Quantitative Structure–Inotropy Relationship Applied to Substituted Grayanotoxins

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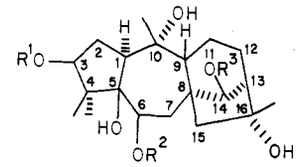
Nine 14β-O-acylated grayanotoxins were synthesized by ozonolysis of 14,16-alkylidenegrayanotoxin III. The correlation between positive inotropic potency (PIE) in guinea pigs and physicochemical parameters (Vw, Mw, and Rm_{50}) in 14 14-substituted grayanotoxins were quantitatively analyzed. It became clear that a parabolic relation existed between the bulkiness of the 14-substituents and PIE and that some electronic factor and the hydrophilic–hydrophobic balance would be related to the development of PIE.

Active principles, such as grayanotoxins or asebotoxins, from some ericaceous plants are diterpenoids having complex pharmacological and toxicological manifestations in the nervous and muscular systems of various kinds of animals. The mechanism by which these toxins produce these effects involves opening Na^+ channels of excitable cell membranes, as shown in several electrophysical studies.[1,2]

In a previous paper,[3] we reported on the relationship between the structure, positive inotropic potency (PIE), and lethal dose of grayanotoxins in guinea pigs. In that study it became clear that the presence of 3β-hydroxy and 10β-methyl groups attached to the grayanane (*A*-nor-*B*-homo-*ent*-kaurane) skeleton was essential for the development of PIE and that the potency was increased by acylation of the 14β-hydroxy group. Kinghorn et al.[4] described the structure–activity relationship of grayanotoxin derivatives using the spasmodic response of brine shrimp. They reported that 6-acylgrayanotoxin III (**2**) had some 20–30 times less activity than the mother substance, grayanotoxin III (G-III, **1**); that 6,14-diacylates (**3**) were 2–3 times less effective than 6-acylates (**2**) in LD_{50} studies; and that the 3,6,14-triacylates 4 were inactive. The toxic activity of 14-acylates **5** was not reported because of difficulty in preparing them. For the elucidation of the structure–activity relationships of grayanotoxins, 14β-acylates were indispensable to get information about the role of side chain at 14β-position. The aim of this paper is to clarify the degree of contribution of these compounds to PIE in isolated guinea pig papillary muscle.

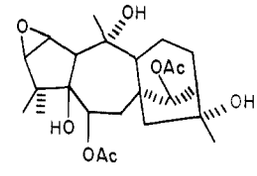
Chemistry. 14-Acylgrayanotoxin III (**5**) could not be obtained in the usual way: acylation of G-III (**1**) by acyl anhydrides and pyridine gave 6-acylates (**2**) or 3,6-diacylates (**6**) but did not yield 14-acylates.[5] In contrast, 3,14-diacetyl-6-benzoylgrayanotoxin III (**7**) or rhodojaponin I (**8**) was hydrolyzed only at the 14-position when treated with dilute alkali.[6,7]

Recently, Terai et al. reported that ozonolysis of 5,6:14,16-diethylidenegrayanotoxin III (**9**) afforded 6,14-diacetylgrayanotoxin III (**10**).[8] This reaction was applied in our laboratory to synthesize 14-acylgrayanotoxin III (**5**). We selected 6-acetylgrayanotoxin III (**11**) as a starting material to avoid acetalization between 5- and 6-hydroxygroups. Compound **11** was treated at room temperature with aldehydes (or acetal in the case of **11f**) in dimethylformamide in the presence of *p*-toluenesulfonic acid to afford 6-acetyl-14,16-alkylidene(or arylidene)-grayanotoxin III (**12a–i**), which in the next step was submitted to alkaline hydrolysis to remove the protected 6-acetyl group. The 14,16-alkylidene(or arylidene)grayanotoxins III (**13a–i**) were then oxidized with ozone at −78 °C



no.	R^1	R^2	R^3
1	H	H	H
2	H	acyl	H
3	H	acyl	acyl
4	acyl	acyl	acyl
6	acyl	acyl	H
7	Ac	Bz	Ac
10	H	Ac	Ac
15	H	H	glucosyl
16	Bz	Bz	H
17	H	H	Ac
18	H	H	lactoyl

acyl = acetyl, propionyl, butyryl, and benzoyl



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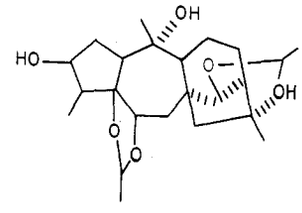

