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HIGH FIELD ^{1}H- AND ^{13}C-NMR ASSIGNMENTS OF GRAYANOTOXINS I, IV, AND XIV ISOLATED FROM *KALMIA ANGUSTIFOLIA*[1]

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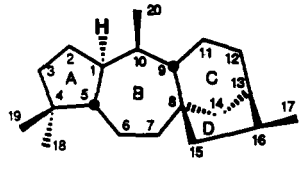
ABSTRACT.—Grayanotoxins I [**2**], IV [**3**], and XIV [**4**] were isolated from *Kalmia angustifolia* var. *caroliniana*, a North American member of the Ericaceae. Their structures were established from physical and spectral data (mp, ir, [α]D, uv, cd, ms, ^{1}H and ^{13}C nmr). High field ^{1}H- and ^{13}C-nmr assignments were made for each compound using 1D and 2D nmr techniques including extensive ^{1}H single-frequency spin-decoupling and nOe difference experiments, one-bond and long-range (COLOC) carbon-hydrogen correlations, and the INADEQUATE carbon-carbon correlation experiment. This is the first report of the presence of grayanotoxins IV and XIV in this source, of complete high field (500 MHz) ^{1}H-nmr assignments for any grayanoid, and of ^{13}C-nmr assignments for grayanotoxins IV and XIV. For grayanotoxin I, ambiguities in the literature ^{13}C-nmr assignments have been removed by definitive assignments made through the use of the INADEQUATE experiment.

The grayanotoxins are toxic diterpenes with a unique tetracyclic A-nor-B-homo-*ent*-kaurane skeleton called andromedane [**1**]. The toxicity of plants containing grayanotoxins is well documented (1–3), and the toxicity of honey made from the nectar of these plants was noted as early as the first century B.C. (4). The harmful effects of grayanotoxins result from their action on sodium channels by increasing the permeability of sodium ions in excitable membranes (5–7).

Grayanotoxins have been obtained only from genera of the Ericaceae (heath family),



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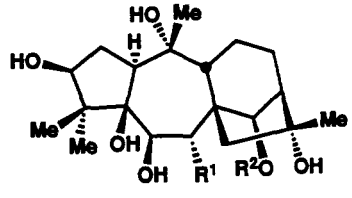
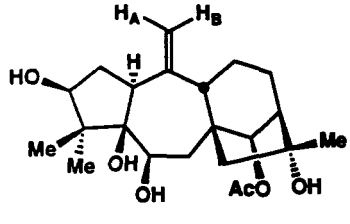


2 R^1=H, R^2=Ac
5 R^1=OH, R^2=H



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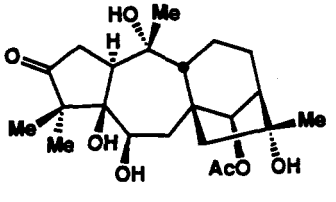

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[1]Taken in part from the Ph.D. dissertation of J.W.B. accepted by the Graduate School, The Ohio State University, in December 1987, and presented in part at the 27th Annual Meeting of the American Society of Pharmacognosy, July 1986, Ann Arbor, MI.

[2]Recipient of a predoctoral fellowship from the American Foundation for Pharmaceutical Education (1984–1987) and a designated Johnson & Johnson Company Fellow (1986–1987).

a family indigenous to temperate zones worldwide. The Ericaceous genera most extensively studied for grayanotoxins are shrubs common to Japan, especially members of the genera *Pieris* (*Andromeda*), *Leucothoe, Rhododendron,* and *Lyonia*. Previously in this laboratory the leaves of *Kalmia latifolia* L., the North American mountain laurel, yielded grayanotoxins as the feeding deterrents for the gypsy moth (*Lymantria dispar* L.) larvae (8). Another member of the same genus found in eastern North America, *Kalmia angustifolia* L. or sheep laurel, is known to be toxic to grazing animals (2). In 1957 (9) it was reported that *K. angustifolia* var. *caroliniana* (Small) Fern. was a source of grayanotoxin I [**2**], and in 1964 (10) grayanotoxins I, II, and III were found in plants collected in New Hampshire.

