NEISOSPOSININE : A NEW OXINDOLE ALKALOID FROM NEISOSPERMA OPPOSITIFOLIA [APOCYNACEAE][1]

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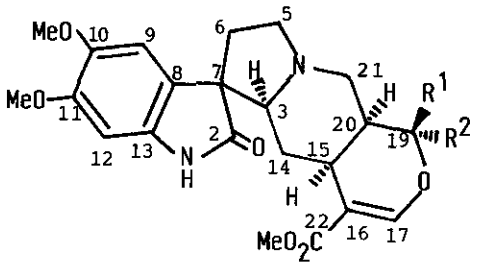
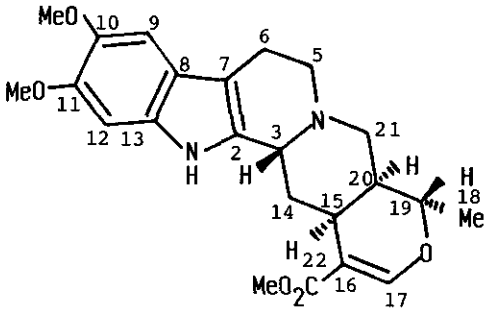
Abstract - A new oxindole alkaloid named neisosposinine was isolated from the stem bark of *Neisosperma oppositifolia* and its structure was elucidated as (**1**) with the aid of spectroscopic data. The C-18 methyl epimer of (**1**), isocarapanaubine (**2**) was also isolated along with three indole alkaloids, reserpiline (**3**), isoreserpiline (**4**) and ochroposinine (**5**).

Neisosperma oppositifolia, a plant occurring in southern coastal region of Sri Lanka has been subjected to several chemical studies as plants of this genus are employed in traditional systems of medicine.[2] Previous chemical investigations of the bark of *N. oppositfolia (= Ochrosia oppositifolia)* have revealed the presence of alkaloids, isoreserpiline,[3-5] reserpiline,[5-7] ochroposinine,[5,8] epirauvanine,[4] bleekerine,[4] 10-hydroxyapparacine,[9,10] 10-methoxy-apparacine,[9,10] 10-methoxydihydrocorynantheol,[5] ochrolofuanine,[5], reserpinine,[5] isoreserpinine,[5], and 9-methoxyellipticine.[10] In continuing our studies on medicinal and related plants of Sri Lanka and minor alkaloids of the plants of Apocynaceae we have investigated the bark of *N. oppositifolia* and in this communication we report the isolation and characterization of a new oxindole alkaloid, neisosposinine (**1**) in addition to isocarapanaubine (**2**), reserpiline (**3**), isoreserpiline (**4**) and ochroposinine (**5**). This constitutes the first report of the occurrence of oxidole alkaloids in the genus *Neisosperma*. The crude alkaloid fraction derived from the hot methanolic extract of the stem bark of *N. oppositifolia* on chromatographic separation afforded five alkaloids. Three of these were found to be indole alkaloids and were identified as reserpiline (**3**), isoreserpiline (**4**) and ochroposinine (**5**) by comparison of their spectral data (uv, ir, ^{1}H nmr and ms) with those



(1); R^1 = Me, R^2 = H

(2); R^1 = H, R^2 = Me



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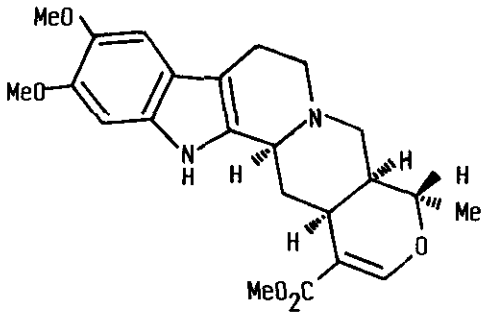