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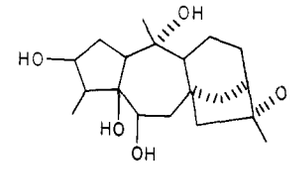

14

to yield 14-acylgrayanotoxins III (**5a–i**) (Scheme I). The physical and analytical data for these compounds (**5**) are summarized in Table I. Compound **5a** was identical with

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(1) T. Narahashi and I. Seyama, *J. Physiol. (London)*, **242**, 471 (1974).
(2) I. Seyama and T. Narahashi, *J. Pharmacol. Exp. Ther.*, **219**, 614 (1981).
(3) Y. Hotta, K. Takeya, S. Kobayashi, N. Harada, J. Sakakibara, and N. Shirai, *Arch. Toxicol.*, **44**, 259 (1980).
(4) A. D. Kinghorn, F. H. Jawad, and N. J. Doorenbos, *Toxicon*, **16**, 227 (1978).
(5) A. D. Kinghorn, F. H. Jawad, and N. J. Doorenbos, *J. Chromatogr.*, **147**, 299 (1978).
(6) J. Sakakibara, K. Ikai, and M. Yasue, *Yakugaku Zasshi*, **94**, 1534 (1974).
(7) H. Hikino, K. Ito, T. Ohta, and T. Takemoto, *Chem. Pharm. Bull.*, **17**, 1078 (1969).
(8) T. Terai, M. Katai, N. Hamanaka, T. Matsumoto, and H. Meguri, *Chem. Pharm. Bull.*, **26**, 1615 (1978).

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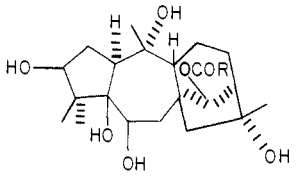
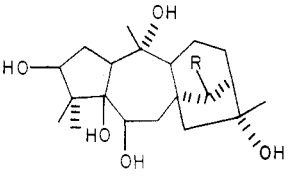
Table I. Physical Properties of Compounds 5a-i[a]



no.	R	reaction time, h	yield,[b] %	mp, °C	formula	anal.[d]
5a	C_2H_5	2	67	194-196	$C_{23}H_{38}O_7$	C, H
5b	n-C_3H_7	2	60	201-204	$C_{24}H_{40}O_7$	C, H
5c	n-C_4H_9	1	45	178-180	$C_{25}H_{42}O_7$	H;[e] C
5d	n-C_5H_{11}	2	72	amorph[c]	$C_{26}H_{44}O_7$	C, H
5e	n-C_6H_{13}	1	22	amorph[c]	$C_{27}H_{46}O_7$	H;[f] C
5f	$ClCH_2CH_2$	6	29	196-200	$C_{23}H_{37}O_7Cl$	C, H
5g	C_6H_{11}	1	45	212-215	$C_{27}H_{44}O_7 \cdot 0.5H_2O$	C, H
5h	C_6H_5	2	63	198-201	$C_{27}H_{38}O_7$	C, H
5i	$(CH_3)_3C$	1	71	amorph[c]	$C_{25}H_{42}O_7 \cdot 0.5H_2O$	C, H

[a] All compounds were recrystallized from n-hexane-AcOEt. [b] Yield from compounds 13. [c] Amorph = amorphous powder. [d] Elemental analyses are within ±0.4% of the theoretical values unless otherwise indicated. [e] C: calcd, 66.05; found, 65.56. [f] C: calcd, 67.19; found, 66.68.

