HETEROCYCLES, Vol. 55, No. 12, 2001, pp. 2405 - 2412, Received, 17th September, 2001

DIPPININES A - D, NEW IBOGA-DERIVED INDOLE ALKALOIDS FROM *TABERNAEMONTANA*

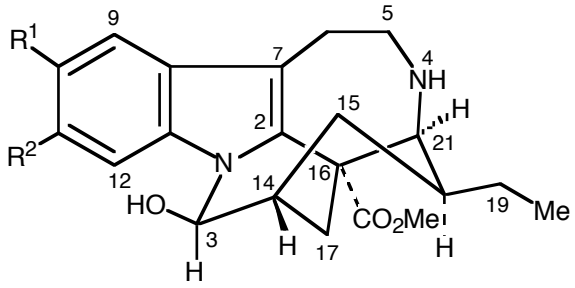
Toh-Seok Kam* and Kooi-Mow Sim

Department of Chemistry, University of Malaya
50603 Kuala Lumpur, Malaysia

Abstract – Four new indole alkaloids, *viz*., dippinines A - D, belonging to the chippiine group were isolated from the leaf and stem extract of *Tabernaemontana corymbosa* and the structures established by spectroscopic analysis.

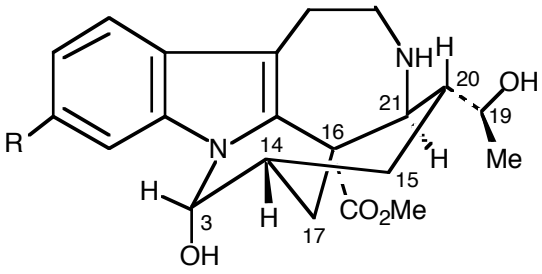
The chippiine group of alkaloids comprises a small group of indoles, of which only two members were previously known.[1,2] The prototype compound, chippiine, was first isolated from the African species, *Tabernaemontana chippii* by Van Beek and coworkers, and due to paucity of material, was only partially characterized, structure (**1**) being proposed as a possible structure for the alkaloid.[1] Another derivative was subsequently reported from the South American species, *T. markgrafiana*, for which the structure was assigned as 10, 11-demethoxychippiine (**2**), based on comparison of the spectral data with those of chippiine.[2] These alkaloids can be considered as having arisen from an iboga-type precursor (for example, 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).[1] We have previously reported in preliminary form the occurrence of two chippiine derivatives from the Malayan species, *T. corymbosa*,[3,4] and now wish to report full details of the isolation and structures of four such alkaloids from the leaf and stem extract of this plant.

Of the four compounds, dippinine A (**3**) was the major constituent isolated. Dippinines A (**3**) and D (**6**) were obtained from the leaf extract, while dippinines B (**4**) and C (**5**) were obtained from the stem extract. Dippinine A (**3**) was obtained as a light yellow oil, $[\alpha]_D$ +19° (*c* 0.67, $CHCl_3$). The IR spectrum showed bands due to NH/OH (3356 cm^{-1}) and ester (1721 cm^{-1}) functions, while the UV spectrum (λ_{max} 231, 280, and 300 nm) is characteristic of an indole chromophore. The EIMS showed an M^+ at *m/z* 400 which analysed for $C_{22}H_{28}N_2O_5$. The ^{13}C NMR spectrum showed a total of 22 carbon resonances in agreement



