Tetrahedron Vol. 47, No 29, pp. 5415-5424, 1991
Printed in Great Britain

0040-4020/91 $3.00+.00

ALKALOIDS FROM DENDROBATID POISON FROGS: FURTHER PUMILIOTOXINS AND ALLOPUMILIOTOXINS AND A REASSIGNMENT OF THE KETO FUNCTION IN PUMILIOTOXIN 307F

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

H.M. GARRAFFO, T.F. SPANDE, J.W. DALY*
National Institute of Diabetes and Digestive and Kidney Diseases,
National Institutes of Health, Bethesda, Maryland 20892 U.S.A.

(*Received in USA* 1 *April* 1991)

Abstract - Skin extracts from the Panamanian poison-frog *Dendrobates pumilio* have afforded further trace alkaloids of the pumiliotoxin-A class (6-alkylidene-8-hydroxy-8-methylindolizidines) and an allopumiliotoxin subclass (7-hydroxy-PTX-As). Structures for pumiliotoxins **209F** and **225F** and allopumiliotoxin **225E**, all with four carbon 6-alkylidene side chains, for allopumiliotoxins **309D**, **325A'** and **325A"**, all with ten carbon 6-alkylidene side chains, and a reassignment of the position of the keto function in the ten carbon 6-alkylidene side chain of pumiliotoxin **307F** are presented. Carbon-13 magnetic resonance assignments for these and the 15-O-methyl ether artefacts of pumiliotoxin **307A** and allopumiliotoxin **323B**, are tabulated.

Dendrobatid alkaloids of the pumiliotoxin-A class exhibit remarkable myotonic and cardiotonic activity (1,2) with activity being optimal in pumiliotoxin B (3). Alkaloids of the pumiliotoxin-A class have been divided into three subclasses, the pumiliotoxins, the allopumiliotoxins and the homopumiliotoxins. The pumiliotoxins exhibit a characteristic mass spectral pattern, showing a loss of OH (M^+-17), and major ions at m/z 194 ($C_{12}H_{20}NO^+$), 166 ($C_{10}H_{16}NO^+$) and 70 ($C_4H_8N^+$). The structural elucidation of pumiliotoxin A and B remained a challenge for nearly a decade after initial isolation and characterization in 1967 (4). The structure of a simpler alkaloid, pumiliotoxin **251D** was defined by x-ray crystallographic analysis of a crystal of **251D**·HCl and the structures of pumiliotoxin A (**307A**) and pumiliotoxin B (**323A**) and of other pumiliotoxins and allopumiliotoxins were deduced from analysis of their mass and NMR spectra, as compared to that of pumiliotoxin **251D** (5). The allopumiliotoxins were hydroxy congeners of the pumiliotoxins, containing a 7-hydroxy group in the indolizidine moiety. The allopumiliotoxins exhibit a characteristic mass spectral pattern, showing a loss of OH (M^+-17), and major ions at m/z 210 ($C_{12}H_{20}NO_2^+$), 182 ($C_{10}H_{16}NO_2^+$) and 70 ($C_4H_8N^+$). Some seventeen pumiliotoxins and fifteen allopumiliotoxins have been identified from extracts of skins of dendrobatid frogs. The structures in many cases, were based only on mass spectral analysis of the alkaloid before and after deuterium exchange with ND_3, and of a

perhydro-derivative of the alkaloid produced by catalytic hydrogenation (6). Only one member of a homopumiliotoxin subclass, **223G**, has been isolated and characterized (7). The homopumiliotoxins differ from the pumiliotoxins in being quinolizidines rather than indolizidines. The mass spectrum of a homopumiliotoxin has major ions at m/z 180 ($C_{11}H_{18}NO^+$), and 84 ($C_5H_{10}N^+$) originating from the 7-alkylidene-9-hydroxy-9-methyl-quinolizidine moiety. Mass, NMR and IR spectral analyses now have defined the structures for additional trace pumiliotoxin-A class alkaloids from extracts of skin of the Panamanian poison frog *Dendrobates pumilio* (For prior spectral analyses of pumiliotoxin-A class alkaloids see ref. 5,7,8). Structures of dendrobatid alkaloids discussed in the present paper are presented in Fig. 1.

