Pseudophrynamine A: An Unusual Prenyl Pyrrolo[2,3-*b*]indole Ester from an Australian Frog, *Pseudophryne coriacea* (Myobatrachidae)

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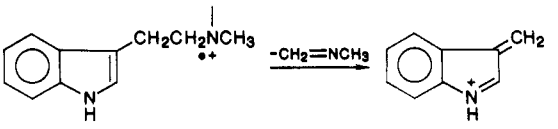
Three new alkaloids having a tricyclic *N*-methyltryptamine structure of the physostigmine type and an isoprene-derived side chain have been isolated from the skins of the Australian frog *Pseudophryne coriacea* and their structures determined by mass spectrometry, NMR spectroscopy, and chemical interconversions.

Amphibian skin has proven a rich source for a variety of unique alkaloids.[1] Such compounds, after secretion from granular glands, appear to serve as a chemical defense against predation. Over 200 such alkaloids have been detected, primarily from so-called poison frogs of the family Dendrobatidae, where bright coloration and distinctive patterns serve as a "warning" to predators that these diurnal frogs are distasteful.[2] Certain of the dendrobatid alkaloids occur in other families of frogs and toads.[3] Thus, the two major lipophilic alkaloids from an Australian burrowing frog, *Pseudophryne semimarmorata*, of the family Myobatrachidae were allopumiliotoxin 323B, an alkaloid common to many species of dendrobatid frogs, and an alkaloid 267D, belonging to the pumiliotoxin A class of dendrobatid alkaloids.[3] Skin extracts of another myobatrachid frog, *Pseudophryne coriacea*, contained an alkaloid closely related in structure and pharmacological activity to the dendrobatid alkaloid pumiliotoxin B.[4,5] An earlier report[6] had suggested that lipophilic alkaloids detected in skin extracts of the brilliant black and yellow myobatrachid frog *Pseudophryne corroboree* were of the samandarine class. Samandarine alkaloids were, however, not detected in extracts of the related *P. semimarmorata*[3] nor in recent samples of *P. corroboree* and *P. coriacea* (unpublished data). Australian burrowing frogs of the genus *Pseudophryne* have also been shown to contain large amounts of serotonin in methanolic skin extracts.[7] Certain species, namely, *P. güntheri*, *P. coriacea*, and *P. bibroni*, contained, in addition to serotonin, significant amounts of unidentified indolic compounds.[7] The present paper reports structural assignments for three of the indolic alkaloids present in skin extracts from *P. coriacea*.

Combined gas chromatographic–mass spectrometric (GC–MS) analysis of a partially purified *P. coriacea* extract (see Experimental Section) revealed a major component (60%) exhibiting an apparent molecular ion at *m/z* 258 (70) and main fragment ions at 185 (46), 173 (100), 171 (33), and 130 (70). Chemical-ionization (CI) mass spectrometry with ammonia confirmed 258 as the parent ion mass (MH^+ at *m/z* 259). A CI mass spectrum with ND_3[8] showed two exchangeable protons (MD^+ at *m/z* 262), only one of these being present in the *m/z* 173 and 130 fragments. Exact mass measurements of the parent ion (*m/z* 258) and major fragment ions at *m/z* 173 and 130 were



m/z 173 *m/z* 130

consistent with the formulas, $C_{16}H_{22}N_2O$, $C_{11}H_{13}N_2$, and C_9H_8N, respectively.[9] These fragments and the CI data suggested the presence of an N^{ω}-methyltryptamine moiety (*m/z* 173) fragmenting to the 3-methylene indolenium ion (*m/z* 130). The absence of an appreciable *m/z* 144 ion (cf. folicanthine[10]) excludes an alternative 1-methyltryptamine structure.

The GC–MS analysis also indicated the presence of a minor alkaloid of slightly longer GC retention time than the *m/z* 258 material. This alkaloid exhibited a parent ion on GC–MS at *m/z* 286 in the EI mass spectrum with major fragment ions at *m/z* 185, 173, and 130, the two latter fragments indicating a close relationship with the *m/z* 258 material. A CI mass spectrum with NH_3 showed a protonated parent ion at *m/z* 287, which changed to *m/z* 289 with ND_3, indicating one exchangeable proton, which was present in the *m/z* 173 and 130 fragments. An exact mass measurement[11] for the parent ion (*m/z* 286) was consistent with the formula $C_{17}H_{22}N_2O_2$.

The *m/z* 258→173 fragmentation and the CI data indicated the loss of a C_5H_9O group, a group with one unit of unsaturation and one exchangeable hydrogen. The *m/z* 286→173 fragmentation and the CI data indicated the loss of a $C_6H_9O_2$ group with no exchangeable hydrogen. A third alkaloid, in addition to the *m/z* 258 and 286 substances, was detected in the extract by direct-inlet EI mass spectrometry. This material, having a molecular ion at *m/z* 512, was observed by GC (see Experimental Section) and