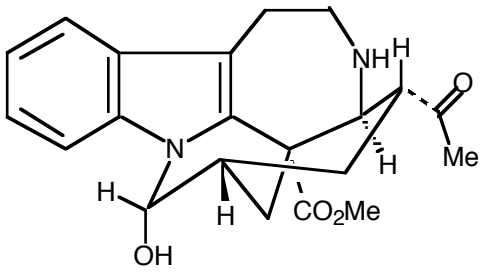
1 $R^1 = R^2 = OMe$

2 $R^1 = R^2 = H$

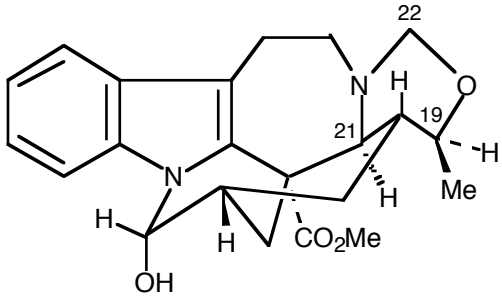


3 R = OMe

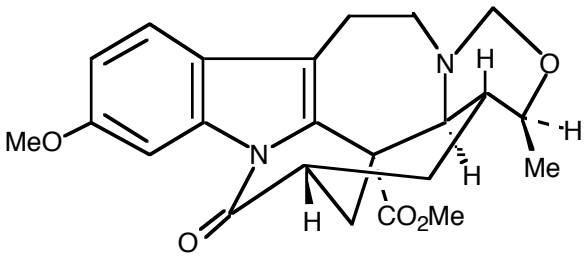
3a R = H



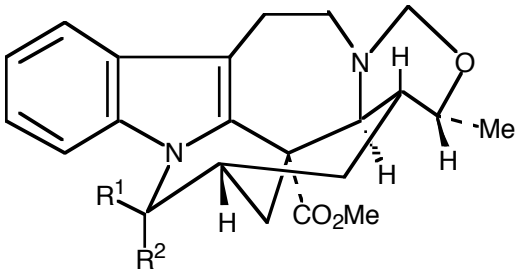
4



5



6



7

with the formula obtained from the mass-spectrum. The ^{1}H NMR spectral data indicated the presence of a aromatic methoxy substituent, which from consideration of the aromatic carbon shifts, is deduced to be at C(11). A conspicuous feature of the ^{1}H NMR spectrum is the absence of the indole NH signal and the presence of a high field signal due to H-15α at δ 1.17 (Table 1). These features coupled with the general similarity of the non-aromatic carbon resonances in the ^{13}C NMR spectrum with those of **2**, indicated that dippinine A (**3**) possesses a similar ring system. The NMR spectral data also showed that in addition, dippinine A possesses an oxidised ethyl side chain, in the form of a hydroxyethyl group (δ 3.50, dq, *J* 7, 6 Hz, H-19; 0.92, d, *J* 6 Hz, CH_3-18), in place of an ethyl side chain at C-20. Application of the standard 2-D experiments allowed complete assignment of the NMR spectra and confirm the basic ring system of **3**. The configuration at C(19) in **3** could not be assigned based on the spectral data, but can be deduced to be *S* from

its relationship to the hexacyclic derivatives, dippinines C (**5**) and D (**6**) (*vide infra*). Irradiation of the H(3) signal resulted in enhancement of the H(15β) as well as the H(12) signals, and *vice versa*. Examination of models revealed that these interactions are only possible if the stereochemistry of H(3) is β. The observed NOE interaction between H(12) and H(3) also provides additional confirmation for the attachment of the aromatic methoxy substituent at C(11).

Dippinine B (**4**) was obtained from the stem extract as a light yellowish oil, $[\alpha]_D$ -54° (*c* 0.06, $CHCl_3$). The UV spectrum (λ_{max} 226, 285, and 294 nm) was similar to that of dippinine A (**3**) while the IR spectrum showed, in addition to bands due to NH/OH (3355 cm^{-1}) and ester (1725 cm^{-1}) groups, a band at 1710 cm^{-1} due to a carbonyl function. The EIMS showed an M^+ at *m/z* 368 which analyzed for $C_{21}H_{24}N_2O_4$. Comparison of the ^1H NMR spectral data (Table 1) suggests that **4** is closely related to **3** except for the following differences. The ^1H NMR spectrum showed the presence of four aromatic hydrogens indicating an unsubstituted indole ring and there was no signal corresponding to a methoxyl group in the upfield region, unlike that of **3**. Another notable difference is the replacement of signals due to the hydroxylethyl side chain at C(20) with those due to an acetyl group (δ_H 2.05, δ_C 210.6, 29.1). Apart from these differences, the NMR spectral data were essentially similar to those of compound (**3**). As in the case of dippinine A, the observed NOE interactions between H(3) and H(15β), H(12) indicated that the stereochemistry at C(3) in dippinine B is the same as that in dippinine A.