Table II. Positive Inotropic Effect, Lethal Dose, and Physicochemical Parameters



no.	R	pD_2 obsd (±SE)[a]	pD_2 calcd[b]	LD_{50}, mg/kg	Rm_{50}	Vw × 10^2, Å^3	Mw
14	H	5.61 (0.06)	5.70	0.16	−0.17	0.013	354.487
1	HO	6.15 (0.06)	5.93	0.45	−0.24	0.081	370.486
17	CH_3COO	6.31 (0.04)	6.64	1.3	−0.16	0.423	412.523
5a	C_2H_5COO	6.67 (0.07)	6.70	0.044	−0.06	0.577	426.550
5b	n-C_3H_7COO	6.81 (0.06)	6.59	0.12	+0.10	0.731	440.577
5c	n-C_4H_9COO	6.41 (0.03)	6.33	0.17	+0.28	0.885	454.604
5d	n-$C_5H_{11}COO$	5.75 (0.06)	5.91	0.34	+0.48	1.039	468.631
5e	n-$C_6H_{13}COO$	5.32 (0.06)	5.33	nt[c]	+0.63	1.193	482.658
5f	$ClCH_2CH_2COO$	6.96 (0.06)	6.61	0.17	−0.04	0.712	460.995
5g	$C_6H_{11}COO$	6.17 (0.04)	5.91	0.31	+0.40	1.039	480.642
5h	C_6H_5COO	5.79 (0.04)	6.14	1.35	+0.23	0.963	474.594
5i	$(CH_3)_3CCOO$	5.78 (0.06)	6.52	0.36	+0.21	0.785	454.604
18	$CH_3(OH)CHCOO$	6.54 (0.09)	6.69	0.08	−0.24	0.595	442.549
15	glucosyl-O ($C_6H_{11}O_5$-O)	<4	5.98	<6	−0.37	1.017	532.627

[a] The mean of pD_2 values (n = 5-6) and standard error of the mean. [b] pD_2 values calculated by eq 6. [c] Not tested.

natural asebotoxin I from *Pieris japonica*.[9]

The synthesis of 14-deoxygrayanotoxin III (14), which has no functional group at the 14β-position, was previously reported by us.[6] In addition, we attempted the synthesis of 14-*O*-glucosylgrayanotoxin III (15) to increase the molecular bulkiness and hydrophilic character. We synthesized 15 from 3,6-dibenzoylgrayanotoxin III (16) and acetobromoglucose in the presence of mercuric bromide and mercuric cyanide.[10]

G-I (17) and G-III (1) were isolated from *Leucothoe grayana*,[11] and asebotoxin X (A-X, 18) was isolated from *Pieris japonica*.[12]

Biological Activity. The force of contraction of the papillary muscle isolated from guinea pig was measured at a driving rate of 1.0 Hz. The positive inotropic effect (PIE) at a given concentration of each test substance was recorded on oscillographic paper. The substances were added cumulatively to the bath containing Krebs-Henseleit solution (Ca^{2+}, 3.2 mM; 30 °C) every 20 min. The molar concentration for 50% of the maximum PIE (pD_2) was determined by depicting the concentration-PIE curve for each test materials in five to six papillary muscles. Acute toxicity was tested in male Hartley strain guinea pigs weighing about 350 g. Cardiac and respiratory arrests were the cause of death by all these test substances. The lethal dose (LD_{50}) was determined by means of the "up and down method".[13] The details of these methods were the same

(9) H. Hikino, K. Ito, and T. Takemoto, *Chem. Pharm. Bull.*, 17, 854 (1969). The preparation of asebotoxin I has neven been reported. The authentic sample was kindly supplied by Dr. Terai, Osaka Institute of Technology.

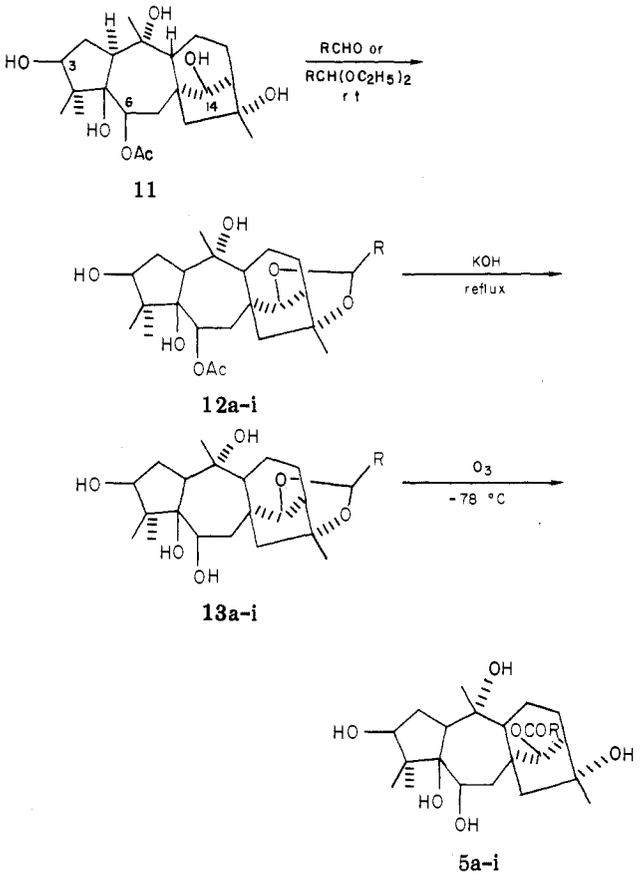
(10) G. Wulff and G. Röhle, *Angew. Chem., Int. Ed. Engl.*, 13, 157 (1974).

