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THIN-LAYER CHROMATOGRAPHIC AND SPECTROSCOPIC CHARACTERIZATION OF SOME DITERPENES OF THE GRAYANOTOXIN TYPE

A. D. KINGHORN*, F. H. JAWAD** and N. J. DOORENBOS

Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, Miss. 38677 (U.S.A.)

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SUMMARY

In a study of toxic polyhydroxylated andromedane diterpenoids, several novel derivatives of grayanotoxin III are described. Their spectral and physical characteristics are compared to ericaceous natural products. Separations on aluminium oxide and silica gel indicated that the absorption affinity of hydroxy groups on the perhydroazulene carbon skeleton was in the order 14β-OH > 3β-OH > 6β-OH > 2α-OH. Partition chromatography using ethylene glycol as stationary phase exhibited migration largely in accordance with the Reversed Traube's rule. This chromatographic–spectroscopic combination procedure is suitable for the micro-screening of these toxins.

INTRODUCTION

The grayanotoxins and related diterpenes have a rigid tetracyclic carbon skeleton variously known as andromedane, grayanane, perhydroazulene and A-*nor*-B-*homo*-*ent*-kaurane[1–3]. These phytotoxins were discovered in genera of the plant family Ericaceae as agents responsible for livestock poisoning[1]. Grayanotoxin III, formerly known as andromedol, and its 14β-acetate, grayanotoxin I, are the most well-known of these compounds, and have a unique range of pharmacological activities. They are hypotensive[4], and cause respiratory depression[4], exitatory activity in muscle spindle afferents[5], and a spasmodic paralysis response of *Artemia salina*[6]. Of particular significance is the resemblance of effect of grayanotoxins to ciguatoxin and batrachotoxin in increasing the permeability of membranes to sodium, in a manner opposed by tetrodotoxin[7,8]. The possibility of using grayanotoxins as neuropharmacological tools is given added impetus by the recent total synthesis of grayanotoxin II[9].

* To whom all correspondence should be addressed. Present address: Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, Ill. 60680, U.S.A.

** Present address: Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

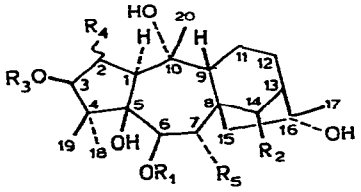
The grayanotoxins are structurally interesting natural products which are polar, neutral, saturated and polyhydroxylated, yet non-saccharides. No detailed account of the spectral data of a range of these compounds has appeared in the literature, while previous chromatographic separations have not been extensive[10–12]. We describe in this communication a comparison of spectroscopic and physical characteristics of grayanotoxins I and III, isolated from *Rhododendron maximum*, to 11 derivatives of grayanotoxin III, eight of which were novel. In a thin-layer chromatographic (TLC) profile of these compounds, and three donated ericaceous toxins, the relative potencies of hydroxy binding sites on the andromedane skeleton are discussed.

EXPERIMENTAL

All melting points are uncorrected. Specific rotations were measured at 25° on a Perkin-Elmer 141 polarimeter. IR spectra were measured on a Beckman IR-33 instrument, using KBr discs. Mass spectra (MS) were recorded at 70 eV on a DuPont 21-492 mass spectrometer with inlet temperatures between 100° and 250°. The mass spectra of compounds II–XII (Table I) were similar to grayanotoxin III (Table I, compound I) below the fragment ion *m/e* 298. The 60-MHz NMR spectra were recorded on a Jeol C-60H instrument, using TMS as internal standard, and C_5D_5N as solvent, unless otherwise stated. Chemical shifts are reported as parts per million on the δ scale (s = singlet; d = doublet; t = triplet; m = multiplet; br = broad).