Dippinine C (**5**) was obtained as a light yellowish oil, $[\alpha]_D$ +19° (*c* 0.07, $CHCl_3$). The UV and IR spectra were similar to those of dippinine A (**3**), indicating an indole chromophore and presence of NH/OH and ester functions. The EIMS showed an M^+ at *m/z* 382 which analyzed for $C_{22}H_{26}N_2O_4$, while the ^{13}C NMR spectrum showed the presence of two methyls, five methylenes, nine methines and six quaternary carbons. The ^1H NMR spectrum of **5** showed a general similarity with that of dippinine A (**3**) and chippiine (**1**) apart from some distinct differences. Firstly, unlike **3** there is no signal due to any aromatic methoxy group and the aromatic region showed the presence of four aromatic hydrogens. Another prominent difference is the presence in the spectrum of **5** of a pair of low field AB doublets at δ 4.41 and 4.62 due to an oxymethylene function, which is also supported by the oxymethylene resonance at δ 88.7 in the ^{13}C NMR spectrum, indicating that it is α to both a nitrogen and an oxygen atom. The molecular formula of **5** yields a DBE value of 11, which is one more than that of chippiine (**1**) and dippinine A (**3**), indicating formation of an additional ring. The HMBC spectrum of **5** showed three-bond correlations from the oxymethylene hydrogens to C(5),

C(19) and C(21) which is consistent with the oxymethylene function being part of the additional tetrahydro-1,3-oxazine ring system as shown in **5**. The configuration at C(3) is similar to that of dippinine A **3** as shown by the observed NOE interactions between H(3) and H(15β), H(12) and *vice versa*.

Dippinine D (**6**) was obtained as a light yellowish oil, $[\alpha]_D$ -152° ($CHCl_3$, *c* 0.06). The UV spectrum (λ_{max} 235, 280, and 290 nm) was that of an indole chromophore as in the previous compounds. However unlike the previous compounds, the IR spectrum showed, in addition to a band due to an ester carbonyl function (1735 cm^{-1}), another band due to a lactam carbonyl function (1707 cm^{-1}). The presence of a lactam function was confirmed by the observed carbon resonance at δ 170.6, in addition to the ester carbonyl resonance at δ 172.6. The EIMS showed an M^+ at *m/z* 410, which analyzed for $C_{23}H_{26}N_2O_5$. Except for some differences, the ^1H NMR spectrum of **6** was generally similar to that of dippinine C (**5**), indicating an essentially similar structure. Unlike **5**, the NMR spectral data indicated the presence of an aromatic methoxy group, which from the coupling pattern of the aromatic hydrogens, the aromatic carbon shifts, and the HMBC spectrum, is deduced to be at C(11). Another departure is the absence of the signal due to H(3), suggesting the location of the lactam function at position-3. This is confirmed by the observed three-bond correlations from H(15) and H(17) to C(3) in the HMBC spectrum. In addition, the signals due to H(12) and H(14) have undergone significant downfield shifts (compared to **3**) due to the proximate presence of the lactam function. As in the hexacyclic derivative (**5**),a pair of low field AB doublets at δ 4.44 and 4.66 due to an oxymethylene function, and the corresponding carbon resonance at δ 88.9, were also observed in the ^1H and ^{13}C NMR spectra, indicating the presence of a similar tetrahydro-1,3-oxazine ring.

The relative configuration of C(19) in both dippinines C (**5**) and D (**6**) was deduced to be *S*, from the observed NOE interactions between H(19) and H(21), H(22α), which would not have been the case in the alternative structure **7** (19*R*), in which the pseudoequatorially oriented H(19) would be directed way from both H(21) and H(22). Since the hexacyclic tetrahydrooxazine containing derivatives (**5**) and (**6**), can be considered as having arisen from the appropriate pentacyclic precursors such as **3a** *via* condensation of formaldehyde onto *N*(4), followed by intramolecular cyclization,[5] it would be reasonable to suppose that similar derivatives such as dippinine A (**3**) should possess the same configuration at C(19), *i.e.*, *S*.

