Novel Hypotensive Agents from *Verbesina caracasana*. 6. Synthesis and Pharmacology of Caracasandiamide[1]

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Caracasandiamide, a second hypotensive agent isolated from *Verbesina caracasana*, is the cyclobutane dimer (truxinic type) of the previously reported 1-[(3,4-dimethoxycinnamoyl)amino]-4-[(3-methyl-2-butenyl)guanidino]butane (caracasanamide) (Delle Monache, G.; et al. *BioMed. Chem. Lett.* **1992**, *25*, 415−418). The structure was confirmed by synthesis starting from β-truxinic acid obtained by photoaddition of 3,4-dimethoxycinnamic acid. The dimer was coupled with 2 mol of prenylagmatine to give caracasandiamide in satisfactory yield. By contrast, the direct photodimerization of caracasanamide was unsuccessful. Caracasandiamide, assayed by the iv route in anesthetized rats at doses ranging from 50 to 3200 μg/kg of body weight, was found to have no appreciable effect on heart rate. At lower doses, the drug stimulates breathing and increases cardiac inotropism, stroke volume, and cardiac output, thus augmenting blood pressure and aortic flow. At higher doses, caracasandiamide depresses breathing likely through central neurogenic mechanisms (not involved in the cardiovascular effects), continues to stimulate cardiac inotropism, and induces, by reducing peripheral vascular resistance, arterial hypotension with reduction of both aortic flow and stroke volume. These cardiovascular effects appear to involve complex interactions at the level of the peripheral β_1-, β_2-, and α_2-adrenoreceptor-dependent as well as M_2- and M_4-cholinergic receptor-dependent transductional pathways both in cardiovascular myocells and at the level of the postganglionic sympathetic endings (with reserpine- and guanethidine-like mechanisms). The cardiovascular effects of caracasandiamide, different from those of caracasanamide, do not depend on significant actions on the central nervous system and on baroreflex pathways. In a similar manner and more effective than caracasanamide, caracasandiamide may be considered a hypotensive and antihypertensive drug. It is devoid of some of the negative side effects, e.g., reflex tachycardia and decreased cardiac inotropism, which are shown by the majority of the most common antihypertensive and vasodilator drugs.

Introduction

Synthetic derivatives of guanidine (e.g., guanethidine,[3] guanabenz,[4] and guanfacine[5]) have been introduced in antihypertensive drug therapy for their ability to block adrenergic nerve activity through central and/or peripheral mechanisms.[6,7] Also pinacidil,[8] an (arylamino)guanidine, is able to lower blood pressure, acting on arterial vasodilation.

The studies on several synthetic guanidines have established that small changes in structure may lead to wide variations of the hypotensive activity.[9−11] Recently, we isolated, by utilizing a biologically controlled purification, a series of active compounds from the crude methanol extract of the Venezuelan plant *Verbesina caracasana* Fries (Compositae), which had been shown to have hypotensive effects on mice. The least polar product was named caracasanamide (G1) and assigned the structure 1-[(3,4-dimethoxycinnamoyl)amino]-4-[(3-methyl-2-butenyl)guanidino]butane (**1)** (Chart 1), as a mixture of (*Z*)- and (*E*)-forms.[2]

The water-soluble (*Z*)-form of **1** was shown to be a hypotensive drug of low-mild potency, devoid of significant tachycardic effects. It also provided, via central and peripheral mechanisms of action, an effect on cardiovascular function and revealed stimulating respiratory effects when administered at nontoxic doses. The pharmacological profile of the (*Z*)-form and the synthesis of the (*E*)-form of caracasanamide (**1**) have been reported in a previous publication.[12]

The ^{1}H and ^{13}C NMR spectra of a second metabolite, named caracasandiamide (G2), showed a close similarity with those of caracasanamide (G1), but the signals of two aliphatic methine protons and carbons replaced those of the olefinic unit in the cinnamoyl moiety. Since the FAB-MS spectrum of caracasandiamide showed a MH^+ peak at *m*/*z* 777 and the molecular weight of

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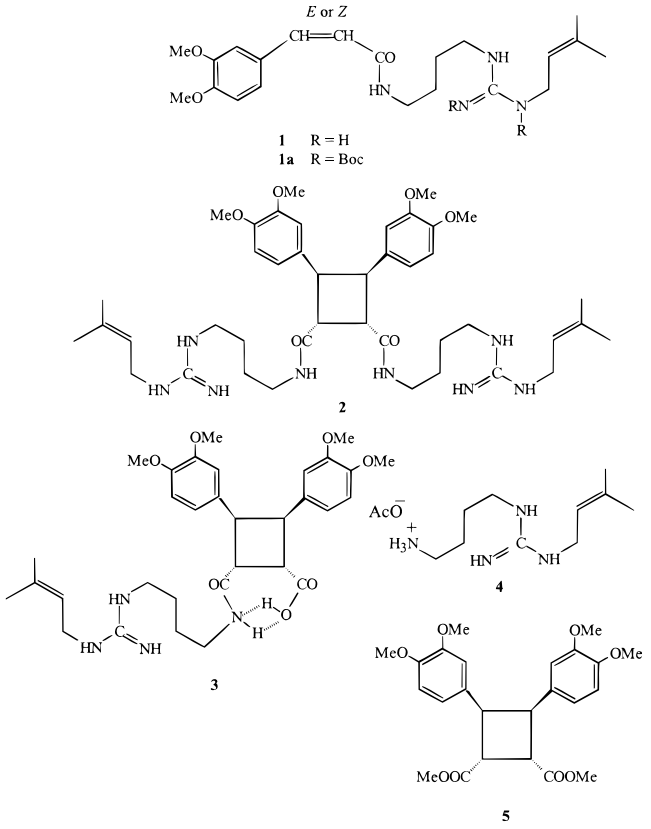
Chart 1



caracasanamide was 388 Da, we concluded that G2 was a dimer of G1, that is, a diamide connected to a cyclobutane skeleton.[13]

A series of hydrolytic experiments allowed the relation of G2 to methyl *β*-3,4-dimethoxytruxinate. The latter was shown to be identical with a synthetic specimen, obtained by photodimerization, in the solid phase of ethyl (*E*)-3,4-dimethoxycinnamate, followed by hydrolysis and methylation.[13] Therefore, caracasandiamide was assigned the structure bis(3′,4′-dimethoxy)-*β*-truxinbis-[[*N*-(3-methylbut-3-enyl)guanidobutyl]amide] (**2**).

This paper deals with the synthesis and the pharmacological profile of caracasandiamide.

Results and Discussion

Structure. The spectral characteristics of caracasandiamide and its hydrolysis products are summarized in Table 1. The alkaline hydrolysis of **2** gave the product **3** and 1-amino-4-(3-methylbut-2-enylguanidino)butane (prenylagmatine, **4**) (Chart 1). The isolation of prenylurea from the reaction mixture confirmed the position of the prenyl group in **4**. The quasi molecular peak (MH^+ at 597 in the FAB-MS spectrum) suggested that compound **3** was an amido acid, as supported by the presence of two distinct carbonyl resonances (at 173.7 and 179.0 ppm) and double signals for the aromatic and cyclobutane carbons in the ^{13}C NMR spectrum. Moreover, the resistance of compound **3** to the hydrolysis of the second prenylagmatine unit, as well as to the methylation of the carboxylic group, was attributed to the presence of a hydrogen bond between the acid and amide groups. As a consequence, the two carbonyl groups must lie on the same side of the cyclobutane ring. A similar *cisoid* disposition, revealed by the high-field signal of the 3′-methoxy groups in the ^1H NMR spectrum, requires the two aromatic rings to be face to face. A strongly acidic hydrolysis (2 N H_2SO_4 in MeOH) of **3** gave again prenylurea, prenylagmatine (**4**), and the methyl ester **5** (Chart 1**)**. The chemical shift (δ 3.68) of the equivalent carbomethoxy groups in the ^1H NMR spectrum indicated that they do not face the aryl rings.[14] Accordingly, DIF NOE experiments revealeded that the H-*β* protons and the aryl rings of **5** are on the same face. Since identical DIF NOE results were obtained for **2** and **3**, the same stereochemistry as for *β*-truxinic acid was established for the three compounds. The mass spectra of **2**, **3**, and **5** are characterized by fragments typical of a truxinic derivative.[15] For instance, the fragmentation of **3** can be rationalized as shown in Scheme 1, the diagnostic peak at *m*/*z* 300 also being present in the mass spectra of **2** and **5**.