Because *K. angustifolia* var. *caroliniana* had never been thoroughly investigated, a systematic phytochemical study was undertaken to establish its grayanotoxin profile, and has yielded a new diterpene, kalmanol, with a unique ring system, a B-homo-C-nor grayanoid skeleton, which possesses pharmacological activity (11). We record here the isolation of grayanotoxins I, IV, and XIV and complete assignment of their high field ^{1}H- and ^{13}C-nmr spectra. Although ^{13}C-nmr assignments have been reported for some grayanotoxins, they are not without ambiguity and were not established by 2D techniques (12–14). A complete ^{1}H-nmr assignment for a grayanotoxin has not heretofore been made.

RESULTS AND DISCUSSION

The EtOH extract residue from the leaves of *K. angustifolia* var. *caroliniana*, on partitioning between pairs of immiscible solvents as detailed in the Experimental section, gave three fractions containing the grayanotoxins, which on tlc show blue-purple zones with *p*-anisaldehyde spray reagent. Chromatography on Sephadex LH-20 separated the terpenes from the phenolics. The terpenes from the EtOAc partition fraction when separated on two Si gel columns gave nine pooled fractions. Grayanotoxin I [**2**] crystallized from the fourth fraction, while grayanotoxins IV [**3**] and XIV [**4**] were obtained in crystalline form after further chromatography of the second fraction.

The structures of the isolated diterpenes were established from spectral data (ir, uv, cd, ms, and ^{1}H and ^{13}C nmr), and comparison of physical data to literature values confirmed the compounds to be grayanotoxins I, IV, and XIV. Grayanotoxin I [**2**], a long-known constituent (15), is widely distributed in the Ericaceae. Its structure was established in 1969 from chemical studies and in 1970 by X-ray crystal analysis (16). Grayanotoxin IV [**3**] was obtained previously from *Rhododendron ponticum* L. (17) and *Leucothoe grayana* Max. (18), and grayanotoxin XIV came from the latter source (19). Our work is the first report of grayanotoxins IV and XIV from *K. angustifolia*, and for the latter compound only the second known source.

The grayanotoxins of this study differ only in the nature of C-3 or C-10; all have an acetate at C-14. Interestingly, not one of these was found in the closely related *K. latifolia*, which provided grayanotoxins II, III, and XVIII, the first two of which contain a hydroxyl at C-14. Also, of the ten grayanoids isolated from that source, only one was acetylated at C-14 (8). Of chemotaxonomic interest, the kalmitoxins, which have a 7α-hydroxyl or 7α-acetate as in kalmitoxin I [**5**] and are obtained from *K. latifolia*, were not found in *K. angustifolia*.

The broad-band (BB) and single-frequency off-resonance decoupled (SFORD) ^{13}C-nmr spectra of grayanotoxin I [**2**] gave the chemical shifts and multiplicities as listed in Table 1. The INADEQUATE (20) experiment gave the carbon-carbon connectivity for grayanotoxin I [**2**] and thereby the complete carbon assignment. The spectrum is given in Figure 1, with the connecting carbons shown by the horizontal lines. The acetate carbonyl carbon is not shown.

TABLE 1. ^{13}C-nmr Chemical Shift Assignments for Grayanotoxins I [2], IV [3], and XIV [4].[a]

Carbon	Compound			
	2	3	3[b]	4
C-1	51.4d	45.3d	44.6d	47.5d
C-2	35.9t	39.7t	38.9t	38.6t
C-3	82.9d	80.8d	81.6d	220.8s
C-4	51.8s	50.4s	50.5s	56.8s
C-5	84.6s	83.5s	82.8s	82.1s
C-6	73.8d	69.3d	69.7d	69.5d
C-7	44.1t	40.8t	40.0t	43.3t
C-8	51.1s	49.0s	48.2s	50.8s
C-9	55.7d	51.5d	53.3d	54.1d
C-10	78.1s	152.0s	150.9s	78.0s
C-11	22.5t	23.7t	23.8t	22.0t
C-12	27.4t	24.9t	24.3t	27.2t
C-13	55.1d	52.7d	52.4d	54.1d
C-14	83.2d	84.1d	83.3d	84.3d
C-15	61.2t	61.3t	61.8t	62.2t
C-16	78.8s	79.5s	80.5s	79.5s
C-17	24.1q	25.9q	24.6q	24.0q
C-18	23.5q	23.9q	23.4q	22.2q
C-19	19.9q	18.7q	18.2q	17.6q
C-20	28.4q	112.6t	113.8t	27.4q
MeCO	170.5s	170.7s	170.0s	170.5s
CO	21.4q	21.3q	21.4q	21.2q

