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ALKALOIDS FROM ROOTS OF *STRYCHNOS POTATORUM*

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Key Word Index—*Strychnos potatorum*; Loganiaceae; root bark; indole alkaloids.

Abstract—Twenty-four compounds have been isolated and identified in the root bark of *Strychnos potatorum*. They are: *Harmane carboxamide*, cantleyine, 18,19-dihydrousambarensine, polyneuridine, norharmane, akuammidine, nor-C-flurorocurarine, ochrolifuanine A, bisnordihydrotoxiferine, ochrolifuanine E, normacusine B, normavacurine, henningsamine, 11-methoxyhenningsamine, *dihydrolongicaudatine*, *dihydrolongicaudatine Y*, antirhine, (20*R*)- and (20*S*)-dihydroantirhine, 11-methoxy-12-hydroxydiaboline, diaboline, 11-methoxydiaboline, desacetylretuline and diaboline *N*-oxide. The alkaloids in italic are novel.

INTRODUCTION

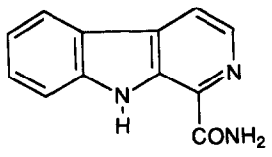
Strychnos potatorum L. is the only *Strychnos* species that grows in Africa and in Asia [1]. Previous phytochemical investigations on the leaves, stem bark and seeds of this plant collected in India yielded diaboline, acetyldiaboline and angustine [2, 3]. Considering the usual complexity of the alkaloid mixtures from *Strychnos* species, it was felt the this work was not complete and it was decided to investigate the most abundant alkaloids from the root bark of *S. potatorum* collected in Zaire.

RESULTS AND DISCUSSION

Alkaloids were extracted in the usual fashion [4] and 880 g of powdered root bark yielded 8.1 g of crude alkaloid mixture (9.2 g kg^{-1}) which was purified by medium pressure liquid chromatography and TLC. From this mixture, 24 pure compounds were separated and fully identified. Their occurrence and characterisation are summarised in Table 1. Among these compounds, 13 have been isolated more than once from *Strychnos* species [5] and four are novel.

The first novel alkaloid is harmane carboxamide (**1**). Its UV spectrum shows an extended chromophore with maxima at 244, 271, 299 and 368 nm. The mass spectrum of **1** is dominated by two ions at *m/z* 211 ($C_{12}H_9N_3O$) and 166. The ^1H NMR spectrum shows signals for three exchangeable resonances at δ 5.65, 7.98 and 10.25; the rest of the spectrum consists of four sets of resonances for six aromatic protons that may be split into an AX pair (δ 8.1, 8.42; $J = 5$ Hz) and an ABMX system similar to the ones present in norharmane (**5**) and in 19,20-dihydrousambarensine (**3**). The ^1H NMR and UV spectra suggest a β-carboline nucleus to which a primary amide function, $CONH_2$, is added to account for the $[M]^+$ *m/z* 211.

Compound **3** belongs to the family of semi-dimeric alkaloids represented in the *Strychnos* series by the ochrolifuanines and usambarensines. It is a dihydrousambarensine derivative as shown by mass spectrometry ($[M]^+$ at *m/z* 434, $C_{29}H_{30}N_4$). Fragmentation β to the nitrogen *N*(4) of the β-carboline unit induces formation of clusters of peaks centred around *m/z* 182 and 251. The ^1H NMR spectrum shows signals for 10 aromatic protons and for an ethyl side chain (triplet at δ 1.02), suggesting



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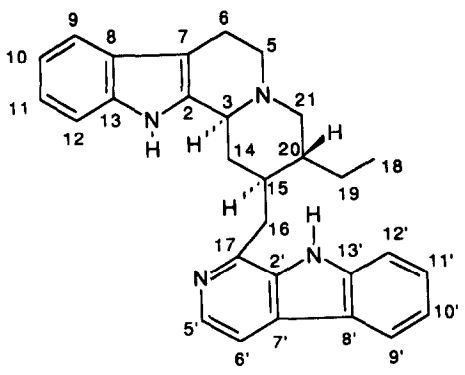