In conclusion, caracasandiamide and its hydrolysis products were assigned the structures summarized in Chart 1.

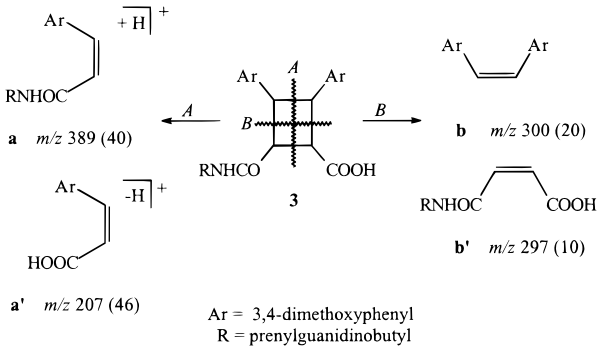
Synthesis. According to the hypotheses put forward on the biogenesis of caracasandiamide,[13] we initially

Table 1. ^1H and ^{13}C NMR Data of Caracasandiamide and Its Hydrolysis Products[a]

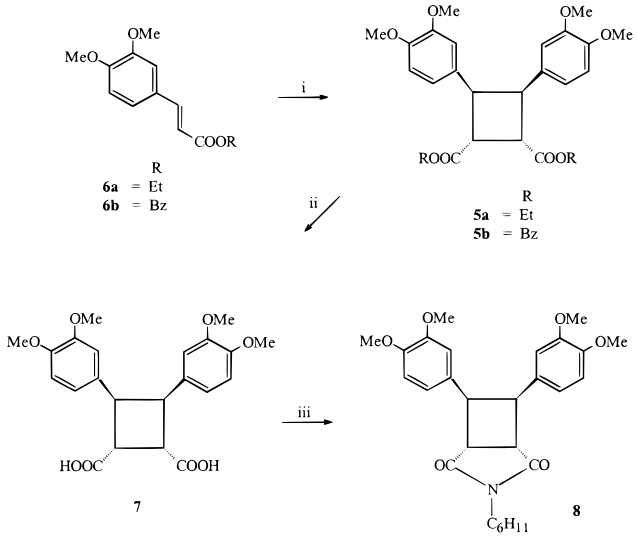
	2	**3**	**4**	**5**
H_2-1	3.35 m	3.22	3.52	
H_2-2	1.70 m	1.68	1.96	
H_2-3	1.61 m	1.52 m	1.96	
H_2-4	3.22 m	3.18 m	3.19	
H-α	4.32 d (6)	4.30		4.24
H-*β*	3.95 d	3.86		3.91
H-2′	6.60 d (2)	6.58, 6.56		6.59
H-5′	6.71 d (8)	6.70, 6.68		6.72
H-6′	6.65 dd	6.66, 6.64		6.68
3′-OMe	3.62 s	3.59, 3.60		3.61
4′-OMe	3.68 s	3.67, 3.66		3.68
H_2-1″	3.94 m	3.63	4.10	
H-2″	5.32 br t (6.5)	5.31	5.52	
H_3-4″	1.73 br s	1.72	1.61	
H_3-5″	1.71 br s	1.69	1.58	
NH	8.10, 7.85, 7.55 br s			
C-1	39.44	38.94	40.81	
C-2	27.35	27.76	27.68	
C-3	27.03	26.18	25.88	
C-4	42.11	42.22	41.22	
C-α	44.18	44.34		44.18
C-*β*	45.54	45.96		45.54
C-1′	133.82	136.23, 135.81		132.57
C-2′	113.36	114.71, 114.58		113.19
C-3′	148.32	148.70, 148.55		148.81
C-4′	149.53	150.12, 150.03		151.44
C-5′	112.03	113.18, 113.13		112.08
C-6′	120.92	121.42, 121.37		120.82
OMe	56.04	56.35 (×2)		55.91
OMe	55.95	56.51, 56.49		55.89
C=O	173.06	179.05, 173.81		173.65
C=NH	157.06	158.23	158.47	
C-1″	40.43	40.31	42.64	
C-2″	120.07	121.00	121.38	
C-3″	137.32	136.43	137.01	
C-4″	25.83	25.69	26.55	
C-5″	18.33	18.17	18.98	

[a] 300 and 75 MHz, TMS as internal standard. Solvents: acetone-d_6-DMSO-d_6, 4:1, **2**, **3** (60 °C); C_5D_5N, **4**; acetone, **5**. The signals in the ^1H NMR spectra showed the appropriate integrate intensities. Multiplicities are indicated in the first row. Coupling constants are given in parentheses (in Hz). Proton and protonated carbon signals were correlated by a HETCOR experiment. The signals for aromatic and truxinic rings in the two moieties of **3** are not equivalent. For compound **5** only the major signal of the *syn*−*anti* pair is reported.

Scheme 1

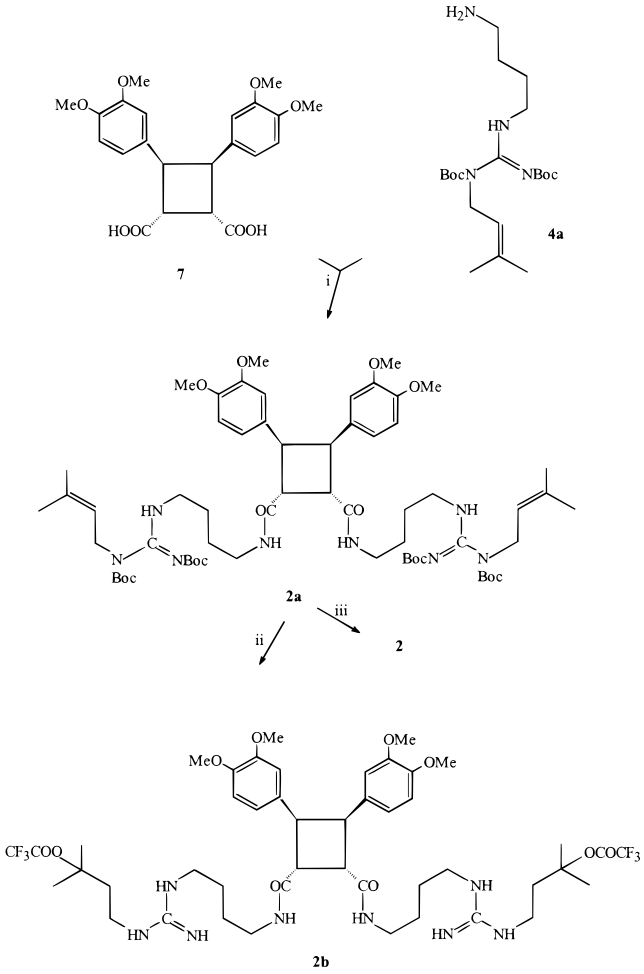


Scheme 2[a]



[a] Reagents: (i) *hν*, rt; (ii) KOH, EtOH (**5b**); H_2, Pd/C (**5c**); (iii) pentylamine, CDI.

Scheme 3[a]



[a] Reagents: (i) CMC metho-*p*-toluenesulfonate, CH_2Cl_2; (ii) CF_3COOH; (iii) CH_3SO_3H, CH_2Cl_2.

focused on the possibility to synthesize **2** by direct photoaddition of caracasanamide (Scheme 2). The photochemical reaction was carried out on the N-Boc-protected caracasanamide **1a**,[12] a more soluble and stable substrate than **1**. Irradiation of **1a** in the solid state[16] with a 125-W mercury lamp for 20 h gave a mixture of photoadducts, which were separated by preparative TLC. However, none of the isolated products corresponded to the N-Boc derivative of **2**.

