Three New Alkaloids from the Leaves of *Uncaria rhynchophylla*

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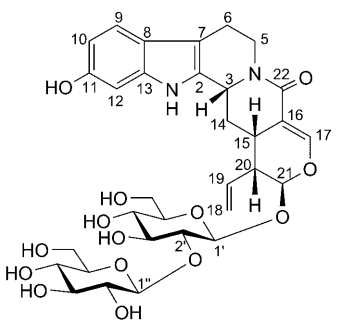
Three new alkaloids, 2′-*O*-*β*-D-glucopyranosyl-11-hydroxyvincoside lactam (**1**), 22-*O*-demethyl-22-*O*-*β*-D-glucopyranosylisocorynoxeine (**2**), and (4*S*)-corynoxeine *N*-oxide (**3**) were isolated from the leaves of *Uncaria rhynchophylla*, together with four known tetracyclic oxindole or indole alkaloids, isocorynoxeine *N*-oxide (**4**), rhynchophylline *N*-oxide (**5**), isorhynchophylline *N*-oxide (**6**), and dihydrocorynantheine (**7**), and an indole alkaloid glycoside, strictosidine (**8**). The structures of **1**–**3** were elucidated by spectroscopic methods including UV, IR, ESI-TOF-MS, 1D- and 2D-NMR, as well as CD experiments. The activity assay showed that **8** (IC_{50} = 8.3 μM) exhibited potent inhibitory activity on lipopolysaccharide(LPS)-induced nitrogen monoxide (NO) release in N9 microglia cells. However, only weak inhibitory activities were observed for **1**–**7** (IC_{50} > 100 μM for **1**–**6** or > 30 μM for **7**).

Introduction. – Some unique tetracyclic or pentacyclic oxindole and indole alkaloids, such as rhynchophylline, isorhynchophylline, corynoxine, corynoxine B, hirsutine, and geissoschizine methyl ether, *etc.*, are the primary constituents of *Uncaria* sp., responsible for a diversity of effects, including lowering blood pressure, vasodilatation, sedation, and protection against ischemia-induced neuronal damage [1–5]. The leaves of *Uncaria rhynchophylla* (MIQ.) JACKS are used as a folk medicine in China for the treatment of hypertension, headache, and stroke [6]. In our previous studies, seven oxindole and indole alkaloids were isolated from the herb, some of which exhibited potent inhibitory activity on LPS-induced NO release in microglia [7][8]. The present study describes the isolation and structure elucidation of the three new alkaloids **1**–**3** together with the five known alkaloids **4**–**8** and their inhibitory activity towards NO release in LPS-activated microglial cells.

Results and Discussion. – The EtOH extract of the leaves of *U. rhynchophylla* was prepared by percolation. The $CHCl_3$- and BuOH-soluble fractions were obtained by partitioning the crude EtOH extract. Three new alkaloids, 2′-*O*-*β*-D-glucopyranosyl-11-hydroxyvincoside lactam (**1**), 22-*O*-demethyl-22-*O*-*β*-D-glucopyranosylisocorynoxeine (**2**), and (4*S*)-corynoxeine *N*-oxide (**3**) were isolated from the $CHCl_3$-soluble fraction, together with three known tetracyclic oxindole alkaloid *N*-oxides, isocorynoxeine *N*-oxide (**4**), rhynchophylline *N*-oxide (**5**), and isorhynchophylline *N*-oxide (**6**) and an indole alkaloid, dihydrocorynantheine (**7**). Moreover, strictosidine (**8**), an indole alkaloid glycoside, was obtained from the BuOH-soluble fraction. The structures of **1**–**3** were elucidated by 1D- and 2D-NMR, UV, IR, MS, and CD techniques, and **4**–**8** were



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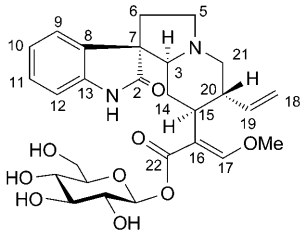

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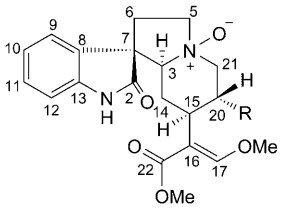
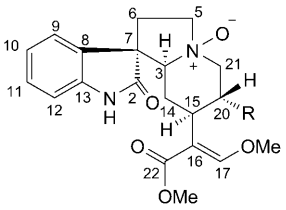
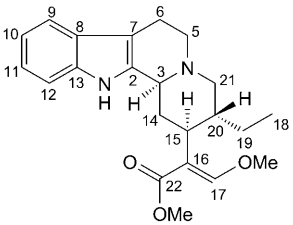


3[1]) R = CH=CH$_2$
5[1]) R = CH$_2$CH$_3$



4[1]) R = CH=CH$_2$
6[1]) R = CH$_2$CH$_3$



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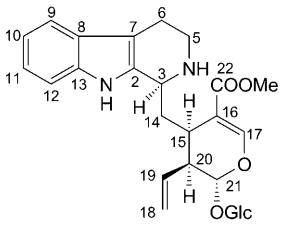

8[1])

identified by comparing their NMR and MS data with reported values [9–14]. The activity of these alkaloids on the activation of LPS-induced N9 cells was assayed for the first time. Only the inhibition by **8** of NO production from LPS-induced N9 cells was confirmed, which was isolated from *Uncaria* sp. for the first time. Alkaloids **1**–**7** showed little activity at the maximum concentration (100 or 30 μM).