TABLE I

STRUCTURES OF GRAYANOTOXINS AND THEIR DERIVATIVES



No.	*Compound*	R_1	R_2	R_3	R_4	R_5
I	Grayanotoxin III (GIII)	H	OH	H	H	H
II	Grayanotoxin I (GIII 14-acetate)	H	$OCOCH_3$	H	H	H
III	GIII 6-acetate	$COCH_3$	OH	H	H	H
IV	GIII 6,14-diacetate (Rhodojaponin IV)	$COCH_3$	$OCOCH_3$	H	H	H
V	GIII 3,6,14-triacetate	$COCH_3$	$OCOCH_3$	CH_3OC	H	H
VI	GIII 6-propionate	$COCH_2CH_3$	OH	H	H	H
VII	GIII 3,6-dipropionate	$COCH_2CH_3$	OH	CH_3CH_2OC	H	H
VIII	GIII 6-propionate 14-acetate	$COCH_2CH_3$	$OCOCH_3$	H	H	H
IX	GIII 6-butyrate	$CO(CH_2)_2CH_3$	OH	H	H	H
X	GIII 3,6-dibutyrate	$CO(CH_2)_2CH_3$	OH	$CH_3(CH_2)_2OC$	H	H
XI	GIII 6-butyrate 14-acetate	$CO(CH_2)_2CH_3$	$OCOCH_3$	H	H	H
XII	GIII 6-isobutyrate	$COCH(CH_3)_2$	OH	H	H	H
XIII	GIII 6-benzoate	COC_6H_5	OH	H	H	H
XIV	Rhodojaponin I	$COCH_3$	$OCOCH_3$	β-epoxy		H
XV	Rhodojaponin VI	H	OH	H	α-OH	H
XVI	Lyoniol A	$COCH_3$	H	β-epoxy		OH

Isolation of grayanotoxins I and III

Authenticated dried *Rhododendron maximum* roots (6 kg) were powdered in a Wiley mill, and extracted with acetone three times. The acetone extract (336 g) was dissolved in methanol–water (1:1), and washed with hexane to remove waxes and sterols. The methanolic residue was exhaustively extracted with warm water at 60°, and chromatographed batchwise on neutral alumina (Activity I) using the solvent system chloroform–isopropanol–water–ether (15:12:1:60). The crude grayanotoxin mixture was subjected to preparative-layer chromatography (PLC) on silica gel G (400 μm) using a combination of systems A–C (Table II), and recrystallization from methanol. Spectral data from the isolated grayanotoxin III (450 mg) and grayanotoxin I (250 mg) was compared to that of reference samples of the compounds and to previously published data[13].

TABLE II

hR_F VALUES OF GRAYANOTOXIN DITERPENES IN VARIOUS SOLVENT SYSTEMS

For solvent systems A–G, see text. For compounds, see Table I.

Compound	*Silica gel G*				*Aluminium oxide G*				*Kieselguhr G*		
	A	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*E*	*F*	*G*
I	30	3	48	8	41	3	57	5	2	10	6
II	41	6	56	22	55	9	66	19	5	24	18
III	39	5	56	19	50	4	64	11	4	24	19
IV	59	18	67	39	66	21	75	39	24	59	56
V	67	35	73	49	75	39	79	60	66	85	88
VI	48	6	61	24	57	6	68	14	10	34	30
VII	65	17	71	41	68	9	75	24	54	80	82
VIII	65	25	70	46	70	25	77	41	43	72	72
IX	53	7	62	25	61	6	73	16	18	53	42
X	75	37	82	60	76	33	82	65	87	91	93
XI	69	27	75	51	73	24	79	51	62	80	81
XII	54	9	62	27	62	7	75	18	19	53	41
XIII	86	76	84	77	82	79	88	88	99	99	99
XIV	66	39	70	54	75	45	77	66	65	83	83
XV	32	2	49	11	29	1	46	3	2	7	3
XVI	45	14	59	31	62	17	70	33	12	40	36

Grayanotoxin III (GIII). M.p., 266–268°; $[\alpha]_D$, −7.7 (c 0.26,methanol).IR, $\nu_{max.}$ 3390–3460, 1040 (OH) cm^{-1}. MS, M^+ $C_{20}H_{34}O_6$, *m/e* 370 missing; diagnostic fragment ions at *m/e* 352 (M − 18), 334 (M − 36), 316 (M − 54); 298 (M − 72), 273, 258, 205, 171, 148, 119, 109, 93, 69, 55, 43 (base peak) and 41. NMR, δ 1.12 (CH_3-19, s); 1.51 (CH_3-18, s); 1.65 (CH_3-17, s) 1.84 (CH_3-20, s); 3.10 (C-1, H, d; J = 8 Hz); 3.89 (C-3, H, m); 4.52 (C-6, H, d; J = 7 Hz); 5.00 (C-14, H, s) and 4.71 (2 OH); 5.08 (OH) and 6.0–6.5 (3 OH) (deuterium exchange) ppm.

