New Alkaloids of the Sarpagine Group from *Rauvolfia serpentina* Hairy Root Culture[⊥]

Yuri Sheludko,[†,‡] Irina Gerasimenko,[†] Heinz Kolshorn,[§] and Joachim Stöckigt*,[†]

Department of Pharmaceutical Biology, Institute of Pharmacy, Johannes Gutenberg-University, Staudinger Weg 5, D-55099 Mainz, Germany, Institute of Cell Biology and Genetic Engineering, National Academy of Science of Ukraine, Zabolotnogo str. 148, DSP-22, 03680, Kyiv, Ukraine, and Institute of Organic Chemistry, Johannes Gutenberg-University, Duesberg Weg 10-14, D-55099 Mainz, Germany

Three new monoterpenoid indole alkaloids, 19(*S*),20(*R*)-dihydroperaksine (**1**), 19(*S*),20(*R*)-dihydroperaksine-17-al (**2**), and 10-hydroxy-19(*S*),20(*R*)-dihydroperaksine (**3**), along with 16 known alkaloids **4**−**19** were isolated from hairy root culture of *Rauvolfia serpentina*, and their structures were elucidated by 1D and 2D NMR analyses. Taking into account the stereochemistry of the new alkaloids and results of preliminary enzymatical studies, the putative biosynthetical relationships between the novel alkaloids are discussed.

During the last few decades hairy root cultures were established for numerous species of medicinal plants. Their biochemical studies have shown that the level of secondary metabolism in hairy roots was often similar to that in nontransformed plants, sometimes surpassing it.[1] However, many novel compounds (about 250) were isolated from callus and suspension cell cultures, while analyses of hairy root cultures resulted in isolation of about 20 new substances.[2] This can be explained by a rather wider variety of cell suspensions and callus cultures compared to hairy roots. By 1999,[2] hairy root cultures were established from about 200 species of higher plants. Hairy roots can be induced by transformation with *Agrobacterium rhizogenes*, sometimes show growth rates comparable to those of cell suspension cultures, and have higher genetical and biochemical stability. They represent an important tool for studying root metabolism as well as for isolation and characterization of root-specific secondary compounds and enzymes involved in their biosynthesis. Moreover, hairy roots are a potentially valuable target for discovery of new natural compounds with interesting biological activities.

The alkaloid patterns of cell and tissue cultures of the Indian medicinal plant *Rauvolfia serpentina* Benth. ex Kurz (Apocynaceae) have been thoroughly investigated, and more than 30 indole alkaloids belonging to different alkaloid groups were identified.[3] Although *R. serpentina* hairy root cultures have been previously investigated for alkaloid occurrence,[4−6] there are still novel structures to be detected. In our recent paper we reported the isolation of 10-hydroxy-*N*(α)-demethyl-19,20-dehydroraumacline, the first naturally occurring indole alkaloid of the rare raumacline type.[7] The present report describes the isolation and structure elucidation of three new monoterpenoid indole alkaloids of the sarpagan group (**1**−**3**) along with 16 known indole alkaloids **4**−**19** (Chart 1).

Results and Discussion

An efficiently growing hairy root culture of *R. serpentina* was established by genetic transformation with *Agrobac-*

terium rhizogenes.[8] Extraction of the hairy roots afforded 19 indole alkaloids. Among them, ajmaline (**4**), 12-hydroxyajmaline (**5**), vinorine (**8**), isomers of vomilenine (**9**), perakine (**10**), 3-epi-α-yohimbine (**14**), and 18β-hydroxy-3-epi-α-yohimbine (**15**) were recently described as constituents of *R. serpentina* hairy root culture.[4,5] In addition, other typical *Rauvolfia* alkaloids were identified, e.g., norajmaline (**6**), 17-O-acetylajmaline (**7**), raucaffrinoline (**11**), vellosimine (**12**), sarpagine (**13**), strictosidine (**16**), strictosidine lactam (**17**), and tetrahydroalstonine (**18**).[3] Isomers of vallesiachotamine (**19**) were found in the fractions containing strictosidine and probably represent the products of its deglucosylation during extraction.

The novel compounds **1** and **2** gave a gray CAS reaction on TLC, which is characteristic for alkaloids of the sarpagine group with an unsubstituted aromatic ring. The mass spectrum of **1** revealed the molecular ion at *m/z* 312. The following intense fragment, registered at *m/z* 281, indicates the loss of an alcoholic group [M − CH_2OH]$^+$.

