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STEROIDAL ALKALOIDS (BATRACHOTOXINS AND 4β-HYDROXYBATRACHOTOXINS), "INDOLE ALKALOIDS" (CALYCANTHINE AND CHIMONANTHINE) AND A PIPERIDINYLDIPYRIDINE ALKALOID (NORANABASAMINE) IN SKIN EXTRACTS FROM THE COLOMBIAN POISON-DART FROG *PHYLLOBATES TERRIBILIS* (DENDROBATIDAE)

T. TOKUYAMA
Faculty of Science, Osaka City University, Osaka, Japan

and

J. W. DALY
National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20205, U.S.A.

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Abstract—Skin extracts from the Colombian poison-dart frog *Phyllobates terribilis* contain three major steroidal alkaloids; Batrachotoxin, homobatrachotoxin and batrachotoxinin A. Minor congeners of batrachotoxin and homobatrachotoxin containing a 4β-OH substituent are identified based on mass spectra and proton and carbon-13 NMR. Two "indole alkaloids" l-calycanthine and *d*-chimonanthine, enantiomeric to the same compounds from plants, and noranabasamine (5-(2-piperidyl)2,3'-dipyridine), a des-N-Me analog of the plant alkaloid anabasamine, are present as minor constituents.

A wide variety of alkaloids have been isolated from skin extracts of neotropical frogs of the family Dendrobatidae.[1,2] The batrachotoxins (Fig. 1) are complex steroidal alkaloids[3] whose presence is characteristic of frogs of the genus *Phyllobates*,[4] while the histrionicotoxins,[5-7] pumiliotoxins,[8-10] and gephyrotoxins[6,11] are diverse compounds all of which contain a piperidine moiety as part of their bicyclic or tricyclic ring systems. Further batrachotoxins and a remarkable additional three classes of alkaloids have now been isolated and characterized from skin extracts of the Colombian frog *Phyllobates terribilis*.

Isolation of alkaloids. Methanolic extracts from 426 skins of *Phyllobates terribilis* were prepared and partitioned between aqueous methanol/chloroform. Alkaloids were then extracted from the chloroform phase into 0.1 N HCl. After adjusting the pH to > 10 with aqueous ammonia, alkaloids were re-extracted into chloroform. The chloroform layer was evaporated *in vacuo* to dryness to afford 780 mg of alkaloids (for details of methods for extraction and partition see Ref. 10). Preparative chromatography of the alkaloid fraction on a reversed phase silica gel column (Merck, prepacked Lobar column RP-8, size B) with tetrahydrofuran: dioxane : water : triethylamine (35 : 10 : 65 : 1), yielded eight fractions (Fractions 1–8, Fig. 2A) corresponding to UV absorption peaks monitored at 254 nm. Each fraction was further purified when necessary on silica gel 60

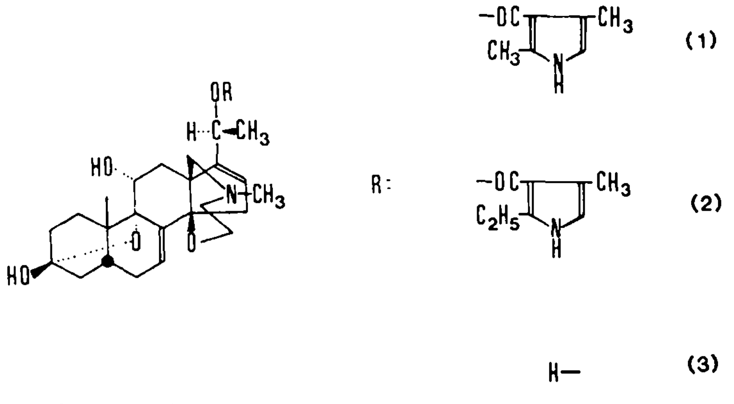


Fig. 1. Structures of batrachotoxin (1), homobatrachotoxin (2), and batrachotoxinin A (3): Major steroidal alkaloids from poison-dart frogs of the genus *Phyllobates* (Dendrobatidae).[4]

