Biologically Active Ibogan and Vallesamine Derivatives from *Tabernaemontana divaricata*

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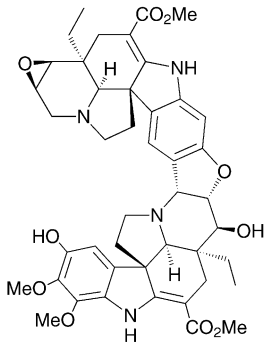
Six new indole alkaloids, *viz*., (3*S*)-3-cyanocoronaridine (**2**), (3*S*)-3-cyanoisovoacangine (**3**), conolobine A (**5**), conolobine B (**6**), conolidine (**7**), and (3*R*/3*S*)-3-ethoxyvoacangine (**8**), in addition to 36 known ones, were obtained from the stem-bark extract of the Malayan *Tabernaemontana divaricata*. The structures were determined by NMR and MS analysis. The CN-substituted alkaloids showed appreciable cytotoxicity towards the KB human oral epidermoid carcinoma cell-line.

Introduction. – Plants of the genus *Tabernaemontana* (Apocynaceae) have a widespread distribution and are rich in alkaloids [1–3]. In our systematic study of the Malaysian representatives of this genus, we have reported many examples of new alkaloids that are distinguished by their structural novelty, as well as useful bioactivity [4–18]. For instance, we recently reported the structures of conodiparine A, a new vobasine-iboga bisindole, which was found to reverse multidrug-resistance (MDR) in vincristine-resistant KB cells [5], and vobatricine, representing the first example of a bisindole of the vobasine-strychnan type [11], from the Malayan species, *T. corymbosa* Roxb. *ex* Wall. We have also reported the full alkaloidal composition of the leaf extract of the Malayan *Tabernaemontana divaricata* (L.) R.Br. *ex* Roem. & Schult (double-flower variety) [18]. The leaf extract provided the novel indoles, voaharine and taberhanine, and the bisindoles conophylline, conofoline, and conophyllinine [18]. The bisindole conophylline (**1**), which is found in both the single-flower as well as the double-flower variety of *T. divaricata*, has been recently found to exhibit important biological activity [19][20]. It was shown to be a potent inhibitor of *ras* functions [19], and very recently, it has also been found to induce morphological change as well as insulin production in pancreatic acinar carcinoma AR42J cells [20]. To complete the chemical study of this plant, we investigated the composition of the stem-bark extract and wish to report the isolation of new, biologically active alkaloids.

Results and Discussion. – A total of 42 alkaloids were obtained from the stem-bark extract of the Malayan *T. divaricata* (double-flower variety), of which six are new alkaloids, *viz*., (3*S*)-3-cyanocoronaridine (**2**), (3*S*)-3-cyanoisovoacangine (**3**), conolobine A (**5**), conolobine B (**6**), conolidine (**7**), and (3*R*/3*S*)-ethoxyvoacangine (**8**). Of these, three are ibogan compounds, while the other three are new vallesamine-apparicine derivatives.



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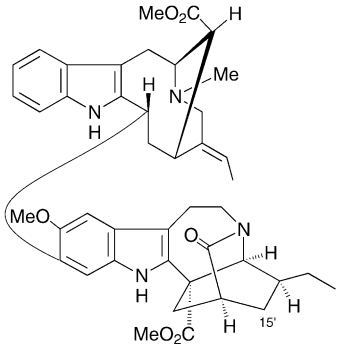

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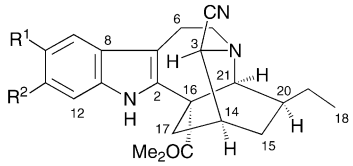
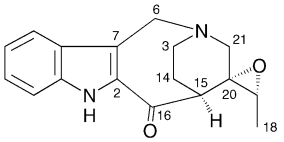


2 R^1, R^2 = H
3 R^1 = H, R^2 = MeO
4 R^1 = MeO, R^2 = H



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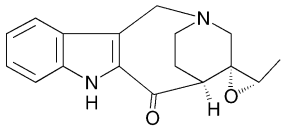

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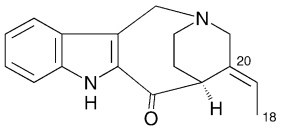

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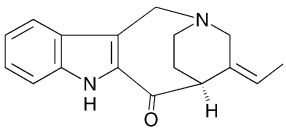

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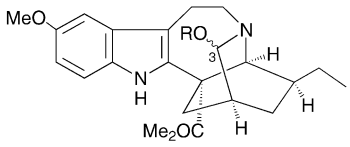


8 R = Et
10 R = Me

Compound **2** was obtained as a light yellow oil, $[\alpha]_D = -64$ ($c = 1.72$, $CHCl_3$). The IR spectrum showed bands at 3375, 2235, and 1728 cm^{-1} due to NH, C≡N, and ester C=O functions, respectively. The UV spectrum showed absorption maxima at 225, 276, 285, and 293 nm characteristic of an indole chromophore. The mass spectrum of **2** showed a molecular ion at *m*/*z* 363, the odd mass indicating the presence of an odd