Pumiliotoxins

The alkaloids **209F** and **225F** exhibit mass spectra characteristic of pumiliotoxins with major fragments at m/z 166 and 70. The NMR spectra also were characteristic of pumiliotoxins and led to definition of the structures of **209F** and **225F**. The ^{13}C-NMR assignments for pumiliotoxins **209F** and **225F** are presented in Table 1, along with those of pumiliotoxin **251D**, which has a seven carbon alkylidene side chain and whose structure has been defined by x-ray crystallographic analysis (5). The ^{1}H-NMR spectrum of **209F** had well separated distinct doublets at δ 0.99 (J = 6.8 Hz) and 0.91 (J = 6.6 Hz) due to the diastereotopic methyls of the gem dimethyl group. The two methyl groups showed NOEs of 2% and 3% with the 10-hydrogen. The ^{13}C- and the ^{1}H-NMR spectra of pumiliotoxin **225F** clearly define the structure as that of a 12-hydroxy-**209F**.

The GC-FTIR spectra of alkaloids of the pumiliotoxin-A class are proving extremely useful for characterization of such dendrobatid alkaloids. FTIR spectra of pumiliotoxin A (**307A**), pumiliotoxin B (**323A**) and allopumiliotoxin B (**323B'**) are presented in Figs. 2 and 3. A GC-FTIR spectrum of **209F** (Fig. 2) revealed a sharp peak at 3544 cm^{-1} typical of the hydroxyl OH stretching frequency of a hydrogen-bonded tertiary alcohol common to the pumiliotoxin-A class. A characteristic Bohlmann band pattern at 2798-2700 cm^{-1} and a sharp absorption at 963 cm^{-1} (C(6)-C(10) trisubstituted double bond) also are typical of alkaloids of the pumiliotoxin-A class. However, the 990 cm^{-1} absorption of PTX-A, which we assign to a carbon-hydrogen out-of-plane vibration of the C(13)-C(14) double bond, is absent in the IR spectrum of **209F**. The IR spectrum of **225F** showed the same hydroxyl stretching absorption as **209F** at 3543 cm^{-1}. In addition, there were two weaker hydroxyl stretching absorptions at approximately 3650 and 3600 cm^{-1}. The latter two absorptions disappear on formation of a mono-O-acetate (Py:Ac_2O (1:1); 2 h), whose IR spectrum showed 3543, 1761, 1229 cm^{-1} among other absorptions (Fig. 2). The spectrum of **225F** also showed Bohlmann bands at 2799-2700 cm^{-1}, a C-O stretching absorption at 1035 cm^{-1} and the trisubstituted double bond C-H deformation absorption at 965 cm^{-1}. The data indicate that the second hydroxyl group at **225F** is acetylatable (i.e. non-tertiary) and that it is partially hydrogen-bonded (see Fig. 2).