(1) Daly, J. W.; Spande, T. F. *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Wiley: New York, 1986; Vol. 4, Chapter 1, pp 1–274.
(2) Myers, C. W.; Daly, J. W. *Sci. Am.* **1983**, *248*, 120.
(3) Daly, J. W.; Highet, R. J.; Myers, C. W. *Toxicon* **1984**, *22*, 905.
(4) Erspamer, V.; Falconieri-Erspamer, G.; Melchiorri, P.; Mazzani, G. *Neuropharmacology* **1985**, *24*, 783.
(5) Falconieri-Erspamer, G.; Erspamer, V.; Melchiorri, P. *Neuropharmacology* **1986**, *25*, 807.
(6) Habermehl, G. *Z. Naturforsch., B: Anorg. Chem., Org. Chem., Biochem., Biophys., Biol.* **1965**, *20B*, 1129.
(7) Roseghini, M.; Erspamer, V.; Endean, R. *Comp. Biochem. Physiol., C: Comp. Pharmacol.* **1976**, *54C*, 31.
(8) ND_3 CI mass spectrometry for determination of number and often position of exchangeable hydrogens has been reported recently: Daly, J. W.; Spande, T. F.; Whittaker, N.; Highet, R. J.; Feigl, D.; Nishimori, N.; Tokuyama, T.; Myers, C. W. *J. Nat. Prod.* **1986**, *49*, 265.
(9) Found: 258.1703 (calcd for $C_{16}H_{22}N_2O$, 258.1732), 173.1083 (calcd for $C_{11}H_{13}N_2$, 173.1079), 130.0671 (calcd for C_9H_8N, 130.0657).
(10) Clayton, E.; Reed, R. I.; Wilson, J. M. *Tetrahedron* **1962**, *18*, 1495.
(11) Found: 286.1701 (calcd for $C_{17}H_{22}N_2O_2$, 286.1681).

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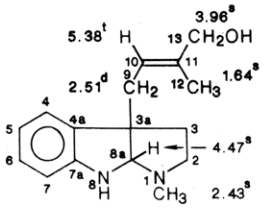
TLC to comprise approximately 75% of the total alkaloid mixture. It could be detected also by GC–MS analysis, although the peak was greatly broadened and weak due to poor transfer through the GC–MS interface.[12]

Sephadex LH-20 chromatography provided a small amount of m/z 258 material, pure as judged by GC and GC–MS analysis (see Experimental Section). A 300-MHz FT ^{1}H NMR spectrum of this material showed a single allylic methyl signal at δ 1.64 and singlets at δ 4.47 and 3.96 (one and two protons, respectively). The aromatic region exhibited signals from an overlapping doublet (δ 7.05) and triplet (δ 7.03), a triplet at δ 6.73, and a doublet at δ 6.59, integrating for 2:1:1 protons, respectively. This was reminiscent of the downfield region of the indoline alkaloid chimonanthine[13] A two-proton doublet at δ 2.51, a one-proton triplet at δ 5.38 showing a long-range coupling ($|J|$ = 1.4), and an N-methyl singlet at δ 2.43 were also observed. A UV spectrum of this material showed the following: $\lambda_{max}^{CH_3OH}$ 242, 295 nm (ratio $\epsilon_{242/295}$ = 2.47), very similar to that expected for an indoline chromophore, again typified by chimonanthine ($\lambda_{max}^{C_2H_5OH}$ 247, 303 nm (ratio $\epsilon_{247/303}$ = 2.43)).[14]



1

The above data permitted structure 1 to be assigned to the m/z 258 material, on the basis of the ^{1}H NMR signals discussed above and indicated on the structure (see Experimental Section for detailed assignments). The assignment of the E configuration to the allylic alcohol side chain is explained later.

A circular dichroism spectrum of 1 showed negative extrema at 242 and 295 nm, strongly suggesting that it possesses the same absolute stereochemistry as physostigmine.[15]

Another fraction obtained on LH-20 chromatography appeared on GC and GC–MS analysis[12] to contain only the alcohol 1, but displayed a much more complex ^{1}H NMR spectrum (see Experimental Section). An EI mass spectrum of this fraction with a direct inlet (probe temperature, 160 °C) revealed no m/z 258 material but, instead, a parent ion at m/z 512 (100). An exact mass for this ion (see Experimental Section) was consistent with the formula $C_{32}H_{40}N_4O_2$, with other major ions at m/z 338, 241, 173, and 130 fitting $C_{21}H_{26}N_2O_2$, $C_{16}H_{21}N_2$, $C_{11}H_{13}N_2$, and C_9H_8N, respectively. The presence of major fragment ions at m/z 173 and 130 again indicated a close structural relationship to alkaloid 1.