Therefore, we followed a different approach, based on the formation of amide bonds between prenylagmatine and the suitably substituted *β*-truxinic acid, which in turn can be obtained by photodimerization of the corresponding cinnammate.[17] Ethyl 3,4-dimethoxycinnamate (**6a)** and benzyl 3,4-dimethoxycinnamate (**6b**) were thus subjected to solid-state photodimerization (Scheme 2) affording the *β*-truxinic esters **5a** (40%) and **5b** (38%), respectively, along with small amounts of other stereoisomers and starting material (ca. 40%). The ethyl ester **5a** by hydrolysis with ethanolic KOH gave the *β*-truxinic acid **7**, which by methylation with diazomethane provided the dimethyl ester **5**, identical with the hydrolysis product (H_2SO_4, MeOH) of the natural compound **2**. Moreover, treatment of **7** with catalytic H_2SO_4/EtOH afforded again the diester **5a**, proving that no epimerization had occurred during the alkaline hydrolysis. The acid **7** was also obtained by the hydrogenolysis of the benzyl ester **5b**. Finally, the acid **7** was converted into the bicyclic imide **8**, by treatment with *n*-pentylamine in the presence of *N*,*N*′-carbodiimidazole (CDI) in excess, thus chemically confirming a *cis* relationship between the *β*-substituents.

Following the procedures successfully employed for the synthesis of caracasanamide,[12] the diacid was activated as the corresponding acyl dichloride or mixed anhydride with diethyl chlorophosphate and reacted with the suitably protected prenylagmatine **4a** (Scheme 3).[12] However, the yield of the acylation reaction never exceeded 13%. This failure may be attributed to the considerable steric hindrance around the carboxy groups. In addition, the preferential formation of the cyclic anhydride of **2**, resulting in compouds such as **3**, may be a factor. The reaction between **7** and **4a** in the presence of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide (CMC) metho-*p*-toluenesulfonate gave the compound **2a**, in fair yield, when the steric congestion of the molecule is considered.

Although the removal of N-Boc protecting groups can be accomplished by trifluoroacetic acid, ensuing from the electrophilic addition of the acid to the prenyl double bond, deprotection of **2a** was better performed with a catalytic amount of methanesulfonic acid at reflux in 1,4-dioxane. Synthetic **2** (61% yield) was identical by comparison of spectral and chromatographic data with an authentic sample of caracasandiamide.

Table 2. Changes[a] in Systolic and Diastolic Blood Pressure, Heart Rate, Maximum Rate of Rise of the Left Ventricular Isovolumetric Pressure (d*P*/d*t*), Mean Aortic Flow, and Stroke Volume following iv Administration of Caracasandiamide (**3**) in Anesthetized Rats

	blood pressure											
	systolic		diastolic		heart rate		d*P*/d*t*		mean aortic flow		stroke volume	
3 (μg/kg)	mmHg	%	mmHg	%	beats/min	%	mmHg/s	%	mL/min	%	μL	%
50	+17 ± 2	+16 ± 2	+10 ± 2	+13 ± 3	-9 ± 3	−3 ± 2	+1070 ± 90	+17 ± 2	+19 ± 3	+26 ± 3	+73 ± 5	+36 ± 4
100	+12 ± 2	+12 ± 2	+10 ± 1	+13 ± 2	−10 ± 2	−3 ± 1	+1129 ± 82	+18 ± 2	+11 ± 2	+15 ± 2	+20 ± 3	+10 ± 2
200	−14 ± 1	−13 ± 2	−13 ± 2	−17 ± 3	−13 ± 1.5	−4 ± 1	+1369 ± 170	+22 ± 3	−14 ± 4	−19 ± 2	+23 ± 2	+11 ± 1
400	−20 ± 2	−19 ± 2	−17 ± 1	−22 ± 2	−16 ± 2	−4 ± 1	+2615 ± 181	+43 ± 5	−14 ± 3	−19 ± 3	−46 ± 4	−23 ± 3
800	−30 ± 4	−29 ± 4	−31 ± 3	−40 ± 4	−16 ± 3	−4 ± 2	+3282 ± 269	+54 ± 4	−25 ± 3	−34 ± 4	−86 ± 6	−43 ± 4
1600	−41 ± 5	−39 ± 4	−43 ± 7	−55 ± 6	−30 ± 2	−8 ± 1	+3676 ± 264	+60 ± 3	−36 ± 2	−49 ± 3	−97 ± 6	−48 ± 3
3200	−48 ± 6.5	−46 ± 5	−53 ± 4	−68 ± 4	−41 ± 5	−11 ± 3	+4708 ± 430	+77 ± 6	−41 ± 3	−56 ± 4	−117 ± 7	−58 ± 5
baseline	104 ± 4		78 ± 5		365 ± 16		+6131 ± 348		73 ± 4		201 ± 15	

[a] Values are means± SE (n = 12 for each dose with the exception of 3200 μg/kg, for which n = 7).

Table 3. Changes[a] in Respiratory Frequency and Tidal Volume following iv Administration of Caracasandiamide (**3**) in Anesthetized Rats

	respiratory frequency		tidal volume	
3 (μg/kg)	beaths/min	%	μL	%
50	+5 ± 1	+5 ± 1	+743 ± 55	+15 ± 2
100	+6 ± 2	+6 ± 2	+1106 ± 61	+22 ± 2
200	+9 ± 3	+9 ± 2	+1442 ± 108	+29 ± 4
400	−3 ± 1	−3 ± 1	−530 ± 48	−11 ± 2
800	−15 ± 2	−16 ± 2	−3191 ± 275	−64 ± 7
1600	−61 ± 5	−64 ± 4	−3654 ± 290	−74 ± 5
3200	−67 ± 3	−70 ± 3	−4229 ± 334	−85 ± 5
baseline	96 ± 7		4970 ± 371	

[a] Values are means ± SE (n = 12 for each dose with the exception of 3200 μg/kg, for which n = 7).

Pharmacology. The cardiovascular and respiratory effects following intravenous (iv) injection of caracasandiamide (G2) in anesthetized rats are reported in Tables 2 and 3, respectively. The drug slightly reduced heart rate (HR) while increasing maximum rate of rise of the left ventricular isovolumetric pressure (d*P*/d*t*), an index of cardiac inotropism, in a dose-related manner. The negative chronotropic effect determined, at the highest tested dose (3200 μg/kg), only an 11% reduction of basal HR (i.e., preceding the administration of G2), whereas cardiac inotropism was markedly augmented by the drug, reaching a 77% maximum increase. On the other hand, G2 showed biphasic effects on systolic and diastolic blood pressure (BP), aortic flow (AF), stroke volume (SV), respiratory frequency (RF), and tidal volume (TV). In this regard, lower doses of the drug slightly increased systolic and diastolic BP and AF (50 and 100 μg/kg) as well as SV, RF, and TV (50−200 μg/kg), whereas higher doses reduced all these indices in a dose-related manner.