number of N-atoms. HR-MS Measurements gave the molecular formula $C_{22}H_{25}N_3O_2$. The ^{13}C-NMR spectrum (*cf. Table 2* in *Exper. Part*) showed a total of 22 separate C-atom resonances, comprising two Me, five CH_2, eight CH groups, and seven quaternary C-atoms, in agreement with the molecular formula. The observed quaternary C-resonance at δ(C) 120.1 provided additional confirmation for the presence of the CN substituent [21][22]. The ^{1}H-NMR spectrum (*cf. Table 1* in *Exper. Part*) showed the presence of four aromatic H-atoms, an indole NH, an ester methyl, and an ethyl side chain. The spectrum is characteristic of iboga compounds, and the use of standard 2-D techniques confirmed the presence of an ibogan ring system. A notable change is observed for the aminomethylene H-atoms at C(3), for which only one H-atom is present in the spectrum of **2**. Comparison with the spectrum of coronaridine, as a representative iboga compound, showed, in addition, that the H–C(14) and H–C(15) resonances were shifted downfield. In the ^{13}C-NMR spectrum, the CH_2(3) group of an iboga compound was replaced by a H–C(3) group, while the signal of the adjacent C(14) was shifted downfield, and the signal of C(15) upfield, compared to coronaridine, consistent with the effect of replacement of a H-atom by a CN group at C(3). The position of substitution at C(3) is also supported by the HMBC spectrum (H–C(3)/CN; H–C(5), H–C(15), H–C(21)/C(3) correlations), as well as from NOE. Irradiation of H–C(3) resulted in enhancement of H_β–C(5), H_β–C(17), and H–C(14), and *vice versa*, providing additional confirmation for the location of the CN substituent, as well as for the configuration at C(3).

Compound **3** was obtained as a light yellow oil, $[\alpha]_D = -69$ ($c = 0.22$, $CHCl_3$). The IR and UV spectra were similar to those of **2**, indicating the presence of an indole chromophore, as well as NH, C≡N, and ester C=O functions. The mass spectrum of **3** showed a molecular ion at *m*/*z* 393, which, corresponding to $C_{23}H_{27}N_3O_3$, differed from **2** by replacement of H by MeO. Likewise, the ^{1}H- and ^{13}C-NMR data were generally similar to those of **2**, except for the presence of a MeO substituent in the aromatic ring. The MeO group was deduced to be at C(11), on the basis of the coupling behavior of the aromatic H-atoms, the ^{13}C shifts, and NOE (NH/H–C(12); H–C(6) and H–C(10)/H–C(9)). Compound **3** is, therefore, the 3-CN derivative of isovoacangine, which is a new alkaloid, although it constitutes the common ibogan unit of the bisindoles tabernaelegantinine C and D [23].

Compound **4**, (3*S*)-3-cyanovoacangine, was also obtained together with compounds **2** and **3**. Since the previous report [24] did not provide detailed NMR data, the NMR data of **4** are also given in *Tables 1* and *2* in *Exper. Part*.

Occurrence of the CN function in indole alkaloids are known, although examples are rare. Besides the ibogan derivatives discussed above, other examples include the 21-substituted aspidofractinine compounds, lahadinines A and B from *Kopsia* [21], a CN-substituted *seco*-stemmadenine derivative from *Alstonia* [22], and the *N*-cyanostrychnine derivatives from *Strychnos* species [25].

Conolobine A (**5**) was obtained as a pale yellow oil, $[\alpha]_D = +24$ ($c = 0.21$, $CHCl_3$). The IR spectrum showed bands at 3340 and 1634 cm^{-1} due to NH and conjugated ketone functions, respectively. The UV spectrum showed absorption maxima at 239 and 313 nm, characteristic of a 2-acylindole chromophore [26]. The EI-MS of **5** showed a molecular ion at *m*/*z* 282, which corresponds to $C_{17}H_{18}N_2O_2$. The ^{13}C-NMR spectrum (*Table 2* in *Exper. Part*) showed a total of 17 C-atom resonances (one Me, four CH_2,

and six CH groups, and six quaternary C-atoms). The ^{1}H-NMR spectrum (*Table 1* in *Exper. Part*) showed the presence of four aromatic H-atoms (δ(H) 7.1–7.6), an indole NH (δ(H) 9.11), and a MeCH group (δ(H) 1.31 ($d, J = 5.5$); 2.91 ($q, J = 5.5$)). Two isolated CH_2 groups were deduced from the presence of two pairs of *AB doublets*. The lower-field *doublets* centered at δ(H) 4.53 and 4.83 are due to the H-atoms at C(6), which is also in α-position to the indole moiety. The H-atoms at C(21) are found upfield from the H–C(6) signals, at δ(H) 2.68 and 3.65. The COSY-NMR spectrum disclosed only one other fragment, *viz.*, NCH_2CH_2CH, in addition to the MeCHO unit mentioned earlier. In the latter fragment, the Me group is coupled to a downfield CH at δ(H) 2.91, the characteristic shift value suggesting a tertiary H-atom of an epoxide. The downfield quaternary C resonance at δ(C) 192.0 confirms the presence of a conjugated ketone C=O group, which is placed at C(16), consistent with the 2-acylindole chromophore indicated earlier by the UV spectrum. After accounting for the corresponding ^{13}C shifts of all the partial structures mentioned above, only one quaternary oxygenated C-atom at δ(C) 59.9 remains, which is assigned to C(20) and, together with the adjacent C(19) resonance at δ(C) 56.6, provided additional confirmation for the presence of an epoxide function. The molecule can be, therefore, assembled to furnish an apparicine-type ring system, with an 19,20-epoxide function, as shown in **5**. The structure is in agreement with the HMBC data (see *Exper. Part*). To determine the relative configuration at C(19) and C(20) of the epoxide function, it is first necessary to establish the preferred conformation adopted by the molecule. Analysis of the coupling interaction for the ring-D H-atoms of **5** indicated that the piperidine ring D adopts a boat conformation. For instance, the $J(3\beta,14\alpha)$, $J(3\beta,14\beta)$, $J(3\alpha,14\alpha)$, and $J(3\alpha,14\beta)$ values of 10, 8.5, 8, and 3 Hz, respectively, are consistent with an approximate boat conformation of the piperidine ring (*Fig. 1*) [27]. The assignment of the relative configurations at C(19) and C(20) are based on two considerations. First, the epoxide configuration is likely to be α, which, from a biogenetic viewpoint, would be consistent with the anticipated, less hindered α-face epoxidation of the precursor olefin. Second, irradiation of the Me(18) signal resulted in enhancement of H–C(15) and *vice versa*, while irradiation of H–C(19) resulted in enhancement of H_β–C(21) (or H_S–C(21)) and *vice versa* (*Fig. 1*). These observations establish the relative configuration of C(19) and C(20) as (*R*) and (*R*) [28].