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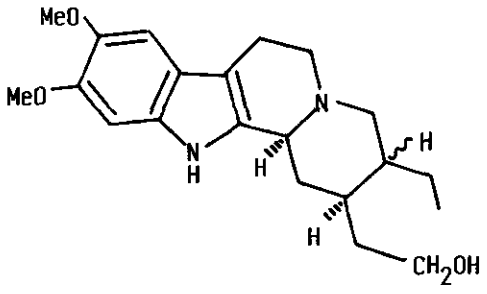

(5)

reported.[3-8] The spectral data of the remaining two alkaloids indicated them to be of oxindole type. One of them was identified as isocarapanaubine (2) from its spectral data, especially ^1H and ^{13}C nmr (Tables 1 and 2, respectively) data.[11]

The new alkaloid, named neisosposinine, mp 228-30°C, had M^+ at m/z 428.19647 ($C_{23}H_{28}N_2O_6$) in its EI-ms. The non-indolic type uv spectrum (λ_{max} 215, 245 and 302 nm) and the presence of an absorption band for an amide carbonyl in its ir spectrum (ν_{max} 1705 cm^{-1}) coupled with down field shift of the NH proton in ^1H nmr (δ 7.59 ppm) suggested the possibility of an oxindole skeleton. This was further confirmed by its characteristic ms fragmentation.[12,13]

The ^1H and ^{13}C nmr of neisosposinine were similar to those observed for isocarapanaubine. In ^1H nmr (Table 1) the signals due to aromatic and the olefinic (17-H) protons have been assigned by comparison with oxindoles reported in the literature.[14] 2D-Homocosy (COSY 45) experiment confirmed the H-H connectivity and the *J* resolved experiment indicated the multiplicty of those