The ^{1}H NMR spectrum of **1** indicated the presence of Nα-H as a broad singlet at δ 12.02. Four signals in the region δ 7.66−7.23 were assigned to aromatic protons. The protons of the hydroxyl groups were observed as broad singlets at δ 6.16 (21-OH) and 5.58 (17-OH). The presence of two hydroxyl groups in the molecule was also proved by acetylation of **1**, which yielded the mixture of di- and triacetate. In the last, Nα-acetylation occurred as demonstrated by mass spectroscopy (data not shown). The protons adjacent to both hydroxyl groups were detected as two-proton multiplets at δ 4.01 and 3.84. The signal seen as a doublet of broadened peaks at δ 4.51 ($J_{3-14\alpha}$ = 9.6) was ascribed to H-3. The irradiation of H-14α led to collapsing the signal of H-3 to a singlet, while irradiation of H-14β did not influence the H-3 signal. Two resonances at δ 3.39 (dd, *J* = 15.1, 4.8) and 3.84 (d, *J* = 15.1) were assigned to H-6 protons. In the COSY spectrum they showed connections only between themselves and with H-5 (δ 3.58). The methyl group (3H-18) was detected as a three-proton doublet at δ 1.52 (*J* = 6.5). The last six signals observed in the area between δ 1.54 and 2.81 were attributed to aliphatic protons H-14−H-16, H-19, and H-20 (Table 1).

The ^{13}C NMR spectra revealed 19 distinct carbon resonances, including the two alcoholic carbons C-17 and C-21 at δ 63.0 and 62.9, respectively, and a high-field resonance

Chart 1

1 R_1=CH$_2$OH, R_2=H
2 R_1=CHO, R_2=H
3 R_1=CH$_2$OH, R_2=OH

4 R_1=CH$_3$, R_2=H, R_3=H
5 R_1=CH$_3$, R_2=H, R_3=OH
6 R_1=H, R_2=H, R_3=H
7 R_1=CH$_3$, R_2=Ac, R_3=H

8 R=H
9 R=OH

10 ,R=CHO
11 R=CH$_2$OH

12 R_1=CHO, R_2=H
13 R_1=CH$_2$OH, R_2=OH

14 R=H
15 R=OH

16

17

18

19

20

21
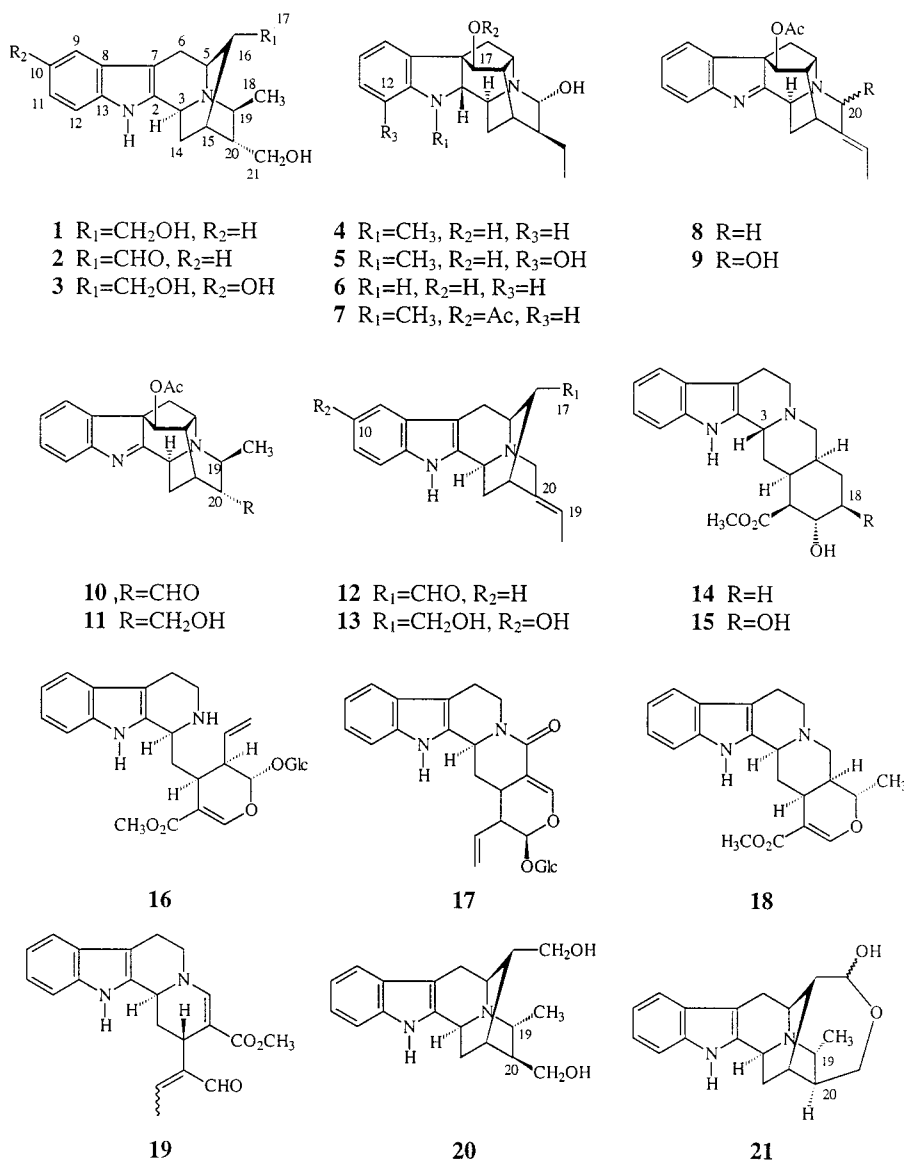
at δ 8.1 assigned to the methyl carbon. The aromatic region displayed eight signals indicating four protonated carbons and four quaternary carbons. The four strong resonances between δ 111.9 and 121.3 belong to the unsubstituted benzene carbons C-9−C-12. The remaining aromatic signals were assigned to quaternary carbons C-2, C-7, C-8, and C-13. The three lowest field resonances (δ 49.7, 53.4, and 57.5) of the eight remaining signals were attributed to the carbons adjacent to N-β (C-5, C-3, and C-19, respectively). Two-proton carbons at δ 27.4 and 29.8 were assigned to C-6 and C-14, respectively; the other three signals in the region between δ 27.7 and 43.9 were designated to aliphatic carbons C-15, C-16, and C-20 (Table 2).