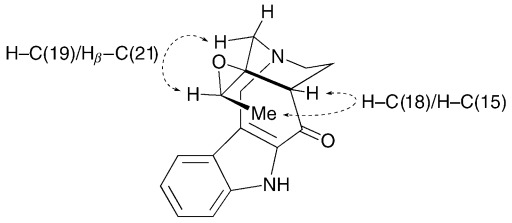


Fig. 1. *Selected NOEs of* **5**

Conolobine B (**6**) was obtained as a pale yellow oil, $[\alpha]_D = +159$ ($c = 0.06$, $CHCl_3$). The IR and UV spectra are similar to those of compound **5**. The EI mass spectrum was also similar to that of **5**, with the same molecular ion, suggesting the presence of an isomer. The ^{13}C-NMR spectral data of **6** were also very similar to those of **5**, except for

differences in chemical shifts for C(15), C(19), and C(21). Likewise, the ^{1}H-NMR spectrum, except for minor differences in the chemical shifts, showed a general similarity to that of **5**. The same behavior is shown by the COSY and HMBC data. These similarities suggested a common C-skeleton as well as conformation for both **5** and **6**, with a change in the configuration at C(19) and C(20). This was confirmed by NOE experiments, which gave results different from those obtained for **5** (*Fig. 2*). In the case of compound **6**, irradiation of H–C(19) resulted in enhancement of H–C(15) and *vice versa*, while irradiation of H–C(18) caused enhancement of H_β–C(21) and *vice versa*. These results established the relative configurations of C(19) and C(20) as (*S*) and (*R*), respectively. The configuration of the epoxide function remains α, as in compound **5**, consistent with the observed NOEs, as well as the sterically preferred, α-face epoxidation of the precursor olefin.

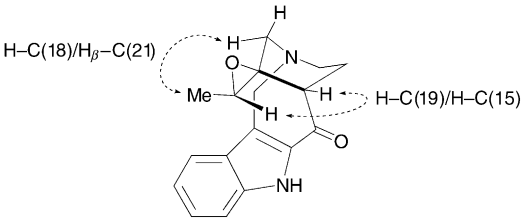


Fig. 2. *Selected NOEs of* **6**

Compound **7**, conolidine, was obtained as a light yellow oil, $[\alpha]_D = +32$ ($c = 0.16$, $CHCl_3$). The IR spectrum showed bands due to NH (3339 cm^{-1}) and conjugated ketone (1630 cm^{-1}) functions, while the UV spectrum (238 and 313 nm) was again characteristic of a 2-acylindole chromophore. The ESI-MS of **7** showed an $[M+H]^+$ ion at *m/z* 267 corresponding to the formula $C_{17}H_{18}N_2O$. The ^{13}C-NMR spectrum (*Table 2* in *Exper. Part*) comprises one Me, four CH_2, and six CH groups, and six quaternary C-atoms, and, in addition, indicated the presence of a conjugated C=O function (δ(C) 193.2), which was assigned to C(16) as in the previous two compounds. The ^{1}H- and ^{13}C-NMR-spectral data are somewhat similar to that of **5**, except for the replacement of signals, due to the epoxide function, by the signals of an ethylidene side chain, corresponding to C(18) and C(19). The H-atoms at C(18) showed characteristic long-range (homoallylic) coupling to H–C(21) and H–C(15). The geometry of the double bond was determined from NOE. Irradiation of H–C(18) (Me) caused enhancement of H–C(19) and H–C(15), while irradiation of H–C(19) resulted in enhancement of H–C(18) and H_α–C(21), indicating that the double bond is (*E*)-configured, as shown in structure **7**. The (*Z*)-isomer **9**, which was named ervaticine, was obtained previously from *Ervatamia coronaria*, and the NOE results, despite the structure drawn, indicated a (*Z*)-configuration for the C(19)=C(20) bond (NOE: H–C(18)/H_β–C(21); H–C(19)/H-C(15)) [29]. Conolobines A (**5**) and B (**6**) are, therefore, the corresponding α-epoxide derivatives of conolidine ((*E*)-isomer) and ervaticine ((*Z*)-isomer), respectively. While the (*E*) compound (conolidine) could be isolated, the (*Z*)-isomer (ervaticine) was not detected in the present investigation.