(11) H. Kakisawa, T. Kojima, M. Yanai, and K. Nakanishi, *Tetrahedron*, 21, 3091 (1965).

(12) J. Sakakibara, T. Kaiya, and N. Shirai, *Yakugaku Zasshi*, 100, 540 (1980).

(13) K. A. Brownlee, J. L. Hodges, Jr., and M. Rosenblatt, *J. Am. Stat. Assoc.*, 48, 262 (1953).

Scheme I



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12a-i

13a-i

5a-i

as reported in the previous paper.[3]

Results and Discussion

All 14 acylgrayanotoxins and related compounds were tested on PIE in isolated guinea pig papillary muscles. Cardiotonic potencies are summarized in Table II. The data in this table suggest that there exists a relationship between carbon chains of the 14-acyl groups and PIE. We previously[3] reported that more bulky substituents at the 14β-position produced more potent cardiotonic activity. The bulkiness of 14-substituents was calculated as van der Waals volume (Vw)[14] and molecular weights (Mw). As a parameter of hydrophobic property of the test substances, Rm_{50} values were obtained from experiments on thin-layer chromatography (TLC) with a methanol–water system as a developing solvent.[15] These data are also listed in Table II.

The correlations between pD_2 and physicochemical parameters (Vw, Mw, and Rm_{50}) were quantitatively analyzed by regression analysis.[16] The statistically significant regression equations are given below, where n is the number of compounds, r is the multiple regression coefficient, s is the standard deviation of the regression, and f is the value of the F test. The number in parentheses under each coefficient is the value of Student's t test for that coefficient. Equations 1 and 2 were obtained when the inactive compound (**15**) was omitted from the calculations, because no pD_2 values can be assigned to it.

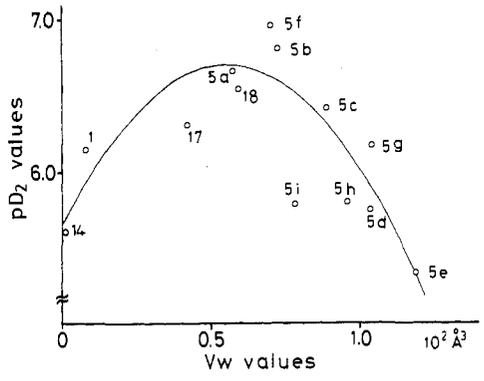


Figure 1. Parabolic relation between Vw and PIE

$$pD_2 = -2.708\ (\pm 1.711)\ Rm_{50} + 1.680\ (\pm 1.372)\ Vw + 5.302 \quad (1)$$
$$(3.53) \qquad (2.73)$$

$n = 13$, $r = 0.753$, $s = 0.361$, $F = 6.548$; $F_{\alpha(1)=0.05,2,10} = 4.10$

$$pD_2 = -2.000\ (\pm 1.292)\ Rm_{50} + 0.011\ (\pm 0.009)\ Mw + 1.725 \quad (2)$$
$$(3.45) \qquad (2.56)$$

$n = 13$, $r = 0.737$, $s = 0.371$, $F = 5.952$; $F_{\alpha(1)=0.05,2,10} = 4.10$

These equations show that the higher potency of the positive inotropy of the compounds is associated with the larger volume of the group attached to the 14-position and with the lower Rm_{50} value indicating hydrophilic properties of the test substances.