On a molar basis and by the iv route, G2 induced higher hypotensive effects than (*Z*)-caracasanamide, guanethidine, hexamethonium, reserpine, and papaverine, being, however, less potent than clonidine and histamine (Table 4). Furthermore, G2 and (*Z*)-caracasanamide increased cardiac inotropism in contrast to the other tested drugs, and only (*Z*)-caracasanamide was able to increase HR (Table 4). The G2-induced hypotension lasted much more than that following administration of guanethidine, histamine, papaverine, hexamethonium, and (*Z*)-caracasanamide. Notably, the dimer G2 was more potent, as a hypotensive and positive inotropic agent, than the monomer (*Z*)-caracasanamide. However, the latter (50−6400 μg/kg by the iv route in the rat) did not show biphasic effects on BP, while having slight and no dose-related tachycardic action as well as stimulatory effects on RF and TV until the dose of 1600 μg/kg (higher doses caused great respiratory depression and 6400 μg/kg was also able to induce irreversible respiratory blockade, bradycardia, and ventricular arrhythmias with final cardiac arrest); (*Z*)-caracasanamide was already found to be more potent than guanethidine in lowering BP; the same drug was as potent as reserpine and papaverine and less potent than clonidine, hexamethonium, and histamine.[12] Like (*Z*)-caracasanamide, G2 did not decrease (at nontoxic doses) cardiac inotropism in contrast with all the tested hypotensive drugs. Furthermore, the hypotensive effect of G2 was longer than that of (*Z*)-caracasanamide, which was already found to last more than those of drugs (like guanethidine, papaverine, and histamine) acting by peripheral vasodilatatory mechanisms.[12] The analysis of the effects of G2 indicated that, if the lower doses of the drug did not significantly change HR while increasing BP, d*P*/d*t*, AF, and SV, doses higher than 200 μg/kg continued to increase only d*P*/d*t* while reducing BP, AF, and SV. Therefore, this reduction of BP, AF, and SV was due to a G2-induced decrease of total peripheral resistance (overcoming the increase of cardiac inotropism) and, only in the case of the doses of 1600 and 3200 μg/kg, also to the slight negative chronotropic effect of the drug. On the other hand, the increase of BP, AF, and SV induced by 50 and 100 μg/kg G2 was explained by an increase of cardiac output due to higher cardiac inotropism. Since BP and AF were reduced by G2 (200 μg/kg) when RF and TV continued to be increased, it was quite unlikely that the depression of respiratory function, induced by the highest doses of the drug, was involved in the arterial vasodilation responsible of both blood hypotension and reduction of AF and SV. In this respect, 5 of the 12 rats treated with the dose of 3200 μg/kg died because of respiratory blockade, which developed within a period of 15−19 s starting from administration of the drug. Also at the dose of 1600 μg/kg, G2 caused, in some cases, short periods of respiratory blockade, ranging from 23 to 35 s, that disappeared spontaneously and were not considered in evaluating the drug-induced depression of respiratory function. Even when G2 (400−3200 μg/kg) depressed RF and TV, there was an initial increase of both RF and TV; this increase started at 4−6 s, reached the maximum within 8−12 s, and disappeared after 17−23 s following iv administration of the drug. G2 (800−1600 μg/kg) reduced RF and TV beginning from 36 ± 4 to 48 ± 5 s and reached the maximum between 68 ± 4 and 93 ± 5 s; the basal respiratory values were restored within a

Table 4. Cardiovascular Responses[a] to Caracasandiamide (**3**) and Several Antihypertensive or Vasodilating Drugs Administered by iv Route in Anesthetized Rats

drug	blood pressure		heart rate (beats/min)	d*P*/d*t* (mmHg/s)
	systolic	diastolic		
caracasandiamide (4.12 μM/kg)	−43 ± 2	−47 ± 3	−35 ± 3	+3991 ± 242
(*Z*)-caracasanamide (4.12 μM/kg)	−24 ± 3	−20 ± 2	+25 ± 2	+3107 ± 190
guanethidine (25 μM/kg)	−29 ± 4	−22 ± 1	−24 ± 3	−1090 ± 84
clonidine (0.108 μM/kg)	−18 ± 2	−16 ± 1	−53 ± 7	−1345 ± 126
hexamethonium (12 μM/kg)	−48 ± 4	−40 ± 4	−46 ± 5	−4421 ± 284
reserpine (8 μM/kg)	−35 ± 5	−29 ± 2	−51 ± 6	−1438 ± 146
papaverine (5 μM/kg)	−20 ± 3	−15 ± 2	−18 ± 3	−1204 ± 49
histamine (0.044 μM/kg)	−28 ± 2	−24 ± 3	−12 ± 2	−1002 ± 68
baseline	113 ± 7	92 ± 6	348 ± 14	6420 ± 374

[a] Values are means ± SE (n = 4 in each group).

period of 348 ± 41 and 408 ± 23 s (n = 7). The hypotensive effect of G2 (800−1600 μg/kg) began concomitantly to those on d*P*/d*t*, AF, and SV (4−5 s), starting from its iv administration, and increased progressively, reaching a maximum at the maximal increase of d*P*/d*t*. Both drug-induced hypotension and positive inotropic effect lasted, in the dose interval 800−1600 μg/kg, from 378 ± 26 to 439 ± 31 s (n = 12) and returned to the values preceding drug administration within the same times of restoration of AF and SV. The slight bradycardic response to G2 (800−1600 μg/kg) developed during the course of the other cardiovascular effects; however, it began later (24 ± 4 to 33 ± 5 s; n = 12) and disappeared concomitantly to the restoration of the respiratory parameters. Interestingly, the G2 (800−3200 μg/kg)-induced decrease of BP, AF, and SV and increase of d*P*/d*t* soon reached simultaneously their maximum value, which was followed by a transient rebound of BP, AF, and SV almost to the basal values and by a contemporary marked decrease of d*P*/d*t*; during this rebound phase, HR continued to be slightly reduced, while RF and TV continued to climb up toward their basal values; the same rebound phase lasted from 22 ± 5 to 41 ± 6 s (n = 7), and afterward, BP, AF, and SV as well as d*P*/d*t* reached again their maximal negative and positive increments, respectively. At the end of the rebound phase, G2 (1600−3200 μg/kg) caused the appearance of sporadic extrasystolia, which rapidly evolved to extrasystolic firing lasting from 8 to 36 s and, in some rats surviving to the respiratory blockade induced by the dose of 3200 μg/kg, also to periods of ventricular fibrillation. At last, in some cases, the G2 (800−3200 μg/kg)-induced hypotensive response was followed by a period (128 − 206 s) of blood hypertension, which was accompanied by increased TV and HR and by reduced d*P*/d*t*, AF, and SV. It was concluded that G2 is a drug that increases markedly cardiac inotropism when administered by iv route in the rat at doses ranging from 50 to 3200 μg/kg. The positive inotropic effect coexists, at lower doses, with a moderate increase of BP, AF, SV, and minute volume and, at higher doses (400−3200 μg/kg), with increasing reduction of BP, AF, and SV and with respiratory depression. Only at doses of 1600 μg/kg or higher, administered by iv injection, G2 may induce reversible or irreversible respiratory blockade, respectively, with occurrence of ventricular arrhythmias including fibrillation.

As far as the mechanisms of action of G2 were concerned, it was noted that the effects of this drug on BP, d*P*/d*t*, AF, and SV preceded and lasted more than the actions on HR, RF, and TV. Moreover, the few seconds required for inducing cardiovascular effects indicated that the drug acts at the cardiac and vascular levels in determining increase of the inotropism and increase (at lower doses) and reduction (at higher doses) of the other cardiovascular indices. According to these observations, there were no central neurogenic components in the cardiovascular effects of G2: either bilateral vagotomy or spinalization (under vagotomy) did not change cardiovascular responses to the drug (200−1600 μg/kg, ratio = 2.0, iv route) which, by itself, failed to change the positive chronotropic and inotropic as well as pressor responses to bilateral carotid occlusion (BCO). On the other hand, G2 (400−1600 μg/kg, ratio = 2.0, iv route) antagonized cardiovascular responses to iv noradrenaline and adrenaline (each at the dose of 1 μg/kg), while increasing those of iv acetylcholine (2.50 μg/kg) and, mostly, iv isoprenaline (0.625 μg/kg); these effects were related to the doses of G2 and remained for at least over 60 min following its administration (Table 5). Moreover, phenylephrine (10 μg/kg/min, a selective α_1-adrenoreceptor agonist) did not influence cardiovascular effects of G2 (400−1600 μg/kg, ratio = 2.0, iv route), while papaverine, lowering BP by relaxing arterial myocells (through a phosphodiesterase-dependent reduced availability of calcium ions for contractile mechanisms), reduced significantly only the diastolic hypotensive effect of G2 (Table 6). Similarly, reserpine, depleting the catecholamine content in both central and peripheral adrenergic synapses, strongly reduced cardiovascular effects of G2, while, on the contrary, the ganglion blockade by the anticholinergic drug hexamethonium potentiated the same effects of G2 (Table 6). Conversely, these effects of G2 were not changed by pretreatment with the $\alpha_{1,2}$-adrenoreceptor-blocking drug phentolamine (2 mg/kg, iv route). All the above tested drugs were ineffective in changing significantly the respiratory effects of G2. On the whole, these results showed that G2 does not affect the central sympathetic and parasympathetic tone (spinalization, vagotomy) as well as the baroreflex activity (BCO) and does not interact with the cardiovascular α_1- and α_2-adrenoreceptors (phenylephrine, phentolamine). On the other hand, considering that reserpine (depleting the content of catecholamines at both postganglionic adrenergic endings and central adrenergic presynaptic terminals by blocking their accumulation into storage vesicles) strongly opposed the G2-induced reduction of BP, AF, and SV, it was deduced that this guanidine compound acts presynaptically at the peripheral (cardiovascular)