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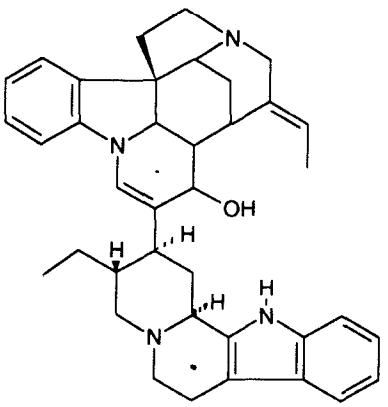
Table 1. Occurence and characterisation of alkaloids from roots of *S. potatorum*

Alkaloid	Yield (%)	CCM*	IR	UV	MS	^{1}H NMR	^{13}C NMR
Harmane carboxamide (**1**)	0.1	–	–	+	+	+	–
Cantleyine (**2**)	0.1	+	–	+	+	+	–
18,19-Dihydrousambarensine (**3**)	<0.1	–	+	+	+	+	+
Polyneuridine (**4**)	0.2	+	–	+	+	+	+
Norharmane (**5**)	0.2	+	–	+	+	+	+
Akuammidine (**6**)	0.2	+	–	+	+	+	–
Nor-C-fluorocurarine (**7**)	0.7	+	–	+	+	+	–
Ochrolifuanine A (**8**)	2.6	+	–	+	.	+	+
Bisnordihydrotoxiferine (**9**)	0.7	+	–	+	+	+	–
Ochrolifuanine E (**10**)	0.9	+	–	+	+	+	+
Normacusine B (**11**)	2.2	–	–	+	+	+	–
Normavacurine (**12**)	0.4	+	+	+	+	+	–
Henningsamine (**13**)	1.8	+	–	+	+	+	–
11-Methoxyhenningsamine (**14**)	0.6	+	–	+	+	+	–
Dihydrolongicaudatine (**15**)	<0.1	–	+	+	+	+	+
Dihydrolongicaudatine Y (**16**)	<0.1	+	+	+	+	+	–
Antirhine (**17**)	0.1	+	+	+	+	+	+
18,19-Dihydro-(20*R*)-antirhine (**18**)	3.0	+	+	+	+	+	+
11-Methoxy-12-hydroxydiaboline (**19**)	0.4	–	–	+	+	+	–
Diaboline **20**	18.0	+	–	+	+	+	–
18,19-Dihydro-(20*S*)-antirhine (**21**)	<0.1	–	+	+	+	+	–
11-Methoxydiaboline (**22**)	5.0	–	+	+	+	+	–
Desacetylretuline (**23**)	0.2	+	–	+	+	+	–
Diaboline *N*-oxide (**24**)	0.2	+	–	+	+	+	–

*A cross means that comparison with an authentic sample was performed.

that compound **3** is a 19, 20-dihydrousambarensine. The 3αH, 15αH, 20βH configuration is given to the corynantheal moiety of this alkaloid to account for the similarities between the ^{13}C NMR spectra of **3** (Table 1) and of the dihydroochrolifuanines A, B [6] and E; the rest of the spectrum is assigned by comparison with the spectrum of usambarensine [7]. Known compounds of the series, ochrolifuanine A **8** and ochrolifuanine E **10** were identified by direct comparison with reference samples [8].

Compounds **15** and **16** were obtained in the same fraction and separated by prep. TLC; on TLC, both coloured blue when sprayed with Ce^{4+}. Their mass spectra showed $[M]^+$ at *m/z* 570 ($C_{38}H_{42}N_4O$) and 572 ($C_{38}H_{44}N_4O$), respectively, and intense clusters of ions centred at *m/z* 251. TLC and mass spectral behaviour suggested their structures to be of the longicaudatine type [4, 9, 10]. The ^{1}H and ^{13}C of compound **15** and of longicaudatine display many similarities, amongst which are signals for H-and C-2, 3, 16, 17, 18 and 19 of the strychnane part. The two sets of spectra differ in the presence of signals for an ethyl side chain in **15** (cf. ethylidene in longicaudatine); this accounts for the two