Compound **8**, (3*R*/3*S*)-3-ethoxyvoacangine, was obtained as a mixture of the (3*R*)- and (3*S*)-epimers, which could not be separated by chromatography. This was revealed

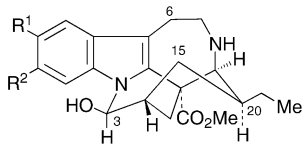
by the ^{1}H-NMR spectra, which showed the presence of a *ca.* 3:1 mixture of the two epimers with the (*R*)-epimer predominating (*Table 1* in *Exper. Part*). The UV spectrum was similar to that of voacangine with absorption maxima observed at 224, 287, and 299 nm. The IR spectrum showed peaks due to NH (3370 cm^{-1}) and ester (1727 cm^{-1}) functions. The ^{1}H- and ^{13}C-NMR-spectral data were consistent with those of an *Iboga* alkaloid and indicated the presence of an EtO substituent at C(3), from the observed H–C(3) (δ(H) 4.11 (*d*, *J* = 2, (3*R*)); 4.43 (*d*, *J* = 8, (3*S*))) and the EtO–C(3) group (δ(H) 1.17, 3.36 (EtO (*R*)); 1.24, 3.35 (EtO, (*S*))) resonances. The presence of the (3*R*)- and (3*S*)-epimers was further confirmed by the distinct ^{13}C resonances for C(3) (*R*) at δ(C) 93.8, and C(3) (*S*) at δ(C) 86.0 [10]. The expected molecular ion (*m*/*z* 412, $C_{24}H_{32}N_2O_4$) was not detected in the EI mass spectrum. The highest mass fragment observed in the EI mass spectrum, which was also the base peak, was at *m*/*z* 367 ($C_{22}H_{27}N_2O_3$, $[M - OEt]^+$), with an accompanying peak at *m*/*z* 366, corresponding to loss of EtOH. When the ESI mass spectrum was obtained with MeOH as solvent, another peak, in addition to the *m*/*z* 367 peak, was observed at *m*/*z* 399 ($C_{23}H_{30}N_2O_4$ + H), indicating the formation of the MeO derivative **10**. Since **8** is stable in MeOH solution and could be recovered intact, formation of **10** must have occurred during the ionization process. This behavior has been previously observed for the related compounds (3*R*/3*S*)-3-ethoxyheyneanine, (3*R*/3*S*)-3-ethoxy-19-epiheyneanine, and (3*R*/3*S*)-3-ethoxycoronaridine, isolated by us from another *Tabernaemontana* species [10]. The possibility that compound **8** (and other 3-alkoxy derivatives) are artefacts arising from 3-hydroxyiboga alkaloid precursors cannot be ruled out, since EtOH was used in the extraction of alkaloids, although this has been previously discounted [30][31]. Another possibility is that compound **8** could have been formed from 3-hydroxyvoacangine *via* iminium ion intermediates generated during silica-gel chromatography, since $CHCl_3$ stabilized with 1% EtOH was used as the eluting solvent [10]. Although this possibility cannot be entirely ruled out, it is, however, rendered unlikely, since 3-hydroxyiboga alkaloids have been isolated intact under similar conditions [30][32].

10,11-Demethoxychippiine [33], a member of the rare chippiine-dippinine group of compounds, was also obtained in this study. The chippiine group of alkaloids comprises a small group of indoles of which only two members were previously known. The prototype compound, chippiine (**11**), was first isolated from the African species *Tabernaemontana chippii* by *Van Beek et al.*, and due to paucity of material, was only partially characterized, structure **11** being proposed as a possible structure for the alkaloid [30]. Another derivative was subsequently reported from the South American species *T. markgrafiana*, for which the structure was assigned as 10,11-demethoxychippiine (**12**), based on comparison of the spectral data with those of chippiine (**11**) [33]. These alkaloids can be considered as having arisen from an iboga-type precursor (*e.g.*, 3-hydroxyconopharyngine in the case of chippiine) *via* cleavage of the N(4)–C(3) bond, followed by bond formation between C(3) and N(1) [30]. We have previously reported the occurrence of four chippiine-like derivatives, dippinines A–D (**13**–**16**), from the Malayan species *T. corymbosa* [9]. Detailed NMR studies of these compounds showed two major differences with respect to the previous two chippiine compounds. First, in contrast to the chippiines **11** and **12**, where the six-membered ring D adopts a boat conformation, the observed *J*(15α,20) and *J*(20,21) values of 12 and

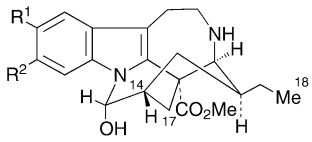
11 Hz, respectively, in the case of the dippinines, require these H-atoms to be *trans*-diaxial with respect to each other, which, in turn, requires the configuration of the Et side chain to be α (20*R*) [9]. The resulting preferred chair conformation of the six-membered ring has the Et group oriented equatorially, with the axially oriented H–C(20) directed towards the indole ring, which accounts for the unusual shielding observed for these H-atoms [9]. A second major difference concerns the configuration at C(3). In the dippinines, **13**–**16**, the configuration of the OH substituent at C(3) is deduced to be α (H_β–C(3)), based on the clear NOEs observed between H–C(3)/H–C(12), H_β–C(15), which differ from those of chippiine (**11**), in which the configuration at C(3) was determined by comparison of the chemical shift of H–C(3) with that of H–C(16) in the eburnamines and 16-descarbomethoxytacamines [30]. With 10,11-demethoxychippiine in hand from the present work, which showed similar NOEs as that observed for the dippinines (H–C(3)/H–C(12), H_α–C(15), and H_β–C(15)), the configuration at C(3) could be ascertained. It is likely, therefore, that the configuration at C(3) for both chippiines, **11a** and **12a**, is similar to that of the dippinines. The configuration at C(20) and the preferred boat conformation of the six-membered ring D in the chippiine compounds, however, remain unchanged.