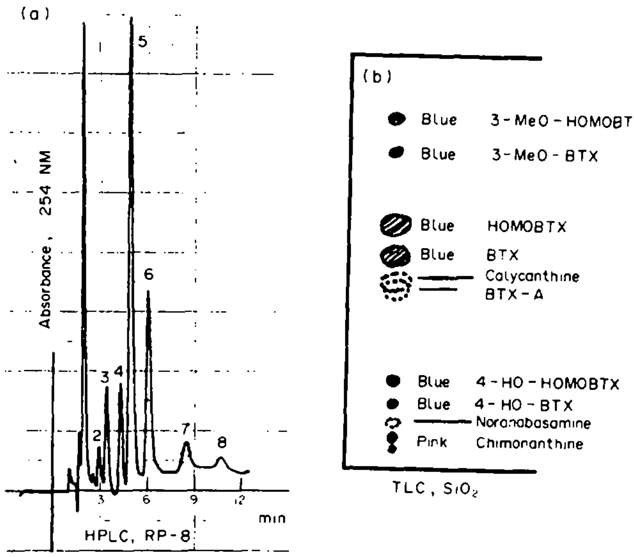


Fig. 2. Chromatographic analysis of the alkaloid fraction from the poison-dart frog *Phyllobates terribilis*. A. High pressure liquid chromatogram: reversal silica gel column, RP-8 solvent, tetrahydrofuran: dioxane: water: triethylamine (42.5 : 10 : 57.5 : 1). Detection at 254 nM. Flow rate 1.0 cm^3/min. Fraction numbers are noted. B. Thin-layer chromatoplate: Silica gel, solvent chloroform : isopropanol : aqueous ammonia (14 : 1 : 0.1). Color reaction given for modified Ehrlich reagent. Spots corresponding to homobatrchotoxin (HOMOBTX), batrachotoxin (BTX), and batrachotoxinin A (BTX-A), 3-O-methyl (3-MeO) derivatives of homobatrachotoxin and batrachotoxin and the 4β-hydroxybatrachotoxins are identified. The low r$_f$ pink-reacting spot is chimonanthine. Noranabasamine and calycanthine and batrachotoxinin A do not give color reaction with Ehrlich reagnet.

column (Merck) with a mixed solvent of chloroform : isopropanol : aqueous ammonia (14 : 1 : 0.08) to yield homogenous material. A thin-layer chromatoplate for the alkaloid fraction with the color of Ehrlich positive spots is depicted in Fig. 2(b).

Two of the major alkaloids of the skin extract, batrachotoxin (175 mg) and homobatrachotoxin (113 mg) eluted in fractions 5 and 6, respectively. The third major alkaloid, batrachotoxin A exhibits no UV absorption at 254 nm^3 and eluted along with a minor UV absorbing alkaloid in fraction 1.

The alkaloids of fraction 8 and 7 were identified as 3-O-methylhomobatrachotoxin (16 mg) and 3-O-methylbatrachotoxin (38 mg), respectively, based on spectral properties and synthesis from the parent alkaloid (*vide infra*). It appears likely that these 3-O-Me compounds are formed as artefacts by reaction of (homo)batrachotoxin with methanol during preparation and partitions of skin extracts.

Fraction 4 contained a single compound (11 mg) which exhibited a molecular ion of $C_{22}H_{26}N_4$ based on high resolution mass spectrometry. This alkaloid was identified as l-calycanthine based on spectral properties and comparison with the *d*-enantiomer from plants (*vide infra*).

Fraction 3 was separated on a silica gel 60 column with a mixed solvent of chloroform : isopropanol ; aqueous ammonia (14 : 1 : 0.1) into two compounds. The first compound (8 mg) exhibited a pink color reaction with modified Ehrlich reagent (*p*-dimethylaminocinnamaldehyde, acid) and a molecular ion of $C_{22}H_{26}N_4$ based on high resolution mass spectrometry. It was identified as *d*-chimonanthidine based on spectral properties (*vide infra*). The second compound (3 mg) exhibited a blue color with modified Ehrlich reagent and a weak molecular ion at $C_{32}H_{44}N_2O_7$. It appeared to be a monohydroxy analog of homobatrachotoxin (*vide infra*). Fraction 2 contained one component (2 mg) which exhibited a blue color with modified Ehrlich reagent and a weak molecular ion at $C_{31}H_{42}N_2O_7$. The compound thus appeared to be the corresponding monohydroxy analog of batrachotoxin (*vide infra*).

Fraction 1 was separated on a Lobar column, RP-8, B size column with a solvent of tetrahydrofuran: dioxane: triethylamine: water (32.5 : 5 : 3 : 1 : 67.5) into two components. The first compound (15 mg) exhibited a molecular ion of $C_{15}H_{17}N_3$ based on high resolution mass spectrometry and was identified as noranabasamine based on spectral properties (*vide infra*). The second and major compound of fraction 1 was batrachotoxinin A (3, 153 mg).