[a]Taken in pyridine-d_5 at 67.9 MHz unless stated otherwise; shifts in ppm and multiplicities by SFORD.

[b]Taken in $CDCl_3$.

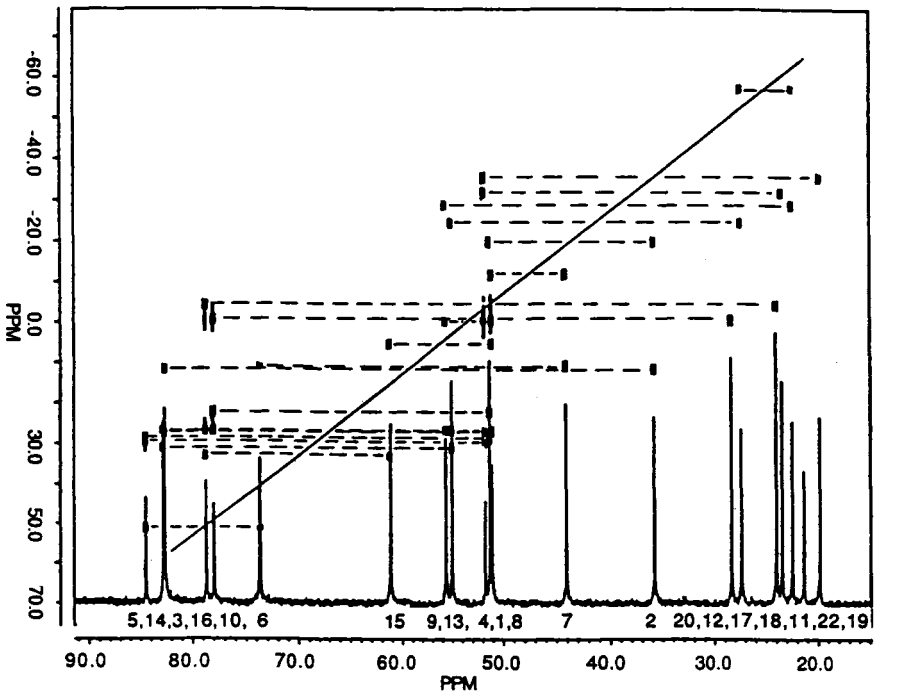

FIGURE 1. Two-dimensional INADEQUATE nmr spectrum of grayanotoxin I [2] at 125 MHz.

Assignment of the protons to the relevant carbons was made by the 2D CH-correlation experiment (21–23), and the stereochemical location of the methylene protons was determined from the values of the coupling constants obtained from simplified patterns by homonuclear spin decoupling, and especially from nOe enhancements as determined by difference experiments (24). The geminal methyls were assigned by the nOe. For example, upon irradiation of the methyl singlet at 1.28 ppm, enhancements were obtained for H-1α (3%), H-3α (10%), and H-6α (10%), thereby identifying the irradiated methyl as Me-18. The ^{1}H-nmr assignments are found in Table 2, and additional nOe results are presented in the Experimental section.