In order to get better correlation, we tried polynomial regression analysis and obtained eq 3–5. Equation 3 is

$$pD_2 = -2.984\ (\pm 3.822)\ Rm_{50}^2 + 0.061\ (\pm 1.056)\ Rm_{50} + 6.431 \quad (3)$$
$$(1.74) \qquad (0.09)$$

$n = 13$, $r = 0.648$, $s = 0.418$, $F = 3.623$, $F_{\alpha(1)=0.05,2,10} = 4.10$

$$pD_2 = -0.0002396\ (\pm 0.0001655)\ Mw^2 + 0.201\ (\pm 0.1394)\ Mw - 35.541 \quad (4)$$
$$(3.22) \qquad (3.21)$$

$n = 13$, $r = 0.714$, $s = 0.384$, $F = 5.202$, $F_{\alpha(1)=0.05,2,10} = 4.10$

$$pD_2 = -3.039\ (\pm 1.606)\ Vw^2 + 3.319\ (\pm 1.967)\ Vw + 5.696 \quad (5)$$
$$(4.22) \qquad (3.76)$$

$n = 13$, $r = 0.806$, $s = 0.324$, $F = 9.289$, $F_{\alpha(1)=0.01,2,10} = 7.56$

not statistically significant, but eq 4 is stastically significant. Equation 5 is highly significant and gave the best correlation.

The discrepancy between the potency of compound **5i** calculated from eq 5 (6.43) and that obtained from the experiment (5.78) is so large that omission of compound **5i** from the calculation resulted in a much better fit (eq 6). This equation was highly statistically significant and

$$pD_2 = -3.371\ (\pm 1.296)\ Vw^2 + 3.751\ (\pm 1.592)\ Vw + 5.653 \quad (6)$$
$$(5.88) \qquad (5.33)$$

$n = 12$, $r = 0.894$, $s = 0.252$, $F = 17.840$, $F_{\alpha(1)=0.005,2,9} = 10.11$

(14) I. Moriguchi, Y. Kanda, and K. Komatsu, *Chem. Pharm. Bull.*, **24**, 1799 (1976).

(15) G. L. Biagi, M. C. Guerra, and A. M. Barbaro, *J. Med. Chem.*, **13**, 944 (1970).

(16) The calculation was performed with ACOS-6 library program and carried out on an NEAC ACOS S-700 computer of the computation Center of this university.

shows that a parabolic relation exists between the bulkiness of 14β substituents and PIE. When Vw is 0.556, the maximum pD_2 value is 6.70 (Figure 1). Substitution of the 14β-position with a *tert*-butyl group (**5i**) resulted in development of steric hindrance by which the inotropic potency seemed to be decreased.

From the above experiments and calculations, the following conclusion can be drawn. A new synthetic method of 14-acylgrayanotoxins has been established. Although we reported in the previous paper that the more bulky substituents at the 14β carbon produced the more potent cardiotonic potency,[3] the compounds showing the highest PIE ($pD_2 > 6.5$) in this study, such as **5a,b,f** and **18**, bear three or four carbons at the 14-position. Their van der Waals volumes lie between 0.55 and 0.75 ($\times 10^2$ Å^3). They show Rm_{50} values between −0.25 and +0.10, and high acute toxicity ($LD_{50} \leq 0.17$ mg/kg). Of the 14 compounds studied, only compound **15**, which has a glucose moiety at the 14-position, is inactive. Compound **15** has an ether linkage and the others have an ester linkage at the 14-position. The former is the most hydrophilic compound ($Rm_{50} = -0.37$). Therefore, some electronic factor at the 14β-position and the hydrophilic–hydrophobic balance of the molecule would be related to the development of PIE.

Experimental Section

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. The ^{1}H NMR spectra were determined with a JEOL MH 100 spectrometer with Me_4Si as an internal standard. The IR spectra were recorded with a Hitachi EPI-G3 spectrometer. TLC was performed with silica gel 60 F_{254} (Merck, No. 5715 and 5747), and column chromatography was carried out with silica gel 60 (Merck, no. 7734). The developed plates were sprayed with Godin's reagent and heated at about 85 °C.[17] The structures of the compounds studied were supported by NMR, IR, and TLC.

General Procedure for the Preparation of 14,16-Alkylidene(or arylidene)grayanotoxin III (13a–i). *p*-Toluenesulfonic acid monohydrate (80 mg, 0.42 mmol) was added to a solution of 6-acetylgrayanotoxin III (**11**; 200 mg, 0.48 mmol) in a mixture of aldehyde (or acetal in the case of **13f**) (5–6 mL) and DMF (6 mL) and stirred for 1.5 h at room temperature. The mixture was poured into 5% $NaHCO_3$ solution and extracted with EtOAc. The EtOAc extract was evaporated in vacuo to give **12**. The crude product was purified by column chromatography on silica gel with 14:1 $CHCl_3$–MeOH as the eluent and/or recrystallization from *n*-hexane–AcOEt.