Carbon-13 *magnetic resonance spectral assignments for batrachotoxin* (1), *homobatrachotoxin* (2), *and batrachotoxinin A* (3)

The ^{13}C NMR peaks of the batrachotoxins are listed in Table 1. Peaks appearing as doublets or quarters in the off resonance spectra of these alkaloids were readily assigned based on selective proton decouplings with the single exception of C-5. Assignment of PMR peaks for batrachotoxinin A have been reported in detail[12] and served as the basis for the proton decoupling experiments. The remaining doublet at δ 36.5 therefore must be due to C-5. The triplet peak at δ 47.8 was assigned to C-15, that at δ 61.9 to C-18 and that at δ 62.8 to C-1′ based again on selective proton decoupling experiments. The triplet peak at δ 59.5 was assigned to C-2′ in view of the chemical shift and J_{CH} value of 132 Hz. The triplet peak at δ 32.7 was assigned to C-6 in view of an observed increase in peak height upon selective decoupling with the H-7 proton (δ 6.23) (LPSEL). The triplet peaks at δ 32.0 and δ 40.1 were assigned to C-2 and C-4

Table 1. Carbon-13 nuclear magnetic resonance spectral assignments for batrachotoxins

	Solvent $CDCl_3$				Solvent CD_3OD	
Position	Batrachotoxinin A	Batrachotoxin	Homobatratoxin	4-hydroxyhomobatrachotoxin	Batrachotoxin	4-Hydroxybatrachotoxin
Steroid						
1	30.8	30.9	30.9	31.7	29.9	31.7
2	32.0	32.9[a]	32.9[a]	26.8	31.73	95.6
3	95.6	95.5	95.6	97.5	94.5	96.5
4	40.1	40.3[b]	40.4[b]	—[d,e]	39.2[b]	77.1
5	36.5	37.0	37.1	46.4	36.8	—[d]
6	32.7	32.9[a']	32.8[a']	29.4	32.4[a']	28.4
7	124.6	124.9	125.0	125.3	123.7	124.8
8	140.9	140.3	140.4	141.3	140.4	141.7
9	78.1	79.3	79.4	80.1	78.7	79.0
10	32.4	32.5	32.5	33.4	31.4	32.5
11	66.9	67.4	67.5	67.0	67.4	66.8
12	41.3	40.3[b']	40.2[b']	40.3	39.5[b']	39.7
13	58.0	57.2	57.3	57.3	56.6	56.6
14	87.9	89.3	89.1	88.7	89.2	89.2
15	47.8	49.1	49.1	49.1	—[d]	—[d]
16	127.3	125.3	125.3	125.3	124.8	125.1
17	152.4	151.1	151.3	151.4	150.2	150.1
18	61.9	59.3	59.4	59.6	58.2	58.2
19	19.4	20.1	20.1	20.2	18.1	18.1
20	66.7	65.4	65.4	65.4	64.2	64.2
21	24.4	19.4	19.4	19.7	17.8	18.1
1'	62.8	62.9	62.9	63.0	61.9	61.9
2'	59.5	58.6	58.6	58.8	57.7	57.7
$N-CH_3$	47.0	47.1	47.2	47.2	—[d]	—[d]
C=O		165.7	165.6	—[c]	165.7	164.6
Pyrrole						
2"		135.9	141.7	141.6	135.9	135.6
3"		110.7	110.1	110.2	110.7	110.8
4"		121.4	121.4	121.6	121.4	120.3
5"		114.3	114.3	114.2	114.3	113.8
$2''-CH_3$		14.3	13.7	13.5	14.3	12.4
$2''-CH_2$			21.2	21.3		
$4''-CH_3$		12.9	13.0	13.0	12.9	11.3

Footnotes:

a, a', b and b': Assignments designated a and a' or b and b' are tentative and may be interchanged within each column.

c: The singlet is too weak for positive detection due to small sample size.

d: The chemical shift coincides with peaks due to the solvent.

e: The signal is observed at δ 76.9 in the methanol solution.

respectively on the basis of the up-field shift upon 3-O-methylation (*vide infra*). The remaining triplet peak at δ 41.3 was assigned to C-12. This resonance peak is about 10 ppm higher field than would be expected from other steroids probably due to an effect (*γ-gauche* substituent) of the 3,9-O atom. The vinyl singlet peaks at δ 140.3 and δ 152.4 were assigned to C-8 and C-17, respectively, based on the low power ^1H-selective decoupling (LPSEL) with irradiation either at H-7 (δ 6.23) or at H-16 (δ 5.65). Assignment of the singlet peaks for the quarternary carbons at C-9 and C-14 were based on dipole-dipole relaxation times in batrachotoxinin A: δ 78.1, T_1^{DD} 9.0 sec for C-9 and δ 89.9, T_1^{DD} 6.4 sec for C-14 in deuteromethanol (relaxation times were obtained through the saturation recovery method). The remaining singlet peak at δ 32.4 was readily assigned to C-10. Assignments of the additional seven or eight peaks in the pyrrole moiety of batrachotoxin and homobatrachotoxin, respectively, were unambiguous.