Grayanotoxin I (GIII 14-acetate). M.p., 259–262°; $[\alpha]_D$, −11.0 (c 0.51, methanol). IR, $\nu_{max.}$ 3590, 3550, 3420, 1040 (OH); 1735, 1235 (acetate); 1370 (*gem* dimethyl) cm^{-1}. MS, M^+ $C_{22}H_{36}O_7$, *m/e* 412 missing, diagnostic fragment ions at *m/e* 394 (M − 18), 376 (M − 36), 358 (M − 54), 334 (M − 60 − 18), 316 (M − 60 − 36) and 298 (M − 60 − 54). NMR, δ 1.17 (CH_3-19, s); 1.45 (CH_3-18, s); 1.57 (CH_3-17, s);

1.77 (CH_3-20, s); 2.09 (CH_3OCO, s); 3.10 (C-1, H, dd; J = 6, 12 Hz); 3.87 (C-3, H, m); 4.17 (C-6, H, m); 6.04 (C-14, H, s) ppm.

Derivative formation of grayanotoxins I and III

In the following acylation procedures excess reagent was hydrolysed in iced water, and the acylate extracted into 50 ml chloroform. This layer was washed with 1 ml 0.5% (v/v) H_2SO_4 and 2 × 10 ml water. After concentration of the chloroform layer under reduced pressure, PLC on silica gel G (400 μm) was carried out on systems A–C (Table II).

Grayanotoxin III 6-acetate[14]. 15 mg GIII in 1.0 ml pyridine was acetylated with 0.5 ml acetic anhydride for 4 h at room temperature. Purification and crystallization from methanol yielded 11 mg needles. M.p., 293–294°; $[\alpha]_D$, −10.7 (c 0.57, methanol). IR, $\nu_{max.}$ 3350–3530 (br), 1045 (OH); 1737, 1250 (acetate); 1380 (*gem* dimethyl) cm^{-1}. MS, M^+ $C_{22}H_{36}O_7$, *m/e* 412, and diagnostic fragment ions at *m/e* 394 (M − 18), 376 (M − 36), 358 (M − 54), 334 (M − 60 − 18), 316 (M − 60 − 36) and 298 (M − 60 − 54). NMR, δ 0.81 (CH_3-19, s); 1.45 (CH_3-18, s); 1.46 (CH_3-17, s); 1.76 (CH_3-20, s); 2.01 (CH_3OCO, s); 3.04 (C-1, H, d; J = 7 Hz); 3.80 (C-3, H, m); 4.93 (C-14, H, s); 5.50 (C-6, H, d; J = 7 Hz) ppm.

Grayanotoxin III, 6,14-diacetate (Rhodojaponin IV)[15]. 15 mg GIII 14-acetate was warmed at 80° in 1.5 ml pyridine and 1.0 ml acetic anhydride for 1 h, and then allowed to stand at room temperature overnight before extraction and purification. Crystallization from methanol yielded 10 mg crystals. M.p., 238–240°; $[\alpha]_D$, −2.5 (c 0.56, methanol); IR, $\nu_{max.}$ 3570, 3440, 1045 (OH); 1737, 1250 (acetate); 1375 (*gem* dimethyl) cm^{-1}. MS, M^+ $C_{24}H_{38}O_8$, *m/e* 454 missing, diagnostic fragment ions at *m/e* 436 (M − 18), 418 (M − 36), 400 (M − 54), 376 (M − 60 − 18), 358 (M − 60 − 36), 340 (M − 60 − 54), 334 (M − 120), 316 (M − 120 − 18), and 298 (M − 120 − 36). NMR, δ 1.04 (CH_3-19, s); 1.53 (CH_3-18, s); 1.53 (CH_3-17, s); 1.86 (CH_3-20, s); 2.04 (CH_3OCO, s); 2.09 (CH_3OCO, s); 3.42 (C-1, H, d; J = 8 Hz); 3.95 (C-3, H, m); 5.35 (C-6, H, m); 6.30 (C-14, H, d; J = 3 Hz) ppm.