These spectral data suggested an indole alkaloid with the pentacyclic sarpagan ring system and bearing two alcoholic groups and a methyl group (**1**). It lacks a double bond at C-19−C-20, which is usual for many of the sarpagine alkaloids such as vellosimine (**12**) or sarpagine (**13**).

The further novel compound **2** was isolated from hairy roots in a minor amount and displayed a mass spectrum similar to that of **1** but with the molecular ion at *m/z* 310. However, the following intense fragment, recorded at *m/z* 281, was not changed in comparison with the respective

fragment of **1**, indicating the loss of an aldehyde group. The ^{1}H NMR spectrum of **2** showed an aldehyde proton as a singlet at δ 9.87, which was assigned to H-17 (Table 1). Accordingly, the signals of H-5, H-15, and H-16 were shifted to lower field at δ 4.17, 2.73, and 2.32, respectively, while positions of H-6β and H-20 were displaced in the high-field area (δ 2.75 and 1.57, respectively) in comparison with the appropriate signals of **1**. The chemical shifts of the H-21 protons adjacent to the remaining hydroxyl group did not change significantly as compared to **1** and were found at δ 3.75 and 3.82. ^{13}C NMR spectra of **2** displayed the only significant differences in positions of C-17 and C-16 (Table 2). The signal of the aldehyde carbon was detected at δ 204.4, and the C-16 resonance was shifted to low field at δ 54.6. These data allowed assignment of the structure **2**, a 17-aldehyde-analogue of **1**.

The third new alkaloid (**3**) exhibited high polarity on TLC and a violet CAS reaction which is specific for alkaloids of the sarpagine group with a hydroxylated aromatic ring. Its mass spectrum displayed close similarity to that of **1**, but the molecular ion peak and the following intense fragments were shifted up by 16 units, indicating an additional OH group. Compared to **1**, the NMR spectra of **3** exhibited significant difference in the aromatic region only (Tables

Table 1. ¹H NMR Data for Compounds **1**–**3** in Pyridine-d_5[a]