3-O-methyl derivatives of batrachotoxin and homobatrachotoxin. Treatment of homobatrachotoxin and batrachotoxin with an anhydrous methanolic solution of hydrochloric acid resulted in facile conversion to 3-O-Me derivatives which were identical with the alkaloids isolated in fractions 8 and 7, respectively. The ease of conversion of batrachotoxins to 3-O-Me derivatives with methanol under acid conditions strongly suggests that the isolated compounds were probably artefacts formed from batrachotoxins during purification. It should be mentioned that in earlier studies condensation artefacts formed by reaction of the pyrrole rings of batrachotoxin and homobatrachotoxin with acetone (present in the methanol) were obtained (see Experimental Section).

The ^{13}C NMR of the 3-O-Me derivatives were analyzed. The 3-O-Me quartet peak was at δ 49.7. The 3-O-Me substitution resulted in a shift of −1.4 ppm and −4.8 ppm for C-2 and C-4, respectively, and a shift of +2.8 for C-3. Increments in the values for ^{13}C chemical shifts caused by 3-O-methylation are presented in Fig. 3 for homobatrachotoxin.

4β-Hydroxyhomobatrachotoxin and 4β-hydroxybatrachotoxin. The compounds of fraction 2 and 3 which exhibited a blue color with modified Ehrlich reagent appeared based on electron impact mass spectra to be a hydroxybatrachotoxin and a corresponding hydroxyhomobatrachotoxin, respectively. The latter compound exhibited a molecular ion at *m/z* 568 ($C_{32}H_{44}N_2O_7$) with very low intesnity. Two major fragment ions were at $C_{24}H_{33}NO_5$ (*m/z* 415.2381) and $C_8H_{11}NO_2$ (*m/z* 153.0766), both typical of fragments to

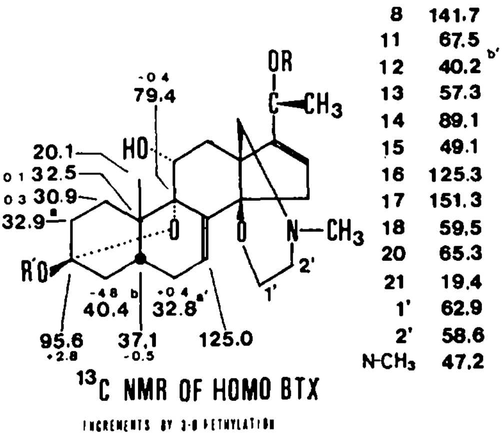


Fig. 3. Carbon-13 magnetic resonance spectral assignments for homobatrachotoxin (see Table 1). Solvent deuterochloroform. The effect of 3-O-methyl substituent on the chemical shift is given as + or – in ppm. The resonance peaks of most carbons including those of the pyrrole carboxylate were unaffected. Similar results were obtained for 3-O-methylbatrachotoxin. Resonances marked a and a' and b and b' may be interchanged.

be expected of a hydroxyhomobatrachotoxin with the additional OH group in the steroid moiety. The alkaloid of fraction 2 exhibited a molecular ion at *m*/*z* 554 ($C_{31}H_{42}N_2O_7$) with very low intensity. Two major fragments were at $C_{24}H_{33}NO_5$ (*m*/*z* 415.2371) and $C_7H_9NO_2$ (*m*/*z* 139.0627). The fragment at *m*/*z* 153 for (hydroxy)homobatrachotoxin and at *m*/*z* 139 for (hydroxy)batrachotoxin derives from the 20α-2,4-dialkylpyrrole-3-carboxylate moiety (Ref. 3). Major fragment ions at *m*/*z* 399, 312 and 294 in (homo)batrachotoxin derive from the steroid moiety and are replaced by fragment ions at *m*/*z* 415, 328 and 310 in the hydroxybatrachotoxins (Fig. 4). Metastable analysis of mass spectra indicated that the fragment ion at $C_{20}H_{22}O_3$ (*m*/*z* 310.1538) was the parent of the ion $C_{13}H_{12}O$ (*m*/*z* 184.0919). In the case of homobatrachotoxin the $C_{13}H_{12}O$ ion appeared to derive from the $C_{20}H_{22}O_2$ ion (*m*/*z* 294) by a retro Diels–Alder type fragmentation of the A-ring. Thus the additional OH group would appear to be in the A-ring of the hydroxybatrachotoxins.