The stereochemistry at C(20) in **3** - **6** can be deduced from the observed coupling constants for the hydrogens on C(15), C(20), and C(21). The observed $J_{15\alpha\text{-}20}$ and $J_{20\text{-}21}$ values of 12 and 11 Hz respectively require these hydrogens to be *trans*-diaxial with respect to each other, which in turn requires the

stereochemistry of the ethyl side chain to be α (20*R*).[6] The resulting preferred chair conformation of the six-membered ring has the ethyl group oriented equatorially, with the axially oriented H(20) directed towards the the indole ring, which accounts for the unusual shielding observed for these hydrogens.[7]

The stereochemistry of the C(3) hydroxyl substituent in dippinines A, B, and C, have been unequivocally established as α from NOE experiments, which differs from that of chippiine (**1**), in which the stereochemistry at C(3) was determined by comparison of the chemical shift of H(3) with that of H(16) in the eburnamines and 16-descarbomethoxytacamines.[1,8,9] In addition to the dippinines, several other indole alkaloids possessing novel carbon skeletons have also been obtained from this plant.[10-12]

EXPERIMENTAL

General. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrophotometer. UV spectra were obtained on a Shimadzu UV-3101PC spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on a JEOL JNM-LA 400 spectrometer at 400 and 100 MHz respectively. EIMS and HREIMS were obtained on a JEOL JMS-AX505H mass spectrometer, courtesy of Dr. K. Komiyama of the Kitasato Institute, Japan.

Plant Material. Plant material was collected in Perak, Malaysia (May, 1996) and were identified by Dr. A. J. M. Leeuwenberg, Laboratory of Plant Taxonomy and Plant Geography, Agricultural University, Waneningen, The Netherlands.[13]

Extraction and Isolation. Extraction of the ground leaf and stem bark material was carried out in the usual manner by partitioning the concentrated EtOH extract with dilute acid as has been described in detail elsewhere.[14,15] The alkaloids were isolated by initial column chromatography on silica gel using $CHCl_3$ with increasing proportions of MeOH followed by rechromatography of appropriate partially resolved fractions using centrifugal TLC. Solvent systems used for centrifugal TLC were Et_2O, EtOAc/cyclohexane (1:1), EtOAc/cyclohexane (1:1, NH_3-saturated), and $CHCl_3$ (NH_3-saturated). The yields (g kg^{-1}) of the alkaloids (**3**-**6**) were as follows: **3** (0.0738), **4** (0.0012), **5** (0.0052), **6** (0.0031).

Dippinine A (**3**): Light yellowish oil; $[\alpha]_D$ +19° (*c* 0.67, $CHCl_3$). UV (EtOH) λ_{max} (log ε) 231 (4.27), 280 (3.67), 300 (3.61) nm. IR (dry film) υ_{max} 3356, 1721 cm^{-1}. ^{1}H-NMR and ^{13}C-NMR data, see Tables 1 and 2. EIMS *m/z* 400 [M]$^+$ (91), 382 (50), 352 (100), 350 (70), 279 (8), 239 (7), 149 (8). HREIMS *m/z* 400.1997 (calcd for $C_{22}H_{28}N_2O_5$, 400.1998).

Dippinine B (**4**): Light yellowish oil; $[\alpha]_D$ -54° (*c* 0.06, $CHCl_3$). UV (EtOH) λ_{max} (log ε) 226 (4.18), 285 (3.65), 294 (3.61) nm. IR (dry film) υ_{max} 3355, 1725, 1710 cm^{-1}. ^{1}H-NMR and ^{13}C-NMR data, see Tables 1 and 2. EIMS *m/z* 368 $[M]^+$ (100), 350 (36), 325 (16), 309 (36), 296 (27), 252 (33), 180 (24), 167 (28), 149 (33). HREIMS *m/z* 368.1707 (calcd for $C_{21}H_{24}N_2O_4$, 368.1736).