Grayanotoxin 3,6,14-triacetate[16]. Diacetylation of 12 mg GIII 14-acetate in 1.0 ml with 1.0 ml acetic anhydride was accomplished by refluxing for 4 h and standing at room temperature for 48 h. Extraction, purification and crystallization from benzene yielded a product, 12 mg. M.p., 111–113°; $[\alpha]_D$, +15.9 (c 0.25, methanol). IR, $\nu_{max.}$ 3400–3600 (br), 1040 (OH); 1755, 1740, 1725, 1250 (acetate); 1372 (*gem* dimethyl) cm^{-1}. MS, M^+ $C_{26}H_{40}O_9$, *m/e* 496, and M − 18 (*m/e* 478) and M − 36 (*m/e* 460) fragment ions missing, diagnostic fragment ions at *m/e* 442 (M − 54), 436 (M − 60), 418 (M − 60 − 18), 400 (M − 60 − 36), 376 (M − 120), 358 (M − 120 − 18), 340 (M − 120 − 36), 322 (M − 120 − 54), 316 (M − 180), and 298 (M − 180 − 18). NMR, δ 1.10 (CH_3-19, s); 1.33 (CH_3-18, s); 1.50 (CH_3-17, s); 1.76 (CH_3-20, s); 2.06, 2.11, 2.39 (3 × CH_3OCO, s); 3.29 (C-1, H, dd; J = 6, 11, Hz); 4.99 (H-3, m); 5.30 (C-6, H, d; J = 7 Hz); 6.19 (C-14, H, s) ppm.

Grayanotoxin III 6-propionate. 15 mg GIII in 1.0 ml pyridine and 0.6 ml propionic anhydride were reacted at room temperature for 24 h. On extraction and crystallization from benzene 12.5 mg of a novel compound was obtained. M.p., 235–238°; $[\alpha]_D$, −7.0 (c 0.61, methanol); IR, $\nu_{max.}$ 3550, 3370, 1050 (OH); 1727, 1205 (propionate); 1390 (*gem* dimethyl) cm^{-1}. MS, M^+, $C_{23}H_{38}O_7$, *m/e* 426, and diagnostic fragment ions at *m/e* 408 (M − 18), 390 (M − 36), 372 (M − 54), 334 (M − 74 −

18), 316 (M — 74 — 36), 298 (M — 74 — 54). NMR, δ 0.85 (CH_3-19, s); 1.14 (CH_3CH_2OCO, t; J = 8 Hz); 1.50 (CH_3-18, s); 1.50 (CH_3-17, s); 1.83 (CH_3-20, s); 2.14 (CH_3CH_2OCO, m); 3.01 (C-1, H, d; J = 6 Hz); 3.90 (C-3, H, m); 5.00 (C-14, H, m); 5.50 (C-6, H, d; J = 6 Hz) ppm.

Grayanotoxin III 3,6-dipropionate. This novel compound was a minor product of the propionation of GIII, as described above. Increased yield (8.3 mg) was obtained from 20 mg GIII by heating at 80° for 2 h and standing for 24 h. The resinous product was purified as described previously. $[\alpha]_D$, +6.0 (c 0.15, methanol); IR, $\nu_{max.}$ 3440, 1050 (OH); 1738, 1720, 1190 (propionate); 1387 (*gem* dimethyl) cm^{-1}. MS, M^+ $C_{26}H_{42}O_8$, *m/e* 482, and diagnostic fragment ions at *m/e* 464 (M — 18), 446 (M — 36), 428 (M — 54), 408 (M — 74), 390 (M — 74 — 18), 372 (M — 74 — 36), 354 (M — 74 — 54), 334 (M — 74 — 74), 316 (M — 74 — 74 — 18) and 298 (M — 74 — 74 — 36). NMR, δ 0.93 (CH_3-19, s) 1.10 (CH_3CH_2OCO, m); 1.25 (CH_3-18, s); 1.42 (CH_3-17, s); 1.70 (CH_3-20, s); 2.41 (CH_3CH_2OCO, m); 3.21 (C-1, H, d; J = 5 Hz); 4.49 (C-3, H, m); 4.97 (C-14, H, m); 5.46 (C-6, H, d; J = 6 Hz) ppm.

Grayanotoxin III 6-propionate 14-acetate. 12 mg GIII 14-acetate in 1.0 ml pyridine was reacted in 0.5 ml propionic anhydride for 6 h at room temperature. After the usual work-up 10.2 mg of a new compound was crystallized from benzene. M.p., 245–247°; $[\alpha]_D$, —1.1 (c 0.35, methanol). IR, $\nu_{max.}$ 3570, 3455, 1045 (OH); 1758, 1742, 1235 (ester) cm^{-1}. MS, M^+ $C_{25}H_{40}O_8$, *m/e* 468, missing, diagnostic fragment ions at *m/e* 450 (M — 18); 432 (M — 36), 414 (M — 54), 390 (M — 60 — 18), 376 (M — 74 — 18), 372 (M — 60 — 36), 358 (M — 74 — 36), 334 (M — 74 — 60), 316 (M — 74 — 60 — 18), and 298 (M — 74 — 60 — 36). NMR, δ 0.98 (CH_3-19, s); 1.16 (CH_3CH_2OCO, t; J = 8 Hz); 1.45 (CH_3-18, s); 1.48 (CH_3-17, s); 1.80 (CH_3-20, s); 2.12 (CH_3OCO, s); 2.38 (CH_3CH_2OCO, m); 3.32 (C-1, H, d; J = 7 Hz); 3.89 (C-3, H, m); 5.48 (C-6, H, d; J = 6 Hz); 6.24 (C-14, H, s) ppm.