The location and configuration of the OH group in the A-ring of the hydroxybatrachotoxins followed from an analysis of ^1H and ^{13}C NMR (Table 1). A doublet peak at δ 77.1 in the ^{13}C spectrum (CD_3OD) of hydroxyhomobatrachotoxin is obviously assigned to the C bearing the (secondary) OH group. The presence of a doublet indicates the new OH group is at either C-1, C-2 or C-4 of the A-rings. The triplet peak at δ 40.3–40.4 in (homo)batrachotoxin disappears and the other peaks are shifted in a manner expected for a 4-OH derivative (Fig. 5). Thus, the doublet peak assigned to C-5 appears at lower magnetic field by 9.3 ppm than C-5 in homobatrachotoxin. The singlet peak assigned to C-3 appears at lower field by 1.9 ppm. Resonance peaks at C-2 and C-6 appear at higher field, with increments of −6.1 and −3.4 ppm, respectively. The configuration of the 4-OH group was deduced from the PMR. The peak corresponding to the H-4 proton appears as a broad singlet (half height width ~8 Hz, Fig. 6). The lack of greater coupling to H-5 is compatible with a 4α-H (equatorial) and not with a 4β-(axial). Thus OH group in the 4-hydroxybatrachotoxins is in the β-configuration.

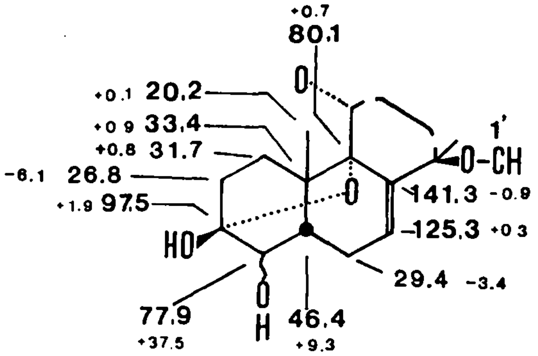


Fig. 5. Carbon-13 magnetic resonance spectral assignments for 4-hydroxyhomobatrachotoxin (see Table 1). Solvent deuterochloroform. The effect of the 4-hydroxy group on the chemical shift is given as + or – in ppm. The remaining resonance peaks were little affected. Similar results were obtained for 4-hydroxybatrachotoxin (see Table 1).

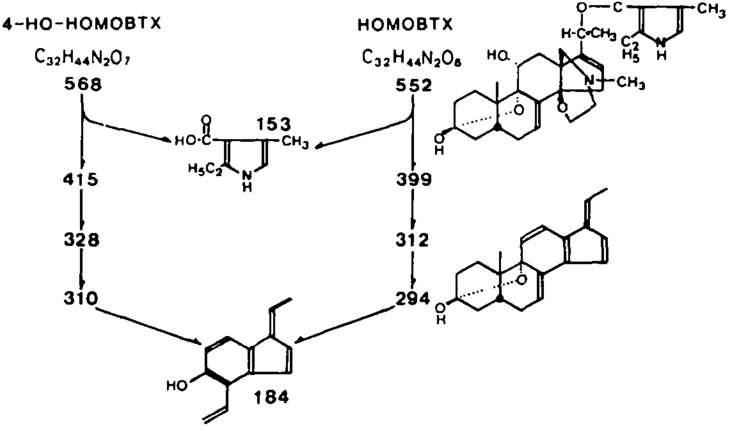


Fig. 4. Mass spectrum and mass spectral fragmentation pathways for 4-hydroxyhomobatrachotoxin and homobatrachotoxin (see Ref. 3 for spectra of (homo)batrachotoxin).