proton	1	2	3
3	4.51 (d, J = 9.6)	4.28 (d, J = 9.4)	4.41 (d, J = 9.4)
5	3.58 (m)	4.17 (dd, J = 8.2, 4.7)	3.47 (m)
6α	3.39 (dd, J = 15.1, 4.8)	3.23 (dd, J = 15.3, 4.7)	3.30 (dd, J = 15, 4.4)
6β	3.11 (d, J = 15.1)	2.75 (d, J = 15.3)	3.05 (d, J = 15.1)
9	7.66 (dd, J = 7.2, 1.4)	7.68 (dd, J = 7.6, 1.2)	7.49 (d, J = 2.4)
10	7.23 (ddd, J = 7.2, 7.2, 1.4)	7.25 (ddd, J = 7.2, 7.2, 1.2)	–
11	7.27 (ddd, J = 7.2, 7.2, 1.4)	7.29 (ddd, J = 7.2, 7.2, 1.2)	7.24 (dd, J = 8.5, 2.4)
12	7.58 (dd, J = 7.2, 1.4)	7.57 (d, J = 7.6, 1.2)	7.50 (d, J = 8.5)
14α	2.33 (ddd, J = 13, 9.6, 1.4)	2.22 (ddd, J = 12.9, 9.4, 1.2)	2.29 (dd, J = 12.9, 9.4)
14β	1.54 (dd, J = 13, 3.1)	1.47 (dd, J = 12.9, 2.4)	1.53 (d, J = 12.9)
15	2.48 (m)	2.73 (m)	2.51 (m)
16	2.12 (m)	2.32 (d, J = 8.2)	2.19 (m)
17a	4.01 (m)	9.87 (s)	4.01 (m)
17b	4.01 (m)		4.01 (m)
18	1.52 (d, J = 6.5)	1.32 (d, J = 6.5)	1.47 (d, J = 6.5)
19	2.81 (ddd, J = 14.7, 6.5, 6.5)	2.51 (ddd, J = 14.7, 6.5, 6.5)	2.74 (ddd, J = 15.3, 6.5, 6.5)
20	2.07 (ddd, J = 14.7, 7.9, 5.8)	1.57 (ddd, J = 14.7, 8.8, 5.3)	2.04 (dd, J = 15.3, 8.2, 5.9)
21a	3.81 (dd, J = 10.9, 7.9)	3.75 (dd, J = 11.4, 8.8)	3.82 (dd, J = 10.9, 8.2)
21b	3.87 (dd, J = 10.6, 5.8)	3.82 (dd, J = 11.4, 5.3)	3.90 (dd, J = 10.9, 5.9)
10-OH			10.72 (br s)
17-OH	5.58 (br s)		5.28 (br s)
21-OH	6.16 (br s)	6.18 (br s)	6.17 (br s)
N(a)-H	12.02 (br s)	11.90 (br s)	11.64 (br s)

[a] Values are in ppm (δ). The multiplicities (s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; coupling constants (J in Hz) are in parentheses.

Table 2. ¹³C NMR Data for Compounds **1**–**3** in Pyridine-d_5[a]

carbon	1	2[d]	3
2	139.3 (s)[b]	140.1 (s)	137.8 (s)
3	53.4 (d)	53.0 (d)	53.6 (d)
5	49.7 (d)	44.5 (d)	49.6 (d)
6	27.4 (t)	27.8[c] (t)	27.7 (t)
7	105.0 (s)	104.4 (s)	104.5 (s)
8	128.5 (s)	128.6 (s)	129.7 (s)
9	118.5 (d)	118.4 (d)	103.8 (d)
10	119.3 (d)	119.4 (d)	152.4 (s)
11	121.3 (d)	121.3 (d)	111.7 (d)
12	111.9 (d)	111.8 (d)	112.2 (d)
13	137.8 (s)[b]	137.7 (s)	132.4 (s)
14	29.8 (t)	28.0[c] (t)	30.0 (t)
15	27.7 (d)	26.6 (d)	27.7 (d)
16	43.9 (d)	54.6 (d)	44.1 (d)
17	63.0 (t)	204.4 (d)	63.1 (t)
18	18.6 (q)	18.7 (q)	18.9 (q)
19	57.5 (d)	55.7 (d)	57.3 (d)
20	40.2 (d)	41.9 (d)	40.2 (d)
21	62.9 (t)	62.3 (t)	63.1 (t)

[a] Values are in ppm (δ). The multiplicities: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet refer to C, CH, CH$_2$, and CH$_3$, respectively; coupling constants (J in Hz) are in parentheses. [b,c] Assignments may be interchanged. [d] ¹³C NMR shifts were retrieved from HMQC and HMBC spectra.

1 and 2). The three observed signals at δ 7.49 (d, J = 2.4), 7.24 (dd, J = 8.5, 2.4), and 7.50 (d, J = 8.5) suggested an indole moiety substituted at the 10 or 11 position. Finding NOEs between Nα-H (δ 11.64) and H-12 (δ 7.50) as well as between H-6β (δ 3.05) and H-9 (δ 7.49) along with data of COSY experiments allowed placement of the hydroxyl at C-10. This was also supported by ¹³C NMR data: the C-10 hydroxylated carbon signal was detected at δ 152.4. The resonances of C-9 at δ 103.8 and C-11 at δ 111.7 were shifted upfield compared to **1** and **2** due to the hydroxyl group at C-10.