A solution of **12** (200 mg, 0.39–0.44 mmol) in a mixture of MeOH (5 mL) and 10% KOH (0.2 mL) was refluxed for 1 h. The mixture was diluted with water and extracted with EtOAc. The extract was evaporated in vacuo to give **13**. The crude product was purified by column chromatography on silica gel with 9:1 $CHCl_3$–MeOH as the eluent and/or recrystallization. Physical data of compounds **13** are summarized in Table III.

Compound **13a**: ^{1}H NMR (C_5D_5N) δ 0.96 (t, J = 7 Hz, 3 H, $CHCH_2CH_3$), 1.18, 1.36, 1.67, 1.83 (s, each 3 H, CH_3), 3.90 (br s, 1 H, C_3 H), 4.34 (dd, J = 4 and 12 Hz, C_6 H), 5.00 (s, 1 H, C_{14} H), 5.26 (t, J = 4 Hz, 1 H, $CH_3CH_2CHC(O-_2)$]; IR (KBr) 3400 (OH) cm^{-1}.

General Procedure for the Preparation of 14-Acylgrayanotoxin III (5a–i). A solution of **13** (ca. 150 mg, 0.32–0.36 mmol) in a mixture of EtOAc (1.5 mL) and MeOH (2.5 mL) was added to a saturated solution of ozone in EtOAc (50 mL) at −78 °C. After the end of the reaction was confirmed by TLC, an excess of ozone was removed by flushing nitrogen into the reaction mixture. The solvent was evaporated in vacuo, and the residue was purified by column chromatography on silica gel with 19:1 $CHCl_3$–MeOH as the eluent and/or recrystallization to yield **5**.

Compound **5a**: ^{1}H NMR (C_5D_5N) δ 1.10, 1.25, 1.46, 1.82 (s, each 3 H, CH_3), 3.25 (t, J = 8 Hz, 1 H, C_1 H), 3.90 (br s, 1 H, C_3 H), 4.12 (dd, J = 5 and 10 Hz, 1 H, C_6H), 6.21 (s, 1 H, C_{14} H); IR (KBr) 3580, 3500, 3400 (OH), 1720 (CO) cm^{-1}.

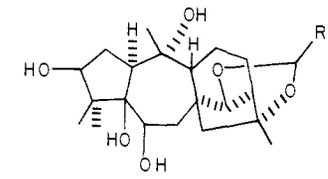
14-*O*-Glucosylgrayanotoxin III (15). 3,6-Dibenzoylgrayanotoxin III (**16**; 300 mg, 0.5 mmol)[6] was added to a mixture of benzene (25 mL) and nitromethane (25 mL) containing $HgBr_2$ (350 mg, 1 mmol), $Hg(CN)_2$ (1.2 g, 4.8 mmol), and 4A molecular sieves (9 g) and stirred for 30 min at room temperature, to which was added a solution of 2,3,4,6-tetraacetyl-β-D-glucopyranosyl bromide (1.2 g, 3 mmol)[18] in benzene at 0 °C and stirred for 22 h at room temperature. Triethylamine (2.1 mL) and benzene (40 mL) were added to the mixture, followed by filtration. The filtrate was washed with KI solution, $NaHCO_3$ solution, and water, successively. The solvent was evaporated in vacuo, and the residue was chromatographed on a Sephadex LH-20 column with MeOH. Glucoside fractions were purified by silica gel column chromatography with 17:3 CH_3CN–CH_2Cl_2 to yield 166 mg of 3,6-dibenzoyl-14-*O*-(tetraacetyl-β-D-glucopyranosyl)grayanotoxin III, which was refluxed with alkaline MeOH for 2 h and neutralized with Amberlite CG-50 (H^+) to yield crude glucoside **15**. It was purified by column chromatography on silica gel with 7:3 $CHCl_3$–MeOH to give 104 mg of **15** (38% from **16**): amorphous; $[\alpha]^{22}_D$ −21.8° (*c* 2.94, MeOH); ^{1}H NMR (C_5D_5N) 1.47, 1.50, 1.69, 1.84 (s, each 3 H, CH_3), 4.98 (d, J = 7 Hz, anomeric H). Acetylation of **15** gave hexaacetate, mp 214–216 °C, recrystallized from isopropyl ether. Anal. ($C_{38}H_{56}O_{17}$) C, H.