The 2′-*O*-*β*-D-glucopyranosyl-11-hydroxyvincoside lactam (**1**) was isolated as a colorless, amorphous solid. The molecular formula was determined to be $C_{32}H_{40}N_2O_{14}$ from the $[M-H]^-$ ion peak at *m*/*z* 675.2465 in the ESI-TOF-MS, which implies that **1** has 14 degrees of unsaturation. ^{13}C- and DEPT-NMR Spectra (*Table 1*) showed the

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

presence of 32 C-atoms, 12 of which were ascribed to two hexose units. The aglycone of **1**, thus, accounted for the remaining molecular formula $C_{20}H_{19}N_2O_3$. The UV absorptions at 227.1, 248.3, and 296.3 nm revealed an indole moiety. The IR spectrum showed absorption bands due to a phenolic OH (3421 cm^{-1}), a C=O (1643 cm^{-1}), and a CH=CH_2 group (983.1 and 918.6 cm^{-1}). The resonances for the C- and H-atoms located at rings B, C, D, and E in the pentacyclic framework had a close resemblance to those of the known vincoside lactam aglycone, and they were assigned according to the literature values of the ^{1}H- and ^{13}C-NMR data of vincoside lactam aglycone [15] as well as according to its own HSQC spectrum (*Table 1*). The ^{1}H-NMR spectrum revealed an *ABX* aromatic-proton system appearing at δ(H) 7.53 (*d*, J = 8.4 Hz, H–C(9)), 7.22 (*dd*, J = 8.4, 2.4 Hz, H–C(10)), and 7.29 (*d*, J = 2.4 Hz, H–C(12)), which suggested a substituent attached to ring A. Compared with the ^{13}C-NMR data of vincoside lactam aglycone [15], the chemical shift of C(11) was shifted downfield by 32.5 ppm, while those of C(10) and C(12) were shifted upfield by 9.8 and 14.0 ppm, respectively. These NMR data pointed to hydroxylation of C(11). The literature values for 11-hydroxyhirsutine [16] and the HMBCs (*Fig. 1*) H–C(9)/C(11) and C(13), H–C(10)/C(12) and C(8), and H–C(12)/C(11) and C(13) also supported the presence of HO–C(11). The pentacyclic moiety and two hexose units accounted separately for the two partial molecular formulas $C_{18}H_{16}N_2O_3$ and $C_{12}H_{21}O_{11}$ with 13 degrees of unsaturation. The remaining partial molecular formula C_2H_3 was assigned to a CH_2=CH unit based on the ^{1}H- and ^{13}C-NMR signals at δ(H) 4.95 (*dd*, J = 10.2, 1.8 Hz), 4.80 (*dd*, J = 16.8, 1.8 Hz), and 5.46 (*dd*, J = 7.2, 3.0 Hz), and δ(C) 119.4 and 133.6. The CH_2=CH is located at C(20) according to the strong HMBCs CH_2(18)/C(20) and H–C(19)/C(20). Thus, the aglycone of **1** is 11-hydroxyvincoside lactam aglycone. Paper-chromatographic (PC) analysis of the acid-hydrolysis products of **1** exhibited only the presence of glucose. The signals of two anomeric protons (δ(H) 5.32 (*d*, J = 7.2 Hz, H–C(1′)) and 5.44 (*d*, J = 4.8 Hz, H–C(1″))) and twelve protons (δ(H) 4.50–3.84) together with the ^{13}C-NMR data indicated the presence of two β-D-glucosyl moieties [17]. The linkage between the anomeric C(1′) and C(21) and the interglucosidic linkage between C(2′) and C(1″) were determined by the HMBCs H–C(1′)/C(21) and H–C(1″)/C(2′).

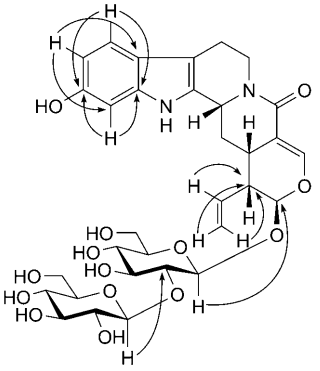

Fig. 1. *Main HMBCs of* **1**

Table 1. *NMR Data of Compound* **1**[1]). Measured in C_5D_5N; δ in ppm, J in Hz.