Table 5. Cardiovascular Responses[a] to iv Doses of Physiological Agonists Before (Control) and After Administration of Caracasandiamide (**3**) in Anesthetized Rats[b]

agonist	**3** (μg/kg)	blood pressure (mmHg) systolic	blood pressure (mmHg) diastolic	heart rate (beats/min)	d*P*/d*t* (mmHg/s)
noradrenaline (1 μg/kg)	−	+43 ± 3	+25 ± 2	+28 ± 3	+5564 ± 420
	400	+31 ± 2*	+15 ± 1*	+23 ± 2	+5136 ± 364
	1600	+24 ± 2*	+10 ± 1*	+13 ± 2*	+2225 ± 174*
adrenaline (1 μg/kg)	−	+25 ± 3	+16 ± 3	+41 ± 3	+8416 ± 147
	400	+21 ± 2	+5 ± 1*	+24 ± 3*	+6848 ± 240*
	1600	+14 ± 1*	+3 ± 1*	+7 ± 2*	+2739 ± 168*
isoprenaline (0.625 μg/kg)	−	−20 ± 2	−31 ± 3	+46 ± 4	+9844 ± 704
	400	−36 ± 3*	−49 ± 3*	+69 ± 5*	+13696 ± 975*
	1600	−43 ± 4*	−66 ± 5*	+79 ± 5*	+14552 ± 1021*
acetylcholine (2.50 μg/kg)	−	−25 ± 1	−31 ± 2	−27 ± 4	−4451 ± 172
	400	−27 ± 2	−45 ± 3*	−30 ± 5	−5820 ± 304*
	1600	−38 ± 4*	−54 ± 3*	−34 ± 5	−6821 ± 491*

[a] Values are means±SE ($n = 8$ in each group). *$p < 0.05$ (compared with the control mean). [b] Cardiovascular responses to the agonists were repeated beginning 30 min after administration of each dose of caracasandiamide.

Table 6. Cardiovascular Responses[a] to iv Doses of Caracasandiamide (**3**) Before (Control), During, and After Administration of Papaverine, Reserpine, and Hexamethonium in Anesthetized Rats

treatment	**3** (μg/kg)	blood pressure (mmHg) systolic	blood pressure (mmHg) diastolic	heart rate (beats/min)	d*P*/d*t* (mmHg/s)
control	200	−11 ± 2	−13 ± 1	−15 ± 3	+1129 ± 152
	400	−16 ± 2	−16.5 ± 3	−17 ± 3	+2436 ± 205
	800	−26 ± 3	−27.5 ± 2	−22 ± 2	+3154 ± 271
	1600	−36 ± 3.5	−40 ± 3	−36 ± 5	+3597 ± 299
papaverine (1 mg/kg/min, iv route)	800	−22 ± 4	−16 ± 3*	−17 ± 3	+2967 ± 147
	1600	−33 ± 4	−29 ± 2*	−31 ± 4	+3321 ± 168
reserpine (5 mg/kg/day, for 2 days, ip route)	800	−11 ± 2*	−12 ± 3*	−9 ± 1*	+720 ± 57*
	1600	−16 ± 5*	−15 ± 3*	−14 ± 4*	+1238 ± 81*
hexamethonium (5 mg/kg, iv route)	−	−25 ± 3*	−27 ± 2*	−34 ± 4*	+3424 ± 212*
	400	−41 ± 5*	−45 ± 6*	−46 ± 4*	+3938 ± 181*
	800	−50 ± 5*	−53 ± 3*	−57 ± 5*	+4965 ± 322*

[a] Values are means ± SE ($n = 6$ in each group). *$p < 0.05$ (compared with the control mean).

sympathetic synapses through a reserpine-like mechanism.[18] In this regard, a central neurogenic "reserpine-like" mechanism of G2 was excluded since the interruption of the central sympathetic pathways by spinalization failed to change the effects of this drug. An explanation of such conclusion may be that G2, differently from (*Z*)-caracasanamide and similarly to guanethidine and guanadrel, does not cross easily the blood−brain barrier.[19] Moreover, it is interesting to note that the guanidine derivative guanethidine (not crossing this barrier) is concentrated within the neurosecretory vesicles of the postganglionic sympathetic endings, where it replaces noradrenaline; then, this drug depletes the normal transmitter with a transient increase of its release which is responsible, for example, for transient blood hypertension; at large acute doses, guanethidine also appears to block excitation-secretion coupling ("bretylium-like" mechanism).[20] These mechanisms of action of guanethidine seem to well explain the biphasic effects of G2 on cardiovascular function: i.e., lower doses may cause a transient increase of the release of noradrenaline, whereas higher doses may first oppose this release by a "bretylium-like" effect and then deplete the normal transmitter ("reserpine-like" effect). As compared to (*Z*)-caracasanamide, only partially lipophilic in the *cis* form,[13,21] G2 has a double molecular weight, a higher steric hindrance, and a lower molecular flexibility.

On the basis of the above considerations, the antagonistic effect of G2 on the cardiovascular responses to noradrenaline and adrenaline (the latter having been used at a dose activating prevalently the α_1- and α_2-adrenoreceptors) may be explained only through interactions of G2 with the intracellular biochemical pathways transducing, in the cardiac and vascular myocells, the α-adrenoreceptor activation toward the related functional effects. The same interpretation may be given for explaining the potentiating effects of G2 on the cardiovascular responses to acetylcholine (activating the M-cholinergic receptors) and isoprenaline (activating the cardiac β_1- and vascular β_2-adrenoreceptors). In this respect, (*Z*)-caracasanamide was found to increase HR and d*P*/d*t* also by interacting at the cardiac β_1-adrenoreceptors.[12] Moreover, the ability of papaverine to reduce the diastolic hypotensive response to G2 seems to confirm a "transductional" level of action of this drug in the vascular myocells, i.e., on the adenosine cyclic 3′,5′-monophosphate (cAMP)-dependent availability of calcium ions for contractile mechanisms (reduced by papaverine).[22] With regard to this, activation of the β_2-adrenoreceptors leads to increased levels of cAMP by stimulation of adenylyl cyclase, thus causing reduced availability of the free calcium in vascular myocells. Conversely, in the heart, stimulation of the β_1-adrenoreceptors increases cAMP, which enhances phosphorylation of contractile proteins and activates voltage-sensitive calcium channels in the plasma membrane, thus leading to positive inotropic and chronotropic responses.[23] On the other hand, stimulation of the cardiac and vascular M(M_2,M_4)-cholinergic receptors results in activation of receptor-operated potassium channels, increased levels of guanosine cyclic 3′,5′-monophosphate (cGMP, reducing the availability of free calcium) with consequent lower or inhibited activity of adenylyl cyclase, and, possibly, suppression of the

activity of receptor-operated calcium channels, thus causing negative inotropic and chronotropic responses, and vasodilation.[24,25] Finally, activation of the $\alpha(\alpha_1,\alpha_2)$-adrenoreceptors in the cardiac and vascular myocells ultimately causes contraction as a result of increased concentrations of intracellular calcium (mainly depending on inhibition of adenylyl cyclase, stimulation of phospholipases, increased polyphosphoinositide hydrolysis, and activation of protein kinase C).[23,26] Since phenylephrine (α_1-adrenoreceptor agonist) did not alter the effects of G2, it may be thought that this drug interacts, in opposing the responses to noradrenaline and adrenaline, only with transductional pathways coupled to activation of the cardiac and vascular α_2-adrenoreceptors.