A ^{13}C NMR spectrum of the m/z 512 alkaloid exhibited a carbonyl carbon (167.5 ppm) in agreement with the IR spectrum which showed a strong carbonyl absorption at 1705 cm^{-1}, two allylic methyls (12.8, 14.2 ppm), and many pairs of similar or overlapping signals, indicating a dimer-like material (see Experimental Section). A 2D COSY

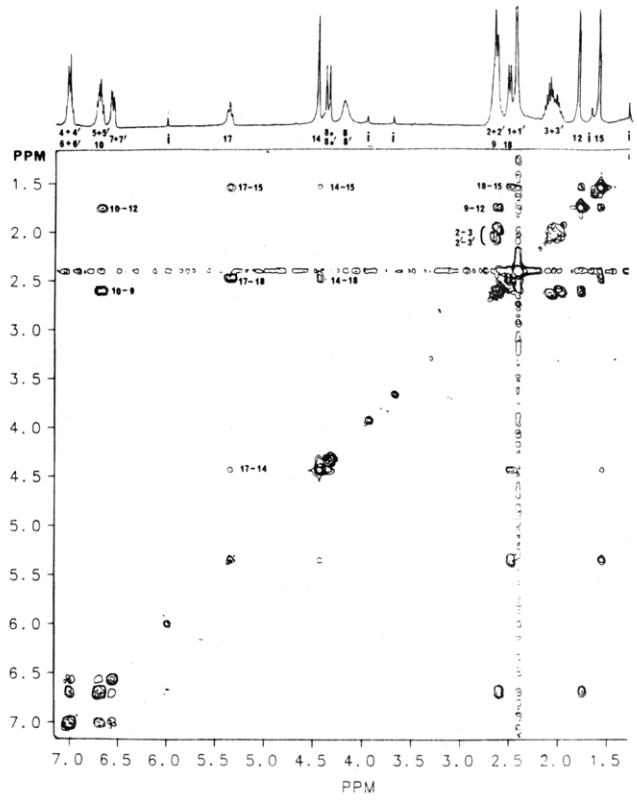


Figure 1. A 2D correlated spectroscopy (COSY) plot for pseudophrynamine A (2); 300 MHz FT ($CDCl_3$). Couplings are indicated. See structure 2 for numbering used; i, impurity.

^{1}H NMR experiment (Figure 1) established several significant scalar couplings, the most important being one between a triplet at δ 6.68 and the allylic methylene at δ 2.63. This indicated the presence of a vinyl proton signal, most likely the β-proton of an α,β-unsaturated carbonyl system (CH_2CH=CCO). Figure 1 also shows the following couplings: (a) the δ 6.68 vinyl triplet coupled with the δ 1.80 allylic methyl singlet; (b) the δ 5.34 vinyl triplet coupled with the δ 2.50 allylic methylene doublet; (c) the δ 1.59 allylic methyl singlet and a weak long-range coupling with the singlet at δ 4.45 (assigned to an acylated allylic hydroxymethylene group[16]); and (d) the multiplet at δ 2.02 (CH_2CH_2N) coupled with signals under the δ 2.63 envelope (CH_2N). Significantly, no coupling was observed involving an apparent doublet at δ 4.33, indicating that this signal must instead be two singlets (δ 4.30, 4.35) and most probably two 8a-protons (NCHN).[17] These data and an interpretation of the ^{13}C chemical shifts based in part on assignments reported by Wenkert, Stothers, and others[18–22] are satisified in every detail by the ester structure 2 below.[23] Assignments in the tricyclic portions of 2 are in

(12) The 512 material partially decomposes to yield 258 material either in the injector regions (250–300 °C) of the GC or GC–MS or on the heated direct-inlet EI probe. With the latter technique, the m/z 512 ion was the base peak at 160 °C, while at 230 °C, the intensity of this ion dropped to only 10% of the new m/z 173 base peak.

(13) Tokuyama, T.; Daly, J. W. *Tetrahedron* **1983**, *39*, 41.

(14) Hall, E. S.; McCapra, F.; Scott, A. I. *Tetrahedron* **1967**, *23*, 4131.

(15) Longmore, R. B.; Robinson, B. *J. Pharm. Pharmacol.* **1969**, *21* (Suppl.), 118s.

(16) Acylation of an allylic alcohol leads to a downfield shift of the hydroxymethylene group. Cf. tiglyl acetate (δ_{CH_2OAc} 4.44[26]) vs tiglyl alcohol (δ_{CH_2OH} 3.85[19b]).

(17) Newkome, G. R.; Bhacca, N. S. *J. Chem. Soc., Chem. Commun.* **1969**, 385. Hino, T.; Kodato, S.; Takahashi, K.; Yamaguchi, H.; Nakagawa, M. *Tetrahedron Lett.* **1978**, 4913. See for representative $\delta_{8a\text{-}H}$ values.

(18) (a) Wenkert, E.; Bindra, J. S.; Chang, C.; Cochran, D. W.; Schell, F. M. *Acc. Chem. Res.* **1974**, *7*, 46. (b) Wenkert, E.; Cochran, D. W.; Hagaman, E. W.; Schell, F. M.; Neuss, N.; Katner, A. S.; Potier, P.; Kan, C.; Plat, M.; Koch, M.; Mehri, H.; Poisson, J.; Kunesch, N.; Rolland, Y. *J. Am. Chem. Soc.* **1973**, *95*, 4990.

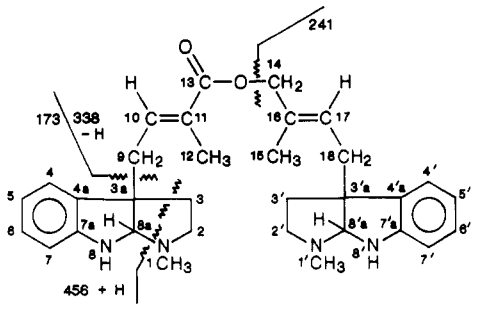
(19) (a) Brouwer, H.; Stothers, J. B. *Can. J. Chem.* **1972**, *50*, 601. (b) Brouwer, H.; Stothers, J. B. *Can. J. Chem.* **1972**, *50*, 1361.