Three bisindole alkaloids were also obtained, *viz*., conofoline [18], voacamine [34][35], and conodusarine (**17**) [36]. Conofoline also occurs in the leaf extract [18], while conodusarine (**17**) is a new biologically active bisindole alkaloid, which was found to reverse multidrug resistance in vincristine-resistant tumor cells [36].

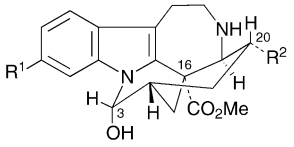
In addition to the new alkaloids discussed above, 35 other known alkaloids were also obtained from the stem-bark extract of *T. divaricata* (including the recently reported novel pentacyclic quinonilic alkaloid voastrictine, isolated from *T. corymbosa* [8]), as detailed in the *Exper. Part*. A notable feature of the alkaloidal composition, in addition to the novel structures discussed above, is the predominance of the ibogan and apparicine–vallesamine skeleton, which is a characteristic of *Tabernaemontana* [2–4].



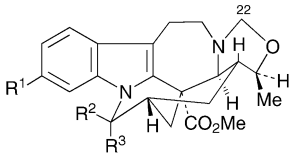
11 $R^1 = R^2 = OMe$
12 $R^1 = R^2 = H$



11a $R^1 = R^2 = OMe$
12a $R^1 = R^2 = H$



13 $R^1 = OMe$, $R^2 = CH_3CH(OH)$
14 $R^1 = H$, $R^2 = CH_3CO$



15 $R^1 = R^2 = H$, $R^3 = OH$
16 $R^1 = OMe$, R^2, $R^3 = O$

The three CN-substituted iboga derivatives **2**, **3**, and **4** showed appreciable *in vitro* cytotoxicity towards the KB human oral epidermoid carcinoma cell line (IC_{50} 2.2, 1.9, and 9.4 μg/ml, resp.).

Experimental Part

General. Optical rotations: *JASCO DIP-370* digital polarimeter or an *Atago Polax-D* polarimeter. UV Spectra: *Shimadzu UV-3101PC* spectrophotometer; λ_{max} in nm (log ε). IR Spectra: *Perkin-Elmer RX1* FT-IR spectrophotometer; in cm^{-1}. ^1H- and ^{13}C-NMR spectra: in $CDCl_3$ on a *JEOL JNM-LA 400* spectrometer at 400 and 100 MHz, resp.; δ in ppm rel. to Me_4Si, *J* in Hz. ESI-MS: *Perkin Elmer API 100* instrument; EI-MS and HR-EI-MS were obtained at the School of Chemistry, University of Nottingham, United Kingdom, courtesy of Professor *G. Pattenden*.

Plant Material. Details of collection, identification, and deposition of voucher specimens have been reported in [17].

Extraction and Isolation. Extraction of the ground stem-bark material was carried out in the usual manner by partitioning the concentrated EtOH extract with dil. acid as described in [37]. The alkaloids were isolated by initial column chromatography on silica gel by using $CHCl_3$ with increasing proportions of MeOH, followed by rechromatography of appropriate partially resolved fractions by centrifugal TLC. Solvent systems used for centrifugal TLC were Et_2O, $CHCl_3$, AcOEt, Et_2O/petroleum ether (4:1; 1:1; 1:5), AcOEt/petroleum ether (4:1), $CHCl_3$/petroleum ether (2:1), MeOH/$CHCl_3$ (2%; 5%), MeOH/AcOEt (2%), AcOEt/petroleum ether (1:1; NH_3-saturated), Et_2O/petroleum ether (5:1, NH_3-saturated; 2:1, NH_3-saturated), and $CHCl_3$/petroleum ether (5:1, NH_3-saturated). The yields ($g \cdot kg^{-1}$) of the alkaloids were as follows: **2** (0.02), **3** (0.005), **4** (0.042), **5** (0.002), **6** (0.0005), **7** (0.0013), **8** (0.056), **12a** (0.0007), **17** (0.0065), coronaridine (0.033), voacangine (0.091), isovoacangine (0.019), 10-hydroxycoronaridine (0.0008), 3-oxocoronaridine (0.0008), 3-oxovoacangine (0.0009), voacangine-7-hydroxyindolenine (0.018), heyneanine (0.028), voacristine (0.058), ibogamine (0.0067), (19*S*)-19-hydroxyibogamine (0.0016), ibogaine (0.0024), iboxygaine (0.0011), (3*R*/3*S*)-3-ethoxycoronaridine (0.0034), apparicine (0.0061), 16,22-dihydro-15-hydroxyapparicine (0.0014), vallesamine (0.0036), *O*-acetylvallesamine (0.0042), voaphylline (0.032), voalenine (0.014), voafinine (0.0014), voafinidine (0.001), (−)-mehranine (0.0003), pachysiphine (0.0014), vobasine (0.097), pericyclivine (0.002), (16*R*,19*E*)-isositsirikine (0.013), 19,20-didehydroervatamine (0.025), methuenine (0.0007), voastrictine (0.011), tubotaiwine (0.017), conofoline (0.0067), and voacamine (0.012).

(3S)-3-Cyanocoronaridine (**2**). Light yellow oil. $[\alpha]_D = -64$ ($c = 1.72$, $CHCl_3$). UV (EtOH): 225 (4.68), 276 (4.02), 285 (4.06), 293 (4.01). IR (dry film): 3375, 2235, 1728. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS: 364 $[M+1]^+$). EI-MS: 363 (8, M^+), 337 (4), 149 (74), 136 (64). HR-EI-MS: 363.1945 (M^+, $C_{22}H_{25}N_3O_2^+$; calc. 363.1947).