TABLE 2. ^{1}H-nmr Spectral Data for Grayanotoxins I [2], IV [3] and XIV [4].[a]

Proton	Compound			
	2[b]	3[c]	3[d]	4[e]
H-1	3.29, dd, J = 10,7	3.16, dd, J = 10,10	2.94, dd, J = 10,10	3.46, dd, J = 10,10
H-2α	~2.57, h m	2.63, ddd, J = 14,10,7	2.54, ddd, J = 15,11,7	3.02, dd, J = 19,9
H-2β	~2.57, h m	2.18, ddd, J = 14,9,2	1.74, h m	3.07, dd, J = 19,10
H-3	3.91, dd, J = 4,3	3.92, dd, J = 5,5	3.66, dd, J = 8,8	
H-6	4.21, m, J = 11,8,4	4.23, br dd, J = 5,5	3.70, dd, J = 8,8	4.39, m, J = 9,4,3
H-7α	2.60, dd, J = 14,4	2.19, dd, J = 14,2	1.69, dd, J = 15,2	2.64, dd, J = 15,3
H-7β	2.51, dd, J = 14,11	2.52, dd, J = 14,9	1.80, dd, J = 15,9	2.55, h dd, J = 15,9
H-9	2.22, d, J = 7	3.19, br dd, J = 5,5	2.76, dd, J = 8,8	2.42, h
H-11α	2.04, dd, J = 14,6	1.77, h m	1.62, h m	2.02, dd, J = 14,6
H-11β	1.62, m	1.64, m	1.56, h m	1.68, m
H-12α	2.67, h m	1.92, m	1.93, m (6pk), J = 8	2.55, h
H-12β	1.67, h m	1.74, h m	1.77, h m	1.68, m
H-13	~2.50, br s	2.46, br s, $\omega_{1/2}$ = 10	2.08, br d, J = 5	2.50, h br s
H-14	6.23, s	5.53, s	5.34, s	6.33, s
H-15α	2.26, d, J = 15	2.52, d, J = 14	2.08, d, J = 14	2.42, h m
H-15β	2.17, d, J = 15	2.33, d, J = 14	2.13, d, J = 14	2.42, h m
H-17	1.48, s	1.51, s	1.37, s	1.52, s
H-18	1.28, s	1.05, s	0.95, s	1.25, s
H-19	1.69, s	1.48, s	1.17, s	1.43, s
H-20	1.87, s	5.16, s (2H)	A 4.99, s B 5.11, s	1.73, s
Ac	1.99, s	2.03	2.12, s	2.01, s

[a]Taken in pyridine-d_5 at 500 MHz unless stated otherwise with chemical shifts in ppm, spin coupling (J) in Hz, and multiplicities designated as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broadened and h = hidden.

[b]D_2O exchanged peaks at δ 6.42 (d, 5 Hz, 3-OH), 5.45 (d, 8, 6-OH), 5.42 (s, 10-OH), 4.76 (s, 5-OH), and 4.36 (s, 16-OH).

[c]D_2O exchanged peaks at δ 5.82 (s, 3-OH), 5.81 (s, 6-OH), 4.80 (s, 5-OH), and 4.64 (s, 16-OH).

[d]In $CDCl_3$ with H-20A cis and H-20B trans to ring A; D_2O exchanged protons at δ 3.06 (s), 2.89 (d, 9 Hz, 3-OH), 2.75 (s), and 2.37 (d, 8, 6-OH).

[e]D_2O exchanged peaks at δ 6.85 (br s, 6-OH), 5.63 and 5.09 (2s, 5-OH and 10-OH), and 4.43 (s, 16-OH).

The low field ^{13}C-nmr spectral designations in the literature contain ambiguities (9–11). These uncertainties can now be removed; they involve (depending on the study) C-3, C-9, C-11, C-12, C-13, C-14, C-18, and C-19.

Extensive nmr studies with grayanotoxin IV [3] resulted in the assignments for the ^{1}H- and ^{13}C-nmr spectra as summarized in Tables 2 and 1, respectively. Homonuclear proton decoupling identified the three different spin systems for protons associated with C-1, C-2, and C-3; C-6 and C-7; and C-9, C-11, C-12, and C-13; while the 2D C-H correlation study associated the protons with the relevant carbons. The use of pyridine-d_5 as solvent was of advantage in achieving spectral dispersal for ^{1}H-nmr patterns. The nOe difference studies permitted assignment of the stereotopic positions for the methylene protons and differentiated the geminal methyls; these results are in the Experimental section. For example, irradiation of H-9 (2.76 ppm) enhanced the signal of the olefinic proton at 5.11 ppm, thus requiring it be placed syn to H-9, with the other olefinic proton (4.99 ppm) placed anti.