	δ(C)	δ(H)	HMBC[a])	NOESY
C(2)	133.3			
H−C(3)	53.6	5.36–5.40 (*m*)	C(2)	H_β−C(14), H−C(15)
H_α−C(5)	40.0	2.91–2.95 (*m*)	C(6), C(7)	H_α−C(6)
H_β−C(5)		5.41–5.43 (*m*)	C(3), C(6), C(16), C(22)	H_β−C(6)
H_α−C(6)	21.7	2.62 (*dd*, *J* = 14.4, 3.0)	C(2), C(5), C(7), C(8)	H_α−C(5)
H_β−C(6)		2.85–2.89 (*m*)	C(2), C(3), C(5), C(7)	H_β−C(5), H−C(9)
C(7)	108.5			
C(8)	121.5			
H−C(9)	119.1	7.53 (*d*, *J* = 8.4)	C(7), C(11), C(13)	H_β−C(6), H_β−C(14)
H−C(10)	110.2	7.22 (*dd*, *J* = 8.4, 2.4)	C(8), C(12)	
C(11)	155.0			
H−C(12)	98.0	7.29 (*d*, *J* = 2.4)	C(8), C(10), C(11), C(13)	
C(13)	139.2			
H_α−C(14)	32.1	1.66 (*d*, *J* = 12.6)	C(2), C(3), C(15), C(16), C(20)	
H_β−C(14)		2.59 (*ddd*, *J* = 12.6, 4.2, 4.2)	C(15), C(16)	H−C(3), H−C(9), H−C(15)
H−C(15)	26.7	3.64–3.69 (*m*)	C(16), C(19)	H−C(3), H_β−C(14), H−C(20)
C(16)	109.1			
H−C(17)	147.5	7.83 (*d*, *J* = 2.4)	C(15), C(16), C(21), C(22)	
H_a−C(18)	119.4	4.95 (*dd*, *J* = 10.2, 1.8)	C(20), C(21)	
H_b−C(18)		4.80 (*dd*, *J* = 16.8, 1.8)	C(19), C(20), C(21)	H−C(20)
H−C(19)	133.6	5.46 (*dd*, *J* = 7.2, 3.0)	C(15), C(20), C(21)	
H−C(20)	43.8	2.51 (*dd*, *J* = 8.4, 5.4)	C(15), C(16), C(18), C(19), C(21)	H−C(15), H−C(18)
H−C(21)	96.3	5.63 (*d*, *J* = 1.8)	C(15), C(17), C(19), C(1′)	
C(22)	163.7			
H−C(1′)	97.7	5.32 (*d*, *J* = 7.2)	C(21)	H−C(5′)
H−C(2′)	81.0	4.33 (*dd*, *J* = 10.2, 2.4)	C(1″), C(3′)	
H−C(3′)	78.4[b])	4.30 (*t*, *J* = 8.4)	C(1″), C(2′), C(4′)	
H−C(4′)	71.2	4.19–4.24 (*m*)		
H−C(5′)	78.4[b])	3.89 (*ddd*, *J* = 9.6, 3.6, 2.4)	C(1′), C(3′), C(4′)	H−C(1′)
CH_2(6′)	62.8	4.35–4.38 (*m*)	C(3′)	
H−C(1″)	105.2	5.44 (*d*, *J* = 4.8)	C(2′), C(2″), C(5″)	H−C(5″)
H−C(2″)	76.4	4.07 (*t*, *J* = 8.4)	C(1″), C(3″), C(4″)	
H−C(3″)	78.4[b])	4.19 (*t*, *J* = 9.0)	C(2″)	
H−C(4″)	71.6	4.19–4.24 (*m*)	C(6″)	
H−C(5″)	78.8	3.84 (*ddd*, *J* = 9.0, 4.2, 2.4)	C(3″), C(6″)	H−C(1″)
CH_2(6″)	62.4	4.50 (*dd*, *J* = 13.8, 1.8), 4.48 (*dd*, *J* = 11.4, 1.8)	C(3″), C(4″)	
H−N(1)		11.5 (*s*)	C(2), C(7), C(8), C(13)	

[a]) HMBC Correlations, optimized for 8 Hz, are from H-atom(s) stated to the indicated C-atom. All chemical shift assignments were carried out on the basis of HSQC and HMBC NMR techniques.
[b]) Signals overlapped by other resonances, chemical shift obtained from 2D correlation.

The absolute configuration of the aglycone of **1** was determined from the CD spectrum and 2D-NOESY correlations (*Fig. 2*). As a pentacyclic indole alkaloid, its (3*R*) configuration was established according to a negative *Cotton* effect (CE) at 270 nm in the CD spectrum [18]. It was also evidenced by the resonance of C(5) shifted upfield by 3 ppm and those of C(14) and C(15) shifted downfield by 5 and 3 ppm relative to corresponding signals of strictosidine lactam with (3*S*) configuration, and by the close match with corresponding signals of vincoside lactam with (3*R*) configuration [19]. The strong NOE correlations H_β–C(3)/H–C(15) and H–C(15)/H–C(20) indicated that both H–C(15) and H–C(20) were cofacial with H_β–C(3). Meanwhile, the coupling constant of H–C(21) (δ(H) 5.63 (*d*, $J = 1.8$ Hz)) implies a dihedral angle of nearly 90° between H_β–C(20) and H–C(21) [20], by which the α-orientation of H–C(21) was determined. Thus, **1** possesses the absolute configurations (3*R*,15*S*,20*R*,21*S*).