(3S)-3-Cyanoisovoacangine (**3**). Light yellowish oil. $[\alpha]_D = -69$ ($c = 0.22$ $CHCl_3$). UV (EtOH): 226 (4.48), 276 (3.73), 298 (3.85). IR (dry film): 3375, 2235, 1727. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS: 394 $[M+1]^+$. EI-MS: 393 (4, M^+), 367 (2), 136 (69). HR-EI-MS: 393.2064 (M^+, $C_{23}H_{27}N_3O_3^+$; calc. 393.2052).

(3S)-3-Cyanovoacangine (**4**). Light yellowish oil. $[\alpha]_D = -70$ ($c = 0.57$ $CHCl_3$). UV (EtOH): 227 (4.19), 284 (3.79), 313 (3.62). IR (dry film): 3378, 2230, 1728. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS: 394 $[M+1]^+$. EI-MS: 393 (6, M^+), 367 (14), 136 (49). HR-EI-MS: 393.2080 (M^+, $C_{23}H_{27}N_3O_3^+$; calc. 393.2052).

Conolobine A (**5**). Pale yellow oil. $[\alpha]_D = +24$ ($c = 0.21$ $CHCl_3$). UV (EtOH): 239 (4.12), 313 (4.26). IR (dry film): 3340, 1634. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. 2-D NMR (HMBC): H−C(6), H−C(15), NH/C(2); H−C(6), H−C(14), H−C(15), H−C(21)/C(3); H−C(3), H−C(21)/C(6); H−C(6), H−C(9)/C(7); H−C(6), H−C(12), NH/C(8); H−C(10), H−C(11)/C(9); H−C(12)/C(10); H−C(9), H−C(10)/C(11); H−C(10)/C(12); H−C(9), H−C(11), *N*H/C(13); H−C(3), H−C(15)/C(14); H−C(3), H−C(14), H−C(19), H−C(21)/C(15); H−C(14), H−C(15)/C(16); H−C(19)/C(18); H−C(15), H−C(18), H−C(21)/C(19); H−C(14), H−C(15), H−C(18), H−C(19), H−C(21)/C(20); H−C(3), H−C(6), H−C(19)/C(21). NOE: H_α−C(6)/H−C(9); H_β−C(6)/H_β−C(21); H−C(9)/H_β−C(6), H−C(10); H−C(15)/H_α−C(14), H_β−C(14), Me(18); Me(18)/H−C(15), H−C(19); H−C(19)/Me(18), H_β−C(21); H_β−C(21)/H_β−C(6), H−C(19); NH/H−C(12). ESI-MS: 283 $[M+1]^+$. EI-MS: 282 (74, M^+), 267 (13), 239 (20), 237 (11), 185 (14), 158 (25), 156 (32), 130 (100). HR-EI-MS: 282.1357 (M^+, $C_{17}H_{18}N_2O_2^+$; calc. 282.1368).

Conolobine B (**6**). Pale yellow oil. $[\alpha]_D = +159$ ($c = 0.06$ $CHCl_3$). UV (EtOH): 239 (4.11), 317 (4.14). IR (dry film): 3340, 1634. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. NOE: H_α−C(6)/H−C(9); H_β−C(6)/H_β−C(21); H−C(15)/H_α−C(14), H_β−C(14), H−C(19); Me(18)/H_β−C(21), H−C(19); H−C(19)/H−C(15),