(20) Vogeli, U.; von Philipsborn, W. *Org. Magn. Reson.* **1975**, *7*, 617.

(21) Wenkert, E.; Gašić, M. J.; Hagaman, E. W.; Kwart, L. D. *Org. Magn. Reson.* **1975**, *7*, 51.

(22) Carlé, J. S.; Christophersen, C. *J. Org. Chem.* **1981**, *46*, 3440.

good agreement with those reported[22] by Carlé and Christophersen for compound 8, which they derived from the marine alkaloid flustramine C (7). Structures of major fragment ions in the EI mass spectrum are indicated.

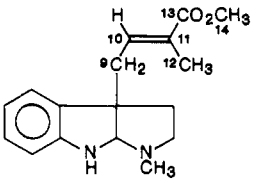


2

The determination of the *E* configuration for the acid portion of the ester is permitted by the clear distinction in chemical shifts reported for the β-proton of methyl tiglate (*E*) (6.72,[24] 6.75[19a]) and methyl angelate (*Z*) (5.97,[24] 5.98[19a]). While not as unambiguous, the C-12 signal of **2** is seen to be identical with that reported for the α-methyl group of the tiglyl stereoisomer (1.80[19a,24]), but slightly upfield of the angelyl stereoisomer (1.85,[19a] 1.87[24]). Unfortunately, the chemical shifts for the vinyl protons of tiglyl and angelyl acetate (5.51–5.53 and 5.43–5.45, respectively)[25,26] are too similar to be used to assign configurations either to **1** or to the alcohol portion of **2**.

The α- and β-carbon chemical shifts in the tiglyl–angelyl system are also too similar to be used to unambiguously assign configurations;[27] however, those of their α-methyl groups do differ enough because of the γ-shielding effect to permit these models to be used to distinguish related *E* and *Z* stereoisomers.[28] Since both allylic methyls (C-12 and C-15) in our ester **2** have ^{13}C chemical shifts upfield by some 6–7 ppm from *Z* (angelyl) models (see ref 19–21), we have assigned the *E* or tiglyl configuration to *both* the acid and alcohol portions of ester **2**. The reported[21] δ_C values for tiglyl acetate were used to assign signals for C-16 and C-17.

LH-20 chromatography failed to yield enough of the minor 286 constituent for NMR analysis. The EI mass spectrum, however, obtained by GC–MS analysis, indicated the material to be a methyl ester. Fragment ions were observed at 255 (5%) and 227 (8%), indicating the losses of OCH_3 and CO_2CH_3 from the molecular ion. The most reasonable structure (**3**) for this ester was confirmed by the demonstration that a sample of **2** exposed to excess $NaOCH_3/CH_3OH$ for 24 h gave two new materials in equivalent amounts by GC and TLC analysis, accompanied



3

by the disappearance of **2**. GC–MS analysis of this mixture on OV-1 and OV-17 capillary columns revealed two peaks, one having a mass spectrum and retention times identical with those of **1**, the other having properties identical with those of **3**. The higher R_f spot seen in the TLC of the methoxide cleavage of **2** corresponds with **3** and indeed can just be discerned with I_2 or the modified Ehrlich reagent on thin-layer chromatograms of either the crude methanol extract of frog skin or the chromatographic fraction from which **1** and **2** were isolated.

A ^1H NMR spectrum of the lower R_f methoxide-cleavage product was virtually identical with that obtained for **1** isolated by LH-20 chromatography. A 2D NOE ^1H NMR experiment on this sample, although giving rise to weak cross-peaks, indicated the *E* configuration for the allylic alcohol side chain in **1**. The following NOE interactions were noted: H_{8a}–H_9, H_9–H_{10}, H_9–H_{12}, H_{10}–H_{13}, and H_{12}–H_{13}. The absence of any off-diagonal resonances between H_9 and H_{13} or H_{10} and H_{12} makes the alternative *Z* configuration unlikely. The ^{13}C NMR resonances observed for C-12 (δ 14.1) and C-13 (δ 68.6), indicating a γ-effect at C-12, provide further support for the *E* configuration in **1**. Angelyl alcohol, a model for the alternative *Z* isomer, exhibits, instead, a γ-effect for the hydroxymethylene carbon (δ 60.5) and a normal allylic methyl group (δ 21.9).[19b]

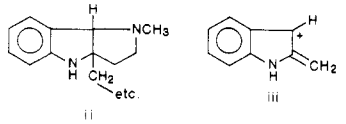
A ^1H NMR spectrum of the higher R_f methoxide-cleavage material (see Experimental Section) corroborated structure **3** in every respect. In particular, a methoxyl singlet (δ 3.70), an allylic methyl (δ 1.81), an allylic methylene doublet (δ 2.66, J = 7.3 Hz), and a vinyl proton triplet (δ 6.69, J = 7.3 Hz), exhibiting allylic coupling ($|J|$ = 1.3 Hz), indicated the presence of a methyl tiglate moiety.[19a,24,29]

The GC–MS analysis of alkaloid fraction 6 (see Experimental Section) also revealed a trace material having a molecular ion at m/z 300 and the same fragment ions as **3**, which is evidently the homologous ethyl ester. This was absent in the chromatogram of the original *P. coriacea* methanol extract and must consequently be an artifact of the alumina chromatography employed to obtain the alkaloid-enriched fraction used in this study. This fraction contains alcohol **1** and ester **2** in roughly a 1:3 ratio as determined by ^1H NMR and TLC. In the methanol extract, before alumina chromatography, **1** and **2** are present in equivalent amounts.