The quaternary carbons were located by the 2D COLOC (COrrelation via LOng

range Coupling) experiment (25) which reveals long-range coupling through two, three, and four bonds, with the three-bond coupling predominant. The geminal methyls (0.94 and 1.17 ppm) showed two-bond coupling to the 50.5 ppm carbon, identifying it as C-4, and three-bond coupling to the 82.8 ppm peak along with H-3 (3.66 ppm) identifying the peak at 82.8 ppm as C-5. C-8 (48.2 ppm) was correlated to H-6 (3.70 ppm, 3-bond) and H-9 (2.76 ppm, 2-bond), while C-16 (80.5 ppm) was coupled to H-14 (5.34 ppm, 3-bond) and to Me-17 (1.37 ppm, 2-bond). The chemical shift value (150.9 ppm) and the long-range coupling from H-1, H-9, and olefinic protons H-20A and H-20B permitted assignment of C-10. The acetate methyl resonance (2.12 ppm) was coupled to the carbonyl carbon (170.0 ppm) as was H-14, allowing for the remaining quaternary carbon to be designated and the position of the acetate confirmed.

Grayantoxin XIV [**4**] showed only 21 of the expected 22 peaks in the broad-band decoupled ^{13}C-nmr spectrum, but the inverse-gated nmr experiment, which gives the nOe-enhanced fully-relaxed spectrum, integrated for 22 carbons with the peak at 54.1 ppm representing two carbons (Table 1). ^{1}H- and ^{13}C-nmr assignments were made as for grayanotoxin IV [**3**]. From extensive ^{1}H-nmr homonuclear decoupling studies the spin-coupled units were identified: two ABX systems, one for protons on C-1 and C-2, the other for protons on C-6 and C-7; and the extended nonfirst-order patterned unit for protons of C-9 and C-11 through C-13; while the usual AB quartet observed for the H-15's was hidden at 2.42 ppm. The 2D CH-correlation study connected the spin systems with the carbons, and the 2D COLOC identified the location for the quaternary carbons. The latter experiment assigned C-8 (50.8 ppm) from 3-bond coupling to H-11 (2.04 ppm) and H-13 (2.50 ppm), and 2-bond coupling to H-9 (2.50 ppm) and H-7 (2.64 ppm). C-10 (78.0 ppm) is 3-bond-coupled to H-11 (2.04 ppm) and 2-bond-coupled to H-1 (3.46 ppm) and Me-20 (1.73 ppm), while C-16 (79.5 ppm) is 3-bond-coupled to H-14 (6.33 ppm) and 2-bond-coupled to Me-17 (1.52 ppm). Finally, C-5 (82.1 ppm) and C-4 (56.8 ppm) showed 2- and 3-bond coupling, respectively, from Me-18 (1.25 ppm) and Me-19 (1.43 ppm). Chemical shift values differentiated C-4 from C-5. The stereochemical identification of the protons was accomplished by nOe difference studies, a summary of which is given in the Experimental section.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points (uncorrected) were determined with a Thomas-Hoover Capillary Uni-melt or a Fisher-Johns hot-stage apparatus. Mass spectra were recorded using α VG 70-250S spectrometer, and ir spectra from a Beckman IR-4230 instrument. Optical rotations, uv spectra, and cd spectra were obtained using a Perkin-Elmer Model 241 polarimeter, a Beckman UV-5260 spectrophotometer, and a Jasco J-500A spectropolarimeter, respectively. All solvents used were spectrophotometric grade.