To investigate the relative stereochemistry of **1**–**3**, measurements of NOE and NOESY were performed. It was found that all three new compounds have similar stereochemical characteristics that suggest a single biogenetical precursor. NOEs were found between H-3 and Nα-H, H-14α, and H-19, indicating an α-position of H-3 and H-19. The NOE signal enhancements between H-5 and H-6α (strong) and H-6β (slight), as well as between H-5 and 3H-18 (strong), indicated an α-position of H-5 and β-position of the methyl group. Finding NOEs signals between H-20 and 3H-18 (strong), H-15 (strong), and H-5 (slight) suggests a β-stereochemistry for H-20, while protons at C-21 exhibited cross-peaks with H-19, H-14α, and H-20, indicating an α-position of the alcoholic group. The R-configuration at C-16 in **1**–**3** was proved by NOEs between H-16 and H14β, H-15, and H-6β. Irradiation of the aldehyde proton in **3** resulted in signal enhancement between H-17 and H-16, H-15, H-5, and H-20, supporting the *exo* position of C-17 and R-configuration at C-20. Moreover, the chemical shift of H-17 (δ 9.87) is in agreement with NMR data of the structurally similar alkaloid vellosimine (**12**), in contrast to 16-epi-vellosimine, where the H-17 proton was found shifted upfield.[9]

Summarizing the data described above, we can conclude that the novel alkaloids **1**–**3** have stereochemical configuration at C-19 and C-20 similar to that of indolenine alkaloids perakine (**10**) and raucaffrinoline (**11**). Deacetylation of **11** under basic conditions yielded the product with TLC, MS, UV, and NMR characteristics identical with **2**. Few alkaloids of the sarpagine group are known to possess a methyl group at C-19.[10] Among them, dihydroperaksine (**20**) was isolated from leaves of *Rauvolfia caffra*.[11] This alkaloid (**20**) was also synthetically prepared from peraksine (**21**).[12] As it was reported, **20** and **21** are structurally similar to **1**, but have different stereochemistry at C-19 and C-20.[10,11] The comparison of dihydroperaksine (**20**) with the diol synthetically formed from perakine (**10**) or raucaffrinoline (**11**) and apparently identical with **1** showed inequality in their chemical properties,[12] suggesting the different stereochemistry. These data indicate that 19(S),20(R)-dihydroperaksine (**1**) and its congeners **2** and **3** represent a novel subgroup of sarpagine alkaloids isolated from a natural source for the first time.

Taking into account the specific stereochemistry of the alkaloids **1**–**3** at C-19 and C-20, we supposed their derivation from *Rauvolfia* alkaloid perakine (**10**) and/or its dihydroderivative raucaffrinoline (**11**), which were both isolated from hairy roots in fair yield. Raucaffrinoline (**11**) can be enzymatically synthesized from **9** in the presence
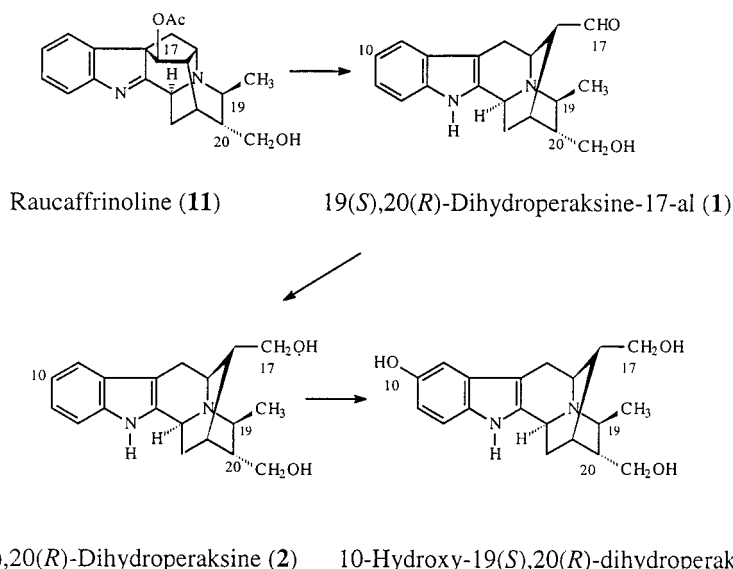
Raucaffrinoline (**11**)   19(*S*),20(*R*)-Dihydroperaksine-17-al (**1**)

19(*S*),20(*R*)-Dihydroperaksine (**2**)   10-Hydroxy-19(*S*),20(*R*)-dihydroperaksine (**3**)