Table 1. *^{1}H-NMR Data*[a]) *of Compounds* **2**–**8**

H-Atoms	**2**	**3**	**4**	**5**	**6**	**7**	(3*R*)-**8**	(3*S*)-**8**
H–C(3)	3.84 (*t*, *J* = 2)	3.83 (*t*, *J* = 2)	3.82 (*t*, *J* = 2)	3.17 (*dddd*, *J* = 14, 10, 8.5, 1)	3.14 (*dtd*, *J* = 14, 8.5, 1)	3.09 (*dddd*, *J* = 14, 10, 8, 1)	4.11 (*d*, *J* = 2)	4.43 (*d*, *J* = 8)
H–C(3)	–	–	–	3.40 (*ddd*, *J* = 14, 8, 3)	3.36 (*ddd*, *J* = 14, 8, 5)	3.40 (*ddd*, *J* = 14, 8.5, 3)	–	–
CH_2(5)	3.32 (*ddd*, *J* = 14, 6.5, 6); 3.48 (*ddd*, *J* = 14, 7, 6.5)	3.30 (*ddd*, *J* = 14, 6.5, 6); 3.44 (*ddd*, *J* = 14, 7, 6.5)	3.30 (*ddd*, *J* = 14, 6.5, 6); 3.46 (*dt*, *J* = 14, 6.5)	–	–	–	3.21 (*dt*, *J* = 14, 7.5); 3.53 (*dt*, *J* = 14, 5)	3.30 (*m*); 3.45 (*dt*, *J* = 14, 5)
CH_2(6)	3.10 (*dt*, *J* = 16, 6.5); 3.17 (*ddd*, *J* = 16, 7, 6)	3.03 (*dt*, *J* = 16, 6.5); 3.10 (*ddd*, *J* = 16, 7, 6)	3.03 (*dt*, *J* = 16, 6.5); 3.10 (*ddd*, *J* = 16, 6.5, 6)	4.53 (*d*, *J* = 18.5); 4.83 (*d*, *J* = 18.5)	4.61 (*d*, *J* = 18.5); 4.71 (*d*, *J* = 18.5)	4.29 (*d*, *J* = 18.5); 4.77 (*d*, *J* = 18.5)	3.05 (*m*); 3.05 (*m*)	3.05 (*m*); 3.05 (*m*)
H–C(9)	7.48 (*dd*, *J* = 7.5, 1)	7.32 (*d*, *J* = 8)	6.90 (*d*, *J* = 2)	7.57 (br. *dd*, *J* = 8, 1)	7.58 (br. *dd*, *J* = 8, 1)	7.57 (br. *dd*, *J* = 8, 1)	6.95 (*d*, *J* = 2)	6.94 (*d*, *J* = 2)
H–C(10)	7.11 (*td*, *J* = 7.5, 1)	6.75 (*dd*, *J* = 8, 2)	–	7.10 (*ddd*, *J* = 8, 6, 2)	7.11 (*ddd*, *J* = 8, 6, 2)	7.11 (*ddd*, *J* = 8, 6, 2)	–	–
H–C(11)	7.18 (*td*, *J* = 7.5, 1)	–	6.18 (*dd*, *J* = 8, 2)	7.32 (*ddd*, *J* = 8.5, 6, 1)	7.33 (*ddd*, *J* = 8.5, 6, 1)	7.33 (*ddd*, *J* = 8.5, 6, 1)	6.81 (*dd*, *J* = 8, 2)	6.81 (*dd*, *J* = 8, 2)
H–C(12)	7.26 (*dd*, *J* = 7.5, 1)	6.73 (*d*, *J* = 2)	7.12 (*d*, *J* = 8)	7.35 (*ddd*, *J* = 8.5, 2, 0.5)	7.36 (*ddd*, *J* = 8.5, 2, 0.5)	7.36 (*ddd*, *J* = 8.5, 2, 0.5)	7.15 (*d*, *J* = 8)	7.14 (*d*, *J* = 8)
H–C(14)	2.19 (*tq*, *J* = 4, 2)	2.16 (*tq*, *J* = 4, 2)	2.16 (*tq*, *J* = 4, 2)	2.09 (*dddd*, *J* = 15, 10, 8, 6)	2.15 (*dddd*, *J* = 15, 8.5, 8, 6)	2.05 (*ddt*, *J* = 15, 8, 3);	1.99 (*m*)	1.87 (*m*)
H–C(14)	–	–	–	2.15 (*ddt*, *J* = 15, 8.5, 3)	2.21 (*dddd*, *J* = 15, 8.5, 5, 3)	2.14 (*dddd*, *J* = 15, 10, 8.5, 6.5)	–	–
H–C(15)	1.52 (*ddt*, *J* = 13, 7, 2)	1.48 (*ddt*, *J* = 13, 7, 2)	1.49 (*ddt*, *J* = 13, 7, 2)	3.23 (*dd*, *J* = 6, 3)	3.04 (*dd*, *J* = 6, 3)	3.98 (br. *d*, *J* = 6.5)	1.50 (*m*)	1.50 (*m*)
H–C(15)	1.79 (*dddd*, *J* = 13, 10, 4, 2)	1.76 (*dddd*, *J* = 13, 10, 4, 2)	1.77 (*dddd*, *J* = 13, 10, 4, 2)	–	–	–	1.58 (*m*)	1.58 (*m*)
CH_2(17)	1.97 (*ddd*, *J* = 14, 4, 2); 2.70 (*dd*, *J* = 14, 2)	1.94 (*ddd*, *J* = 14, 4, 2); 2.64 (*dd*, *J* = 14, 2)	1.94 (*ddd*, *J* = 14, 4, 2); 2.67 (*dd*, *J* = 14, 2)	–	–	–	1.89 (*m*); 2.76 (*dd*, *J* = 14, 2)	1.97 (*m*); 2.72 (*dd*, *J* = 14, 2)
Me(18)	0.93 (*t*, *J* = 7)	0.90 (*t*, *J* = 7)	0.91 (*t*, *J* = 7)	1.31 (*d*, *J* = 5.5)	1.27 (*d*, *J* = 5.5)	1.51 (*ddt*, *J* = 7, 2, 1)	0.90 (*t*, *J* = 7.5)	0.93 (*t*, *J* = 7.5)
H–C(19)	1.55 (br. *dq*, *J* = 14, 7);	1.52 (br. *dq*, *J* = 14, 7);	1.52 (br. *dq*, *J* = 14, 7)	2.91 (*q*, *J* = 5.5)	2.95 (*q*, *J* = 5.5)	5.47 (*qt*, *J* = 7, 1)	1.50 (*m*)	1.50 (*m*)
H–C(19)	1.65 (br. *dq*, *J* = 14, 7)	1.61 (br. *dq*, *J* = 14, 7)	1.62 (br. *dq*, *J* = 14, 7)	–	–	–	1.66 (*m*)	1.66 (*m*)
H–C(20)	1.40 (*dq*, *J* = 10, 7)	1.36 (*dq*, *J* = 10, 7)	1.37 (*dq*, *J* = 10, 7)	–	–	–	1.33 (*m*)	1.33 (*m*)
H–C(21)	3.67 (br. *s*)	3.62 (br. *s*)	3.64 (br. *s*)	2.68 (*dd*, *J* = 15, 1)	2.91 (*dd*, *J* = 15, 1)	3.31 (br. *d*, *J* = 15)	3.80 (br. *s*)	3.77 (br. *s*)
H–C(21)	–	–	–	3.65 (*d*, *J* = 15)	3.61 (*d*, *J* = 15)	3.86 (br. *d*, *J* = 15)	–	–
MeO	3.73 (*s*)	3.70 (*s*)	3.71 (*s*)	–	–	–	3.69 (*s*)	3.69 (*s*)
10-MeO	–	–	3.83 (*s*)	–	–	–	3.85 (*s*)	3.82 (*s*)
11-MeO	–	3.81 (*s*)	–	–	–	–	–	–
NH	7.82 (br. *s*)	7.69 (br. *s*)	7.76 (br. *s*)	9.11 (br. *s*)	9.02 (br. *s*)	9.05 (br. *s*)	7.89 (br. *s*)	7.89 (br. *s*)
EtO	–	–	–	–	–	–	1.17 (*t*, *J* = 7)	1.24 (*t*, *J* = 7)
	–	–	–	–	–	–	3.36 (*q*, *J* = 7)	3.35 (*q*, *J* = 7)