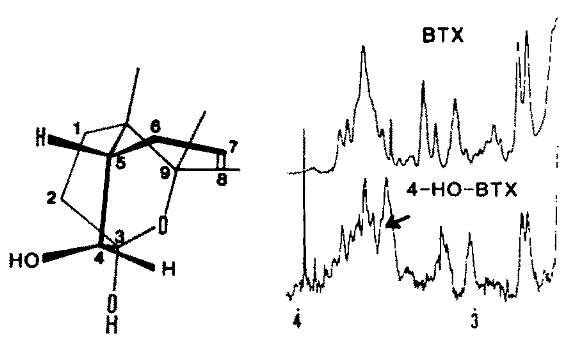


Fig. 6. Proton magnetic resonance peaks in 4-hydroxybatrachotoxin. A relevant portion of the spectra of batrachotoxin (BTX) and 4-hydroxybatrachotoxin (4-HO-BTX) is shown with the axis in δ values. The broad singlet due to the 4-H in 4-hydroxybatrachotoxin is shown with the arrow. The 4β-hydroxy configuration, consonant with a broad singlet, is shown in the structure.

4β-hydroxyhomobatrachotoxin was much less toxic than (homo)batrachotoxin. A minimum lethal dose of about 200 μg/kg was estimated for subcutaneous injection in white mice, compared to minimum lethal doses for homobatrachotoxin and batrachotoxin of 2–3 μg/kg.

Noranabasamine. The minor alkaloid isolated from fraction 1 had a molecular ion of $C_{15}H_{17}N_3$ (*m*/*z* 239.1427). Only two major fragment ions were present corresponding to $C_{10}H_9N_2$ (*m*/*z* 157.0746) and $C_5H_{10}N$ (*m*/*z* 84.0792). Analysis of the ^1H NMR through decoupling indicated a 2,3′-dipyridyl structure with a 2-piperidyl group on the 5-position (Fig. 7). Such a compound corresponds to a N-desmethyl analog of anabasamine, a plant alkaloid from *Anabasis aphylla*[14] (Chenopodiaceae). The UV absorption spectrum with max at 244 nm (ε 11,000) and 275 nm (ε 10,000) in methanol supported a 2,3′-dipyridyl structure. The ^{13}C NMR also supports the noranabasamine structure: The resonance peaks have been assigned as shown in Fig. 7 through decoupling and coupling constants (J_{CH}). The assignment of a singlet peak at δ 140.1 to C-5 is based on the chemical shift of the corresponding carbons of pyridine alkaloids such as nicotine, nornicotine and anabasine.[15]

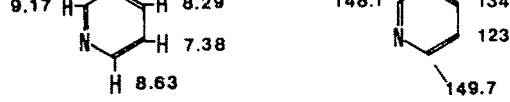


Fig. 7. Structure and magnetic resonance spectral assignments for the frog alkaloid noranabasamine. Proton resonance assignments are at the left and carbon-13 resonance assignments are at the right. Solvent deuterochloroform.

The optical rotation of this alkaloid—to be termed noranabasamine—isolated from frog skin was $[\alpha]_D^{25} - 14.4°$ in methanol. It is uncertain as to whether this alkaloid has the same absolute configuration as the plant alkaloid anabasamine or not. Anabasine [(2S)-2-(3-pyridyl)piperidine], the parent member of the anabasine alkaloids, has a negative optical rotation of $[\alpha]_D^{20} - 82°$.[16] Noranabasamine has been identified by combined gas chromatography-mass spectrometry in the alkaloid fraction from skins of two other closely related poison-dart frogs, namely *Phyllobates aurotaenia* and *P. bicolor*. In both cases, it was a trace constituent. Noranabasamine was not identified as a constituent of alkaloid fractions from various other frogs of the family Dendrobatidae.

1-*calycanthine.* The alkaloid isolated from fraction 4 exhibited a molecular ion corresponding to $C_{22}H_{26}N_4$ (*m*/*z* 346.2152). The mass spectrum and PMR of this alkaloid are shown in Fig. 8. The ^{13}C NMR (δ 145.3^s, 126.5^d, 125.0^s, 124.4^d, 116.4^d, 112.0^d, 71.0^d, 46.6^t, 42.6^q, 36.0^s, 31.7^t) showed only eleven peaks indicating that the alkaloid was dimeric in nature. The "monomer" subunit would contain, a N-methyl carbon appearing at δ 42.6; six carbons as a disubstituted aromatic ring; a quarternary C appearing as a singlet at δ 36.0; two methylene

