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FURTHER HYPOTENSIVE METABOLITES FROM *VERBESINA CARACASANA*.[1]

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Abstract. After the isolation of caracasanamide and caracasandiamide, further hypotensive components of *Verbesina caracasana* were shown to be N^3-prenylagmatine, N^1-3',4'-dimethoxycinnamoylagmatine, agmatine and galegin (prenylguanidine). The structures were assigned on the basis of the spectral data of both metabolites and products from their alkaline hydrolyses. A pharmacological analysis of these products is also presented.
Keywords: Natural products; vasodilators; antihypertension; cardiovascular activity

A crude methanol extract of the Venezuelan plant *Verbesina caracasana* Fries (Compositae), intravenously administered to mice, was found to induce biological effects such as erection of hair and initial stimulation and subsequent blockade of breathing. Biologically controlled purification, culminating in silica gel chromatography, yielded a series of active compounds, the first of which was named caracasanamide and assigned the structure 1-[(3,4-dimethoxycinnamoyl)amino]-4-[(3-methyl-2-butenyl)guanidino]butane (**1**), as a mixture of (Z)- and (E)-forms.[2] The pharmacological profile of the water-soluble (Z)-form and the synthesis of the (E)-form of **1** have been reported in a previous paper.[3] A second metabolite, named caracasandiamide (G2), was shown to be the cyclobutane (truxinic-type) dimer of **1**[4], for which a complete pharmacological analysis has been previously reported.[5] This paper deals with structure and pharmacology of further hypotensive components, isolated from the methanol extract of *Verbesina caracasana* and based on a common guanidine structure.[6]

The metabolites were obtained by extended chromatography of the more polar fractions of the extract.[2,3] The compounds were named G3 to G7 according to the elution order, but the product G4 was obtained as a complex mixture, preventing a definite structure elucidation, and will be not described. The IR absorbance at 1655 cm^{-1} and a signal at 155.6 ppm in the ^{13}C NMR spectrum suggested that all these compounds contained a guanidino group. ^{1}H- and ^{13}C-NMR spectral data (Table 1) and a molecular peak at m/z 198 in the EI-MS spectrum of G3 indicated the structure of a prenylated agmatine (aminoguanidinobutane).

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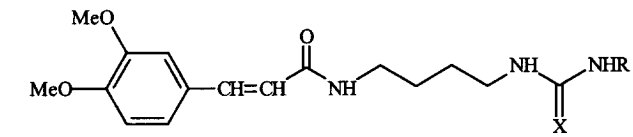
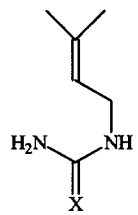
Alkaline hydrolysis of G3, with $Ba(OH)_2$, gave *N*-(3-methyl-2-butenyl)urea (**2**), 1-amino-4-ureido- butane (**3**) and urea. The new compound was thus assigned the structure 1-amino-4-[(3-methyl-2-butenyl)guanidino]butane (**4**). Notably, the isolation of **2** established the location of the isoprenyl chain and excluded the isomeric structure 1-[(3-methyl-2-butenyl)guanidino]-4- amino- butane (sperophysine), which was reported from *Sphaerophysia salsula*.[6]

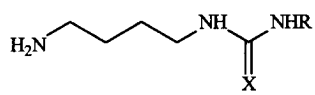
Compound G5 showed ^{1}H- and ^{13}C-NMR spectra (Table 1) very similar to those of G1 (**1**), but not the signals of the 3-methyl-2-butenyl chain. The M^+ ion in the EI-MS spectrum appeared at *m/z* 320, that is 68 mu (C_5H_8) lower than G1, revealing that G5 should be assigned the structure 1-(3,4-dimethoxycinnamoyl)amino-4-guanidinobutane (**5**). Accordingly, the alkaline hydrolysis of G5 gave a mixture of (*Z*)- and (*E*)-3,4-dimethoxycinnamic acids, 4-[(3,4-dimethoxycinnamoyl)amino]butylurea (**6**), also as a mixture of (*Z*)- and (*E*)-forms, and 1-amino-4-ureidobutane (**3**). In summary, G5 gave the same product as did G1, but only urea instead of prenylurea (**2**).[2,3] Compound **5** was a mixture of (*Z*)- and (*E*)-forms (in a ratio 2:1) such as G1 (**1**).



	X	R
1	NH	
5	NH	H
6	O	H



2	X=O
8	X=NH



	X	R
3	O	H
4	NH	
7	NH	H

The natural product G6 gave 1-amino-4-ureidobutane (**3**, 12 mg) and urea (4 mg) by hydrolysis with $Ba(OH)_2$, and was identified with agmatine (**7**) by comparison with a commercial sample. The last guanidino derivative G7 (M^+ at *m/z* 127) was assigned the structure *N*-(3-methyl-2-butenyl)guanidine (**8**) on the basis of ^{1}H- and ^{13}C-NMR spectral data (Table 1). The compound **8**, also named galegine, has been reported as the toxic principle of *Verbesina enceloioides*[7] and *Galega officinalis* (goats rue).[8]

The natural compounds G3, G5 - G7 were administered by "fast" i.v. route to adult male Wistar rats under general anaesthesia (10% ethyl urethane). The general experimental conditions were the same as

Table I. ^{1}H- and ^{13}C-NMR Data of Metabolites from *Verbesina caracasana*[a].