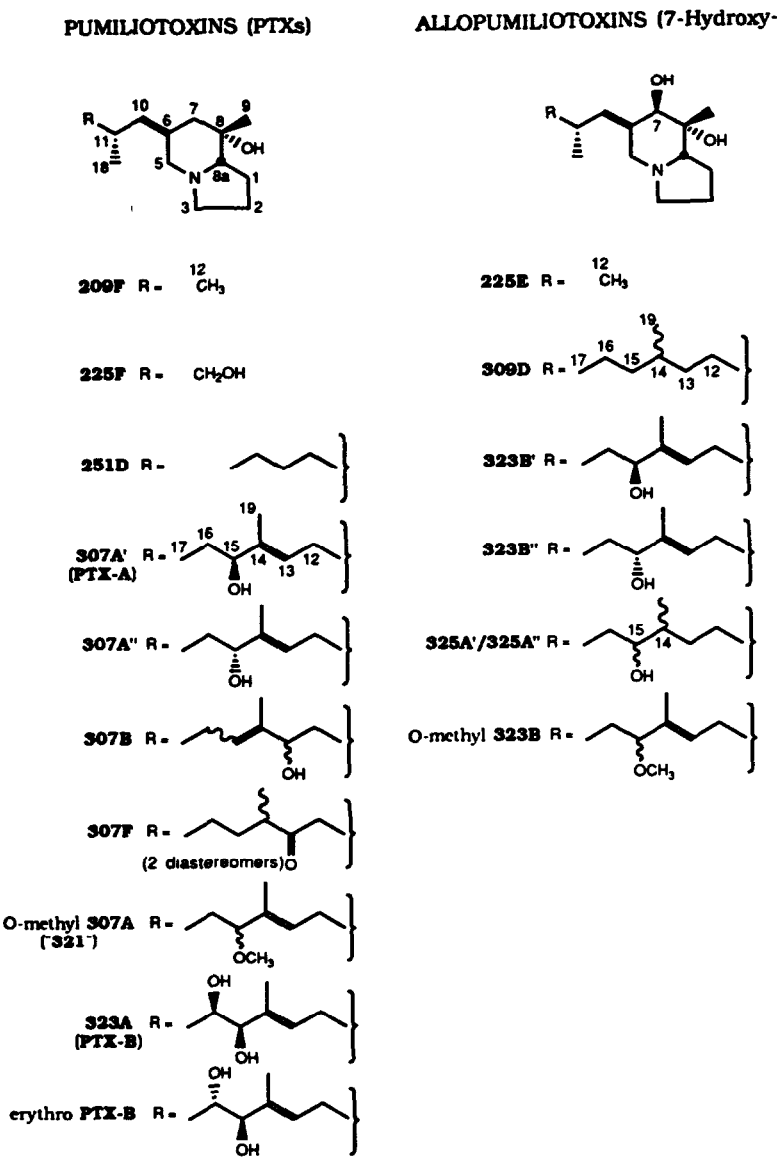

Fig. 1. Proposed structures of pumiliotoxins 209F and 225F, allopumiliotoxins 225E, 309D, 325A' and 325A" and a revised structure for pumiliotoxin 307F. A tentative structure for pumiliotoxin 307B, the structures of pumiliotoxins 251D, 307A', 307A", 323A and erythro-PTX-B, allopumiliotoxins 323B' and 323B", and the 15-O-methyl ethers of 307A (alkaloid "321", ref. 7) and 323B also are shown. The numbering of the simpler alkaloids, 209F, 225F, etc., retains the numbering of the C-18 methyl group for NMR comparison purposes only.

TABLE 1 Carbon 13-magnetic resonance assignments for pumiliotoxins **209F**, **225F** and **251D**. Assignments for **251D** from ref. 8. Assignments for **209F** and **225F** were based on hetero- and homo-nuclear shift correlation spectroscopies (Solvent $CDCl_3$, 40°C, 100 MHz). Values marked with asterisks may be transposed. The values for **209F** agree with those of synthetic material (L.E. Overman, personal communication).

Carbon	209F	225F	251D
1	23.3,t	23.1,t	23.4,t
2	21.2,t	21.1,t	21.2,t
3	54.6,t	54.3,t	54.7,t
5	53.0,t	53.3,t	53.3,t
6	129.4,s	133.9,s	130.0,s
7	48.8,t	49.0,t	49.0,t
8	68.4,s	68.4,s	68.4,s
8a	71.8,d	72.0,d	71.8,d
9	24.3,q	24.5,q	24.3,q
10	135.7,d	130.7,d	134.7,d
11	26.8,d	35.1,d	32.1,d
12	23.4,q*	17.1,q	37.6,t
13			29.8,t
14			22.9,t
15			14.2,q
18	23.5,q*	67.9,t	21.8,q

Allopumiliotoxins

Allopumiliotoxin **225E** was not isolated in quantities sufficient for ^{13}C-NMR assignments, but the mass spectrum indicates it has a structure compatible with 7-hydroxy-**209F**. The ^{1}H-NMR spectrum for **225E** confirms this assignment and demonstrates the usual 7,8-trans-diaxial hydroxyl groups of the allopumiliotoxins (data not shown). A GC-FTIR spectrum of **225E** was not obtained.

Three new allopumiliotoxins with ten carbon alkylidene side chains were isolated in small quantities from the *Dendrobates pumilio* extracts. These were allopumiliotoxins **309D**, **325A'** and **325A"** (Fig. 1). The latter two alkaloids appear, based on NMR spectral analysis, to be 13,14-dihydro analogs of allopumiliotoxins **323B'** and **323B"**. The stereochemistry at C-14 and C-15 is unknown for **325A'** and **325A"**. ^{13}C-NMR assignments are presented in Table 2, along with previous assignments for **323B'** and **323B"**. A tentative structure for **309D** based on NMR spectral analysis and comparison of its 6-alkylidene moiety to that of the **325A** isomers, is presented in Fig. 1 (see Table 2 for ^{13}C-NMR assignments).

An FTIR spectrum of **325A'** shows two equivalent, sharp hydroxyl OH stretching frequencies at 3649 and 3522 cm^{-1}, the latter typical of the tertiary hydrogen-bonded hydroxyl in the allopumiliotoxins (Fig. 3). A sharp Bohlmann band at 2802 and a pair of absorptions at 1013 and 977 cm^{-1} are seen. The absorption at 977 cm^{-1} is consonant with the presence of the exocyclic C(6)-C(10) double bond. Unfortunately the C-O stretching frequency apparently overlaps the δ_{C-H} of the C(13)-C(14) double bond so the absence of the C(13)-C(14) double bond is not clear from the FTIR spectrum. The intensity of this