Figure 1. Putative biosynthetic pathway in *R. serpentina* hairy root culture leading to 19(*S*),20(*R*)-dihydroperaksine-17-al (**2**), 19(*S*),20(*R*)-dihydroperaksine (**1**), and 10-hydroxy-19(*S*),20(*R*)-dihydroperaksine (**3**) from raucaffrinoline (**11**).

of NADPH$_2$ or from **10**.[3] Another possibility is to consider **1** and **2** as artifacts formed during the extraction procedure, as was recently proposed by Lounasmaa.[13] However, no appropriate precursor of **3** was detected in most broadly investigated *Rauvolfia* plants[14] or cell cultures.[3]

To clarify this question, we incubated **11** with a crude enzyme preparation from the hairy root culture. As a control, we used an assay containing the enzyme mixture inactivated by boiling. After overnight incubation of the crude enzyme mixture with **11** a product with TLC and MS data identical with those of **2** was isolated, while in the control sample **11** was not converted. HPLC analysis of the reaction mixture after 120 min incubation revealed the enzyme activity of 0.18 pkat/μg protein.

The experiment indicates that in the hairy roots enzymatical deacetylation of **11** causes destabilization of the C-17 hydroxylated indolenine skeleton followed by cleavage of the C-7, C-17 bond. This process leads to the formation of 19(*S*),20(*R*)-dihydroperaksine-17-al (**2**). Further reduction of the aldehyde group of **2** can be supposed, and, as a late step, hydroxylation of the indole moiety of **1** might occur to form **3** (Figure 1). Final proof of the mechanisms of biosynthesis of **1**−**3** will be possible only after further purification and characterization of the appropriate enzymes. In vivo NMR may also be a helpful approach for direct identification of the biosynthetic products.[15]

In conclusion, the isolation of three novel indole alkaloids along with 16 known compounds from *R. serpentina* hairy root culture displays its potential as a source of new natural products. Alkaloids detected in the hairy roots include substances (e.g., 12-hydroxyajmaline (**5**)) that have never been found in other *Rauvolfia* plant or cell systems. Several known but rare alkaloids, such as **8** and **9**, found in *Rauvolfia* cell cultures in trace amount only, were isolated from the hairy root culture in much higher yields. These alkaloids might have an important potential as substrates for detection of novel enzymes involved in the biosynthesis of the typical constituent of *Rauvolfia*, ajmaline, and structurally related compounds. They also could be useful for ultrahigh-field in vivo NMR monitoring of alkaloid formation by *R. serpentina* cell suspension cultures. Examples of successful applications of the alkaloids isolated from hairy roots of *R. serpentina* are described in our recent publications.[15,16]

Experimental Section

General Experimental Procedures. Melting points were determined using a Wagner & Munz (München) apparatus and a Büchi apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 MC polarimeter. UV spectra were measured using a Perkin-Elmer Lambda 2 spectrophotometer. EIMS measurements were performed with a quadrupole instrument (Finnigan MAT 44S) at 70 eV. HREIMS were recorded on a JEOL JMS-700 mass spectrometer. ^{1}H and ^{13}C NMR, DEPT, NOE, COSY, and HETCOR spectra of **1** and **3** in pyridine-*d$_5$* were recorded using a Bruker AM 400 instrument (400.13 MHz for ^{1}H and 100.6 MHz for ^{13}C). ^{1}H NMR and phase-sensitive NOESY, DQF-COSY, HSQC, and HMBC spectra of **2** were recorded using a Bruker DRX 600 instrument (600.13 MHz for ^{1}H and 150.6 MHz for ^{13}C). HPLC was performed as described recently.[17] Flash chromatography was performed with silica gel 60 (230−400 mesh) (Merck, Darmstadt). For TLC precoated 0.5, 0.25, and 0.2 mm silica gel 60 F$_{254}$ plates, 20 × 20 cm (Merck, Darmstadt) were used. Solvent systems used for TLC and flash chromatography: Me$_2$CO−petroleum ether−diethylamine (7:2:1, SS1), CHCl$_3$−cyclohexane−diethylamine (6:3:1, SS2), CHCl$_3$−MeOH−ammonia (8:2:0.1, SS3), EtOAc−MeOH−H$_2$O−ammonia (7:2:1: 0.1, SS4), EtOAc−cyclohexane−MeOH−amonia (6:2:2:0.1, SS5), CHCl$_3$−MeOH−ammonia (9:1:0.05, SS6), CHCl$_3$−MeOH−ammonia (9:1:0.1, SS7), EtOAc−MeOH−H$_2$O−ammonia (5:4:1:0.1, SS8), EtOAc−MeOH−ammonia (6:4:0.1, SS9), EtOAc−MeOH−ammonia (6:4:0.05, SS10), CHCl$_3$−MeOH−H$_2$O−ammonia (50:45:5:1, SS11), CHCl$_3$−MeOH−H$_2$O−ammonia (5:4:1:0.1, SS12), CHCl$_3$−MeOH−ammonia (5:5:0.1, SS13), CHCl$_3$−MeOH−ammonia (6:4:0.1, SS14). Alkaloids were detected by their absorption at 254 nm; the plates were sprayed with ceric ammonium sulfate (CAS) reagent.