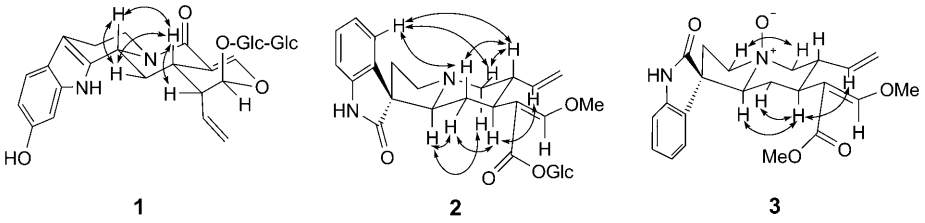

Fig. 2. *Key NOESY correlations of* **1**–**3**

The 22-*O*-demethyl-22-*O*-β-D-glucopyranosylisocorynoxeine (**2**) was isolated as a colorless, amorphous solid. The molecular formula was determined to be $C_{27}H_{34}N_2O_9$ from the $[M+H]^+$ ion peak at *m*/*z* 531.2337 in the ESI-TOF-MS. The ^{13}C-NMR spectra showed the signals for 27 C-atoms, 6 of which were attributed to a hexose unit. The positive-mode ESI-MS/MS gave a product-ion peak at *m*/*z* 369 ($[M+H-162]^+$) corresponding to the loss of a hexose from a precursor ion at *m*/*z* 531 ($[M+H]^+$). The aglycone of **2**, thus, accounted for the remaining molecular formula $C_{21}H_{23}N_2O_3$, indicating the loss of a CH_2 group (14 mass units) from corynoxeine or isocorynoxeine (*m*/*z* 382). The UV absorptions at 210.1, 248.9, and 286.2 nm revealed the presence of an oxindole moiety. The IR spectrum showed absorption bands due to NH (3437 cm^{-1}), C=O (1714 cm^{-1}), C=C (1619 cm^{-1}), and CH_2=CH groups (990.4 and 931.9 cm^{-1}). The ^{1}H- and ^{13}C-NMR data of **2** (*Table 2*) suggested that the tetracyclic framework and the C(20) substituent of its aglycone were similar to those of isocorynoxeine [21][22]. Only two differences in the signals of the C(15) substituent were displayed, *i.e.*, the disappearance of a MeO signal and the signal of a glycosyloxy-substituted C(22) (δ(C) 165.0) shifted upfield by 3 ppm relative to the C(22) signal of isocorynoxeine. Thus, the aglycone of **2** should be 22-*O*-demethylisocorynoxeine, which has not been reported up to now. PC Analysis of the acid-hydrolysis product of **2**, the signals of an anomeric proton (δ(H) 5.19 (*d*, $J = 7.8$ Hz)) and six protons (δ(H) 3.06–3.69) together with the ^{13}C-NMR data indicated the presence of a β-D-glucosyl moiety [17]. The glycosidation at C(22) was conform with the literature values of the ^{1}H- and ^{13}C-NMR data for isomitraphyllic acid [16-1] β-D-glucopyranosyl ester [23].

Table 2. *NMR Data of Compound* **2**[1]). Measured in (D_6)DMSO; δ in ppm, J in Hz.

	δ(C)	δ(H)	HMBC[a])	NOESY
C(2)	180.2			
H−C(3)	71.6	2.25 (*d*, *J* = 11.4)		H_α−C(14), H_α−C(21)
H_α−C(5)	53.3	2.31 (*dd*, *J* = 16.2, 8.4)		H_α−C(21)
H_β−C(5)		3.23 (*t*, *J* = 7.8)	C(6), C(7)	H_β−C(6), H−C(9)
H_α−C(6)	34.8	1.85–1.88 (*m*)	C(2), C(7), C(8)	
H_β−C(6)		2.18 (*dd*, *J* = 12.0, 9.6)	C(5)	H_β−C(5), H−C(9)
C(7)	56.1			
C(8)	133.7			
H−C(9)	124.5	7.27 (*d*, *J* = 7.2)	C(7), C(11), C(13)	H_β−C(5), H_β−C(6), H_β−C(14), H−C(20), H_β−C(21)
H−C(10)	121.6	6.96 (*dd*, *J* = 7.8, 6.6)	C(8), C(12)	
H−C(11)	127.5	7.13 (*dd*, *J* = 7.8, 6.6)	C(9)	
H−C(12)	109.2	6.80 (*d*, *J* = 7.2)	C(8), C(10)	
C(13)	141.4			
H_α−C(14)	29.6	0.78 (br. *d*, *J* = 11.4)		H−C(3), H−C(15)
H_β−C(14)		1.48 (*d*, *J* = 12.0)		H−C(9), H−C(20)
H−C(15)	36.8	2.35–2.38 (*m*)		H_α−C(14), H−C(19)
C(16)	110.4			
H−C(17)	161.2	7.37 (*s*)	C(15), C(16), C(22), MeO	MeO, CH_2(6′)
H_a−C(18)	115.8	4.89 (*d*, *J* = 10.2)		H−C(19)
H_b−C(18)		4.97 (*d*, *J* = 17.4)	C(19), C(20)	H−C(19)
H−C(19)	139.4	5.40–5.46 (*m*)		H−C(15), H−C(18), H_α−C(21)
H−C(20)	41.2	2.70–2.90 (*m*)		H−C(9), H_β−C(14), H_β−C(21)
H_α−C(21)	58.3	1.82–1.86 (*m*)	C(3), C(5), C(15), C(19), C(20)	H−C(19)
H_β−C(21)		3.09–3.11 (*m*)	C(20)	H−C(9), H−C(20)
C(22)	165.0			
MeO	61.6	3.78 (*s*)	C(17)	H−C(17)
H−C(1′)	93.6	5.19 (*d*, *J* = 7.8)		
H−C(2′)	72.5	3.06–3.10 (*m*)[b])		
H−C(3′)	77.7	3.17–3.19 (*m*)	C(2′), C(4′)	
H−C(4′)	69.8	3.06–3.10 (*m*)[b])		
H−C(5′)	76.5	3.06–3.10 (*m*)[b])		
CH_2(6′)	60.8	3.69 (br. *s*), 3.57–3.62 (*m*)		H−C(17)
H−N(1)		10.35 (*s*)		

[a]) HMBC Correlations, optimized for 8 Hz, are from H-atom(s) stated to the indicated C-atom. All chemical shift assignments were carried out on the basis of HSQC and HMBC NMR techniques.
[b]) Signals overlapped by other resonances, chemical shift obtained from 2D correlation.

The absolute and relative configuration of **2** was determined from the CD spectrum and 2D-NOESY correlations (*Fig. 2*). The (7*S*) configuration and α-orientation of H−C(3) were deduced from a negative CE at 285 nm, a positive CE at 220 nm, and a negative CE at 265 nm in the CD spectrum [24][25]. The α-orientation of H−C(15) was confirmed by the strong NOE correlations H_α−C(3)/H_α−C(14) (δ(H) 0.78) and

H_α–C(14)/H–C(15). Furthermore, the β-orientation of H–C(20) was indicated by the substantial NOE correlations H_β–C(14) (δ(H) 1.48)/H–C(20) and H–C(20)/aromatic H–C(9). Thus, **2** possesses the absolute configurations (3*S*,7*S*,15*S*,20*R*), which are consistent with the 'normal' A configuration of isocorynoxeine. The *trans* geometry of the C(16)=C(17) bond was confirmed on the basis of a downfield shift of the olefinic-proton signal relative to the corresponding signals of the *cis* compounds [26]. The tetracyclic oxindole alkaloid glycosides have not been reported although the pentacyclic ones have been isolated from the leaves of *U. sinensis* [23].