^{1}H- and ^{13}C-nmr spectra were recorded at 11.75 Tesla (500 MHz for ^{1}H and 125 MHz for ^{13}C) using a Bruker AM-500 spectrometer and at 6.35 Tesla (270 MHz for ^{1}H and 67.9 MHz for ^{13}C) using an IBM AF-270 spectrometer, both instruments having ASPECT 3000 Data Systems. All spectra were referenced to a residual solvent peak as internal standard: for pyridine-d_5, 7.19 ppm for ^{1}H and 123.5 ppm for ^{13}C, and for $CHCl_3$, 7.26 ppm for ^{1}H and 77.2 ppm for ^{13}C.

Tlc was performed on glass plates with 0.30 mm of Kieselgel 60 G (E. Merck). Terpenes were visualized by spraying with *p*-anisaldehyde–H_2SO_4–MeOH (1:1:18) followed by heating at 110–120° to yield blue or purple spots. For cc, Si gel 60 (70–230 mesh and 230–400 mesh, E. Merck) and Sephadex LH-20 (25–100 μm particle size, Pharmacia) were used. All solvents were analytical grade and redistilled before use.

PLANT MATERIAL.—Above-ground parts of *K. angustifolia* var. *caroliniana* were collected in North Carolina during autumn 1981 by Dr. E.M. Croom, Jr., (presently at the University of Mississippi) and authenticated by him. A voucher specimen is filed at the College of Pharmacy, The Ohio State University. The leaves from the air-dried plants were separated from the stems and twigs, hand-crushed, and sieved into small pieces (through 5 mm mesh).

PLANT EXTRACTION AND ISOLATION OF TOTAL TERPENOIDS.—Pulverized leaves (5.4 kg) were continuously extracted by percolation at room temperature with 95% EtOH. Evaporation of the EtOH extract at 40° under reduced pressure yielded a residue (2.4 kg), which was partitioned between $CHCl_3$ and H_2O; the aqueous phase was extracted successively with EtOAc and *n*-BuOH. Solvents were used in equal volumes, and the residue was kept to 10% or less (w/v) of the combined solvent volume. The dried EtOAc residue in 30–40 g portions was chromatographed on Sephadex LH-20 (250 g) with MeOH, which separated the terpenes as the fastest-eluting components from the phenolics to give a combined terpene fraction of 48 g.

ISOLATION OF GRAYANOTOXINS I, IV, AND XIV.—Chromatography of a 45-g terpenoid fraction on 1.76 kg of Si gel (102 cm × 6.6 cm) and elution with $CHCl_3$ and increasing amounts of MeOH (0–20%) in $CHCl_3$ gave nine pooled fractions. The fourth pooled fraction (3.3 g) crystallized from MeOH/$CHCl_3$ to give grayanotoxin I [**2**] (2.2 g, 4.1×10^{-2}% yield). Yield figures represent percent yield from weight of dried leaves. The second pooled fraction (0.9 g) was rechromatographed on 23 g Si gel (33 cm × 1.4 cm) and eluted with $CHCl_3$ and 0.5% and 1% MeOH in $CHCl_3$. The last solvent system eluted, first, a less polar fraction containing grayanotoxin IV [**3**], and then a more polar fraction containing grayanotoxin XIV [**4**]. The less polar fraction yielded **3** (319 mg, 5.9×10^{-3}% yield) upon crystallization from MeOH/Et_2O, and **4** (60 mg, 1.1×10^{-3}% yield) was obtained from the more polar fraction by crystallization from $CHCl_3$/Et_2O.