Both **1** and **3** could conceivably arise from the methanolysis of **2** during the initial frog-skin extraction or the LH-20 chromatography.[30] The following observations rule out this possibility.

1. The amount of **1** in the crude extract is roughly equivalent to **2**, while **3** is much less. Methanolysis of **2** should yield equivalent amounts of **1** and **3**, as was indeed the case for the methoxide cleavage of **2** (see above).

(23) An alternative structure ii for ester **2** cannot be rigorously excluded by the NMR data alone at this time, although such an indole alkaloid would be highly unusual from a biosynthetic standpoint and would be less likely to generate a significant mass spectral fragment at m/z 130, in this case of structure iii.



ii iii

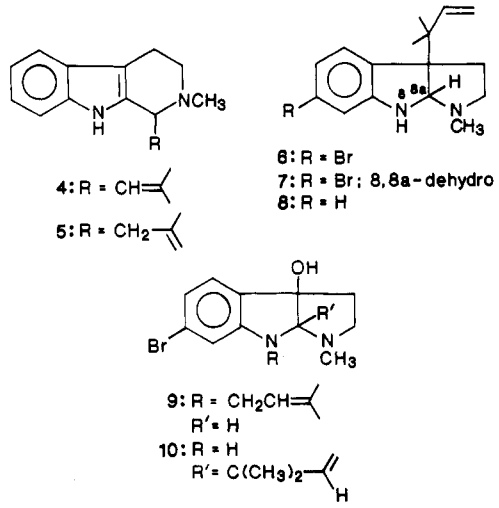
(24) Löffler, A.; Pratt, J. R.; Rüesch, H. P.; Dreiding, A. S. *Helv. Chim. Acta* **1970**, *53*, 383.
(25) Tsukeda, K.; Ito, M.; Ikeda, F. *Chem. Pharm. Bull.* **1973**, *21*, 248.
(26) Spahić, B.; Schlosser, M. Z. *Helv. Chim. Acta* **1980**, *63*, 1242.
(27) See, however: Rappe, C.; Lippmaa, E.; Pehk, T.; Andersson, K. *Acta Chem. Scand.* **1969**, *23*, 1447 where ^{13}C chemical shift differences have been used to distinguish *E*/*Z* configurations in related systems.
(28) Stothers, J. B. *Carbon-13 NMR Spectroscopy*; Academic: New York, 1972; pp 408–411.
(29) Chan, K. C.; Jewell, R. A.; Nutting, W. H.; Rapoport, H. *J. Org. Chem.* **1968**, *33*, 3382.
(30) We have ruled out the possibility that significant amounts of **3** arise from **2** in the GC injector by injecting samples of the alkaloid fraction with CD_3OD on the GC syringe. Only trace amounts of a m/z 289 material were detected, ruling out vapor-phase solvolysis of **2** as a source of appreciable amounts of **3**.

2. Variable amounts of **1** and **3** are found in six different populations of *P. coriacea* collected in Australia on a recent field trip (J. W. Daly, 1987). In some populations, **1** was a major alkaloid, while **3** was a minor or trace alkaloid, while in other populations the converse was true.

3. Solutions of **2** in methanol are stable for months. It should also be noted that, prior to the present studies, fraction 6 had been stored for months in 95% ethanol, yet contained only traces of the ethanolysis product (see above).

The very real possibility remains, however, that **3** is the methanolysis product of some labile acyl intermediate and does not occur naturally in the frog skin. Experiments to test this possibility will require further field work.

Both **2** and **1** are clearly naturally occurring. We name these previously unreported alkaloids pseudophrynamine A and pseudophrynaminol, respectively.



Many alkaloids exist with a terpene (C_{10}) unit attached to a tricyclic *N*-methyltryptamine moiety.[31] Alkaloids with a simple isoprenoid unit are much less common, borrerine (**4**),[32] isoborrerine (**5**),[33] and four recently reported marine alkaloids,[22,34] the flustramines (**6**, **7**) and flustraminols (**9**, **10**), being the only examples of which we are aware.

Experimental Section

Gas chromatography: A Hewlett-Packard Model 5890 gas chromatograph fitted with a 25-m bonded OV-1 fused silica column (Alltech 0.25-mm i.d.) was employed with He carrier (20 cm/s) and flame-ionization detection (300 °C). The injector temperature was 300 °C and split ratio approximately 1:50. The chromatograph was programmed from 150 to 300 °C at 10 °C/min and then held at 300 °C for 10 min. Retention times (t_R) given are for this program unless indicated otherwise. A Hewlett-Packard 3390A recorder–integrator was used.