Having excluded central neurogenic components (sympathetic, parasympathetic, baroreflex) in the cardiac effects of G2, these effects then appear to be related, more than to direct actions of this drug on receptors, to modulations on peripheral transductional pathways physiologically activated by β_1- and β_2-adrenoreceptors as well as α_2-adrenoreceptor and $M_{(2,4)}$-cholinoreceptor agonists (adrenaline, noradrenaline, and acetylcholine, respectively).[23−27] The peripheral reserpine-like component of G2 may only be involved in the duration of the effects of G2 concerning the reduction of BP, HR, AF, and SV, but not in the induction of the same effects because reserpine requires some time for depleting the catecholamine content in the postganglionic sympathetic endings.[18] In this way, "lower" and "higher" doses of G2 are likely to modulate the above transductional pathways in a quantitatively different manner. So, a "β_1-adrenoreceptor-like component" may explain, through an increased cardiac inotropism, the lower dose-induced increase of BP, d*P*/d*t*, AF, and SV; this component may also explain the increase of d*P*/d*t* induced by higher doses of G2, as was found earlier for (*Z*)-caracasanamide.[12] Analogously, "β_2-adrenoreceptor- and M-cholinoreceptor-like components" as well as an "α_2-antagonistic-like" component are likely to cooperate in contributing (by systemic arterial vasodilation) to decrease total peripheral resistance (to levels overcoming the increase of cardiac inotropism) and, only the cholinergic component, to reduce HR. Moreover, the increased effects of G2 during the hexamethonium-induced ganglionic blockade (reducing or impeding the release of catecholamines from adrenals into blood and from sympathetic endings in both heart and vessels as well as from parasympathetic endings in the heart) may be referred to the above α_2-, β_1-, and β_2-adrenoreceptor antagonistic (α_2) and -like (β_1,β_2) components (involved in either vasodilation or the positive inotropic effect) as well as to the above M-cholinoreceptor-like component (involved in the negative chronotropic response) through a mechanism of receptor hypersensibility (up-regulation) due to the reduced levels of both adrenergic and cholinergic neurotransmitters at the autonomous neuroeffector synapses.[23,28−30]

Between (*Z*)-caracasanamide and G2 there are pharmacodynamic analogies and differences, besides differences in potency and duration of action (as hypotensive and positive inotropic drugs) and in the quality of some effects (on HR and respiratory function). Both drugs do not interact with the peripheral α_1- and α_2-adrenoreceptors, while potentiating the β_1-adrenoreceptor-dependent cardiac reactivity.[12] (*Z*)-Caracasanamide reduces central sympathetic tone and baroreflex reactivity and relaxes vascular myocells by a direct effect not involving a β_2-adrenoreceptor component.[12] G2 is not provided with central neurogenic effects, while also having the above vascular β_2-adrenoreceptor-like and cardiac M-cholinoreceptor-like components.

As observed with (*Z*)-caracasanamide, the biphasism of the respiratory effects of G2 is not related to the cardiovascular ones. In fact, G2 increases d*P*/d*t* and decreases HR in the presence of either stimulation or depression of RF and TV. Moreover, the cardiovascular effects of G2 precede and last more than those on RF and TV. Only at subtoxic or toxic doses (like 1600 and 3200 μg/kg, respectively), greatly depressing breathing, G2 reverses for a short period the reduction of BP, AF, and SV. The relatively long latency time for observing the effects of G2 on breathing is likely to depend on the difficulty to cross the blood−brain barrier and to reach significant concentrations in the central nervous system. As for (*Z*)-caracasanamide, the obtained data do not allow us to hypothesize on the mechanisms by which G2 stimulates, and then depresses, the central respiratory pathways, in the latter case with consequent cardiac arrhythmias and possible cardiac arrest.

G2 is the second compound of a series of six guanidine derivatives, isolated from *Verbesina caracasana*, for which a pharmacological profile has been defined. A structure−activity relationship among all these compounds will be established when the pharmacological studies of the remaining four drugs have been completed. The present study shows that, at low dosage (50−100 μg/kg, by iv route), G2 is a positive inotropic drug increasing cardiac output without significant chronotropic effects. At dosages ranging from 200 to 800 μg/kg, G2 becomes a hypotensive drug of mild potency not affecting significantly the chronotropism and mantaining a high positive inotropic action. At doses of 1600 μg/kg or higher, G2 is a hypotensive compound of high potency that, despite the high positive inotropic effect and the poor chronotropic action, may associate effects of excessive respiratory depression leading to severe cardiac arrhythmias. As a cardiovascular agent, G2 appears to act preponderantly by peripheral mechanisms of action.

Experimental Section

General. Melting points were determined with a Gellenkamp apparatus and are uncorrected. The NMR spectra were determined with a Varian Gemini 300 spectrometer (300 MHz) using tetramethylsilane as internal standard. IR spectra were recorded on a Perkin Elmer 298 instrument. Electron impact (EIMS) spectra were recorded with a Kratos MS 80 instrument. High-resolution FABMS were determined on a VG7070EQ spectrometer. Microanalyses were performed by Dipartimento Farmaco Chimico Tecnologico, Siena, Italy.

Plant Material. Leaves of *V. caracasana* were collected in Valencia (Venezuela). A voucher specimen was deposited at the herbarium of the Departamento de Physiologia Vegetal, Universidad Central de Venezuela, Maracaibo (Venezuela).

Extraction and Fractionation. The crude extract (26 g) from leaves (5 kg), obtained as described in our previous paper,[12] was fractionated on silica gel with chloroform−methanol mixtures. The second fraction, eluted with $CHCl_3$-MeOH, 8:2, gave caracasandiamide (**2**; 1.2 g) after extensive chromatographic separation.

3β,4β-Bis(3′,4′-dimethoxyphenyl)-1α,2α-bis[[*N*-(3-methylbut-2-enyl)guanidino]butyl]cyclobutanecarboxamide (caracasandiamide, 2): foam; IR ν_{max} 3320, 3220, 1655, 1638, 1515, 1260, 1205, 1138, 1020 cm^{-1}; ^{1}H and ^{13}C NMR in Table 1; FABMS *m/z* (rel int) 777 $[MH]^+$ (100), 747 $[M - OCH_2]^+$ (6), 709 $[MH - C_5H_8]^+$ (11), 650 [M − prenylguanidine]$^+$ (3), 596 [M − prenylguanidinobutane]$^+$ (3), 579 [MH − prenylagmatine]$^+$ (25), 389 $[a]^+$ (15), 300 $[b]^+$ (8), 191 $[ArCH{=}CHCO]^+$ (57).

Hydrolysis of Caracasandiamide. A solution of caracasandiamide (300 mg) and 2 N NaOH in MeOH (15 mL) was left under stirring for 4 days. The reaction mixture was poured into water, and MeOH was evaporated.The aqueous solution was acidified with concd H_2SO_4 and extracted with EtOAc (×3). The residue of the pooled organic extracts, after purification on silica gel with $CHCl_3$−MeOH, 99:1, gave the monoamide **3** (158 mg). The H_2O lyophilysate by chromatography on LH-20 with MeOH gave 1-amino-4-[(3-methyl-2-butenyl)-guanidino]butane acetate (**4**) (53 mg) and prenylurea (12 mg).