GRAYANOTOXIN I [**2**].—White needles (MeOH/$CHCl_3$), mp 240–241° $[\alpha]^{26.6}D$ −12° ($c = 0.4$, MeOH); ir (KBr) ν max 3590, 3535 and 3420 (OH), 1735 (ester C=O), 1473, 1454, 1404, 1372, 1246 (ester C-O), 1102, 1040, 938 cm^{-1}; uv (MeOH) λ 220 nm (end abs) (log ϵ 1.85); cd ($C = 1.0 \times 10^{-3}$ M, MeOH) $[\theta]_{240}$ 0, $[\theta]_{210}$ +300 (max); fabms (glycerol) *m/z* $[MH - H_2O]^+$ 395 (13%), $[MH - 2H_2O]^+$ 377 (10), 359 (11), $[MH - H_2O - HOAc]^+$ 335 (8), $[MH - 2H_2O - HOAc]^+$ 317 (41), $[MH - 3H_2O - HOAc]^+$ 299 (100), $[MH - 4H_2O - HOAc]^+$ 281 (71), 273 (68), 255 (34); fabms (glycerol, NaCl) $[MNa]^+$ 435 (48); fabms (glycerol, KCl) $[MK]^+$ 451 (34); ^1H nmr see Table 2; ^{13}C nmr see Table 1; nOe (pyridine-d_5, 270 MHz) irradiation at 1.48 ppm (Me-17) observed 1.66 ppm (4% enhancement, H-12β), ~2.50 (3, H-13), 2.17 (3, H-15β); irradiation at 1.28 ppm (Me-18) 3.28 (3, H-1), ~ 2.55 (3, H-2's + H-7's region), 3.91 (10, H-3), 4.20 (10, H-6), 1.69 (3, Me-19); irradiation at 1.69 ppm (Me-19) 3.91 (5, H-3), 1.28 (2, Me-18); irradiation at 1.87 ppm (Me-20) ~2.55 (8, H-2's + H-7's region), 2.22 (10, H-9), 2.04 (6, H-11α).

GRAYANOTOXIN IV [**3**].—White needles (MeOH/Et_2O), mp 167–168° $[\alpha]^{23.5}D$ −20° ($c = 0.3$, MeOH); ir ($CHCl_3$) ν max 3570 and 3480 (OH), 1740 (C=O), 1632 (C=C), 1443, 1390, 1375, 1220 (ester C-O), 1080, 1046, 1021, 942 cm^{-1}; uv (MeOH) λ 220 nm (end abs) (log ϵ 2.32); cd ($C = 4.1 \times 10^{-4}$ M, MeOH) $[\theta]_{255}$ 0, $[\theta]_{223}$ +900 (max), $[\theta]_{217}$ 0, $[\theta]_{205}$ −12,900 (min), $[\theta]_{200}$ 0; eims *m/z* $[M - H_2O]^+$ 376 (4%), $[M - 2H_2O]^+$ 358 (5), 348 (8), $[M - H_2O - HOAc]^+$ 316 (15), $[M - 2H_2O - HOAc]^+$ 298 (23), $[M - 2H_2O - HOAc - Me]^+$ 283 (8), $[M - 3H_2O - HOAc]^+$ 280 (11), 273 (13), 270 (13), 255 (20), 119 (20), 93 (23), 69 (62), 55 (21), $[Ac]^+$ 43 (100); ^1H nmr see Table 2; ^{13}C nmr see Table 1; nOe ($CDCl_3$, 270 MHz) irradiation at 2.94 ppm (H-1) observed 2.54 ppm (5% enhancement, H-2α), 3.66 and 3.70 (3, H-3 and H-6), 5.34 (8, H-14), 0.94 (3, Me-18); irradiation at 2.76 ppm (H-9) 1.62 (4, H-11α), 1.56 (5, H-11β), 5.11 (4, H-20B); irradiation at 5.34 ppm (H-14) 2.94 (8, H-1), 3.70 (4, H-6), 2.08 (7, H-13); irradiation at 1.37 ppm (Me-17) ~1.80 (4, H-12β), ~2.10 (8, H-13 + H-15's region); irradiation at 0.94 ppm (Me-18) 2.94 (8, H-1), 3.66 (14, H-3), 3.70 (14, H-6), 1.17 (7, Me-19); irradiation at 1.17 ppm (Me-19) 3.66 (8, H-3), 0.94 (7, Me-18); irradiation at 4.99 ppm (H-20A) 1.74 (10, H-2β), 5.11 (28, H-20B); irradiation at 5.11 ppm (H-20B) 2.76 (8, H-9), 1.62 (3, H-11α), 4.99 (30, H-20A); nOe (pyridine-d_5, 270 MHz) irradiation at 3.92 ppm (H-3) 2.63 (6, H-2α), 1.05 (5, Me-18), 1.48 (1, Me-19); irradiation at 4.23 ppm (H-6) 1.05 (7, Me-18), 1.48 (2, Me-19), 3.16 (3, H-1), 2.19 (3, H-7α), 2.52 (2, H-7β), 5.53 (H-14); irradiation at 5.53 ppm (H-14) 2.46 (7, H-13), 1.77 (2, H-11α), 1.92 (3, H-12α), 3.16 (7, H-1), 4.23 (3, H-6); irradiation at 2.33 ppm (H-15β) 2.52 (21, H-15α), 3.19 (11, H-9); irradiation at 1.51 ppm (Me-17) 2.46 (3, H-13), 3.19 (2, H-9), 2.33 (4, H-15β); irradiation at 1.05 ppm (Me-18) 4.23 (11, H-6), 3.16 (8, H-1), 3.92 (12, H-3), 1.48 (6, Me-19); irradiation at 5.16 ppm (H-20A + H-20B) 2.18 (8, H-2β), 1.48 (1, Me-19), 3.19 (6, H-9), 1.77 (3, H-11α), 1.92 (2, H-12α); irradiation at 1.48 ppm (Me-19) 4.23 (4, H-6), 3.92 (4, H-3), 1.05 (5, Me-18).

