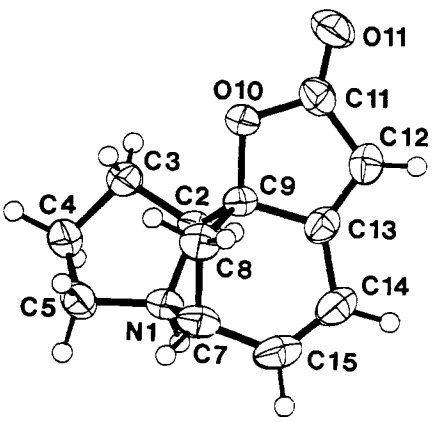

FIGURE 2. Conformation of the molecule in the correct absolute configuration.

As in the structure of securinine hydrobromide (18) the plane involving C9, O10, C11, O11, C12, C13 is inclined by 11.3° to the plane involving C9, C13, C14, C15, C7 implying that the C(12)=C(13) bond and the C(14)=C(15) bond in the diene part are not in a plane. The dihedral angle between these two planes is 10° in securinine molecule. the best plane through the pyrrolidine ring N1, C2, C3, C4, C5 is not coplanar with the plane through the α,β-unsaturated-γ-lactone ring, the dihedral angle between them being 31.2°.

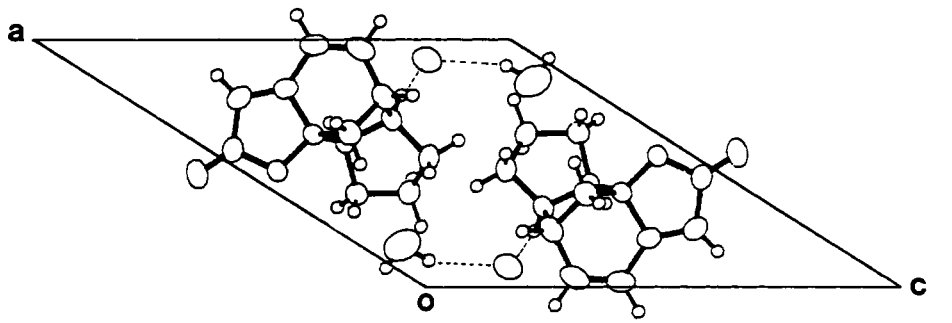

FIGURE 3. Crystal packing is viewed down the b-axis. The dashed lines indicate the hydroxy-bonding between the chlorine atom and the N-H and water molecule.

Because of the useful CNS stimulant and other pharmacological activities of the securinega alkaloids (19,20,21), a number of synthetic routes have been described both in publications and patents (22,23). Norsecurinines however have not been synthesized and recently some attempts have been made toward the synthesis of *ent*-norsecurinine (24).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv and ir spectra were taken on Beckman model DK-2A and Perkin-Elmer model 421 spectrophotometers, respectively. Mass spectra were recorded on an Atlas Varian Mat CH-7 spectrometer using a direct inlet system. ^{1}H-nmr spectra were measured on Varian A-60 and 360 MHz FT-NMR spectrometers and ^{13}C-nmr spectra were recorded on JEOL FT model FX-60 spectrometer with TMS as an internal reference. Ord and cd were measured on JASCO J-20 spectropolarimeter.

EXTRACTION OF *P. NIRURI*.—The plant was collected in July 1976 at Goregaon on the Ciba-Geigy Research Centre Campus. It was identified by Mr. M.R. Almeida. A voucher specimen is on deposit at the Ciba-Geigy Research Centre. The dried and powdered whole plant (5.0 kg) was extracted at room temperature with hexane (10 liters × 3) and the solvent removed to give a dark colored oil (45 g). The plant was further extracted with MeOH (10 liters × 3) at room temperature and the solvent evaporated under vacuum to give a green viscous mass. This was stirred with 10% HCl, the tarry residue discarded and the acidic extract shaken with $CHCl_3$ to remove the neutral fraction. The acidic solution was basified with NH_3 and extracted with $CHCl_3$. Evaporation of the solvent gave the crude alkaloid as a brown oil (2.6 g).

ISOLATION OF *ENT*-NORSECURININE (**4**).—The crude alkaloid (1 g) in C_6H_6 (5 ml) was chromatographed on 10 g silica gel with C_6H_6, C_6H_6-$CHCl_3$, and $CHCl_3$-MeOH. The fraction eluted with $CHCl_3$/1% MeOH yielded 250 mg of *ent*-norsecurinine (4).

The hydrochloride (see below) on regeneration gave the base as a pale yellow oil showing a single spot on tlc (Silica gel, $CHCl_3$/3% MeOH, Rf 0.25); $[\alpha]^{20}D$ +255.8° (c 4.2, MeOH); ms *m/z* 203 (M^+, 40%), 134(20), 106(60), 78(80), 70(100). *Ent*-Norsecurinine was found to be unstable when stored at room temperature or in the refrigerator and turned dark brown. An ethanolic solution was treated with ethereal HCl to afford a crystalline hydrochloride. This was recrystallized from MeOH/Et_2O to give colorless plates; mp 275-276°; $[\alpha]^{20}D$ +188.6° (*c*, 0.93, H_2O); ord (c 0.26, 0.0114, MeOH), [ϕ]589 +391, [ϕ]268 +28958, [ϕ]234 −38018, [ϕ]213 −29846, [ϕ]205 −33399; cd [θ]325° 0, [θ]306 +23, [θ]303 +21.5, [θ]294 +79.8, [θ]290 +53.7, [θ]252 +44414, [θ]205 −1776. Found:C, 59.91, H, 5.83, N, 5.76. $C_{12}H_{13}NO_2$·HCl requires: C, 60.12, H, 5.84, N, 5.84%).

TETRAHYDRO *ENT*-NORSECURININE.—A solution of the free base **4** (250 mg) in EtOH (30 ml) was hydrogenated at 22° and atmospheric pressure over platinum oxide catalyst (50 mg). The catalyst was col-

lected and the solvent evaporated under vacuum to afford an oil (250 mg). Tetrahydro *ent*-norsecurinine gave the hydrochloride as colorless crystals; mp 308-310° (dec.); ir (KBr) ν max 1785, 1490, 1460, 1420, 1290, 1250, 1230, 1210, 1185, 1170, 1160, 1130, 1080, 1060, 1030, 980, 960, 940, 930, 900, 870, 750, cm^{-1}; ms (free base) *m/z* 207 (M^+, 100%), 206(50), 179(60), 178(25), 162(15), 134(40), 122(50), 110(65), 96(35), 70(50). Found: C, 58.67; H, 7.65; N, 5.94, $C_{12}H_{17}NO_2 \cdot HCl$ requires: C, 59.12; H, 7.39; N, 5.74%.

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