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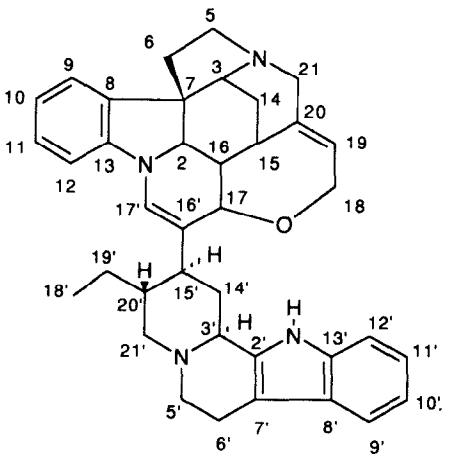
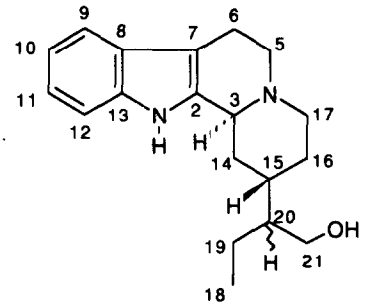


15

extra m.u. in **15**. The configuration of the corynane part of the molecule is 3α-H, 15α-H, 20β-H as in compounds **3**, **8** and **10**. The main difference between the ^{1}H NMR spectra of compounds **15** and **16** concerns the signals of H-18 and H-19 of the strychnane part. In compound **16** H-19 appears as a broadened quartet at δ5.6 (J = 7 Hz) and H-18 as a three-proton doublet at δ1.77. The structures of 19,20-dihydrolongicaudatine and 19,20-dihydrolongicaudatine Y [10] are therefore assigned to alkaloids **15** and **16**.

Compounds **17**, **18** and **21** are antirhine and dihydro-(20*R*)- and -(20*S*)-antirhine, respectively. Antirhine is a known compound from *Strychnos* species [11, 12] and dihydroantirhine was known as a synthetic compound [13, 14] before being identified from a natural source [15]. As pointed out in a recent article [12], it is not easy to distinguish dihydroantirhine from dihydrocorynantheol. Our structural assignment for compounds **18** and **21** rests on mass spectra which are superimposable on the one described in ref. [15], on ^{13}C NMR, where C-3 (δ54.6 for **18**) and C-6 (δ17.9) have typical values for the antirhine series and on a chemical correlation. The spectral and physical properties of compounds **18** and **21** are similar and they are assigned the 3α-H, 15β configuration. Compound **18** is the 20*R* isomer; this is the configuration of antirhine from which **18** was prepared by catalytic hydrogenation. Compound **21** is therefore the 20*S* isomer.



18, 21

Table 2. ^{13}C NMR data for alkaloids **3**, **15** and **18** (75 MHz, $CDCl_3$, central line of $CDCl_3$ at 77 ppm as reference

Carbon	3		15		18
2	—	—	—	60.5	133.2
3	59.3	—	60.5	60.2	54.6
5	53.2	138.8	53.3	51.4	51.4
6	21.6	112.8	21.7	39.1	17.9
7	—	—	—	51.0	107.1
8	—	—	—	—	127.4
9	120.2	121.7	118.1	122.6	117.8
10	121.1	119.2	120.0	119.4	121.1
11	117.9	128.5	121.4	129.0	119.2
12	110.8	111.8	110.6	108.5	111.1
13	—	—	—	—	136.0
14	25.5	—	—	27.0	31.3
15	39.9	—	40.8	31.7	31.8
16	37.4	—	37.0	42.0	28.5
17	—	—	127.2	84.0	46.8
18	11.2	—	11.6	64.8	11.9
19	23.9	—	24.0	129.0	21.0
20	42.6	—	41.9	—	44.7
21	60.3	—	61.0	53.2	61.9

Missing assignments are those of undetected quaternary carbon.