Position	G1 (**1**)	G5 (**5**)	G3 (**4**)	G6 (**7**)	G7 (**8**)
H_2-1	3.27 m	3.26 t (6.5)	2.88 t (6.5)	2.89 m	-
H_2-2	1.57 m	1.57 p (5)	1.62 m[b]	1.60 m	-
H_2-3	1.64 m	1.65 p (5)	1.65 m[b]	1.68 m[c]	-
H_2-4	3.21 m	3.17 t (6.5)	3.16 t (6.5)	3.20 m	-
H-2'	7.35 d (2)	7.34 d (2)	-	-	-
H-5'	6.95 dd (8,2)	7.08 dd (8,2)	-	-	-
H-6'	7.15 d (8)	6.68 d (8)	-	-	-
OMe	3.85 s, 3.82 s	3.98 s, 3.88 s	-	-	-
H-α	5.98 d (12.5)	5.91 d (12.5)	-	-	-
H-β	6.78 d (12.5)	6.68 d (12.5)	-	-	-
H_2-1"	3.74 d (7)	-	3.75 d (6.5)	-	3.66 d (7)
H_2-2"	5.18 br t (7)	-	5.22 br t (6.5)	-	5.07 br t (7)
H_3-4"	1.76 br s	-	1.76 br s	-	1.70 br s
H_3-5"	1.76 br s	-	1.71 br s	-	1.70 br s
NH				-	6.86 br s
C-1	42.3	41.3	41.0	40.7	-
C-2	27.2	26.3	25.2	24.2	-
C-3	27.7	26.8	25.8	25.2	-
C-4	39.8	39.0	39.5	39.4	-
C-1'	129.3	128.6	-	-	-
C-2'	111.6	111.3	-	-	-
C-3'	150.8	148.9	-	-	-
C-4'	152.1	150.0	-	-	-
C-5'	112.9	113.3	-	-	-
C-6'	122.6	123.8	-	-	-
C-α	141.7	138.0	-	-	-
C-β	119.4	122.0	-	-	-
C-1"	40.5	-	39.7	-	40.0
C-2"	123.3	-	118.7	-	118.4
C-3"	138.2	-	137.9	-	136.8
C-4"	25.8	-	25.7	-	25.9
C-5"	18.1	-	18.0	-	18.3
C=NH	157.4	-	156.4	157.2	157.6
C=O	169.1	169.1 -	-	-	-
OMe	56.5	56.3 -	-	-	-

[a]300 (^{1}H) and 75 (^{13}C) MHz, respectively. TMS as int. standard. Solvents: ($CDCl_3$-CD_3OD, 3:1) G1, G5, G3; G7; (D2O) G6.

The assignments were supported by HETCOR and COSY experiments.

The coupling constants (in Hz) are reported in parentheses.

The proton signals showed the appropriate integrate intensity.

Due to the presence of amido and guanido groups, some signals are doubled; only the signal with major intensity is reported.

For G1 and G5, only the values of the (Z) major form are reported.

reported for caracasanamide (G1)[3] and caracasandiamide (G2).[5] Systolic, diastolic and mean blood pressure (BP; mmHg), heart rate (HR; beats/min), maximum rate of rise of the left ventricular isovolumetric pressure (dP/dt, an index of cardiac inotropism; mmHg/sec), respiratory frequency (RF; beats/min) and tidal volume (TV; μL) were monitored continuously under spontaneous breathing, as previously described.[3,5].

At lower doses (200 – 400 μg/kg), G3 slightly increased dP/dt, RF and TV (TV>RF) without significant changes of BP and HR; at higher doses (800 – 1600 μg/kg), the drug appreciably increased dP/dt, RF and TV and decreased BP, while poorly reducing HR; at doses from 3200 to 6400 μg/kg, G3 markedly inhibited breathing and, only in the case of respiratory blockade, was responsible for an irreversible fall of BP, HR and dP/dt.

G5 (200 – 12800 μg/kg, ratio = 2.0) did not show significant cardiovascular and respiratory effects in the dose range 200 – 1600 μg/kg, with the exception of a slight not dose-related reduction of BP, dP/dt, HR and TV. The dose of 3200 μg/kg did not change HR, while slightly decreasing BP and increasing dP/dt, RF and TV. The doses of 6400 – 12800 μg/kg were able to decrease BP and HR and to increase dP/dt and RF without a clear dose-response relationship. The most evident effects of G5 were on dP/dt and TV with, at the dose of 12800 μg/kg, a 56% and 74% increase, respectively; a very high dose (102800 μg/kg) did not change significantly these effects.

G6 (200 – 51200 μg/kg, ratio =2.0) showed effects similar to those of G5: HR was not affected until the dose of 3200 μg/kg; BP and HR were reduced and dP/dt, RF and TV were increased but with a potency lower than that of G5 (*e.g.*, the dose of 12800 μg/kg induced a 48% and a 31 % increase of dP/dt and TV, respectively).