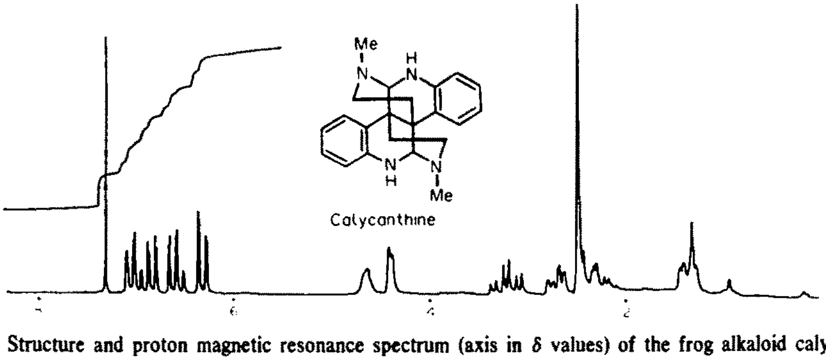


Fig. 8. Structure and proton magnetic resonance spectrum (axis in δ values) of the frog alkaloid calycanthine. Solvent deuterochloroform.

carbons at δ 31.7 and 46.6; a methine C, probably between two nitrogens, at δ 71.0. The PMR supported these assignments and indicated an *ortho*-disubstituted aromatic ring. The protons of the two methylene groups exhibited an AA′-XX′ pattern in the PMR spectrum. The data permitted the formulation of partial structures and led to a tentative structure corresponding to the alkaloid calycanthine known from plants of the genus *Calycanthus*[17] (Calycanthaceae). A comparison based on magnetic resonance and mass spectra of the frog alkaloid to authentic calycanthine (Prof. S. Kobayashi, Gakushuin University) confirmed their identity. However, the optical rotation of the frog calycanthine was $[\alpha]^{25}_{D} - 570°$ (MeOH) which is opposite in sign to that of plant calycanthine ($[\alpha]^{25}_{D} + 550°$). The frog l-calycanthine is, thus, the enantiomer of the plant alkaloid.

d-*chimonanthine.* One of the alkaloids isolated from fraction 3 exhibited the same molecular ion as calycanthine, namely $C_{22}H_{26}N_4$ (*m/z* 346.2131). An intense fragment ion at $C_{11}H_{12}N_4$ (*m/z* 172.0998) was present. The mass spectrum and PMR is shown in Fig. 9. Unlike calycanthine, this alkaloid exhibited a pink color with modified Ehrlich reagent. The ^{13}C NMR (δ 150.6^{s}, 133.1^{s}, 128.1^{d}, 124.4^{d}, 118.7^{d}, 109.4^{d}, 85.2^{d}, 63.2^{s}, 52.7^{t}, 37.2^{q}, 36.5^{t}) showed only eleven peaks indicating that the compound was dimeric in nature. The "monomer" subunit would contain an N-Me carbon appearing at δ 37.2; six carbons as a disubstituted aromatic ring, a quarternary C appearing as a singlet at δ 63.2, two methylene groups at δ 36.5 and 57.7; a methine C, probably between two nitrogens, at δ 85.2. The PMR supported these assignments and indicated an *ortho*-disubstituted aromatic ring. One possible structure consonant with the spectral data was that of another *Calycanthus* alkaloid, namely chimonanthine. Comparison of the spectral data with reported properties[17,18] of plant chimonanthine indicated that the frog alkaloid was, indeed, chimonanthine. However, the optical rotation of frog chimonanthine was $[\alpha]^{25}_{D} + 280°$ (MeOH) which is opposite in sign to that reported for the plant chimonanthine ($[\alpha]^{25}_{D} - 329°$).[19] Thus, as in the case of frog calycanthine, the frog chimonanthine is the enantiomer of the plant alkaloid.

The isomeric indole alkaloids calycanthine/chimonanthine were tentatively identified by combined gas chromatography-mass spectrometry as a trace constituent in the alkaloid fraction from another poison-dart frog, ***Phyllobates bicolor***. The isomeric indole alkaloids were not detected in alkaloid fractions of various other frogs of the family Dendrobatidae.

CONCLUSIONS

The origin of such a remarkable set of alkaloids from a single species of a vertebrate confounds the imagination. There is, of course, the possibility that the "plant" alkaloids of dietary origin and have been accumulated as trace constituents in skin of ***Phyllobates terribilis***. However at least in the case of calycanthine and chimonanthine, the alkaloids from the frog are the opposite enantiomers to those reported from the plant *Calycanthus*. This is particularly remarkable since the absolute stereochemistry of such alkaloids might be expected to be dictated from precursor L-tryptophan. Chimonanthine has been proposed to be the biological precursor of calycanthine[18] and indeed cimonanthine can be converted to calycanthine by heating under acid conditions.[18,19] Isomerization of dextro-rotatory calycanthine yields levorotatory chimonanthine.