Diaboline (**20**) and 11-methoxydiaboline (**22**) are the most abundant alkaloids from this species and may be considered as typical *Strychnos* alkaloids, along with nor-C-fluorocurarine (**7**), bisnordihydrotoxiferine (**9**), henningsamine (**13**), 11-methoxyhenningsamine (**14**), 11-methoxy-12-hydroxydiaboline and desacetylretuline (**23**). The isomeric $C_{19}H_{22}N_2O$ alkaloids normacusine B(**11**) and normavacurine (**12**) are easily distinguished from their spectral properties. This latter alkaloid was formerly believed to be 1,2-dehydrodesacetylretuline [9] but a new isolation from *S. minfiensis* [16] allowed correction of the mistake.

Most alkaloids from *S. potatorum* are typical *Strychnos* alkaloids and none of them represents a new skeleton. A particular feature of *S. potatorum* is its ability to produce 18,19-dihydro compounds (**3**, **8**, **10**, **15**, **16**, **18**, **21**), which is relatively rare in the genus *Strychnos*.

EXPERIMENTAL

General. Plant material was collected near Lubumbashi (Zaire) in clear forest. A herbarium specimen is deposited in the Brussels National Botanical Garden under ref. HB 3670.

Extraction procedure. Dried powdered root bark (880 g) was wetted with 530 ml of NH_4OH half dil. in H_2O and lixiviated overnight with 25 l EtOAc. The organic soln was extrd with 2% H_2SO_4 and the resulting acidic soln basified with NH_4OH and extrd with $CHCl_3$. The extr. was dried (Na_2SO_4) and evapd to dryness under red. press. to yield 9.2 g crude alkaloid mixt.

Separation. The alkaloid mixt. (10 g) was fractionated on 1.3 kg silica gel H-60 (elution pressure 10 bar). After 1.4 l 'dead volume', 30 ml frs were collected. Solvents were $CHCl_3$ (2.4 l, frs 1–80), mixts of $CHCl_3$–MeOH (99:1) (frs 81–150), (49:1) (frs 151–255), (19:1) (frs 256–430), (9:1) (frs 471–780), (4:1) (frs 781–1035), (1:1) (frs 1036–1210) and MeOH (frs 1211–1380 plus one fr. of 1.4 l). Harmane carboxamide (**1**) was in frs 255–270, cantleyine (**2**) in frs 357–377, 18,19-dihydrousambarensine (**3**), polyneuridine (**4**) and norharmane (**5**) in frs 428–456, akuammidine (**6**) in frs 515–542, nor-C-fluorocurarine (**7**) in frs 552–573, ochrolifuanine A (**8**) in frs 574–655, bisnordihydrotoxiferine (**9**) and ochrolifuanine E (**10**) in frs 706–725, normacusine B(**11**) was the major alkaloid of frs 726–775, normavacurine (**12**) in frs 776–834, henningsamine (**13**) in frs 835–854, frs 855–934 contained 11-methoxyhenningsamine (**14**), dihydrolongicaudatine (**15**), dihydrolongicaudatine Y (**16**), antirhine (**17**) and dihydroantirhine (**18**) diaboline (**20**) and 11-methoxy-12-hydroxydiaboline (**19**) in frs 935–960, diaboline (**20**) was the major compound of frs 961–1380 and of the MeOH residue, (20*S*)-dihydroantirhine in frs 961–1243, 11-methoxydiaboline (**22**) in frs 1136–1243, desacetylretuline (**23**) and (**21**) in the MeOH residue and diaboline *N*-oxide (**24**) was in frs 1316–1380.

Harmane carboxamide (**1**). No Ce^{4+} reaction. UV λ_{max}^{MeOH} nm: 212, 244, 271, 299, 368. MS *m/z* (rel. int.): 211 (95), 194 (25), 168 (30), 167 (28), 166 (100), 140 (25), 139 (20), 114 (18). ^{1}H NMR (300 MHz, $CDCl_3$) δ10.25 (*br s*, 1H), 8.42 (*d*, J = 5Hz, 1H), 8.17 (*d*, J = 8Hz, H-1), 8.10 (*d*, J = 5Hz, 1H), 7.98 (*br s*, 1H), 7.6 (*m*, 2H), 7.35 (*m*, 1H), 5.65 (*br s*, 1H).