G7 (50 – 51200 μg/kg, ratio = 2.0) was able to reduce BP and HR and to increase dP/dt, RF and TV in the dose-range 50 – 12800 μg/kg; no dose-response relationship was found for the effects of G7 on BP, HR, RF and TV; however, the effects of G7 on dP/dt were related to the doses of the drug. Only the highest doses (25600 and 51200 μg/kg) markedly depressed RF and TV with next fall of the cardiovascular parameters; the dose of 51200 μg/kg caused irreversible respiratory blockade.

The effects of the tested guanidine compounds on BP (lasting few seconds from their i.v. administration) preceded those on HR, dP/dt, RF and TV, thus suggesting a vascular level of action in determining systemic arterial vasodilation. This level was not shared by G1, which only showed a peripheral (cardiac) β_1-adrenoreceptor-like action (thus causing increase of both HR and dP/dt) and reduced both central sympathetic tone and baroreceptor reflex activity (thus causing reduction of BP).[3] On the other hand, the cardiovascular effects of G2, not depending on central neurogenic components, were explained by peripheral mechanisms including reserpine- and guanethidine-like actions, β_1- and β_2-adrenoreceptor – and $M_{2,4}$-cholinergic receptor-like components as well as α_2-adrenoreceptor antagonistic properties.[5] Moreover, the cardiovascular effects of G1 and G2 were not related to the respiratory ones, since both G1 and G2 increased RF and TV (50 – 1600 μg/kg for G1, 50 – 200 μg/kg for G2; ratio = 2.0) while reducing the same parameters

at higher doses (3200 – 6400 μg/kg for G1, 400 – 3200 μg/kg for G2; ratio = 2.0). Similarly, G3 and G5-G7 increased dP/dt, RF and TV, and only the highest (toxic) doses of G3 (6400 μg/kg) and G7 (51200 μg/kg) were able to reduce (drastically) these indices. Finally, G3 and G5-G7 resembled both G1 and G2 in lowering BP, G1 in increasing RF and TV, and G2 in reducing HR. In this regard, Table II shows that these guanidine compounds differ in pharmacological potency, being G2 the most active drug in lowering BP and increasing cardiac inotropism (only G2 depressed breathing), and G1 and G7 in stimulating breathing.

Table II. Cardiovascular and respiratory responses[a] to several compounds obtained from Verbesina caracasana (G1-G3; G5-G7) and to various antihypertensive or vasodilating drugs administered by i.v. route in anesthetized rats.

DRUGS (μM/kg b.wt.)	BLOOD PRESSURE		HEART RATE	dP/dt	RF	TV
	SYSTOLIC	DIASTOLIC				
	(Δ%)	(Δ%)	(Δ%)	(Δ%)	(Δ%)	(Δ%)
G1 (4.12)	-26 ± 5	-29 ± 5	+5 ± 1	+57 ± 6	+21 ± 5	+41 ± 7
G2 (4.12)	-46 ± 5	-68 ± 4	-11 ± 3	+77 ± 6	-70 ± 3	-85 ± 5
G3 (4.12)	-11 ± 1	-17 ± 4	-4 ± 1	+23 ± 3	+4 ± 1	+11 ± 3
G5 (4.12)	-8 ± 3	-8 ± 2	0	+11 ± 4	+4 ± 1	+12 ± 3
G6 (4.12)	-8 ± 2	-10 ± 2	0	+16 ± 3	+12 ± 4	+14 ± 2
G7 (4.12)	-27 ± 4	-33 ± 4	-27 ± 6	+32 ± 5	+70 ± 8	+31 ± 3
Guanethidine (25)	-26 ± 4	-24 ± 1	-7 ± 3	-17 ± 3	=	=
Clonidine (0.108)	-16 ± 2	-17 ± 1	-15 ± 2	-21 ± 4	=	=
Papaverine (5)	-18 ± 3	-16 ± 2	-5 ± 1	-19 ± 2	=	=
Histamine (0.044)	-25 ± 2	-26 ± 3	-3 ± 1	-16 ± 5	=	=
Reserpine (8)	-31 ± 5	-32 ± 2	-15 ±	-22 ± 1	=	=
Hexamethonium (12)	-42 ± 4	-43 ± 4	-13 ±	-69 ± 3	=	=

[a]Values are means±SE (n=8 for each drug). The effects are expressed as ± percent change (Δ%) with reference to the basal values (i.e., preceding drug administration). dP/dt, maximum rate of rise of the left ventricular isovolumetric pressure; RF, respiratory frequency; TV, tidal volume.

Some of the tested natural compounds were more potent, on molar basis, than guanethidine, papaverine, reserpine or hexamethonium. Although it seems likely that G3 and G5-G7 act by some of the above cardiac and vascular mechanisms shared by G1 and/or G2, further studies are required to establish the single pharmacodynamic profiles, thus making possible to evaluate the structure-activity relationship.

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References and Notes

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