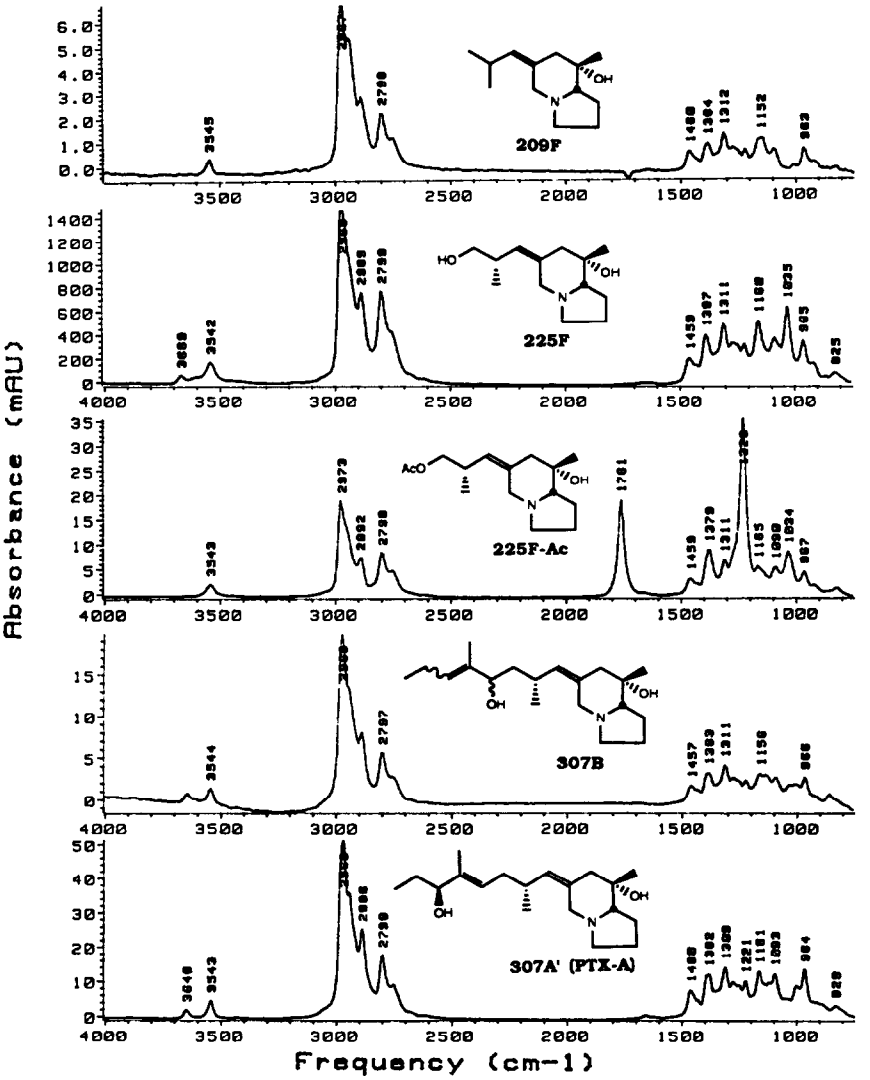

Fig. 2 FTIR spectra of pumiliotoxins **209F**, **225F**, **307B**, pumiliotoxin A (**307A'**) and the 12-O-acetate of **225F**.

absorption (1011 cm^{-1}), however, does appear to be reduced in **325A'** relative to **323B'** (see Fig. 3). The most unambiguous evidence for the saturation of this bond rather than the C(6)-C(10) double bond in **325A'** is the mass spectrum, which shows an m/z 182 base peak. FTIR spectra of **325A"** and **309D** were not obtained.