19,20-*Dihydrousambarensine* (**3**). UV λ_{max}^{MeOH}nm: 227, 280, 290, 354. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3160, 3060, 1625, 1450, 1325, 1240. MS *m/z* (rel. int.): 434 (70), 368 (15), 263 (40), 252 (45), 251 (75), 223 (20), 221 (15), 184 (25), 183 (25), 182 (100), 169 (25), 168 (22). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.4 (*d*, *J* = 5Hz, 1H), 8.32 (*br s*, 1H), 8.13 (*d*, *J* = 8Hz, 1H), 7.95 (*br s*, H), 7.77 (*d*, *J* = 5Hz, 1H), 7.5 (*m*, 2H), 7.4 (*m*, 1H), 7.3 (*m*, 1H), 7.12 (*m*, 1H), 7.03 (*m*, 2H), 3.5 (*dd*, *J* = 12, 2Hz, 1H, H-21), 3.2 (*m*, 1H, H-3), 2.2 (*t*, *J* = 10Hz, 1H, H-14), 1.8 (*m*, 1H, H-19), 1.35 (*m*, 1H, H-19), 1.05 (*t*, *J* = 7Hz, 3H, H-18).

Dihydrolongicaudatine (**15**). Blue Ce^{4+} reaction. $[\alpha]_D = +83°$ (MeOH; *c* 0.25). UV λ_{max}^{MeOH} nm: 226, 283, 290, 310 (sh). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 1630, 1595, 1480, 1460, 1290, 1110, 1030. MS *m/z* (rel. int.): 570 (60), 555 (2), 320 (20), 319 (15), 318 (55), 251 (50), 250 (55), 249 (50), 248 (50), 248 (80), 235 (100). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.75 (*br s*, 1H), 7.47 (*d*, *J* = 7 Hz, 1H), 7.25 (*d*, *J* = 7 Hz, 1H), 7.1 (*m*, 4H), 6.8 (*t*, *J* = 7 Hz, 1H), 6.6 (*d*, *J* = 7 Hz, 1H), 6.58 (*s*, 1H, H-17), 5.93 (*br t*, *J* = 7 Hz, 1H, H-19), 4.2 (*dd*, *J* = 12, 7 Hz, H-18), 4.1 (*dd*, *J* = 12, 5 Hz, H-18).

Dihydrolongicaudatine Y (**16**) Blue Ce^{4+} reaction. $[\alpha]_D = +262°$ (MeOH; *c* 0.1). UV λ_{max}^{MeOH}nm: 226, 283, 290 (sh). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 1720, 1650, 1590, 1570, 1480, 1460. MS *m/z* (rel. int.): 572 ([M]$^+$, 10), 554 (100), 525 (2), 432 (10), 355 (20), 277 (10), 251 (60), 225 (62), 197 (15), 184 (15), 170 (20), 144 (10), 122 (10). ^{1}H NMR (300 MHz, $CDCl_3$–CD_3OD) δ: 7.75 (*br s*, 1H), 7.48 (*d*, *J* = 8 Hz, 1H), 6.8 (*t*, *J* = 8 Hz, 1H), 6.55 (*d*, *J* = 8 Hz, 1H), 6.48 (*s*, 1H), 5.6 (*br q*, *J* = 7 Hz, 1H),4.02 (*d*, *J* = 10 Hz, 1H), 1.77 (*br d*, *J* = 7 Hz, 3H), 1.0 (*t*, *J* = 7 Hz, 3H).

Catalytic hydrogenation. Antirhine (10 mg) was dissolved in 2 ml MeOH and 3 mg PtO_2 added. The suspension was stirred for 24 hr in an atm. of H_2 (1 bar). Filtration over filter-aid and evapn yielded 11 mg of a mixt. which was purified by prep. TLC ($CHCl_3$–MeOH, 17:3, NH_4OH). The most polar compound (5 mg) was identical with compound **18**.

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