(4*S*)-Corynoxeine *N*-oxide (**3**) was isolated as a colorless, amorphous solid. The molecular formula was determined to be $C_{22}H_{26}N_2O_5$ from the $[M+Na]^+$ ion peak at *m*/*z* 421.1734 in the ESI-TOF-MS, which was the same as that of isocorynoxeine *N*-oxide [9]. In the ESI-MS, **3** showed a molecular-ion peak at *m*/*z* 399 ($[M+H]^+$), suggesting the addition of an O-atom to corynoxeine or isocorynoxeine. The resonances for the C-atoms and protons of **3** had a close resemblance to those of corynoxeine [7][27], except for those of C(3), C(5), and C(21) as well as of related protons (*Table 3*). The downfield shifts of C(3) (δ(C) 80.4, +5 ppm), C(5) (δ(C) 67.3, +12 ppm), and C(21) (δ(C) 66.4, +7 ppm) relative to the corresponding signals of corynoxeine revealed the formation of an N(4) oxide, which is also supported by the corresponding signals of isorhynchophylline *N*-oxide (**6**) [11]. For **3**, the (7*R*) configuration and the α-orientation of H–C(3) was established on the basis of a negative CE at 220 nm, a positive CE at 285 nm, and a negative CE at 265 nm [24][25]. The aromatic H–C(9) (δ(H) 7.60) did not appear downfield at δ(H) > 8.00, which indicates that it is on the opposite side of the molecule with respect to the O → N(4) moiety [10]. Thus, **3** possesses the (4*S*) absolute configuration. The strong NOE correlation H_α–C(3)/H–C(15) indicated that both protons are cofacial (*Fig. 2*). The NOE H_α–C(15)/H–C(19) implied that the 20-ethenyl group is in α-orientation because its β-orientation would make H–C(19) exist above the plane of ring D and lose its NOE correlation to H_α–C(15). The downfield shift by 0.8 ppm of H_β–C(20) relative to the corresponding signal of corynoxeine indicated the close proximity of H–C(20) to O → N(4) possessing the absolute configuration (4*S*). Moreover, the chemical shift of H–C(20) is similar to that of isorhynchophylline *N*-oxide possessing the absolute configuration (20*R*) [11]. Thus, **3** possesses the absolute configurations (7*R*,3*S*,4*S*,15*S*,20*R*), which are consistent with the '*normal*' B configuration of corynoxeine [24][25].

Microglia are the primary immune cells of the central nervous system. They are activated to release various neurotoxic factors in response to infection or brain injury, including nitrogen monooxide (NO) and proinflammatory cytokines, such as TNF-α and IL-1β, that are associated with several neurodegenerative diseases including *Alzheimer*'s disease, *Parkinson*'s disease, and multiple sclerosis [28][29]. The inhibitory activity of **1**–**8** and rhynchophylline (Rin) on LPS-induced NO release in N9 microglial cells was investigated. The cytotoxic activity of these compounds on N9 cells in the presence of LPS was assessed by the MTT assay (MTT = 2-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl-2*H*-tetrazolium bromide). Cotreatment of both unstimulated and stimulated N9 cells with **1**–**6** at concentrations up to 100 μM and with **7** and **8** at concentrations up to 30 μM did not affect the cell viability (data not shown). As shown in *Table 4*, only **8** showed a potent inhibitory effect on NO release in LPS-activated N9

Table 3. *NMR Data of Compound* **3**[1]). Measured in $CDCl_3$; δ in ppm, J in Hz.

	δ(C)	δ(H)	HMBC[a])	NOESY
C(2)	179.2			
H–C(3)	80.4	4.45 (br. *s*)		H–C(15)
H_α–C(5)	67.3	4.60 (br. *s*)		
H_β–C(5)		4.28 (br. *s*)		H_β–C(6), H_β–C(21)
H_α–C(6)	33.3	2.42–2.50 (*m*)		
H_β–C(6)		2.85 (br. *s*)		H_β–C(5)
C(7)	55.0			
C(8)	130.6			
H–C(9)	123.5[b])	7.60 (br. *s*)		
H–C(10)	123.5[b])	7.03 (*t*, *J* = 7.8)	C(11), C(12), C(13)	
H–C(11)	129.4	7.20 (*t*, *J* = 7.8)	C(9), C(10), C(13)	
H–C(12)	111.5	7.10 (*d*, *J* = 7.2)	C(10), C(11)	
C(13)	142.3			
H_α–C(14)	23.5	1.33 (*t*, *J* = 7.2)		H–C(15)
H_β–C(14)		2.67 (*dd*, *J* = 12.0, 6.6)		H_α–C(21)
H–C(15)	36.6	2.80 (*td*, *J* = 11.4, 3.6)	C(14), C(16), C(17), C(19), C(20)	H–C(3), H_α–C(14), H–C(19)
C(16)	109.0			
H–C(17)	160.8	7.30 (*s*)	C(16), MeO	MeO
H_a–C(18)	118.6	5.07 (*d*, *J* = 17.4)	C(19), C(20), C(21)	H–C(19)
H_b–C(18)		5.04 (*dd*, *J* = 11.4, 1.2)	C(19), C(20), C(21)	H–C(19)
H–C(19)	135.4	5.44–5.50 (*m*)	C(20), C(21)	H–C(15), H–C(18)
H–C(20)	37.7	3.62–3.65 (*m*)[b])	C(18), C(19), C(22)	
H_α–C(21)	66.4	3.62–3.65 (*m*)[b])		
H_β–C(21)		3.94 (*d*, *J* = 7.8)	C(22)	H_β–C(5)
C(22)	168.3			
MeO	61.9	3.76 (*s*)	C(17)	H–C(17)
COO*Me*	51.3	3.61 (*s*)	C(16), C(22)	
H–N(1)		8.70 (*s*)		