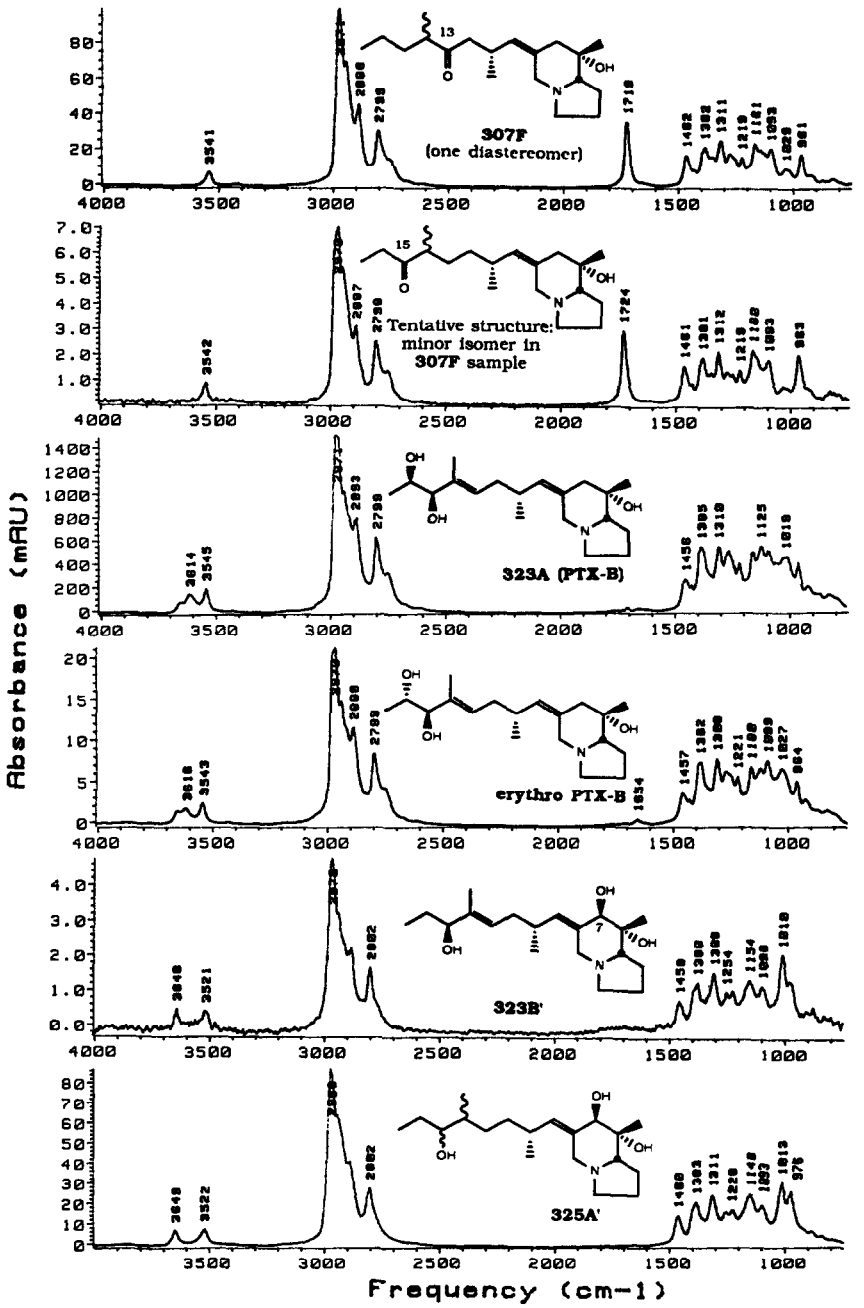

Fig. 3 FTIR spectra of pumiliotoxin **307F** and a minor isomer of **307F**, pumiliotoxin B (**323A**), erythro-pumiliotoxin B, and allopumiliotoxins **323B'** and **325A'**.

TABLE 2 Carbon 13-magnetic resonance assignments for allopumiliotoxins 309D, 323B', 323B", 325A', 325A" and 15-O-methyl-323B. Assignments for 323B' and 323B" are from ref. 8. (Solvent $CDCl_3$, 40°C, 100 MH_z)

Carbon	309D	323B'	323B"	325A'	325A"	15-O-methyl-323B
1	22.7,t	22.7,t	22.7,t	22.7,t	22.7,t	22.9,t
2	21.3,t	21.3,t	21.2,t	21.3,t	21.3,t	21.5,t
3	54.3,t	54.3,t	54.3,t	54.3,t	54.3,t	54.4,t
5	49.0,t	49.3,t	49.0,t	49.1,t	49.2,t	49.1,t
6	133.8,s	133.8,s	133.7,s	133.8,s	133.8,s	133.3,s
7	81.0,d	80.9,d	80.8,d	80.9,d	80.9,d	80.8,d
8	70.3,s	70.4,s	70.4,s	70.3,s	70.3,s	70.4,s
8a	65.3,d	65.2,d	65.3,d	65.3,d	65.3,d	65.4,d
9	20.6,q	20.6,q	20.6,q	20.6,q	20.6,q	21.0,q
10	138.8,d	137.3,d	137.8,d	138.4,d	138.3,d	137.7,d
11	32.3,d	32.6,d	32.4,d	31.8,d	32.5,d	32.7,d
12	29.7,t	35.4,t	35.1,t	30.5,t	29.7,t	35.4,t
13	34.8,t	125.8,d	124.2,d	34.1,t	35.2,t	126.3,d
14	32.5,d	137.6,s	138.0,s	37.4,d	37.6,d	137.6,s
15	39.4,t	80.2,d	79.0,d	77.2,d	76.7,d	89.2,d
16	20.2,t	27.8,t	27.8,t	26.9,t	26.7,t	26.5,t
17	14.4,q	10.2,q	10.2,q	10.7,q	10.7,q	10.7,q
18	21.3,q	20.9,q	20.9,q	21.5,q	21.3,q	21.1,q
19	19.7,q	10.9,q	12.2,q	15.3,q	15.3,q	11.1,q
						-OMe 56.0,q