Grayanotoxin III 6-butyrate. 15 mg GIII were treated with 0.4 ml butyric anhydride and 0.5 ml pyridine overnight at room temperature. Purification and crystallization from methanol yielded 9 mg of a novel crystalline compound. M.p. 258–260°; $[\alpha]_D$, —1.0 (c 0.32, methanol); IR, $\nu_{max.}$ 3525, 3370, 3295, 1045 (OH); 1725, 1200 (butyrate), 1385 (*gem* dimethyl) cm^{-1}. MS, M^+ $C_{24}H_{40}O_7$ *m/e* 440 missing; diagnostic fragment ions at *m/e* 422 (M — 18), 404 (M — 36), 386 (M — 54), 368 (M — 72), 334 (M — 88 — 18), 316 (M — 88 — 36) and 298 (M — 88 — 54). NMR, δ 0.84 (CH_3-19, s); 0.94 ($CH_3(CH_2)_2OCO$, t; J = 3 Hz); 1.47 (CH_3-18, s); 1.49 (CH_3-19, s); 1.70 ($CH_3CH_2CH_2OCO$, m); 1.80 (CH_3-20, s); 2.31 ($CH_3CH_2CH_2OCO$, m); 3.10 (C-1, H, d; J = 5 Hz); 3.97 (C-3, H, m); 4.88 (C-14, H, m); 5.50 (C-6, H, d; J = 6 Hz) ppm.

Grayanotoxin III 3,6-dibutyrate. 20 mg GIII in 1 ml pyridine and 1 ml butyric anhydride were heated under reflux for 30 min and allowed to stand for 48 h at room temperature. A mixture of GIII 6-butyrate and the minor novel compound, GIII 3,6-dibutyrate was purified by PLC. The dibutyrate (9 mg) was resinous, $[\alpha]_D$, +7.8 (c 0.32, methanol); IR, $\nu_{max.}$ 3460, 1045 (OH); 1740, 1722, 1185 (butyrate); 1390 (*gem* dimethyl) cm^{-1}. MS, M^+ $C_{28}H_{46}O_8$, *m/e* 510, and diagnostic fragment ions at *m/e* 492 (M — 18), 474 (M — 36), 456 (M — 54), 422 (M — 88), 404 (M — 88 — 18), 386 (M — 88 — 36), 368 (M — 88 — 54), 334 (M — 88 — 88), 316 (M — 88 — 88 — 18) and 298 (M — 88 — 88 — 36). NMR, δ 0.77 (CH_3-19, s); 0.85 ($CH_3(CH_2)_2OCO$, t; J = 3 Hz); 1.26 (CH_3-18, s); 1.43 (CH_3-17, s); 1.67 (CH_3-20, s); 1.69 ($CH_3CH_2CH_2OCO$,

m); 2.36 ($CH_3CH_2CH_2OCO$, m); 3.13 (C-1, H, d; J = 6 Hz); 4.65 (C-3, H, m); 4.91 (C-14, H, m); 5.49 (C-6, H, m) ppm.