Mass spectrometry: A Finnigan 4500 mass spectrometer, scanning from mass 50 to 450 once per second, attached to an INCOS data system was used for all mass spectrometry. Gas chromatography used Alltech bonded FSOT columns of polydimethylsiloxane (OV-1, 30 m × 0.32 mm) or polyphenylmethylsiloxane (OV-17, 25 m × 0.25 mm) phases with a 1:40 split, programmed from 50 to 300 °C at 10 °C/min. Solid probe samples were applied to the Finnigan direct-exposure probe and evaporated with a heating rate of 10 mA/s. Chemical ionization used either NH_3 or ND_3[8] as reagent gas. Exact masses were measured by peak matching with a VG 7070F mass spectrometer in the electron-impact mode (70 eV). A Finnigan TSP46 mass spectrometer was used for chemical-ionization-type thermospray mass spectrometry.

^{1}H NMR spectra were measured with either a Varian HR 220 or XL-300 instrument. ^{13}C NMR spectra were measured at 75.429 MHz. Proton and carbon chemical shifts are in parts per million from internal tetramethylsilane in $CDCl_3$. UV spectra were recorded in methanol with Beckman DU-8B or DU-7 spectrophotometers; IR spectra were obtained in $CHCl_3$ (0.2-mm cell) with a Perkin-Elmer Infracord.

Thin-layer chromatography: Silica gel GHLF plates (2.5 cm × 10 cm) (Analtech, 0.25 mm) after spotting were exposed to ammonia vapor for 10 s, allowed to stand in air for a few seconds, and developed with $CH_3OH/CHCl_3$ (1:9). Detection used one or more of the following: iodine vapor (I), iodoplatinic acid (IP, Alltech), Dragendorff's reagent (D, Alltech), modified Ehrlich's reagent (E, 0.5% *p*-(dimethylamino)cinnamaldehyde in 0.1 N HCl), or charring (C) with 10% H_2SO_4 + heat.

Isolation of Compounds 1 and 2. Dried skins of *P. coriacea* (13.7 g from 166 frogs obtained in Queensland, Australia in 1972–1974) were cut in small pieces and extracted twice with 20 times their weight of 80% methanol. An amount of the combined extracts corresponding to 10 g of dried skins was evaporated under reduced pressure. The residue was redissolved in 200 mL of 95% ethanol, applied to an alkaline alumina (Merck, Darmstadt, 180 g) column, and then eluted with 95% ethanol followed by ethanol/water mixtures of increasing water composition. Fraction 6 (95% ethanol, equivalent to 200–300 mg of dry skins/mL), in which indole-like alkaloids had been detected by paper chromatography using *p*-(dimethylamino)benzaldehyde (Ehrlich's reagent),[7] was examined by GC–MS (EI) and yielded a chromatogram showing the following major peaks (retention times (min) are for a 10 °C/min, 150→280 °C program): methyl octadecenoate (14.80, 58% relative ion current), compound **1** (*m/z* 258) (14.85, 100), and a pumiliotoxin B like material (*m/z* 323) (17.18, 47). The following minor (<25% RIC) peaks were also noted: methyl hexadecanoate (13.05), ethyl hexadecanoate (13.73), methyl octadecadienoate (14.58), unidentified trace material (*m/z* 256, 173, 130), possibly the aldehyde related to **1** (14.60), methyl octadecanoate isomer (14.70), methyl octadecanoate (15.00), ethyl octadecadienoate (15.22), compound **3** (*m/z* 286) (15.33), the ethyl ester homologue of **3** (*m/z* 300) (15.78), bis(2-ethylhexyl) phthalate (18.28), and cholesterol (22.25).

A 5.0-mL portion of fraction 6 was evaporated to dryness under water-aspirator pressure and then vacuum to afford 73.6 mg of tan residue. It was dissolved in a minimum volume of methanol, applied to a stepped column (1–2 cm × 45 cm) of LH-20 Sephadex, and eluted with methanol, with collection of 1-mL fractions (3 min/fraction), which were examined by GC and GC–MS. Four initial fractions (15–18) had a nearly pure pumiliotoxin B like material (t_R 11.37 min, *m/z* 323, total 3.1 mg). Fractions 11–14 (2.9 mg) did not yield any GC peaks and may represent inorganic material. Fractions 19–29 contained some pumiliotoxin B like material (t_R 11.37 min), but appeared to contain by GC–MS analysis mainly a compound (*m/z* 258) with t_R 9.18 min and lesser amounts of fatty acid esters. Fractions 30–40 appeared to contain mainly the 9.18-min material, accompanied by small amounts of a material (*m/z* 286) with t_R 9.68 min and cholesterol. In actuality, fractions 19–40, with t_R 9.18 min material, contained mainly ester **2** (*m/z* 512), which decomposed thermally in the GC injector (275–300 °C), giving spuriously high indications of *m/z* 258 material. Cholesterol crystallized slowly from fractions 33–38 (*m/z* 386, t_R 16.69 min) with small amounts of water assisting the crystallization to yield a total of 2.0 mg. Fractions 41–81 contained 1.5 mg of authentic *m/z* 258 material (9.18 min) (**1**) used for the 300-MHz ^{1}H NMR spectrum.