Plant Material. Hairy roots were cultivated in 0.3 L Erlenmeyer flasks containing 75 mL of liquid MS-medium[18] without phytohormones, with half reduced salt and sugar concentrations at 25 °C and 75 rpm (gyratory shaker) in the dark for 10 days.

Extraction and Isolation. For alkaloid extraction the protocol of Smith[19] was used with minor modifications. Typical protocol: freeze-dried cells (50 g, 10 days cultivation period) were extracted with 5 L of MeOH under sonication for 30 min at 40 °C. After filtering, the extract was evaporated to dryness under vacuum at 40 °C. The residue was taken up in 1 L of 50 mM bicarbonate buffer (pH 10.0) and partitioned two times into equal volumes of ethyl acetate. Organic fractions were combined and evaporated under vacuum at 40 °C, yielding 4.1

g of crude extract. The extract was then divided in two equal parts, and each part was fractionated by flash chromatography on 140 g of silica gel using SS2 (800 mL)−SS3 (500 mL)−MeOH (200 mL), sequentially yielding six fractions. All fractions were repeatedly chromatographed by flash chromatography on 55 g of silica gel and/or by preparative TLC (PTLC) to finally afford **1** (9 mg) and **2** (1.1 mg) along with 16 known alkaloids. The known alkaloids were characterized by $[\alpha]_D$, UV, MS, NMR, TLC, and HPLC data and by comparison with published data[4,5,10,12,20,21] and with authentic samples whenever possible.

Fraction 1 (between ~200 and ~350 mL) was refractionated by flash chromatography using SS1 (450 mL) followed by PTLC with SS1, SS2, and SS3 and contained **8**−**10**, **12**, and **18**.

Fraction 2 (between ~350 and ~900 mL) was refractionated by PTLC with SS2, SS3, SS4, SS5, and SS6 and contained **4**, **7**−**10**, **11**, **12**, and **14**.

Fraction 3 (between ~900 and ~1000 mL) was refractionated by flash chromatography using SS7 (450 mL) followed by PTLC with SS3, SS4, and SS7 and contained **2, 4**, **6**, **11**, **13**, and **15**.

Fraction 4 (between ~1000 and ~1100 mL) was refractionated by flash chromatography using SS7 (500 mL) followed by PTLC with SS3 and SS4 and contained **5**, **6**, **11**, **13**, **15**−**17**, and **19**.

Fraction 5 (between ~1100 and ~1250 mL) was refractionated by PTLC with SS3, SS4, SS8, SS9, and SS10 and contained **1**, **3**, **5**, **13**, **16**, **17**, and **19**.

Fraction 6 (between ~1250 and ~1500 mL) was rechromatographed by PTLC with SS4, SS11, and SS12 and contained **1**, **3**, **16**, **17**, and **19**.

For additional isolation of **3** cells were extracted as described above, and the extract was fractionated by flash chromatography using SS3 (800 mL)−SS13 (300 mL) sequentially. Fractions between 1000 and 1100 mL were collected and chromatographed over silica gel plates with SS11, SS4, and SS14 sequentially, and this yielded 8.2 mg of pure **3**.

Preparation of 2 from raucaffrinoline (11). Raucaffrinoline (6 mg in 500 μL of MeOH) was incubated with 500 μL of 10 N NaOH for 1 h. The mixture was extracted with 1 mL of CH_2Cl_2, and the extract was chromatographed over a silica gel plate with SS10, yielding 3.2 mg of pure **2**. MS, ^{1}H NMR, COSY, and HMQC data of the synthetic **2** were identical with those of the alkaloid isolated from hairy roots. We used the synthetic sample for performing NOESY and HMBC measurements of **2**.