[a]) HMBC Correlations, optimized for 8 Hz, are from H-atom(s) stated to the indicated C-atom. All chemical shift assignments were carried out on the basis of HSQC and HMBC NMR techniques. [b]) Signals overlapped by other resonances, chemical shift obtained from 2D correlation.

cells, with the IC_{50} value being 8.3 μM. Resveratrol, a naturally occurring polyphenol present in red wine, was used as a positive compound in the parallel experiment. It showed a significantly suppressed NO production induced by LPS, with the IC_{50} value being 24.8 μM, which was consistent with our previous report [30]. The result that **8** showed more potent inhibitory activity than resveratrol suggests that it is of interest in studying the structure–activity relationship due to its characteristic D-ring cleavage as well as of therapeutic potential for neurodegenerative diseases related to microglial activation. Nevertheless, **1**–**7** were not active at the maximum tested concentration (100 μM for **1**–**6** and 30 μM for **7**). Compound **5** is an N(4)-oxide derivative of rhynchophylline that is a major bioactive alkaloid of *Uncaria* sp. Rhynchophylline potently inhibited NO release in LPS-activated N9 cells ($IC_{50} = 20.9$ μM), which is similar to its activity on LPS-activated rat primary microglial cells [7]. This result

Table 4. *Effect of* **1**–**8** *and Analogues on NO Release by LPS-Activated N9 Cells*[a])

	NO [% of LPS control]							IC_{50} [μM]
	control[b])	conc. 0[c])	1 μM	3 μM	10 μM	30 μM	100 μM	
1	41.9 ± 5.0	100.0 ± 14.6[d])	114.2 ± 5.5	119.6 ± 5.1	106.0 ± 4.3	86.7 ± 9.5	88.8 ± 1.7	> 100
2	33.6 ± 2.8	100.0 ± 7.7[d])	100.4 ± 3.6	106.8 ± 6.5	123.3 ± 19.8	103.5 ± 2.6	100.0 ± 3.2	> 100
3	41.9 ± 4.9	100.0 ± 14.6[d])	98.2 ± 3.6	114.6 ± 3.6	90.9 ± 8.4	100.0 ± 2.7	91.3 ± 7.2	> 100
4	20.9 ± 2.0	100.0 ± 2.7[d])	100.3 ± 2.9	106.8 ± 4.9	113.8 ± 1.4	104.1 ± 10.0	98.0 ± 4.9	> 100
5	26.4 ± 4.0	100.0 ± 3.6[d])	86.2 ± 3.3	110.7 ± 9.6	97.8 ± 1.8	103.9 ± 5.2	106.3 ± 4.9	> 100
6	27.5 ± 3.6	100.0 ± 6.5[d])	105.3 ± 6.4	99.3 ± 2.6	112.0 ± 0.7	103.1 ± 2.7	99.0 ± 3.6	> 100
7	24.3 ± 1.0	100.0 ± 5.9[d])	96.7 ± 0.8	89.8 ± 0.9	100.2 ± 5.2	86.8 ± 2.3	–	> 30
8	24.3 ± 2.0	100.0 ± 3.9[d])	92.8 ± 2.7	90.3 ± 3.5	68.2 ± 2.6[f])	31.2 ± 2.8[f])	–	8.3
		conc. 0	0.3 μM	1 μM	3 μM	10 μM	30 μM	
Rin	18.1 ± 0.6	100.0 ± 1.5[d])	86.4 ± 1.8[e])	83.8 ± 1.8[f])	78.5 ± 3.5[f])	67.1 ± 3.2[f])	51.9 ± 2.4[f])	20.9
Res	11.1 ± 2.2	100.0 ± 1.1[d])	98.4 ± 2.3	92.1 ± 1.9	85.3 ± 2.4	72.3 ± 1.3[e])	55.2 ± 3.2[f])	24.8

[a]) N9 Microglial cells were treated with serial dilutions of compounds in the presence of LPS (1 μg/ml) and then incubated for 48 h. Absorbance of 540 nm was determined after mixing the culture supernatants with *Griess* reagent as described in the *Exper. Part*. Data are represented as mean ± s.e.m. of triplicate cultures. [b]) In unstimulated microglial cells, only small amounts of NO_2^- (4.5 ± 0.7 μM) could be detected in the medium. [c]) Stimulation of microglial cells with LPS resulted in a marked increase in NO_2^- production (23.8 ± 2.5 μM). [d]) $p < 0.001$ *vs.* control group (cultured in medium alone). [e]) $p < 0.01$ and [f]) $p < 0.001$ *vs.* the groups treated with LPS alone. Resveratrol (Res) was used as positive control. Rin = rhynchophylline.

suggests that N(4)-oxide formation causes the loss of the inhibitory activity of rhynchophylline-type oxindole alkaloids.

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Marine Chemical Ltd.*, Qingdao, P. R. China), *Sephadex LH-20* (*GE Healthcare*, Sweden), and *MDS-5 RP* (200–300 mesh; *Beijing Medicine Technology Center*, P. R. China). Prep. HPLC: *Shim-pack PRC-ODS* (*Shimadzu Co. Ltd.*, Japan); *Shimadzu-LC-8A* solvent delivery pump; *Shimadzu-SPD-10AVP* detector. For optical rotation, UV (λ_{max} (log ε)), IR ($\tilde{\nu}_{max}$ in cm^{-1}), NMR, ESI-MS, and CD ([θ] (λ [nm])), see [7]. ESI-TOF-MS: *Micro-TOF-Bruker-Daltonics* mass spectrometer.