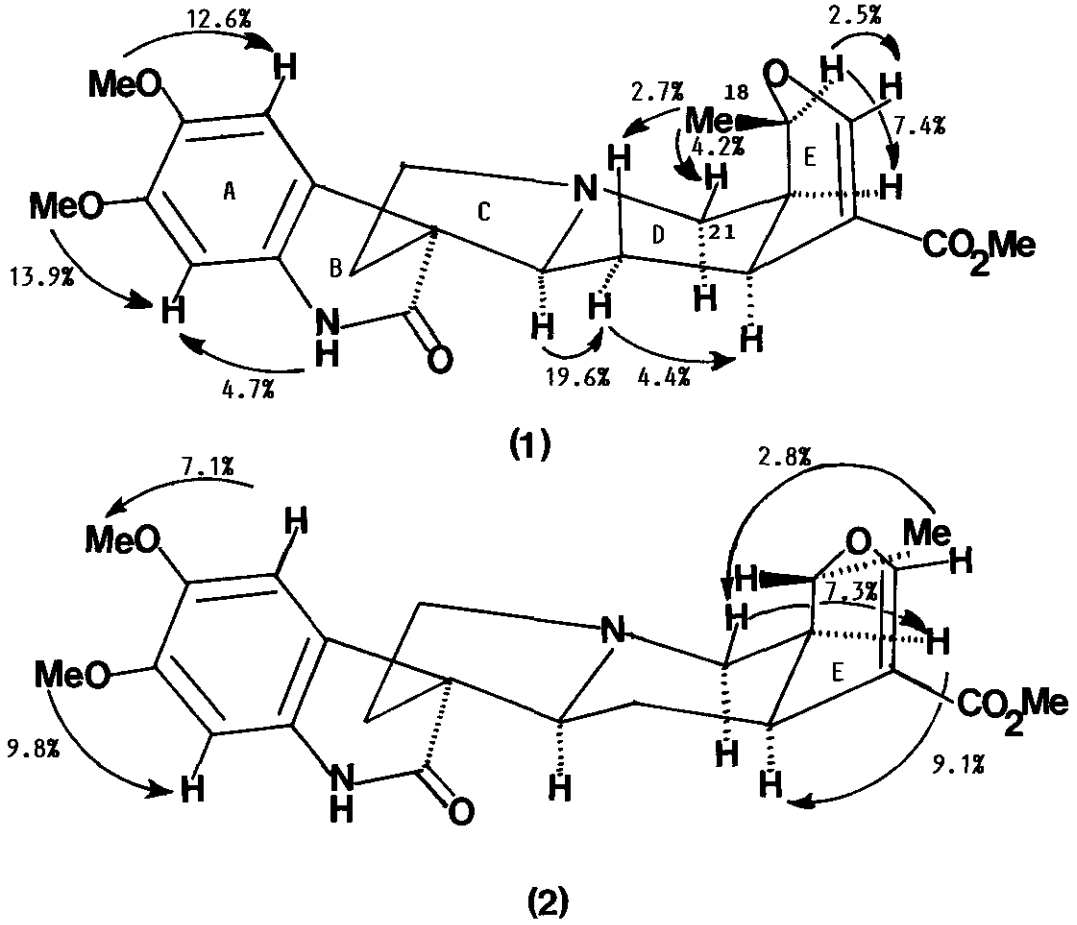

Fig. 1. NOE s observed for neisosposinine (**1**) and isocarapanaubine (**2**).

coupled protons. The 15-H was assigned as α based on biogenetic arguments.[15,16] Appearance of the 3-H signals at δ3.10 in both isocarapanaubine (**2**) and neisosposinine (**1**) and the coupling pattern of 14-H and 15-H with 3-H indicated the presence of C/D *trans* ring junction.[17] In order to avoid unfavourable axial orientation of the 19-Me, the E-ring of (**2**) may adopt half-chair conformation whereas in (**1**) it may adopt boat conformation [18](see Fig. 1). The structure (**1**) proposed for neisosposinine was further confirmed by the nOe data especially that observed between 18β-Me and 21β-H. The nOe data observed for neisosposinine (**1**) and isocarapanaubine (**2**) are presented in Fig. 1.

Table 1. ^{1}H Nmr data of neisosposinine (**1**), isocarapanaubine (**2**), isoreserpiline (**4**) and ochroposinine (**5**) [300 MHz/$CDCl_3$/TMS/ δ (ppm)]

Proton	**1**	**2**	**4**	**5**
NH	7.51 s	7.60 s	7.61 s	8.43 s
3-H	3.28 m	3.25 m	3.30 dd (*J*= 10,2 Hz)	-
5-H_2 6-H_2	1.95 - 2.48 m	1.90 - 2.47 m	2.40 - 3.20 m	-
9-H	6.73 s	6.88 s	6.88 s	6.89 s
12-H	6.47 s	6.49 s	6.80 s	6.85 s
14α-H	2.33 m	2.41 m	2.43 m	-
14β-H	1.71 m	1.65 m	1.56 m	-
15-H	2.43 m	2.48 m	2.72 m	-
17-H	7.47 s	7.40 s	7.54 s	3.65 m*
18-H_3	1.40 d (*J*= 6 Hz)	1.39 d (*J*= 6 Hz)	1.40 d (*J*= 6 Hz)	0.88 m
19-H	4.50 dq (*J*= 11,6 Hz)	4.32 dq (*J*= 11,6 Hz)	4.48 dq (*J*= 12,6 Hz)	-
20-H	1.57 m	1.60 m	1.68 m	-
21α-H	2.33 m	2.40 m	2.68 m	-
21β-H	3.31 m	3.25 m	3.08 dd (*J*= 12,2 Hz)	-
10-OCH_3	3.89 s	3.85 s	3.89 s	3.90 s
11-OCH_3	3.86 s	3.82 s	3.88 s	3.88 s
16-CO_2CH_3	3.61 s	3.60 s	3.73 s	-

* Signal due to two protons.