Enzyme Test. For preparation of a crude enzyme mixture hairy roots (10 g) were frozen in liquid nitrogen, powdered, and thawed in 10 mL of buffer (100 mM Tris/HCl, pH 8.0; 20 mM β-mercaptoethanol). The supernatant after centrifugation (10000g at 4 °C for 10 min) was used for enzymatic studies. For HPLC analysis an assay containing 1 mM raucaffrinoline and 52 μg of protein in total volume of 0.3 mL of 0.1 M potassium phosphate buffer (pH 7.0) was incubated for 120 min at 30 °C. To improve HPLC separation, the reaction mixture was reduced with $NaBH_4$. For TLC analysis a solution containing 0.01 M raucaffrinoline and 180 μg of protein in total volume of 1 mL of 0.1 M potassium phosphate buffer (pH 7.0) was incubated overnight (ca. 16 h) at 30 °C. Before extraction the pH of the reaction mixture was adjusted to approximately 9 with 1 N NaOH. Alkaloids were extracted with an equal volume of CH_2Cl_2, and the extract was analyzed by HPLC or chromatographed on a silica gel plate with SS4. Identity of the obtained product with **2** was proved by co-TLC and by MS. A control assay containing enzymes inactivated by 20 min boiling was treated in the same way.

19(S),20(R)-Dihydroperaksine (1): white crystals (MeOH); SS3 $R_f = 0.25$; SS4 $R_f = 0.65$; SS8 $R_f = 0.68$, SS9 $R_f = 0.55$; mp 208−212 °C (some sublimation at 192 °C); $[\alpha]^{25}_D$ +35.26° (*c* 0.12; Pyr); UV (MeOH) λ_{max} (log ϵ) 225 (4.49), 279 (3.83), 289 (3.73); EIMS *m/z* (rel int %) 312 [M]$^+$ (68), 311 [M − H]$^+$ (100), 281 [M − CH$_2$OH]$^+$ (29), 239 (55), 209 (24), 197 (19), 182 (18), 169 (96), 168 (76), 156 (16), 143(11), 130 (13), 115 (18); ^{1}H and ^{13}C NMR: Tables 1 and 2; HREIMS *m/z* 312.1819

(calcd for C$_{19}$H$_{24}$N$_2$O$_2$, 312.1837), 311.1754 (calcd for C$_{19}$H$_{23}$N$_2$O$_2$, 311.1759) [M − H]$^+$, 281.1631 (calcd for C$_{18}$H$_{21}$N$_2$O, 281.1654) [M − CH$_2$OH]$^+$.

19(S),20(R)-Dihydroperaksine-17-al (2): white powder; SS3 $R_f = 0.40$, SS4 $R_f = 0.45$, SS6 $R_f = 0.08$, SS7 $R_f = 0.11$; mp 156−159 °C; $[\alpha]^{25}_D$ +50.0° (*c* 0.07; MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (4.21), 279 (3.55), 289 (3.45); EIMS *m/z* (rel int %) 310 [M]$^+$ (24), 309 [M − H]$^+$ (20), 281 [M − CHO]$^+$ (49), 237 (17), 209 (32), 195 (10), 182 (20), 169 (85), 168 (100), 156 (12), 154 (17), 143(10), 130 (10), 115 (22); ^{1}H and ^{13}C NMR, Tables 1 and 2; HREIMS *m/z* 310.1659 (calcd for C$_{19}$H$_{22}$N$_2$O$_2$, 310.1681), 309.1591 (calcd for C$_{19}$H$_{21}$N$_2$O$_2$, 309.1603) [M − H]$^+$, 281.1627 (calcd for C$_{18}$H$_{21}$N$_2$O, 281.1654) [M − CHO]$^+$.

10-Hydroxy-19(S),20(R)-dihydroperaksine (3): white crystals (MeOH); SS4 $R_f = 0.42$, SS11 $R_f = 0.55$, SS14 $R_f = 0.31$; mp 323−335 °C; $[\alpha]^{25}_D$ +42.6° (*c* 0.15; Pyr); UV (MeOH) λ_{max} (log ϵ) 226 (4.14), 277 (3.71), shoulder 294−298 nm; EIMS *m/z* (rel int %) 328 [M]$^+$ (63), 327 [M − H]$^+$ (92), 297 [M − CH$_2$OH]$^+$ (30), 255 (58), 225 (26), 213 (23), 198 (20), 196 (12), 185 (100), 184 (75), 172 (14), 170 (13), 159 (14), 156 (16), 146 (14); ^{1}H and ^{13}C NMR, Tables 1 and 2; HREIMS *m/z* 328.1792 (calcd for C$_{19}$H$_{24}$N$_2$O$_3$, 328.1787), 327.1716 (calcd for C$_{19}$H$_{23}$N$_2$O$_3$, 327.1709) [M − H]$^+$, 297.1584 (calcd for C$_{18}$H$_{21}$N$_2$O$_2$, 297.1603) [M − CH$_2$OH]$^+$.

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