Dippinine C (**5**): Light yellowish oil; $[\alpha]_D$ +19° (*c* 0.07, $CHCl_3$). UV (EtOH) λ_{max} (log ε) 228 (4.46), 285 (3.91), 294 (3.86) nm. IR (dry film) υ_{max} 3358, 1728 cm^{-1}. ^{1}H-NMR and ^{13}C-NMR data, see Tables 1 and 2. EIMS *m/z* 382 $[M]^+$ (100), 368 (27), 364 (12), 353 (16), 338 (18), 323 (19), 309 (20), 296 (56), 180 (18),167 (18), 149 (27). HREIMS *m/z* 382.1877 (calcd for $C_{22}H_{26}N_2O_4$, 382.1892).

Dippinine D (**6**): Light yellowish oil; $[\alpha]_D$ -152° (*c* 0.06, $CHCl_3$). UV (EtOH) λ_{max} (log ε) 235 (4.35), 280 (4.05), 290 (4.01) nm. IR (dry film) υ_{max} 1735, 1707 cm^{-1}. ^{1}H-NMR and ^{13}C-NMR data, see Tables 1 and 2. EIMS *m/z* 410 $[M]^+$ (100), 396 (10), 366 (19), 351 (9), 324 (30), 284 (6), 183 (17), 125 (7). HREIMS *m/z* 410.1845 (calcd for $C_{23}H_{26}N_2O_5$, 410.1842).

Table 1. ^{1}H NMR spectral data for compounds (**3 - 6**) (400 MHz, $CDCl_3$)

H	**3**	**4**	**5**	**6**
3	5.47 m	5.61 m	5.58 m	-
5	2.70 td (14, 1.5)	2.59 br t (12)	2.79 m	2.80 m
	2.79 dt (14, 3)	2.83 br dd (12, 3.5)	2.93 m	2.98 br d (13)
6	2.54 td (14, 3)	2.65 td (12, 1.5)	2.74 m	2.80 m
	2.66 ddd (14, 3, 1.5)	2.76 br dd (12, 3.5)	2.79 m	2.80 m
9	7.32 d (8)	7.52 ddd (7.3, 1.5, 0.7)	7.56 ddd (7, 1.5, 0.6)	7.38 d (8.6)
10	6.84 dd (8, 2)	7.20 td (7.3, 1.5)	7.20 td (7, 1.5)	6.98 dd (8.6, 2)
11	-	7.24 td (7.3, 1.5)	7.23 td (7, 1.5)	-
12	7.00 d (2)	7.48 ddd (7.3, 1.5, 0.7)	7.53 ddd (7, 1.5, 0.6)	7.94 d (2)
14	2.34 m	2.49 m	2.46 m	3.04 m
15α	1.17 ddd (14, 12, 6)	1.81 ddd (13, 11.5, 6)	1.23 ddd (14, 11, 6)	1.38 m
15β	1.64 ddt (14, 6, 2.5)	1.74 ddt (13, 6, 2.5)	1.67 ddt (14, 5.5, 2.5)	2.03 ddt (14, 5, 2.5)
17α	2.56 dt (13, 2.5)	2.64 dt (13, 2.5)	2.68 dt (13, 2.5)	2.58 dt (13, 2.5)
17β	1.74 br dd (13, 4)	1.98 ddd (13, 4, 0.7)	2.02 br dd (13, 4)	2.19 dd (13, 3)
18	0.92 d (6)	2.05 s	0.93 d (6)	0.99 d (6)
19	3.50 dq (7, 6)	-	3.27 dq (9, 6)	3.36 dq (9, 6)
20	0.89 dddd (12, 11, 7, 2.5)	2.08 tdl (11.5, 6)	0.67 tdd (11, 9, 5.5)	1.13 m
21	3.19 d (11)	3.49 d (11.5)	3.44 d (11)	3.56 d (11)
22α	-	-	4.62 d (10.5)	4.66 d (10.6)
22β	-	-	4.41 d (10.5)	4.44 d (10.6)
11-OMe	3.87 s	-	-	3.91 s
CO_2Me	3.77 s	3.82 s	3.80 s	3.81 s