GIII 6-butyrate 14-acetate. 10 mg of GIII 14-acetate were reacted overnight with 1.0 ml pyridine and 0.6 ml butyric anhydride at room temperature. On PLC and crystallization from benzene, a novel compound (9 mg) was obtained. M.p., 225–226°; $[\alpha]_D$, +3.5 (c 0.26, methanol); IR, $\nu_{max.}$ 3570, 3440, 1040 (OH); 1757, 1742, 1230 (esters); 1390 (*gem* dimethyl) cm^{-1}. MS, M^+ $C_{26}H_{42}O_8$ *m/e* 482 missing, diagnostic fragment ions at *m/e* 464 (M — 18), 446 (M — 36), 428 (M — 54), 404 (M — 60 — 18), 386 (M — 60 — 36), 376 (M — 88 — 18), 368 (M — 60 — 54), 358 (M — 88 — 36); 340 (M — 88 — 54), 334 (M — 88 — 60), 316 (M — 88 — 60 — 18) and 298 (M — 88 — 60 — 36). NMR, δ 0.92 ($CH_3(CH_2)_2OCO$, t; J = 3 Hz); 0.97 (CH_3-19, s); 1.47 (CH_3-18, s); 1.49 (CH_3-17, s); 1.77 ($CH_3CH_2CH_2OCO$, m); 1.78 (CH_3-20, s); 2.11 (CH_3OCO, s); 2.31 ($CH_3CH_2CH_2OCO$, m); 3.23 (C-1, H, d; J = 5 Hz); 3.88 (C-3, H, m); 5.37 (C-6, H, m); 6.23 (C-14, H, m) ppm.

GIII 6-isobutyrate. 15 mg GIII in 1.0 ml pyridine was refluxed 4 h with 0.6 ml isobutyric anhydride. The previously undescribed compound (10 mg) was obtained on purification and crystallization from methanol. M.p. 258–260°; $[\alpha]_D$, +3.6 (c 0.11, methanol); IR, $\nu_{max.}$ 3560, 3430, 1045 (OH); 1730, 1725, 1200 (isobutyrate); 1385 (*gem* dimethyl) cm^{-1}. MS, M^+ $C_{24}H_{40}O_7$ *m/e* 440 missing, diagnostic fragment ions at *m/e* 422 (M — 18), 404 (M — 36), 386 (M — 54), 368 (M — 72), 334 (M — 88 — 18), 316 (M — 88 — 36) and 298 (M — 88 — 54). NMR, δ 0.92 (CH_3-19, s); 1.20 ($CH_3)_2CHOCO$, d; J = 5 Hz); 1.40 (CH_3-18, s); 1.44 (CH_3-17, s) 1.72 (CH_3-20, s); 2.63 ($(CH_3)_2CHOCO$, m); 3.13 (C-1, H, d; J = 6 Hz); 3.89 (C-3, H, m); 4.90 (C-14, H, m); 5.50 (C-6, H, m) ppm.

GIII 6-benzoate. Benzoylation of GIII (20 mg) according to previously published reaction conditions[17] yielded two apparant products, which were separated by PLC on silica gel G (400 μm) in benzene–ether (5:1) (triple development). NMR and MS studies indicated the less polar band (hR_F 45) to be a mixture of GIII 3-benzoate and GIII 3,6-dibenzoate, while the more polar band (hR_F 35) was GIII 6-benzoate. A similar benzoylate mixture was obtained in recent work starting from GIII 14-acetate[18]. Attempts to separate GIII 3-benzoate and GIII 3,6-dibenzoate were unsuccessful. GIII 6-benzoate (11 mg) which has not been characterised previously, was crystallized from benzene: m.p., 240° (decomp.); $[\alpha]_D$, +69.5 (c 0.22, methanol); IR, $\nu_{max.}$ 3420 (OH); 1722, 1278 (benzoate); 3070, 1603, 1505, 705 (aromatics) cm^{-1}. MS, M^+ $C_{27}H_{40}O_7$, *m/e* 474 missing; diagnostic fragment ions at *m/e* 456 (M — 18), 438 (M — 36), 420 (M — 54), 402 (M — 72), 368 (M — 106), 350 (M — 106 — 18), 316 (M — 122 — 36), 298 (M — 122 — 54); 220, 175, 134, 122, 105 (base peak), 91, 77, 57, 55, 43, 41. NMR ($CDCl_3$), δ 0.95 (CH_3-19, s); 1.18 (CH_3-18, s); 1.27 (CH_3-17, s); 1.33 (CH_3-20, s); 3.23 (C-1, H, d; J = 8 Hz); 3.60 (C-3, H, m); 5.13 (C-6, H, m, br); 5.92 (C-14, H, s); 7.33–8.13 (5 H, m, br; aromatic protons) ppm.

Donated ericaceous toxins

Rhodojaponin I. MS, M^+ $C_{24}H_{36}O_8$, *m/e* 452, diagnostic fragment ions at *m/e* 434 (M — 18), 416 (M — 36), 374 (M — 60 — 18), 356 (M — 60 — 36), 332 (M — 120), 314 (M — 120 — 18), 299 (M — 120 — 15), 296 (M — 120 — 18), 265, 248, 230, 187, 135, 116, 95, 85, 71, 55, 43 (base peak).