Fractions 19–29 and the mother liquors from fractions 33–38 were rechromatographed on LH-20 (2-mL fractions, 5 min each). The ester **2** appeared in tubes 13–30, still contaminated with fatty acid esters and cholesterol. These tubes were combined and triturated with petroleum ether (35–60 °C) to remove most of the latter materials. The residue (48.5 mg) was rechromatographed to afford fractions that were mainly **2** (tubes 32–38). The NMR measurements were obtained on fraction 34 (13 mg). Fractions 35–38 also contained cholesterol.

As mentioned above, several earlier LH-20 fractions showed small amounts of a material apparently identical with pumiliotoxin

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B[1,35] on GC coinjection, but whose ^{13}C NMR and ^{1}H NMR spectra differed slightly from those of pumiliotoxin B. This alkaloid is not identical with the substance responsible for the potent pumiliotoxin B like activity reported from *P. coriacea* extracts.[4,5] The characterization of this material will be reported elsewhere. It has proven to be the 15*R*,16*S* erythro isomer of pumiliotoxin B.

Preparation of 1 and 3. To 10.6 mg (0.0207 mmol) of **2** in absolute methanol (2.0 mL) was added over 0.5 min with rapid stirring 0.75 mL (0.103 mmol, 5 equiv) of a freshly prepared 0.138 M solution of sodium methoxide in methanol. The reaction mixture was kept at room temperature for 24 h, at which time TLC (I and C detection) indicated that only traces of **2** (R_f 0.32) remained and roughly equivalent amounts of **1** (R_f 0.30) and **3** (R_f 0.53) had formed. Then 1 N HOAc (0.10 mL) was added and 50 μL withdrawn for GC and GC–MS analysis. These indicated a 46:54 ratio of the *m/z* 258 (**1**) and 286 (**3**) materials having identical mass spectra and retention times (30-m OV-1 and OV-17 capillary columns) with these components in chromatographic fraction 6. In addition, a thermospray mass spectrometric assay (60% acetonitrile/40% aqueous ammonium acetate (0.05%)) using direct injection showed two MH^+ peaks at *m/z* 259 and 287 with no *m/z* 513 (i.e., **2**) detectable. The reaction residue, after removal of solvent, was extracted with three portions of CH_2Cl_2, and these were applied to a 2 mm × 20 cm × 20 cm silica gel plate (GHLF, Analtech) and developed with 7.5% $CH_3OH/CHCl_3$. Fluorescence-absorbing zones at R_f ~0.15 and ~0.38 were excised and eluted with 8% $CH_3OH/CHCl_3$ (100 mL) to provide 4.4 mg (85%) and 4.5 mg (79%), respectively, of **1** and **3**. NMR spectra were measured in $CDCl_3$ deacidified by passage through alumina (Woëlm N-Super 1).

The ^{1}H NMR spectrum of **1** was identical with that previously isolated by LH-20 chromatography with the exception of a slight change in the chemical shift (δ 4.57) of the 8a-proton. Traces of DCl in the $CDCl_3$ used initially might be responsible for this difference.

Characterization. (Proton or carbon assignments are in brackets. See structures for numbering.)

Compound 1. ^{1}H NMR (300 MHz): 7.05 (d, *J* = 7.3, 1 H) [4], 7.03 (t, *J* = 7.6, 1 H) [6], 6.73 (t, *J* = 6.8, 1 H) [5], 6.59 (d, *J* = 7.6, 1 H) [7], 5.38 (t × m, *J* = 7.4, 1.4, 1 H), 4.47 (s, 1 H), 3.96 (s, 2 H), 2.80–2.50 (m, 2 H) [2], 2.51 (d, *J* = 7.6, 2 H), 2.43 (s, 3 H), 2.3–2.0 (m, 2 H) [3], 1.64 (s, 3 H). Impurities were noted at 3.49 (s, CH_3OH), 1.26 (s), and in the 1.20–0.80 region. δ values for H-10, H-12, and H-13 are in good agreement with values reported for tiglyl alcohol[19b] or *homo* tiglyl alcohol.[29] ^{13}C NMR: 37.0 [1], 52.3 [2], 38.2 [3], 57.9 [3a], 134.9 [4a], 123.2 [4], 119.2 [5], 127.9 [6], 109.2 [7], 149.8 [7a], 86.2 [8a], 36.4 [9], 120.9 [10], 137.8 [11], 14.1 [12], 68.6 [13]. IR: 3400–3200, 1610 (sharp), 1490 (sharp), 1479 cm^{-1} (sharp). TLC: R_f 0.30 detection I, IP, C, D, E. GC: t_R 9.18 min (see introduction of Experimental Section for program). MS (EI): 258 (35), 241 (7), 227 (5), 207 (7), 199 (8), 185 (18), 173 (100), 157 (20), 144 (16), 130 (90), 117 (16), 103 (20); (CI) (NH_3), *m/z* 259, $(ND_3)^8$ 262. UV: $\lambda_{max}^{CH_3OH}$ 210 nm (ε 11 000), 243 (4800), 297 (1800). CD: $\lambda_{max}^{CH_3OH}$ 242, 295 nm (Δε, −0.01, −0.004 respectively). See ref 15 for an ORD of physostigmine and related alkaloids where negative extrema are reported at 260–270 and 305–320 nm.