3β,4β-Bis(3′,4′-dimethoxyphenyl)-1α-carboxy-2α-[[*N*-(3-methylbut-2-enyl)guanidino]butyl]cyclobutanecarboxamide (3): mp 179−180 °C; IR ν_{max} 3320, 3220, 1655, 1638, 1515, 1260, 1205, 1138, 1020 cm^{-1}; ^{1}H and ^{13}C NMR in Table 1; FABMS *m/z* 597 MH^+ (71), 389 $[a]^+$ (40), 300 $[b]^+$ (20), 297 $[b']^+$ (10), 207 $[a']^+$ (46), 191 $[ArCH{=}CHCO]^+$ (100); HRMS found 279.1594, $C_{16}H_{23}O_4$ requires 279.1596.

1-Amino-4-[(3-methylbut-2-enyl)guanidino]butane acetate (prenylagmatine, 4): oil; IR (KBr) ν_{max} 3450, 3360, 1665, 1310, 1150, 838 cm^{-1}; ^{1}H and ^{13}C NMR in Table 1; FABMS *m/z* 199 MH^+.

Acidic Hydrolysis. The monoamide **3** (100 mg) was refluxed in 2 N H_2SO_4 (MeOH, 1 mL) for 3 h. After evaporation of MeOH, the reaction mixture was extracted with EtOAc. The residue of the organic layer chromatographed on silica gel with $CHCl_3$ gave methyl ester **5a** (42 mg). The aqueous layer was lyophilized and chromatographed on Sephadex LH-20 to give prenylagmatine (**4**) (21 mg) and prenylurea (6 mg).

Methyl 3β,4β-bis(3′,4′-dimethoxyphenyl)-1α,2α-cyclobutanedicarboxylate (5): oil; ^{1}H and ^{13}C NMR in Table 1; EIMS *m/z* (rel int) 444 $[M]^+$ (9), 413 $[M - OMe]^+$ (49), 300 $[b']^+$ (100), 285 $[b' - Me]^+$ (32), 222 $[a]^+$ (100), 207 $[a - Me]^+$ (100), 191 $[a - OMe]^+$ (100); m* 351.5 (413 → 381), 270.8 (300 → 285), 193.0 (222 → 207), 164.3 (222 → 191).

Synthesis. Ethyl 3β,4β-Bis(3′,4′-dimethoxyphenyl)-1α,2α-cyclobutanedicarboxylate (5a). Powdered ethyl (*E*)-3,4-dimethoxycinnamate (**6a**) was dissolved in the minimum volume of CH_2Cl_2 and spread between pairs of glass plates (20 × 20 cm). After complete evaporation of the solvent, the plates were irradiated for 6 h with a 125-W mercury lamp (Hanovia) at room temperature. The oily reaction mixture by silica gel column and preparative TLC chromatographies (hexane−EtOAc, 3:2) afforded **5a** (100 mg, 40%): oil; IR ($CHCl_3$) ν_{max} 1720 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.65 (2H,d, J = 8.3 Hz, H-5′), 6.58 (2H, dd, J = 8.3 and 2 Hz, H-6′), 6.30 (1H, d, J = 2 Hz), 4.25 (2H, m, H-α), 4.14 (4H, q, J = 7 Hz, CH_2), 3.73 (6H, s, 4′-OMe), 3.72 (2H, m, H-β), 3.58 (6H, s, 3′-OMe), 1.24 (6H, t, J = 7 Hz, Me); EIMS *m/z* (rel int) 472 $[M]^+$ (2), 427 $[M - OEt]^+$ (5), 300 $[b]^+$ (100), 236 $[a]^+$ (100), 221 $[a - Me]^+$ (6), 191 $[a - OEt]^+$ (100); m* 340.0 (427 − 381), 207.0 (236 − 221), 154.5 (236 − 191). Anal. ($C_{26}H_{32}O_8$) C, H.

Benzyl 3β,4β-Bis(3′,4′-dimethoxyphenyl)-1α,2α-cyclobutanedicarboxylate (5b). Benzyl (*E*)-3,4-dimethoxycinnamate (**6b**) by the same procedure as above (but with 20-h irradiation time) gave **5b** (38%): oil; IR ($CHCl_3$) ν_{max} 1720 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.33 (10H, br s, Ph), 6.65 (2H, d, J = 8.3 Hz, H-5′), 6.54 (2H, dd, J = 8.3 and 2 Hz, H-6′), 6.33 (1H, d, J = 2 Hz), 5.05 (4H, m, OCH_2), 4.34 (2H, m, H-α), 3.82 (6H, s, 4′-OMe), 3.79 (2H, m, H-β), 3.59 (6H, s, 3′-OMe); EIMS *m/z* (rel int) 596 $[M]^+$ (2), 505 $[M - OBn]^+$ (5), 300 $[b]^+$ (100), 298 $[a]^+$ (100), 283 $[a - Me]^+$ (6), 191 $[a - OBn]^+$ (100). Anal. ($C_{36}H_{36}O_8$) C, H.

3β,4β-Bis(3′,4′-dimethoxyphenyl)-1α,2α-cyclobutanedicarboxylic Acid (7). Ethyl ester **5a** (447 mg, 0.95 mmol) was added to a solution of KOH (533 mg, 9.5 mmol) in 95% EtOH (20 mL), and the mixture was held at reflux for 6 h, concentrated to a small volume, and diluted with H_2O. The solution was washed with EtOAc, acidified with concd HCl to pH 2, and extracted with EtOAc. The organic layer was washed with water, dried (Na_2SO_4), and evaporated to give the product **5** (383 mg, 97%; homogeneous on TLC with 1% AcOH in EtOAc): glassy solid, softens at 99−125 °C; IR ($CHCl_3$) ν_{max} 1720 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.84 (2H, br s, COOH), 6.66 (2H, d, J = 8.3 Hz, H-5′), 6.56 (2H, dd, J = 8.3 and 2 Hz, H-6′), 6.32 (1H, d, J = 2 Hz), 4.33 (2H, d, J = 6.3 Hz, H-a), 3.84 (2H, d, J = 6.3 Hz), 3.77 (6H, s, 4′-OMe), 3.61 (6H, s, 3′-OMe); EIMS *m/z* (rel int) 416 $[M]^+$ (2), 300 $[b]^+$ (100), 208 $[a]^+$ (100), 191 $[a - OH]^+$ (62). Anal. ($C_{22}H_{24}O_8$) C, H.

Methyl and Ethyl Derivatives of 7. The diacid **7**, when treated with CH_2N_2, gave the dimethyl ester **5**, identical with an authentic sample of the hydrolysis product, whereas with catalytic H_2SO_4/EtOH it afforded again the diethyl ester **5a**.

***N*-(*n*-Pentyl)-3β,4β-bis(3′,4′-dimethoxyphenyl)-1α,2α-cyclobutanedicarboximide (8).** *n*-Pentylamine (0.02 mL, 0.2 mmol) was added to a refluxing solution of **7** (62 mg, 0.15 mmol) and CDI (71 mg, 0.44 mmol) in dry 1,4-dioxane. After heating for 8 h, the reaction mixture was cooled to room temperature; further CDI (35 mg, 0.22 mmol) was added and refluxed overnight. After removal of the solvent the residue was dissolved in EtOAc. The solution was washed with 1 N HCl, 10% $NaHCO_3$, and water, then concentrated to give an oil, which was purified by column chromatography on silica gel with CH_2Cl_2−EtOAc, 1:1, to give **8** (56 mg, 80%): mp 93−95 °C (Et_2O); IR (Nujol) ν_{max} 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.69 (2H, d, J = 8.4 Hz, H-5′), 6.60 (2H, dd, J = 8.3 and 1.5 Hz, H-6′), 6.32 (1H, d, J = 1.5 Hz), 4.03 (2H, d, J = 3.8 Hz, H-α), 3.79 (6H, s, 4′-OMe), 3.62 (4H, m, H-β, N-CH_2), 3.60 (6H, s, 3′-OMe) 1.67 (2H, m, CH_2), 1.36 (4H, m, 2xCH_2), 0.92 (3H, t, J = 7 Hz, Me); EIMS *m/z* (rel int) 467 $[M]^+$ (22), 300 $[b]^+$ (46), 167 $[b]^+$ (100). Anal. ($C_{27}H_{33}NO_6$) C, H, N.