Rhodojaponin VI. MS, M^+ $C_{20}H_{34}O_7$, *m/e* 386 missing, diagnostic fragment

ions at *m/e* 368 (M — 18), 350 (M — 36), 332 (M — 54), 314 (M — 72), 296 (M — 90), 248, 230, 187, 149, 135, 133, 119, 109, 105, 93, 91, 85, 71, 55, 43 (base peak).

Lyoniol A. IR, $\nu_{max.}$ 3590, 3505, 1025 (OH); 1705, 1270 (acetate); 1380 (*gem* dimethyl) cm^{-1}. MS, M^+ $C_{22}H_{34}O_7$, *m/e* 410; diagnostic fragment ions at *m/e* 392 (M — 18), 374 (M — 36), 356 (M — 54), 350 (M — 60), 332 (M — 60 — 18), 314 (M — 60 — 36), 299 (M — 60 — 36 — 15), 296 (M — 60 — 54), 248, 230, 215, 148, 116, 109, 85, 55, 43 (base peak).

Thin-layer chromatography

Adsorption chromatography was carried out on 20 × 20 cm glass plates spread with silica gel G (400 μm) (Merck, Darmstadt, G.F.R.) and aluminum oxide G (400 μm; Merck), which were activated at 100° for 45 min before use. The following solvent systems were employed: (A) chloroform–isopropanol–water–ether (15:12:1:60); (B) ether–benzene (2:1) (double development); (C) ethyl acetate–isopropanol–water (80:24:6); (D) hexane–benzene–ether–acetone (1:3:9:2). Visualization was effected by spraying with reagent A, 60% (w/v) H_2SO_4 (110°, 5 min), and reagent B, Godin's reagent, 1% (w/v) vanillin in ethanol oversprayed with 3% ethanolic perchloric acid (80°, 4 min).

Partition chromatography was performed on 20 × 20 cm plates spread with Kieselguhr G UV/G_{254} (Macherey-Nagel, Düren, G.F.R.), activated as described above, and eluted their full length in 20% ethylene glycol in acetone. Plates were ready for use after air-drying for 15 min in: (E) toluene–ether (1:2); (F) ethyl acetate–ether (3:2); and (G) ethyl acetate–cyclohexane (3:1). To visualize the plates, reagent A was used as described for adsorption chromatography, and also used was reagent C, 1% vanillin in ethanol, oversprayed with 60% (w/v) H_2SO_4 (110°, 5 min).

Plates were developed in the above solvent systems after the application of 5–10 μg of each pure compound. Visualization was undergone after development, allowing adequate solvent drying time.

RESULTS AND DISCUSSION

Table II shows the separation of sixteen grayanotoxin-type compounds by adsorption chromatography on silica gel G and aluminium oxide G (systems A–D), and by partition chromatography in ethylene glycol on Kieselguhr (systems E–G). Marginally improved separation was obtained on aluminium oxide compound with silica gel, but the resolution was greatly increased by partition chromatography. Separation in ethylene glycol was dependent on molecular weight, and generally can be accounted for by the Reversed Traube's rule. One striking deviation from this was the effect of the presence of an epoxide group in rhodojaponin I and lyoniol A, which showed far greater hR_F values than the related compounds of about the same molecular weight, rhodojaponin IV and grayanotoxin III 6-acetate with a 3β-OH.

The colors in UV and visible light of the grayanotoxins are shown in Table III, employing three acidic visualizing reagents. For any particular reagent, this data varies from absorbent to adsorbent, and thus the table embraces both adsorbents and the partition method used. The color range is not significantly differential between these compounds, but this data serves rather to charaterize them as a group based on the perhydroazulene carbon skeleton.

The inclusion of acylated derivatives of GIII in Table II enables conclusions

TABLE III

COLORS OF GRAYANOTOXIN DITERPENES WITH ACID SPRAYS VIEWED IN DAYLIGHT (DL) AND UV LIGHT (366 nm)

For reagents A–C, see text.