The ^{13}C-NMR assignments for an O-methyl derivative of allopumiliotoxin 323B also are presented in Table 2. Such O-methyl derivatives form easily from certain of the pumiliotoxin-A class alkaloids that contain an allylic 15-hydroxy group. For example, pumiliotoxin A (307A) yields an O-methyl ether, previously thought to occur naturally as pumiliotoxin 321 (7), but now recognized as an artefact. Such O-methyl derivatives do not form from alkaloids that contain the 15,16-dihydroxy moiety, as in pumiliotoxin B (323A) and allopumiliotoxin 339A (unpublished results). Epimerization at the 15-position occurs readily under acid conditions for 307A and for 323B and has led to isolation of both 15-epimers (307A', 307A", 323B', 323B") for these alkaloids (8). Epimerization at the 15-position of pumiliotoxin B (323A) has not yet been attained, in spite of strenuous conditions, presumably due to a stabilizing effect of the 16-hydroxyl group (see ref. 9).

Pumiliotoxin A (307A) presumably occurs naturally as 307A' with the same configuration of the 15-hydroxyl group as in pumiliotoxin B (323A). Epimerization at C(15) during isolation yields varying amounts of 307A", which can be separated from 307A' only by HPLC and which has a mass spectrum identical to 307A'. Since much of our preliminary characterization of frog skin extracts is done with capillary GC-MS where 307A' and 307A" co-chromatograph, we reserve the 307A identification to refer to material that may or may not be a mixture of 15-hydroxy diastereomers. The same situation holds for allopumiliotoxin-B (323B) and the methyl ether artefact, 15-O-methyl-323B. In addition to epimerization at C(15) during isolation, it appears likely that pumiliotoxin A

TABLE 3 Carbon 13-magnetic resonance assignments for pumiliotoxins **307A'**, **307A"** and **307F**. Assignments for **307A'** and **307A"** are from ref. 5. (Solvent $CDCl_3$, 40°C, 100 MHz)

Carbon	307A'	307A"	307F
1	23.3,t	23.3,t	23.1,t
2	21.2,t	21.2,t	21.0,t
3	54.6,t	54.6,t	54.4,t
5	53.3,t	53.3,t	53.0,t
6	130.3,s	130.4,s	130.6,s
7	48.9,t	48.9,t	48.7,t
8	68.4,s	68.4,s	68.3,s
8a	71.7,d	71.7,d	71.6,d
9	24.3,q	24.3,q	24.2,q
10	133.8,d	133.8,d	132.6,d
11	32.6,d	32.6,d	28.0,d
12	35.6,t	35.6,t	48.3,t
13	125.0,d	125.0,d	213.6,s
14	137.7,s	137.7,s	46.6,d
15	79.6,d	79.5,d	34.8,t
16	27.8,t	27.8,t	20.3,t
17	10.2,q	10.2,q	14.1,q
18	21.3,q	21.4,q	21.5,q
19	11.4,q	11.4,q	15.9,q

also undergoes allylic rearrangement to yield another isomer. Such an isomer has been isolated in trace amounts by HPLC from the *Dendrobates pumilio* extract. The mass spectrum and the slightly shorter retention time on packed or capillary (OV-1) columns than either **307A'** or **307A"** indicate that it is **307B**, an alkaloid often reported as a trace constituent in dendrobatid extracts that contain major amounts of pumiliotoxin A (see ref. 6). The mass spectrum of **307B** had a ratio of m/z 193 to m/z 194 of two to one (see ref. 6) similar to that observed with the present trace alkaloid. In contrast, the m/z 194 peak for **307A** was much larger than the m/z 193 peak. The FTIR of this trace alkaloid is very similar to that of **307A'** (see Fig. 2). At present we consider this trace alkaloid, **307B**, to correspond to an acid-catalyzed allylic rearrangement product of **307A** and perhaps, like the 15-O-methyl ethers to be an artefact of the isolation procedure.

Alkaloids **307B** and **307F** have similar mass spectra and cochromatograph on the packed gas chromatography columns used in earlier analyses. Thus, it is likely that the designation **307B** applied to some extracts may have been used to characterize an alkaloid peak comprised of a mixture of **307B** and **307F** or even of **307F** rather than **307B**. Indeed, "**307B**" in some extracts showed only one exchangeable hydrogen rather than two (6). FTIR spectra clearly distinguish **307B** and **307F** and moderately polar capillary columns allow their separation.