Table 2. ^{13}C Nmr data of neisosposinine (**1**), isocarapanaubine (**2**), and isoreserpiline (**4**) [75 MHz/$CDCl_3$/TMS/ δ (ppm)]

Carbon	**1**	**2**	**4**
C-2	181.5	181.3	133.3
C-3	72.3	72.2	60.0
C-5	53.8	54.0	53.7
C-6	29.6	30.1	21.9
C-7	-*	57.2	108.1
C-8	123.8	124.6	120.2
C-9	108.4	109.5	100.8
C-10	149.5	149.2	146.7
C-11	145.3	145.1	145.1
C-12	95.6	95.9	95.2
C-13	134.3	133.6	130.4
C-14	34.4	34.5	34.5
C-15	31.3	30.4	31.4
C-16	109.3	110.0	109.7
C-17	155.4	155.1	155.7
C-18	19.1	18.4	18.5
C-19	74.4	72.3	72.5
C-20	37.9	38.0	38.6
C-21	55.2	56.3	56.3
OCH_3	57.0	56.8	56.6
OCH_3	56.5	56.3	56.5
$CO_2\underline{C}H_3$	51.0	51.0	51.1
$\underline{C}O_2CH_3$	167.8	167.7	168.0

* Signal not recognizable.

Further evidence for the structure of neisosposinine (**1**) came from its ^{13}C nmr data and by comparison of the chemical shifts observed for (**1**) with those observed for isocarapanaubine (**2**) (Table 2). The ^{13}C nmr spectra of the two alkaloids were similar except for the chemical shifts of the carbon atoms, C-18 and C-19. These data confirm that in neisosposinine the methyl group at C-18 has β-orientation [δ_{C-18}, 19.1; δ_{C-19}, 74.4] whereas in isocarapanaubine it has α-orientation [δ_{C-18}, 18.4;δ_{C-19}, 72.3]. These ^{13}C chemical shift data are in agreement with those observed for the corresponding isomeric indole alkaloids, runiticine with C-18-βMe [δ_{C-18}, 19.1; δ_{C-19}, 76.4][19] and tetrahydroalstonine with C-18-αMe [δ_{C-18}, 18.4; δ_{C-19}, 72.3].[20] The configuration of the C-7 spiro carbon of both (**1**) and (**2**) was determined as S based on the ^{13}C chemical shifts of C-3 (72.3 and 72.2, respectively, see Table 2) which is comparable with δ_{C-3}(72.2) of isorhyncophylline.[21] For the R isomer, rhyncophylline, δ_{C-3} is 75.3.[21]

EXPERIMENTAL

All melting points were determined on a Kofler hot stage apparatus and are uncorrected. Tlc involved Silica gel G; visualisation was by spraying with Dragendorff's reagent. Plc used 0.5 mm layers of GF silica. Column chromatography involved silica gel of mesh 30-70. Ir spectra were recorded in $CHCl_3$ solutions with a JASCO A 302 grating spectrophotometer. Uv spectra were determined in ethanol with Shimadzu UV 240 spectrophotometer. Ms were recorded on Finnigan MAT 312 mass spectrometer connected to PDP 11/34 (DEC) computer system. The ^1H and ^{13}C nmr spectra were recorded in $CDCl_3$ at 300 MHz and 75 MHz, respectively, on a Brucker AM 300 nmr spectrometer and the multiplicity of carbon signals established by using DEPT measurements. An aspect 3000 computer (12 bits) was used for recording the data.

Extraction and separation of alkaloids

Dried and powdered bark of *Neisosperma oppositifolia* (40 kg), collected in the Southern coastal zone of Sri Lanka was exhaustively extracted with hot methanol. The methanol extract was concentrated and the concentrate extracted three times with 20% acetic acid. The acidic extract was washed several times with hexane and ethyl acetate. Subsequent basification of the acidic extract with conc. ammonia successively to pH 4, pH 6 and pH 9 and exhaustive extraction at each pH with ethyl acetate followed by evaporation to dryness yielded extracts weighing 8 g, 30 g and 10 g, respectively. The pH 6 fraction (30 g, 0.075%) which contained most of the alkaloids was chromatographed over a column of silica gel (600 g) made up in hexane and eluted with increasing amounts of acetone in hexane.