It has been assumed that the batrachotoxins, pumiliotoxins, histrionicotoxins and gephyrotoxins which are widespread in dendrobatid frogs and appear to provide a character correlated with othe taxonomic characters[4,20] are elaborated by the frogs themselves. However, even for these alkaloids further study on biogenesis is needed. Thus, frogs of the present species *Phyllobates terribilis* do not elaborate sufficient batrachotoxins for positive identification skin extracts when hatchlings are raised to maturity in captivity.[21] As yet no successful bisoynthetic studies on dendrobatid alkaloids have been reported (Ref. 22).

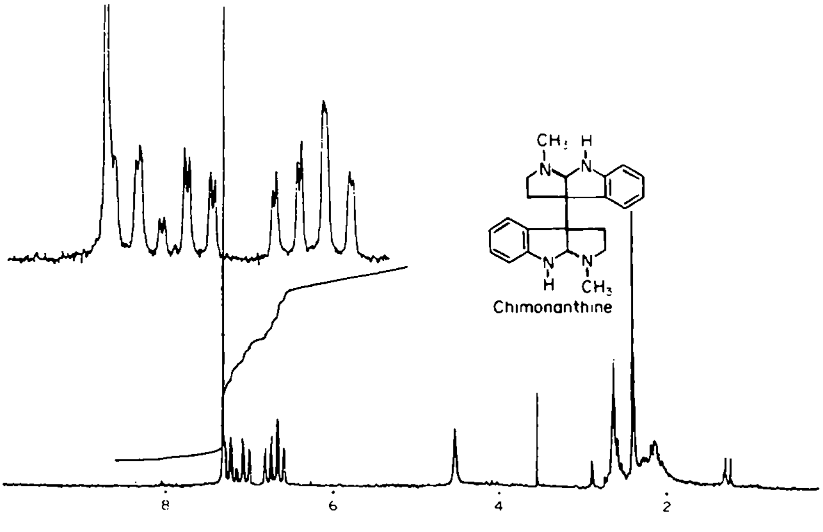
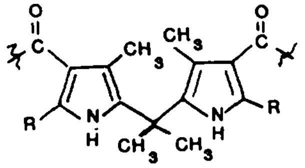


Fig. 9. Structure and proton magnetic resonance spectrum (axis in δ values) of the frog alkaloid chimonanthine. Solvent deuterochloroform.

EXPERIMENTAL

High-resolution mass spectral data were obtained on JEOL D-300 mass spectrometer electron impact (70 eV). Combined gas chromatography-mass spectrometry was on a 1.5% OV-1 chromasorb G AW-DMCS column programmed from 150–280° at 10°/min with a Finnegan 1015 mass spectrometer. NMR were obtained on JEOL FX-100 spectrometer. PMR were determined at 99.60 MHz using a 16K Fourier transform and 1 KHz spectra range for a digital resolution of 0.12 Hz. Typically, free induction decays from a 45° pulse were collected at 6 sec intervals. ^{13}C NMR spectra were determined at 25.05 MHz using a 16 K or 8 K Fourier transform and 5 KHz spectra range for a digital resolution of 0.61 or 1.22 Hz. Typically, 2000 free induction decays from a 45° pulse were collected at 1.5 sec intervals to obtain a completely decoupled spectra.

Condensation of homobatrachotoxin with acetone Homobatrachotoxin (58 mg) was dissolved in a mixed solvent of 0.5 N HCl (50 ml) and acetone (30 ml), and allowed to stand at 0° for 1 hr. After neutralization with dilute aqueous ammonia, the mixture was concentrated *in vacuo* and extracted with CHCl$_3$. The CHCl$_3$ extract was chromatographed on a Sephadex LH-20 column with a mixed solvent of benzene, cyclohexane, isopropanol and triethylamine (35 : 10 : 5 : 1). Fractions were 5 ml. Fraction 16–25 gave an acetone-batrachotoxin condensation product (40 mg). Unreacted alkaloid (20 mg) was recovered from Fraction 34–42. The typical PMR signal assigned to 5-position of the pyrroles (δ 6.33) was absent in the condensation product. The proton on the pyrrole N (δ 8.05) was quite resistent to exchange with D. An additional Me signal originating from the acetone moiety was observed at δ 1.66. In the ^{13}C NMR the pyrrole 5-C was shifted downfield by 15.7 ppm. Other ^{13}C resonances are little affected. The quaternary C of the acetone moiety was at δ 36.0, the Me's at δ 28.3. The results are compatible with a pyrrole condensation product of the following structure (R = either Me or Et):



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