Tetrakis(Boc-caracasandiamide) (2a). CMC metho-*p*-toluenesulfonate (93 mg, 0.22 mmol) was added to a solution of **5** (45 mg, 0.11 mmol) and protected prenylagmatine **4a** (90 mg, 0.22 mmol) in dry CH_2Cl_2 (8 mL). The mixture was stirred at room temperature for 2 h, then washed with water, dried (Na_2SO_4), and concentrated under reduced pressure. The residue, by column chromatography on silica gel with EtOAc, afforded **2a** (39 mg, 30%; homogeneous on TLC with EtOAc): glassy solid; IR (Nujol) ν_{max} 3250, 1720, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.12 (2H, t, J = 8 Hz, exchg D_2O, NHCO), 6.68 (2H, br s, H-2′), 6.65 (1H, d, J = 8 Hz, H-5′), 6.55 (2H, br d, J = 8Hz, H-6′), 5.45 (2H, t, J = 7.5 Hz, exchg D_2O, NH), 5.15 (2H, dd, J = 10 and 6 Hz, H-2″), 4.22 (4H, m, H-α, H-β), 3.76 (6H, s, 4′-OMe), 3.60 (6H, s, 3′-OMe), 3.23 (8H, br t, J = 7 Hz, H_2-1, H2-4), 3.04 (4H, m, H_2-1″), 1.70 (12H, br s, 2 *x* Me), 1.69−1.56 (8H, m, CH_2), 1.49, 1.45 (18H each, s, Me_3); FABMS (TDEG/Gly) *m/z* 1178 $[MH]^+$. Anal. ($C_{62}H_{96}N_8O_{14}$) C, H, N.

Caracasandiamide (2). A mixture of **11** (20 mg, 0.017 mmol) and methanesulfonic acid (0.2 μL) in dry 1,4-dioxane (1 mL) was refluxed under N_2 for 4 h. The residue from evaporation, by preparative TLC ($CHCl_3$−MeOH, 8:2), gave caracasandiamide (8 mg, 61%), identical with an authentic sample of the natural product. Anal. ($C_{42}H_{64}N_8O_6$) C, H, N.

Pharmacology. Animals. Adult male Wistar rats, weighing 299 + 3 g (mean + SE; n = 118), were housed in stainless steel cages and fed a standard laboratory diet. They received "ad libitum" deionized drinking water and were kept undisturbed for 2 weeks in controlled conditions of dampness, light, temperature, and noise.

Cardiovascular and Respiratory Determinations. Rats were anesthetized with 10% (w/v) ethylurethane (1 mL/kg of body weight), which was dissolved in 0.9% NaCl solution (saline) and administered with a single ip injection. The trachea was cannulated to allow spontaneous breathing. Polyethylene catheters (PE 20 tubing) were placed in the left femoral artery for recording aortic blood pressure (BP) and into the femoral veins for drug administration. A calibrated 3F catheter-tip pressure transducer (Millar Instruments, Houston, TX), inserted in the right common carotid artery and

advanced in the left ventricle, was used for determining the maximum rate of rise of the left ventricular isovolumetric pressure (d*P*/d*t*), an index of cardiac inotropism.[31,32] Systolic BP and diastolic BP were measured by a P23Db Statham pressure transducer (Statham Medical Instruments, Los Angeles, CA) and averaged electronically. Heart rate (HR) was obtained by a 9875B Beckman cardiotachometer coupler (Beckman Instruments, Inc., Schiller Park, IL), which was triggered by the R-peak of the lead II electrocardiogram.[33] A Biotronex derivative computer (model BL622; Biotronex Laboratories, Inc., Kensington, MA) was used for determining d*P*/d*t*, by differentiating the pulsatile BP registered in the left ventricle. The computer was adjusted to minimize the expression of preload and afterload, as previously described.[34] The unit of measurement of d*P*/d*t* was mmHg/s.

Systolic BP, diastolic BP, or mean aortic flow (AF) was measured electromagnetically by inserting, after having performed a laparatomy of about 3 cm, a probe (1-mm i.d.) around the abdominal aorta (about 1 cm above iliac bifurcation), using a Statham K2002 flowmeter. To obtain an index of stroke volume (SV), the pulsatile AF was integrated by using a BL620 Biotronex apparatus.[35] BP, HR, d*P*/d*t*, AF, and SV were continuously monitored on a Beckman RM dynograph recorder.[36] The body temperature of the animals was kept constant at 37 °C by using an electrically heated table. Each rat received by iv route 1 mL of 0.9% saline solution containing 100 USP of sodium heparin.

Respiration was monitored by means of a pneumotachograph adapted to a Biotronex BL 620 integrator to yield the full respiratory wave.[37] Respiratory frequency (RF) and tidal volume (TV) were assessed under spontaneous breathing by connecting the tracheal cannula to the pneumotachograph. RF and TV were monitored polygraphycally along with the cardiovascular parameters. After completion of the surgical procedure, the rats were kept undisturbed for 60 min to allow for the stabilization of all cardiovascular and respiratory parameters.

Protocol. Twelve rats were used to determine the dose−response relationship for caracasandiamide (G2). In this respect, saline solutions of the compound were prepared daily and injected by iv route in a volume of 50 μL. The doses of the drug ranged from 50 to 3200 μg/kg of body weight (ratio = 2.0). Bilateral carotid occlusion (BCO) at the neck was performed for 30 s in basal conditions and 1 min after having administered each dose of G2.

Twelve rats were randomly divided into two equal groups for determining the effects of either bilateral vagotomy (1st group), carried out at the neck below the nodose ganglion, or spinalization under vagotomy (2nd group), carried out by making a complete transversal section of the spinal cord at the level of the first cervical metamere, on the cardiovascular effects induced by iv administration of G2 (50−1600 μg/kg, ratio = 2.0). After spinalization, the respiration was maintained through a respiratory pump, which was regulated in order to assure the minute volume calculated for each rat in basal conditions (i.e., preceding administration of the first dose of the drug). The data were compared with those obtained in the rats used for determining the dose−response relationship for G2.

Sixteen rats were randomly divided into two equal groups in order to test the effects of 400 μg/kg (1st group) and 1600 μg/kg (2nd group) of G2, injected by iv route, on the cardiovascular responses induced by iv administration of noradrenaline (1 μg/kg), adrenaline (0.625 μg/kg), and acetylcholine (2.50 μg/kg) before and 5−10 min after having administered G2.

Ten rats were randomly divided into two equal groups for determining the cardiovascular responses to G2 (400−1600 μg/kg, ratio = 2.0) before (control) and during iv infusion of phenylephrine (10 μg/kg/min) or 10 min after iv administration of phentolamine (2 mg/kg).

Thirty-six rats were randomly divided into four equal groups in order to evaluate the effects of 200−1600 μg/kg (ratio = 2.0) of G2 alone (control), during continuous iv infusion of papaverine (1 mg/kg/min), and after treatment with reserpine (5 mg/kg/day for 2 days, by ip route) or hexamethonium (5 mg, iv route).

Thirty-two rats were randomly divided into eight groups in order to compare on a molar basis the cardiovascular effects of some antihypertensive or vasodilating drugs with those of G2. These rats received, by iv injection under the above experimental conditions, (*Z*)-caracasanamide (4.12 μM/kg of body weight), G2 (4.12 μM/kg), guanethidine (25 mM/kg), clonidine (0.108 mM/kg), hexamethonium (12 mM/kg), reserpine (8 mM/kg), papaverine (5 mM/kg), or histamine (0.044 mM/kg). All drugs were dissolved in saline solution, and all doses were expressed in terms of free bases. The control administration of solvent alone caused insignificant changes in both cardiovascular and respiratory parameters. Peak effects were considered for each assay. Each of the consecutive tests was not made until the parameters had returned to the values preceding the previous administration of (*Z*)-caracasanamide and had stabilized.

Statistics. Data were expressed as means ± SE and compared by analysis of variance.[38] Only a *P* value less than 0.05 was considered to be significant.

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