A Revised Structure for Pumiliotoxin **307F**

The structure proposed (7) for **307F** has proven on further nuclear magnetic resonance analysis to be incorrect. The keto group of this pumiliotoxin is at the 13-position,

rather than at the 15-position as initially proposed. The reassignment to the 13-position is based on HOHAHA experiments, which exhibited mutual magnetization transfer relay between both the C-17 and C-19 methyl groups. The revised ^{13}C-NMR assignments for pumiliotoxin **307F** are presented in Table 3.

The GC-FTIR chromatogram of **307F** shows two partially resolved peaks with identical IR spectra, both exhibiting a strong OH stretching absorption at 3542 cm^{-1} and a carbonyl absorption at 1720 cm^{-1} (see Fig. 3). The Bohlmann band pattern at 2799-2700 cm^{-1} and olefin absorption at 963 cm^{-1} are typical of the pumiliotoxin-A class of dendrobatid alkaloids. The two partially resolved GC peaks (~1:1) are assumed to comprise a mixture of the 14-methyl epimers of **307F**. A minor peak following the main pair also has a very similar IR spectrum and may be the 15-ketone originally proposed as the structure for **307F**, as shown in Fig. 3.

A sample of **307F** exposed to excess $NaOCD_3$ in CD_3OD (1 h, RT) gave an approximately 1:1 mixture of bis and tris deuterium-exchanged material whose E.I. M.S. cleavage pattern supports the location of the ketone at C-13. In particular, fragments at m/z 237/238 (undeuterated **307F** had m/z 236) and m/z 208/209/210 (m/z 208 in undeuterated **307F**) indicate ketonic cleavage at the C(13)-C(14) and C(12)-C(13) bonds, respectively.

EXPERIMENTAL

High-resolution mass spectral data were obtained on a JEOL D-300 mass spectrometer (electron impact, 70 eV). Gas chromatographic mass spectral analyses were with a 2% OV-225 Chromosorb G AW-DMCS column programmed from 100° to 250° at 10°/min (electron impact, 30 eV). Nuclear magnetic resonance spectra were obtained on JEOL FX-100 and GX-400 spectrometers. Phase-sensitive 2D-NMR, homo- and hetero-nuclear shift correlation spectroscopies were routinely used for structure analysis. HOHAHA and NOE effects were observed through 2D-spectra and 1D-differential spectra. A Hewlett-Packard model 5965A FTIR instrument with a narrow band (4000-750 cm^{-1}) detector and a 59970 IRD ChemStation was used to record FTIR spectra of GC peaks. A Hewlett-Packard model 5890 gas chromatograph fitted with an HP-5 (bonded 5% diphenylsiloxane : 95% dimethylsiloxane) fused silica capillary column (30 m x 0.32 mm) programmed from 100° to 280° at 10°/min was used to generate the total response chromatogram.

Isolation of Alkaloids

A methanolic extract from 1200 skins of *Dendrobates pumilio* from Isla Bastimentos, Bocas, Panama (1987) was partitioned between aqueous methanol-chloroform. Alkaloids were then extracted from the chloroform layer with 0.05 N HCl. After adjusting to pH > 10 with ammonia, the aqueous layer was extracted with hexane and then chloroform. The hexane and chloroform layers were evaporated *in vacuo* to afford 0.13 g and 0.18 g, respectively. The hexane extract residue was treated with hexane containing 0.3% triethylamine. The soluble portion was chromatographed on a DIOL column (Merck, prepacked Lobar column, size B) with hexane-0.3% triethylamine to afford five main fractions (H-1 to H-5) as monitored by refractive index. GC-MS analysis revealed the following main alkaloids from each fraction: Fraction H-1, 20 mg, cis-**195A** and **205B**; H-2, 20 mg, cis-**195A**; H-3, 8 mg, **209F** and **205A**; H-4, 5 mg, mainly **235B**; H-5, 5 mg, mainly **321**. Fraction H-3 was further purified by HPLC on an ODS column with acetonitrile-water-triethylamine (60:40:1) to provide 2 mg **209F** and 3 mg **205A**. The chloroform extract residue was chromatographed on a reversed phase silica gel column (Merck, prepacked Lobar column, RP-8, size B) with acetonitrile-water-triethylamine (40:60:1) to afford five main fractions (C-1 to C-5). GC-MS analysis revealed the following: Fraction C-1, 14 mg, mainly the O-methyl ether of **323B**; C-2, 12 mg, **225E** and **225F**; C-3 110 mg, **323A**; C-4, 16 mg, **323B**; C-5, 23 mg, cis-**211A**,

323B and **325A**. Rechromatography of C-2 with hexane-chloroform-isopropanol-triethylamine (60:40:1:1) afforded almost pure samples of 2 mg **225E** and 3 mg **225F**. Rechromatography of C-5 with hexane-chloroform-triethylamine (50:50:1) yielded almost pure samples of cis-**211A** (3 mg), **323B** (4 mg) and **325A** (3 mg). The latter material on rechromatography provided **325A'** and **325A"**.