Plant Material. Leaves of *U. rhynchophylla* were collected in October 2005 in Fujian Province, China. Species identification was confirmed by Prof. *Zerong Jiang*, Shenyang Pharmaceutical University. A voucher specimen (syphu-20051105) is deposited with the Department of Traditional Chinese Medicines, Shenyang Pharmaceutical University.

Extraction and Isolation. The air-dried leaves (2.5 kg) of *U. rhynchophylla* were percolated with 75% aq. EtOH. The EtOH extract (670 g) was suspended in H_2O and partitioned successively with hexane (3 × 4 l), $CHCl_3$ (3 × 4 l), and BuOH (3 × 4 l) to obtain a hexane extract (25 g), a $CHCl_3$ extract (55 g), and a BuOH extract (250 g), resp. The dried $CHCl_3$-soluble fraction was subjected to CC (silica gel (2.0 kg), $CHCl_3$/MeOH 20:1 → 2:1): *Frs. 1–10*. *Fr. 2* (1 g) was subjected to CC (*Sephadex LH-20*, $CHCl_3$/MeOH 50:50): *Frs. 11–14*. *Fr. 14* (350 mg) was further subjected to CC (*MDS-5*, MeOH/H_2O 40:60 → 100:0): *Frs. 14.1–14.6*). *Fr. 14.4* (120 mg) was subjected to prep. HPLC (MeOH/H_2O 70:30

plus 0.03% Et_2NH): **7** (15.0 mg). *Fr. 4* (3 g) was subjected to CC (*MDS-5*, MeOH/H_2O 40:60 → 100:0): *Frs. 15–22*. *Fr. 20* (200 mg) was subjected to prep. HPLC (MeOH/H_2O 70:30 plus 0.03% Et_2NH): *Fr. 20.1* which was further separated by prep. HPLC (MeOH/H_2O 52:48 plus 0.03% Et_2NH): **4** (6.0 mg) and **6** (13.0 mg). *Fr. 5* (2 g) was subjected to CC (*Sephadex LH-20*, $CHCl_3$/MeOH 50:50): *Frs. 23–30*. *Fr. 26* (1.0 g) was further subjected to CC (*MDS-5*, MeOH/H_2O 50:50 → 100:0): *Frs. 26.1–26.6*. *Fr. 26.4* (170 mg) was subjected to prep. HPLC (MeOH/H_2O 54:46 plus 0.03% Et_2NH): **3** (16.0 mg) and **5** (7.5 mg). *Fr. 6* (2 g) was subjected to CC (*MDS-5*, MeOH/H_2O 40:60 → 100:0): *Frs. 31–38*. *Fr. 36* (80 mg) was subjected to prep. HPLC (MeOH/H_2O 51:49 plus 0.03% Et_2NH): **1** (7.0 mg) and **2** (8.0 mg). The BuOH extract was subjected to CC (silica gel (2.5 kg), $CHCl_3$/MeOH 20:1 → 2:1): *Frs. 39–48*. *Fr. 45* (2 g) was subjected to CC (*MDS-5*, MeOH/H_2O 30:70 → 100:0): *Frs. 49–53*. *Fr. 51* (380 mg) was subjected to CC (silica gel, $CHCl_3$/MeOH 10:1 → 4:1): *Frs. 51.1–51.4*. *Fr. 51.3* (45 mg) was subjected to CC (*Sephadex LH-20*, $CHCl_3$/MeOH 50:50): **8** (8 mg).

*2′-*O*-β-*D*-Glucopyranosyl-11-hydroxyvincoside Lactam* (=*(3β,15β,16α,17β)-19,20-Didehydro-16-ethenyl-17-[(2-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)oxy]-11-hydroxyoxayohimban-21-one*; **1**): Amorphous solid. $[\alpha]_D^{26} = -63.3$ ($c = 0.40$, MeOH). UV (MeOH): 227.1 (3.90), 248.3 (3.55), 296.3 (3.17). CD (0.25 mM, MeOH, 25°): +2.7 (220), −1.0 (230), −2.0 (240), −2.0 (250), −1.0 (260), −0.5 (270), −0.2 (280), −0.3 (290), −0.4 (300). IR (KBr): 3421, 1643, 1157, 1077, 983.1, 918.6. ^{1}H- and ^{13}C-NMR (C_5D_5N): *Table 1*. ESI-MS: 699 ($[M+Na]^+$), 675 ($[M-H]^-$). ESI-TOF-MS: 675.2465 ($[M-H]^-$, $C_{32}H_{39}N_2O_{14}^-$; calc. 675.2401).

*22-*O*-Demethyl-22-*O*-β-*D*-glucopyranosylisocorynoxeine* (=*(αE,1′S,6′R,7′S,8′aS)-6′-Ethenyl-1,2,2′,3′,6′,7′,8′,8′a-octahydro-α-(methoxymethylene)-2-oxospiro[3*H*-indole-3,1′(5′*H*)-indolizine]-7′-acetic Acid β-*D*-Glucopyranosyl Ester*; **2**): Amorphous solid. $[\alpha]_D^{26} = -20.0$ ($c = 0.42$, MeOH). UV (MeOH): 210.1 (3.90), 248.9 (3.77), 286.2 (2.78). CD (0.26 mM, MeOH, 25°): +6.5 (220), +4.6 (230), +4.5 (240), +1.8 (250), −2.0 (260), −2.2 (270), −1.8 (280), −1.0 (290), −0.5 (300). IR (KBr): 3437, 2923, 2854, 1714, 1697, 1619, 990.4, 931.9. ^{1}H- and ^{13}C-NMR ((D$_6$)DMSO): *Table 2*. ESI-MS/MS: 369 ($[M+H-162]^+$). ESI-MS: 531 ($[M+H]^+$), 553 ($[M+Na]^+$), 529 ($[M-H]^-$), 565 ($[M+Cl]^-$). ESI-TOF-MS: 531.2337 ($[M+H]^+$, $C_{27}H_{35}N_2O_9^+$; calc. 531.2342).