Isolation of isoreserpiline (**4**)

Elution of the above column with 15% acetone in hexane yielded fractions containing crude isoreserpiline. Purification by flash chromatography (on neutral alumina; elution with 20% acetone in hexane) followed by plc (silica gel; eluent-30% acetone in hexane; R_f 0.6) afforded

isoreserpiline (**4**) (42 mg, 1.05×10^{-3}%) as a colourless crystalline solid, mp 209-211°C (from acetone) (lit.[3] mp 211-212°C). Uv λ_{max} nm (log ε): 228 (4.52), 304 (4.02). Ir ν_{max} cm^{-1}: 2900, 1710, 1690, 1610, 1480, 1460, 1440, 1340, 1300, 1210, 1190, 1080, 1030, 850, 770. Ms m/z (%): 412 (M^+, 100), 411 (71), 397 (63), 353 (15), 328 (29), 327 (39), 311 (36), 283 (59), 230 (13), 229 (17), 223(27), 216 (42). For ^1H and ^{13}C nmr spectral data see Tables 1 and 2, respectively.

Isolation of neisosposinine (**1**)

Elution of the column with 25% acetone in hexane followed by plc (silica gel; eluent-40% acetone in hexane; R_f, 0.7) gave neisosposinine (**1**) (17 mg, 4.2×10^{-4}%) as colourless plates, mp 228-230°C (from acetone). High resolution ms: Calcd. for $C_{23}H_{28}N_2O_6$ (M^+, m/z): 428.19472. Found: 428.19647. Uv λ_{max} nm (log ε): 215 (4.54), 245 (infl. 4.21), 302 (infl. 3.87). Ir ν_{max} cm^{-1}: 3450, 2800, 1710, 1630, 1315, 1130. Ms m/z (%): 428 (M^+, 83), 223 (96), 222 (30), 208 (49), 204 (25), 190 (20), 180 (58), 165 (32). For ^1H and ^{13}C nmr spectral data see Tables 1 and 2, respectively.

Isolation of reserpiline (**3**)

Continued elution of the column with the same solvent system (25% acetone in hexane) followed by flash chromatography (neutral alumina; eluent-40% acetone in hexane) and plc (silica gel; 40% acetone in hexane, R_f, 0.55) afforded reserpiline (**3**) (21 mg, 5.2×10^{-4}%) as a colourless crystalline solid, mp 105-107°C. Uv λ_{max} nm (log ε): 229 (4.50), 302 (4.00). Ir ν_{max} cm^{-1}: 3450, 2840, 1700, 1618, 1453, 1352, 1128, 1088. Ms m/z (%): 412 (M^+, 100%), 411 (70), 397 (60), 353 (15), 328 (25), 327 (36), 311 (35), 283 (55), 230 (15), 229 (18), 223 (27), 216 (40). The above spectral data agreed well with those reported[3] for reserpiline (**3**).

Isolation of isocarapanaubine (**2**)

Elution of the column with 30% acetone in hexane followed by plc (silica gel; 50% acetone in hexane; R_f, 0.65) afforded isocarapanaubine as a solid (18 mg, 4.5×10^{-4}%) which resisted crystallization. Uv λ_{max} nm (log ε): 220 (4.52), 245 (infl. 4.19), 300 (infl. 3.91). Ir ν_{max} cm^{-1}: 2815, 1710, 1630, 1360, 1310, 1140. Ms m/z (%): 428 (M^+, 92), 413 (5), 411 (8), 397 (4), 223 (100), 219 (23), 208 (50), 205 (16), 204 (24), 190 (18), 180 (36), 166 (11), 164 (16), 69 (85). For ^1H and ^{13}C nmr spectral data see Tables 1 and 2, respectively.

Isolation of ochroposinine (**5**)

Elution of the column with 35% acetone in hexane followed by plc (silica gel: 40% acetone in hexane; R_f, 0.4) afforded ochroposinine (**5**) (12 mg, 3.0×10^{-4}%). Uv λ_{max} nm (log ε): 229 (4.30), 278 (3.65), 299 (3.85). Ir ν_{max} cm^{-1}: 3350, 2750, 1700, 1610. Ms m/z (%): 358 (M^+, 32), 357 (34), 313 (3), 285 (7), 230 (10), 229 (6), 216 (3). For ^1H nmr spectral data see Table 1. These spectral data agreed well with those reported[22] for ochroposinine.

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