Rm Determination. The stationary phase was precoated TLC plates of silica gel 60 F_{254} silanized (layer thickness 0.25 mm, Merck no. 5747), and the mobile phase was aqueous MeOH solutions of various concentrations, e.g., 40, 45, 50, 55, 60 v/v %. The compounds tested were dissolved in MeOH (3 mg/mL), and 1 μL of the solution was spotted in randomized allocations in order to avoid any systematic error. The plates were developed about 15 cm from the spotted line at room temperature, dried, sprayed with Godin's reagent, and heated at about 85 °C.[17] The Rm values were calculated by means of the following formula:

$$Rm = \log (1/R_f - 1)$$

Higher and/or positive Rm values indicate compounds more hydrophobic than those represented by a lower and/or negative Rm value. The mean Rm values of five experiments were plotted

Table III. Physical Properties of Compounds 13a–i



no.	R	mp, °C	formula	anal.[c]
13a	C_2H_5	170–171[a]	$C_{23}H_{38}O_6$	C, H
13b	n-C_3H_7	180–181[a]	$C_{24}H_{40}O_6$	C, H
13c	n-C_4H_9	149–151[b]	$C_{25}H_{42}O_6$	C, H
13d	n-C_5H_{11}	165–167[b]	$C_{26}H_{44}O_6$	C, H
13e	n-C_6H_{13}	184–185[b]	$C_{27}H_{46}O_6$	C, H
13f	$ClCH_2CH_2$	173–175[b]	$C_{23}H_{37}O_6Cl$	C, H
13g	C_6H_{11}	173–175[b]	$C_{27}H_{44}O_6$	C, H
13h	C_6H_5	177–181[b]	$C_{27}H_{38}O_6$	C, H
13i	$(CH_3)_3C$	219–221[b]	$C_{25}H_{42}O_6$	H;[d] C

[a] Recrystallized from AcOEt. [b] Recrystallized from *n*-hexane–AcOEt. [c] Elemental analyses are within ±0.4% of the theoretical values unless otherwise indicated. [d] C: calcd, 68.46; found, 68.93.

Table IV. Squared Correlation Matrices for Variables of Equations 1 and 2

	Rm	Mw	Vw
Rm	1.0000	0.7673	0.8817
Mw		1.0000	0.9737
Vw			1.0000

(17) P. Godin, *Nature (London)*, **174**, 134 (1954).

(18) C. E. Redeman and C. Niemann, "Organic Syntheses", Collect. Vol. III, E. C. Horning, Ed., Wiley, New York, 1955, p 11.

against the composition of the mobile phase, which showed a linear relationship. According to Boyce and Milborrow,[19] the Rm values in the range of linearity were considered to be the most satisfactory ones. By means of the least-squares method, the equations of the straight lines were calculated. On the other hand, the equation of G-I (17) from 20 experiments had been derived as $Rm_c = -0.0172c + 0.7018$ (c = concentration; $r = 0.998$), from which the Rm_{50} of 17 could be calculated as −0.16. This was defined as a standard Rm_{50} value. Whenever TLC of other compounds were developed, G-I (17) was spotted simultaneously as a standard. And when Rm_{50} values of other grayanotoxins were calculated from their equations, the concentration corresponding to the standard Rm_{50} value (−0.16) was used instead of 50 in order to avoid any error from experimental conditions. Rm_{50} values thus obtained were listed in Table II.

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Registry No. 1, 4678-45-9; **5a**, 23984-17-0; **5b**, 84849-09-2; **5c**, 84849-10-5; **5d**, 84849-11-6; **5e**, 84849-12-7; **5f**, 84849-13-8; **5g**, 84849-14-9; **5h**, 84849-15-0; **5i**, 84849-16-1; 11, 54781-72-5; **13a**, 84849-17-2; **13b**, 84849-18-3; **13c**, 84849-19-4; **13d**, 84849-20-7; **13e**, 84849-21-8; **13f**, 84849-22-9; **13g**, 84849-23-0; **13h**, 84849-24-1; **13i**, 84849-25-2; 14, 54781-61-2; 15, 84863-60-5; 16, 84849-26-3; 17, 4720-09-6; 18, 84893-93-6; 2,3,4,6-tetraacetyl-β-D-glucopyranosyl bromide, 6919-96-6; 3,6-dibenzoyl-14-*O*-(tetraacetyl-β-D-glucopyranosyl)grayanotoxin III, 84863-61-6.

(19) C. B. C. Boyce and B. V. Milborrow, *Nature (London)*, **208**, 537 (1965).