*(4*S*)-Corynoxeine* N*-Oxide* (=*(αE,1′R,4′S,6′R,7′S,8′aS)-6′-Ethenyl-1,2,2′,3′,6′,7′,8′,8′a-octahydro-α-(methoxymethylene)-2-oxospiro[3*H*-indole-3,1′(5′*H*)-indolizine]-7′-acetic Acid Methyl Ester 4′-Oxide*; **3**): Amorphous solid. $[\alpha]_D^{26} = -36.3$ ($c = 1.18$, MeOH). UV (MeOH): 211.2 (4.39), 244.2 (4.17), 288.3 (3.04). CD (1.88 mM, MeOH, 25°): −3.0 (220), +6.0 (230), +21.0 (240), +13.0 (250), −2.0 (260), −1.5 (270), +1.5 (280), +3.5 (290), +2.0 (300). IR (KBr): 3431, 2949, 1705, 1637, 991.5, 931.0. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Table 3*. ESI-MS: 399 ($[M+H]^+$), 421, 437 ($[M+K]^+$), 797 ($[2M+Na]^+$), 819 ($[2M+K]^+$), 433 ($[M+Cl]^-$). ESI-TOF-MS: 421.1734 ($[M+Na]^+$, $C_{22}H_{26}N_2O_5Na^+$; calc. 421.1739).

*(4*S*)-Isocorynoxeine* N*-Oxide* (=*(αE,1′S,4′S,6′R,7′S,8′aS)-6′-Ethenyl-1,2,2′,3′,6′,7′,8′,8′a-octahydro-α-(methoxymethylene)-2-oxospiro[3*H*-indole-3,1′(5′*H*)-indolizine]-7′-acetic Acid Methyl Ester 4′-Oxide*; **4**): Amorphous solid. UV (MeOH): 210.7 (4.25), 246.6 (4.12), 288.0 (3.14). ^{1}H-NMR: consistent with data in [9]. ^{13}C-NMR ($CDCl_3$, 100 MHz): 181.5 (C(2)); 167.7 (C(22)); 160.2 (C(17)); 141.7 (C(13)); 136.5 (C(19)); 130.7 (C(8)); 128.5 (C(11)); 128.4 (C(9)); 122.8 (C(10)); 117.7 (C(18)); 110.0 (C(16)); 109.6 (C(12)); 80.3 (C(3)); 68.3 (C(21)); 68.1 (C(5)); 61.7 (MeO); 55.0 (C(7)); 51.3 (COO*Me*); 38.0 (C(15)); 37.4 (C(6)); 35.9 (C(20)); 25.2 (C(14)); data assigned for the first time. ESI-MS: 399 ($[M+H]^+$).

*(4*S*)-Rhynchophylline* N*-Oxide* (=*(αE,1′R,4′S,6′R,7′S,8′aS)-6′-Ethyl-1,2,2′,3′,6′,7′,8′,8′a-octahydro-α-(methoxymethylene)-2-oxospiro[3*H*-indole-3,1′(5′*H*)-indolizine]-7′-acetic Acid Methyl Ester 4′-Oxide*; **5**): Amorphous solid. UV (MeOH): 217.7 (4.30), 243.0 (4.17), 290.3 (3.00). ^{1}H-NMR: consistent with data in [10]. ^{13}C-NMR ($CDCl_3$, 100 MHz): 180.5 (C(2)); 168.9 (C(22)); 160.8 (C(17)); 133.8 (C(13)); 128.9 (C(8)); 128.9 (C(11)); 122.2 (C(9)); 121.5 (C(10)); 112.5 (C(16)); 109.8 (C(12)); 81.5 (C(3)); 68.5 (C(21)); 68.5 (C(5)); 62.3 (MeO); 55.1 (C(7)); 51.5 (COO*Me*); 38.8 (C(15)); 33.8 (C(6)); 33.6 (C(20)); 24.1 (C(14)); 23.3 (C(19)); 10.7 (C(18)); data assigned for the first time. ESI-MS: 401 ($[M+H]^+$).

Biological Materials. Fetal bovine serum (FBS), *Iscove*'s modified *Dulbecco*'s medium (IMDM) and LPS (E5:055) were the same as those used in our previous study [7]. Rhynchophylline was isolated from the leaves of *U. rhynchophylla* in our laboratory [7]. Resveratrol was purchased from *Xi'an Guanyu Biotech Co. Ltd.* (China). The purity of **1**–**8**, rhynchophylline, and resveratrol was >97% (by HPLC). They were dissolved initially in DMSO and then diluted with PBS (phosphate-buffered saline) for

experiments. DMSO at the highest concentration possibly present under the experimental conditions used (0.1%) was not toxic to cells.

Microglial Cell Cultures. The murine microglial cell line N9 was a kind gift from Dr. *P. Ricciardi-Castagnoli* (Universita degli Studi di Milano-Bicocca, Milan, Italy). The cells were grown in IMDM supplemented with 5% heat-inactivated FBS, 2 mM glutamine, 100 U/ml of penicillin, 100 µg/ml of streptomycin, and $5 \cdot 10^{-5}$ M 2-mercaptoethanol. The cells were plated at a density of 5×10^5 cells/cm^2 onto 96-well microtiter plates for MTT and nitrite assay.

Cell Viability and Nitrite Assays. The measurements of cell viability and nitrite assays have been described in detail in our previous studies [7].

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