Compound	*Silica gel G*				*Aluminium oxide G*		*Kieselguhr G*			
	Reagent A		*Reagent B*		*Reagent A*		*Reagent A*		*Reagent C*	
	UV	*DL*	*UV*	*DL*	*UV*	*DL*	*UV*	*DL*	*UV*	*DL*
I	Orange	Orange	Orange	Blue-violet	Orange	Purple-brown	Orange	Purple	Orange-yellow	Purple
II	Orange	Orange	Orange	Blue-violet	Orange	Purple-brown	Orange	Pink	Yellow	Blue
III	Yellow	Orange-brown	Yellow	Blue	Orange	Purple-brown	Orange	Pink	Yellow	Purple
IV	Yellow	Orange	Yellow	Yellow	Orange	Purple-brown	Orange	Pink	Orange-yellow	Blue
V	Yellow	Orange	Yellow	Yellow	Orange	Purple-brown	Orange	Pink	Yellow	Blue
VI	Orange	Purple-brown	Yellow	Blue	Orange	Purple-brown	Orange	Pink	Yellow	Blue
VII	Orange	Purple-brown	Orange-yellow	Red	Orange	Purple-brown	Orange	Pink	Yellow	Blue
VIII	Yellow	Orange-brown	Yellow	—	Orange	Purple-brown	Orange	Pink	Yellow	Blue
IX	Orange	Purple-brown	Yellow	Blue	Orange-red	Purple-brown	Yellow	Pink	Yellow	Blue
X	Orange	Purple-brown	Orange-yellow	—	Orange	Purple-brown	Yellow	Pink	Yellow	Blue
XI	Orange	Orange	Yellow	Blue	Orange-red	Purple-brown	Yellow	—	Yellow	Blue
XII	Yellow	Orange-brown	Yellow	Blue	Yellow	Purple-brown	Yellow	—	Yellow	Blue
XIII	Yellow	Lt. Brown	—	—	Yellow	—	Yellow	—	Yellow	—
XIV	Orange-brown	Yellow	Yellow	Blue-violet	Yellow	—	Yellow	Brown	Yellow	Blue
XV	Yellow	Yellow	Yellow	Blue-violet	Yellow	—	Yellow	Blue	Yellow	Blue
XVI	Yellow	Gray	Yellow	—	Yellow	—	Yellow	—	Yellow	—

to be drawn about the potency of adsorption sites by considering isomeric pairs of compounds, and small structural changes from one compound to another. The migration of GIII in adsorption systems may be regarded as being determined by the 3β-, 5β-, 6β-, 10α-, 14β- and 16α-OH groups. In systems A–D GIII 14-acetate migrates further than GIII 6-acetate, implying that the 14β-OH has more affinity for the adsorbent than the 6β-OH. A possible explanation may be made by considering the three-dimensional structure of GIII[19,20]. The 14β-OH and 16α-OH are non-adjacent and are located on the same side of the molecule, and would be expected to mutually reinforce adsorption. In contrast, while the 6β-OH and 5β-OH are also orientated similarly, they are adjacent groups. Hence, it is feasible that intramolecular hydrogen bonding could reduce the contribution of the 6β-OH in the adsorption process. This also accounts for the observation that GIII 3-benzoate is less polar than GIII 6-benzoate, since the 3β-OH is not influenced by any oxygen functionality. However, the 14β-OH has more adsorption affinity than the 3β-OH, because GIII 6-butyrate 14-acetate migrates further than its isomer GIII 3,6-dipropionate. While the acetylation of the 6β-OH in GIII 6-acetate results in separation from GIII in all solvent systems, on silica gel rhodojaponin VI is virtually inseparable from GIII, indicating that the 2α-OH has a very weak effect on adsorption. Hence, the order of adsorption affinity of hydroxy groups on the perhydroazulene skeleton is 14β-OH $>$ 3β-OH $>$ 6β-OH $>$ 2α-OH.

Other influences of structural variation on migration are evident in adsorption systems. Increases in hR_F values occurs with increase of esterification and in higher representatives of homologous series. The inclusion of an epoxide group and the introduction of aromaticity results in decreased polarity. Chain branching in GIII 6-isobutyrate involving an increase in electron density relative to the straight-chain isomer GIII 6-butyrate is reflected in a slight polarity increase in the latter compound.

Extensive work over the last decade by Japanese workers has shown the co-existence of complex mixtures of ericaceous diterpenes in several species[21], and the results in Tables II and III would compliment the necessary spectroscopic work to characterize a particular compound. Work by our group and others[7] indicate the interaction of grayanotoxins with tetrodotoxin in more than one physiological system. Accordingly, a portion of the great interest accorded tetrodotoxin in recent years may well be extended towards the isolation and synthesis of grayanotoxin-type diterpenes.

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