Antihypertensive 9-Substituted 1-Oxa-4,9-diazaspiro[5.5]undecan-3-ones[1]

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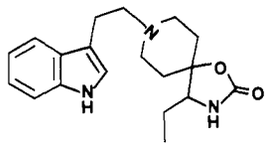
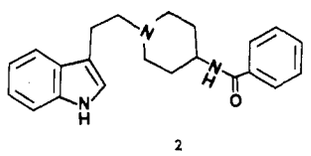
Forty-one 9-substituted 1-oxa-4,9-diazaspiro[5.5]undecan-3-ones were prepared for antihypertensive screening in the spontaneously hypertensive rat (SHR). For the 9-(2-indol-3-ylethyl) series, the parent compound, 9-(2-indol-3-ylethyl)-1-oxa-4,9-diazaspiro[5.5]undecan-3-one (21), was the most potent antihypertensive agent. Substitution of lower alkyl groups on the spirolactam ring gave compounds close in activity to 21, while substitution with large alkyl or aryl groups led to a significant decrease in activity. Ring-opened analogues of 21 that contained the same functionality were markedly less active. Several 1-oxa-4,9-diazaspiro[5.5]undecan-3-ones substituted at the 9 position with 1,4-benzodioxan-2-ylmethyl, 1,4-benzodioxan-2-ylhydroxyethyl, and 2-phenylethyl groups also demonstrated significant activity. Compound 21 was chosen for a detailed pharmacological evaluation. Its antihypertensive activity appears to be predominantly due to peripheral α_1-adrenoceptor blockade.

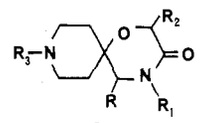
The synthesis and antihypertensive activity of a series of spiropiperidinyloxazolidones exemplified by 1 have been



1



2

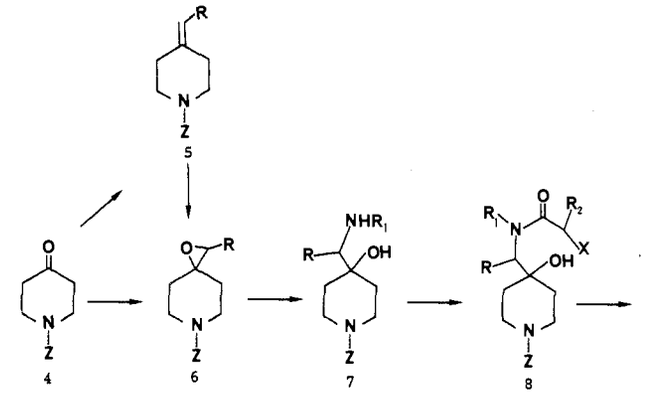


3

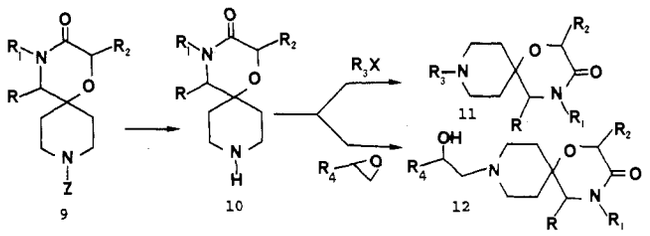
recently described.[2] The antihypertensive activity of this series was due to postsynaptic (α_1) adrenoceptor blockade as has been found for the structurally related antihypertensive agent indoramin (**2**).[3] It was thus of interest to examine the related 9-substituted 1-oxa-4,9-diazaspiro-[5.5]undecan-3-ones of general formula **3**,[4] and we now report that a number of compounds from this series display significantly greater antihypertensive activity relative to the earlier series. With the activity of the spiro compounds established, it was of further interest to prepare acyclic analogues that contain the same basic functionality in order to establish the exact structural requirements for activity.

Chemistry. The 1-oxa-4,9-diazaspiro[5.5]undecan-3-one ring system was prepared as shown in Scheme I. Epoxides

Scheme I



(Z = $CO_2CH_2C_6H_5$)



6 were prepared from 1-carbobenzoxypiperidone **4**[2] either directly with a sulfur ylide for compounds in which R =

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(2) Caroon, J. M.; Clark, R. D.; Kluge, A. F.; Nelson, J. T.; Strosberg, A. M.; Unger, S. H.; Michel, A. D.; Whiting, R. L. *J. Med. Chem.* 1981, *24*, 1320.

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