Extracts from Panamanian poison frog *Dendrobates pumilio* (Isla Bastimentos, Bocas, Panama) contain more than forty alkaloids (ref. 6,7 and unpublished data). These include pumiliotoxin A (**307A**) and pumiliotoxin B (**323A**) and other pumiliotoxins, allopumiliotoxins, decahydroquinolines, 5-substituted-8-methylindolizidines and alkaloids of unknown classes. Properties of **209F**, **225E**, **225F**, **309D**, **325A'**, **325A"** and the 15-O-methyl ether of **323B** from *Dendrobates pumilio* are presented below. The empirical formulas as determined by high resolution mass spectrometry are followed by the electron impact mass spectra with intensities relative to a base peak set equal to 100 in parentheses and the optical rotations if known.

Pumiliotoxin **209F**, $C_{13}H_{23}NO$, m/z 209 (22), 166 (70), 70 (100), $[\alpha]_D$ -11.6° (c 0.10 $CHCl_3$).

Allopumiliotoxin **225E**, $C_{13}H_{23}NO_2$, m/z 225 (40), 208 (86), 182 (41), 138 (41), 114 (34), 112 (60), 70 (100).

Pumiliotoxin **225F**, $C_{13}H_{23}NO_2$, m/z 225 (21), 194 (28), 166 (79), 112 (19), 84 (26), 70 (100), $[\alpha]_D$ -87.4° (c 0.23, $CHCl_3$).

Allopumiliotoxin **309D**, $C_{19}H_{35}NO_2$, m/z 309 (13), 292 (34), 182 (37), 123 (22), 114 (26), 112 (35), 70 (100). This alkaloid was isolated from a 1983 collection of *D. pumilio* at the same site (7), but was lost during purification after initial NMR analyses. It was not detected in the 1987 extract.

Allopumiliotoxin **325A'**, $C_{19}H_{35}NO_3$, m/z 325 (12), 308 (22), 182 (100), 114 (25), 112 (21), 70 (73). An isomer allopumiliotoxin **325A"** ($C_{19}H_{35}NO_3$), was also isolated. The mass spectrum was virtually identical to that of **325A'**.

15-O-methyl ether of allopumiliotoxin **323B**, $C_{20}H_{35}NO_3$, m/z 337 (16), 322 (11), 306 (69), 288 (23), 262 (10), 222 (17), 209 (59), 192 (29), 182 (100), 114 (44), 70 (73), $[\alpha]_D$, + 24.0° (c 0.2, $CHCl_3$).

REFERENCES

1. Mensah-Dwumah, M.; Daly, J. W. Toxicon, **1978**, 16, 189.
2. Albuquerque, E. X.; Warnick, J. E.; Maleque, M. A.; Kauffman, F. C.; Tamburini, R.; Nimit, Y.; Daly, J. W. Mol. Pharmacol. **1981**, 19, 411.
3. Daly, J. W.; Gusovsky, F.; McNeal, E. T.; Secunda, S.; Bell, M.; Creveling, C. R.; Nishizawa, Y.; Overman, L. E.; Sharp, M. J.; Rossignol, D. P. Biochem. Pharmacol. **1990**, 40, 315 and references therein.
4. Daly, J. W.; Myers, C. W. Science **1967**, 156, 970.
5. Daly, J. W.; Tokuyama, T.; Fujiwara, T.; Highet, R. J.; Karle, I. L. J. Am. Chem. Soc. **1980**, 102, 830.
6. Daly, J. W.; Myers, C. W.; Whittaker, N. Toxicon **1987**, 25, 1023.
7. Tokuyama, T.; Nishimori, N.; Shimada, A.; Edwards, M. W.; Daly, J. W. Tetrahedron **1987**, 43, 643.
8. Tokuyama, T.; Daly, J. W.; Highet, R. J. Tetrahedron **1984**, 40, 1183.
9. Garraffo, H. M.; Edwards, M. W.; Spande, T. F.; Daly, J. W.; Overman, L. E.; Severini, C.; Erspamer, V. Tetrahedron **1988**, 44, 6795.