Compound 2. ^{1}H NMR (220 MHz, FT): 7.02 (m, 4 H) [4,4′,6,6′], 6.70 (d × t, *J* = 1.0, 7.0, 2 H) [5,5′], 6.68 (t, 1 H) [10], 6.55 (d × d, *J* = 3.0, 8.3, 2 H) [7,7′], 5.34 (t, *J* = 7.3, 1 H) [17], 4.45 (s, 2 H) [14], 4.35 (s, 1 H) [8a or 8a′], 4.30 (s, 1 H) [8a or 8a′], 4.15 (br, CD_3OD-exchangeable, 2 H) [8,8′], 2.63 (m, 6 H) [2,2′,9], 2.50 (d, *J* = 7.3, 2 H) [18], 2.45 (s, 6 H) [1,1′], 2.02 (m, 4 H) [3,3′], 1.80 (s, 3 H) [12], 1.59 (s, 3 H) [15]. ^{13}C NMR (assignments designated with the superscript "a" may be interchanged): 36.9 [1,1′], 52.3, 52.4 [2,2′], 38.7[a], 38.8[a] [3,3′], 57.2, 57.6 [3a,3a′], 134.4, 135.0 [4a,4a′], 123.2, 123.3 [4,4′], 118.9, 119.0 [5,5′], 127.7, 128.0 [6,6′], 109.3 [7,7′], 150.0, 150.1 [7a,7a′], 87.0, 87.2 [8a,8a′], 39.0[a] [9], 138.3 [10], 129.7 [11], 12.8 [12], 167.5 [13], 69.8 [14], 14.2 [15], 132.5 [16], 124.5 [17], 37.6[a] [18]. Carbon multiplicities were determined by using the distortionless enhancement by polarization transfer technique and are as follows: CH_3 [1,1′,12,15], CH_2 [2,2′,3,3′,9,14,18], CH [4,4′–7,7′,8a,8a′,10,17]. The sample used for NMR also contained 6.8% **1**, 0.4% **3**, and <1% fatty acid esters. IR: 3400 (fairly sharp, NH stretching), 1705 (sharp, 1730 shoulder, conjug CO), 1620 (sharp, C=C), 1490, 1470 cm^{-1}. TLC: R_f 0.38, detection I, IP, C, D, E. GC: t_R 29.4 min (program 150→300 °C, 10 °C/min, then 20 min at 300 °C); also affords **1** (t_R 9.18 min) due to decomposition on GC injector. MS (EI, direct inlet): 512 (56), 456 (23), 455 (13), 340 (55), 338 (100), 273 (20), 241 (40), 225 (10), 211 (17), 197–199 (20–23), 182–185 (22–25), 173 (80), 172 (60), 144 (20), 130 (38); ratio 512/513 = 37.7 (theor 38.0), (EI, direct inlet, exact masses), 512.3170 (calcd for $C_{32}H_{40}N_4O_2$, 512.3151), 456.2665 (calcd for $C_{29}H_{34}N_3O_2$, 456.2651), 338.1979 (calcd for $C_{21}H_{26}N_2O_2$, 338.1994), 241.1711 (calcd for $C_{16}H_{21}N_2$, 241.1705), 173.1077 (calcd for $C_{11}H_{13}N_2$, 173.1079), 130.0683 (calcd for C_9H_8N, 130.0657), (CI, NH_3, direct inlet), *m/z* 513.

Compound 3. ^{1}H NMR (300 MHz): 7.04 (t, 1 H) [6] and 7.03 (d, *J* = 7.3, 1 H) [4] overlapping, 6.74 (t, *J* = 7.1, 1 H) [5] and 6.69 (t × m, *J* = 7.3, 1.3, 1 H) [10] overlapping, 6.60 (d, *J* = 7.8, 1 H) [7], 4.48 (s, 1 H) [8a], 3.70 (s, 3 H) [14], 2.74–2.62 (m, 2 H) [2], 2.66 (d, *J* = 7.3, 2 H) [9], 2.45 (s, 3 H) [1], 2.17–2.01 (m, 2 H), [3], 1.81 (s, 3 H) [12]; impurities noted at 3.48 (CH_3OH) and 1.26. ^{13}C NMR (assignments designated with the superscript "a" may be interchanged): 36.7 [1], 52.2 [2], 38.6[a] [3], 57.3 [3a], 134.2 [4a], 123.2 [4], 119.2 [5], 128.1 [6], 109.4 [7], 149.9 [7a], 87.0 [8a], 39.0[a] [9], 138.0 [10], 129.7 [11], 12.8 [12], 168.4 [13], 51.8 [14]. IR: 3400, 1705, 1650 (w), 1610, 1490, 1470, 1440, 1265 cm^{-1}. TLC: R_f 0.53. GC: t_R 9.66. MS (EI): 286 (38), 255 (5), 242 (4), 227 (8), 199 (20), 185 (32), 173 (100), 157 (62), 156 (52), 130 (97). UV: $\lambda_{max}^{CH_3OH}$ 212 nm (ε 14 000), 240 sh (7700), 299 (2200).

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