Table 2. ^{13}C NMR spectral data for compounds (**3 - 6**) (100 MHz, $CDCl_3$)[a]

C	**3**	**4**	**5**	**6**
2	132.0	133.3	132.6	131.3
3	79.1	79.1	79.4	170.6
5	42.0	41.4	47.6	47.1
6	25.2	26.1	23.2	23.2
7	112.9	114.0	113.9	120.5
8	122.1	128.3	128.1	124.3
9	119.0	110.0	109.9	118.9
10	110.1	120.9	120.8	113.2
11	156.1	122.0	121.8	158.1
12	93.9	118.7	118.7	101.8
13	136.8	136.6	136.8	136.2
14	35.7	35.7	36.0	39.5
15	30.0	29.7	31.3	30.4
16	50.5	50.5	47.7	47.5
17	29.7	29.8	29.0	35.0
18	20.6	29.1	18.5	18.7
19	75.1	210.6	79.4	78.8
20	36.4	46.1	33.1	33.7
21	60.8	58.1	64.6	63.8
22	-	-	88.7	88.9
11-OMe	55.7	-	-	55.8
CO_2Me	52.6	52.8	52.6	52.9
CO_2Me	174.8	174.2	173.5	172.6

[a]Assignments based on HMQC and HMBC

ACKNOWLEDGEMENT

We would like to thank Dr. A. J. M. Leeuwenberg, Laboratory of Plant Taxonomy and Plant Geography, Agricultural University, Waneningen, The Netherlands, for identification of plant material, Dr. K. Komiyama, The Kitasato Institute, Japan, for MS measurements, and the University of Malaya and IRPA for financial support.

REFERENCES AND NOTES

1. T. A. Van Beek, R. Verpoorte, A. B. Svendsen, and R. Fokkens, *J. Nat. Prod.*, 1985, **48**, 400.
2. H. B. Nielsen, A. Hazell, R. Hazell, F. Ghia, and K. B. G. Torssell, *Phytochemistry*, 1994, **37**, 1729.
3. T. S. Kam and K. M. Sim, *Nat. Prod. Lett.*, 1999, **13**, 143.
4. T. S. Kam and K. M. Sim, *Heterocycles*, 1999, **51**, 345.
5. M. Bert, G. Baudouin, F. Tillequin, and M. Koch, *Heterocycles*, 1985, **23**, 2505.

6. It is to be noted that the present determination of the stereochemistry of the C(20) side chain as α represents a revision of the stereochemistry at C(20) previously reported by us for dippinines A and C (reference 3 and 4 respectively).
7. The stereochemistry at C(20) in **3** - **6**, deduced from the observed coupling constants for the hydrogens on C(15), C(20), and C(21), is also supported by the observed NOE interactions in dippinine A (**3**) (H19/H21; H15β/H20) and dippinine C (**5**) (H19/H21, H22α; H21/H19, H22α, H17β).
8. X. Z. Feng, C. Kan, H. P. Husson, P. Potier, S. K. Kan, and M. Lounasmaa, *J. Nat. Prod.*, 1984, **47**, 117.
9. T. A. Van Beek, R. Verpoorte, and A. B. Svendsen, *Tetrahedron*, 1984, **40**, 737.
10. T. S. Kam, K. M. Sim, and T. M. Lim, *Tetrahedron Lett*., 1999, **40**, 5409.
11. T. S. Kam, K. M. Sim, and T. M. Lim, *Tetrahedron Lett*., 2000, **41**, 2733.
12. T. S. Kam, K. M. Sim, and T. M. Lim, *Tetrahedron Lett*., 2001, **42**, 4721.
13. Herbarium voucher specimens (GK 604) are deposited at the Herbarium of the Department of Chemistry, University of Malaya, Malaysia, and at Waneningen.
14. T. S. Kam and P. S. Tan, *Phytochemistry*, 1990, **29**, 2321.
15. T. S. Kam, K. Yoganathan, C. H. Chuah, and W. Chen, *Phytochemistry*, 1993, **32**, 1343.