GRAYANOTOXIN XIV [**4**].—White needles ($CHCl_3$/Et_2O), mp 204–205° $[\alpha]^{25.1}D$ −44° ($c = 0.5$, MeOH); ir (KBr) ν max 3590, 3560, 3525 and 3440 (OH), 1745 (ketone C=O), 1730 (ester C=O), 1480, 1450, 1380, 1245 (ester C-O), 1164, 1101, 1060, 1030, 977 cm^{-1}; uv (MeOH) λ 220 nm (end abs) (log ϵ 2.29), 286 (ϵ 1.70); cd ($C = 1.1 \times 10^{-3}$ M, MeOH) $[\theta]_{330}$ 0, $[\theta]_{298}$ −2700 (min, $[\theta]_{240}$ 0; fabms (glycerol, NaI) *m/z* $[MNa]^+$ 433 (1%), $[M]^+$ 410 (1), $[MH - H_2O]^+$ 393 (9), $[MH - 2H_2O]^+$ 375 (9), $[MH - 3H_2O]^+$ 357 (3), $[MH - H_2O - HOAc]^+$ 333 (10), $[MH - 2H_2O - HOAc]^+$ 315 (20), 309 (9), $[MH - 3H_2O - HOAc]^+$ 297 (18), 275 (4), 269 (5), 255 (5), 155 (48), 135 (39), 119 (100), 101

(31), 85 (99.7), 71 (21), 47 (31); ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; nOe (pyridine-d_5, 270 MHz) irradiation at 3.46 ppm (H-1) observed 3.04 ppm (4% enhancement, H-2's region), 4.39 (5, H-6), 2.63 (5, H-7α), 6.33 (13, H-14α), 1.25 (3, Me-18); irradiation at 3.04 ppm (H-2's region) 3.46 (2, H-1), 1.73 (1, Me-20); irradiation at 4.39 ppm (H-6) 3.46 (4, H-1), ~2.60 (4, H-7's region), 6.33 (1, H-14), 1.25 (5, Me-18), 1.43 (4, Me-19), 2.01 (1, Ac); irradiation at 6.33 ppm (H-14) 3.46 (9, H-1), 4.39 (3, H-6), ~2.55 (4, H-12α), 2.50 (6, H-13), 2.01 (1, Ac); irradiation at 1.52 ppm (Me-17) ~1.67 (8, H-11β + H-12β), 2.50 (4, H-13), 2.42 (4, H-15's region); irradiation at 1.25 ppm (Me-18) 3.46 (10, H-1), 4.39 (12, H-6), 1.43 (2, Me-19); irradiation at 1.43 ppm (Me-19) 4.39 (4, H-6), 1.25 (3, Me-18); irradiation at 1.73 ppm (Me-20) 3.04 (11, H-2's region), 2.42 (2, H-9), 2.04 (6, H-11α).

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