[a]) $CDCl_3$, 400 MHz; assignments based on COSY and HMQC.

Table 2. *^{13}C-NMR Data* (110 MHz, $CDCl_3$)[a]) *for Compounds* **2**–**8**

C-Atoms	**2**	**3**	**4**	**5**	**6**	**7**	(3*R*)-**8**	(3*S*)-**8**
C(2)	135.3	133.9	136.1	130.9	130.5	133.4	137.4	137.0
C(3)	53.3	53.1	53.2	43.6	44.4	44.2	93.8	86.0
C(5)	51.9	51.8	51.9	–	–	–	52.2	51.2
C(6)	21.5	21.6	21.6	53.7	53.8	53.3	21.9	21.8
C(7)	109.9	109.7	109.6	119.8	120.0	120.1	109.8	109.6
C(8)	128.3	122.6	128.8	127.7	127.7	127.9	128.5	128.5
C(9)	118.5	119.2	100.6	120.8	120.8	120.8	100.4	100.4
C(10)	119.6	109.4	154.2	120.0	120.1	120.5	153.9	156.3
C(11)	122.5	156.8	112.5	126.5	126.6	126.4	111.7	111.9
C(12)	110.5	94.2	111.3	111.8	111.8	111.8	111.1	111.1
C(13)	135.5	136.3	130.6	136.5	136.6	136.3	130.6	130.6
C(14)	31.3	31.3	31.2	23.4	24.4	22.9	30.7	34.4
C(15)	28.5	28.6	28.5	46.1	52.3	48.1	25.0	24.6
C(16)	54.3	54.2	54.3	192.0	192.7	193.2	54.4	54.2
C(17)	35.7	35.6	35.7	–	–	–	35.3	35.3
C(18)	11.5	11.5	11.5	13.2	13.8	12.7	11.6	11.6
C(19)	26.6	26.5	26.6	56.6	61.4	122.9	26.5	26.7
C(20)	38.3	38.3	38.2	59.9	59.4	130.2	37.8	37.6
C(21)	56.1	56.2	56.1	54.9	50.2	55.0	55.9	56.1
MeO	52.9	52.9	52.9	–	–	–	52.5	52.6
CO	174.5	174.5	174.4	–	–	–	175.0	175.0
10-MeO	–	–	56.0	–	–	–	55.8	55.6
11-MeO	–	55.7	–	–	–	–	–	–
C≡N	120.1	120.2	120.0	–	–	–	–	–
EtO	–	–	–	–	–	–	15.6	18.3
EtO	–	–	–	–	–	–	61.4	58.2

[a]) Assignments based on HMQC and HMBC.

Me(18); H_β–C(21)/H_β–C(6), Me(18); NH/H–C(12). ESI-MS: 283 $[M+1]^+$. EI-MS: 282 (100, M^+), 267 (8), 239 (13), 238 (12), 185 (7), 158 (9), 156 (10), 130 (42). HR-EI-MS: 282.1379 (M^+, $C_{17}H_{18}N_2O_2^+$; calc. 282.1368).

Conolidine (**7**). Light yellowish oil. $[\alpha]_D = +32$ ($c = 0.16$ $CHCl_3$). UV (EtOH): 238 (4.15), 313 (4.22). IR (dry film): 3339, 1630. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS: 267 ($[M+1]^+$, $C_{17}H_{19}N_2O^+$).

(3R/3S)-3-Ethoxyvoacangine (**8**). Light yellowish oil ((3*R*)/(3*S*) *ca.* 3:1). UV (EtOH): 224 (4.40), 287 (4.09), 299 (4.06). IR (dry film): 3370, 1727. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS: 367 ($[M-OEt]^+$). EI-MS: 367 (100, $[M-OEt]^+$), 366 (60, $[M-EtOH]^+$), 136 (18), 122 (13). HR-EI-MS: 367.2022 ($[M-OEt]^+$), $C_{22}H_{27}N_2O_3^+$; calc. 367.2021).

Cytotoxicity Assays. Cytotoxicity assays were carried out on KB cells by a slight modification of the method described in [38]. Human oral epidemoid carcinoma KB cells were maintained in culture flasks in *Eagle*'s MEM, supplemented with 10% fetal calf serum and kanamycin (60 μg/ml). KB Cells (1.5 × 105 ml) were seeded in 0.2 ml of culture medium/well in 96-well plates (*Corning Glass Works*). The cells were treated in triplicate with graded concentrations of 5 μl test samples and then incubated in a 5% CO_2 atmosphere at 37° for 72 h. The MTT assay was used to measure the cytotoxicity effect. The activity was shown as the IC_{50} value, which was the concentration [μg/ml] of test compound to give 50% inhibition of KB cell growth.

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