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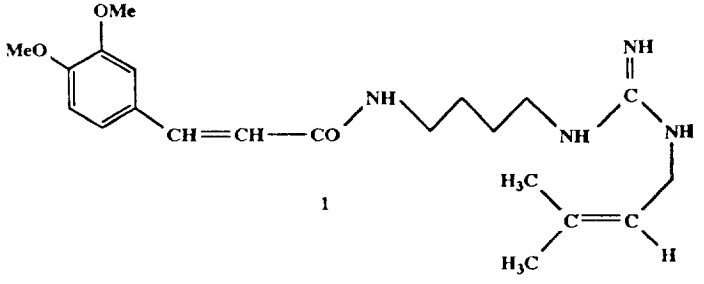
CARACASANDIAMIDE, A TRUXINIC HYPOTENSIVE AGENT FROM *VERBESINA CARACASANA*[1]

Giuliano Delle Monache,[a]* Bruno Botta,[a]* Franco Delle Monache,[a] Romulo Espinal,[b] Stella C. De Bonnevaux,[b] Carlo De Luca,[c] Maurizio Botta, [d]* Federico Corelli,[d] Donata Dei,[d] Eszter Gacs-Baitz,[e] and Marco Carmignani[f]

[a] *Centro Chimica dei Recettori, Università Cattolica, Largo F. Vito 1, 00168 Roma, Italy*
[b] *Departamento de Farmacologia, Universidad de Carabobo, Carabobo, Venezuela*
[c] *Centro Elettrochimica e Chimica Fisica delle Interfasi, Roma, Italy*
[d] *Dipartimento Farmaco Chimico Tecnologico, Università di Siena, Siena, Italy*
[e] *Central Research Institute for Chemistry, Hungarian Academy of Sciences, Budapest, Hungary*
[f] *Dipartimento di Biologia e Fisiologia Cellulare, Università di L'Aquila, L'Aquila, Italy*

Abstract. A second hypotensive agent from *Verbesina caracasana* is shown to be the truxinic type dimer of the previously isolated 3,4-dimethoxycinnamoyl guanidinoamide. The new compound gave by alkaline hydrolysis a crystalline monoamide, which in turn by acid hydrolysis gave 3',4'-dimethoxy-β-truxinic acid, identical with a synthetic specimen.

Biologically controlled purification of the methanol extract of the Venezuelan plant *Verbesina caracasana* Fries (Compositae) yielded a novel hypotensive agent named caracasanamide (G1).[2] The compound, chemically a mixture of (Z)- and (E)-forms of 1-(3,4-dimethoxycinnamoyl)amino-4-(3-methylbut-2-enyl)guanidinobutane (**1**), was shown to be a hypotensive drug of low-mild potency, devoid of significant tachicardic effects, provided with central and peripheral mechanisms of action in affecting cardiovascular function, and with stimulating respiratory effects when administered at nontoxic doses.[3]



1

A second compound named caracasandiamide (G2) was isolated either by silica gel or Sephadex LH-20 chromatography of the partially purified extract.[2] The ^{1}H- and ^{13}C-NMR of G2 were very similar with those of **1** (Table 1), except for the signals of two aliphatic methines, which substituted those of the olefin group in the cinnamoyl moiety. This variation was also reflected by the downfield shifts of the signals of amide carbonyl and C-1' aromatic carbon, both being attributable to the saturation of the C-α, C-β bond. G2 gave no interpretable result by EIMS, whereas in FABMS spectrum it showed a *quasi* molecular peak at m/z 777.[4] On consideration of the molecular weight of G1 (388 D), we may conclude that G2 is a dimer with a cyclobutane skeleton.

Cyclobutane derivatives with two phenyl rings and two carboxylic groups as substituents are named truxinic or truxillic acids depending on the kind of junction (head to head or head to tail, respectively). Truxinic and truxillic derivatives can be obtained by photodimerization of cinnamates[5] or by cycloaddition of stilbenes to alkenes.[6]
The two classes of compounds can be easily distinguished by their mass fragmentations because of the two cleavages **a** and **b** in truxillic acids give ions of the a' type, whereas truxinic acids may yield also fragments of the b' and b" kinds (Scheme I). Actually, a diagnostic peak at *m/z* 300 was found in the mass spectrum of G2 and it was attributed to structure b' (Ar = 3,4-dimethoxyphenyl). Therefore G2 was assigned structure **2**, while the stereochemistry of the cyclobutane ring was not yet defined (*vide infra*).

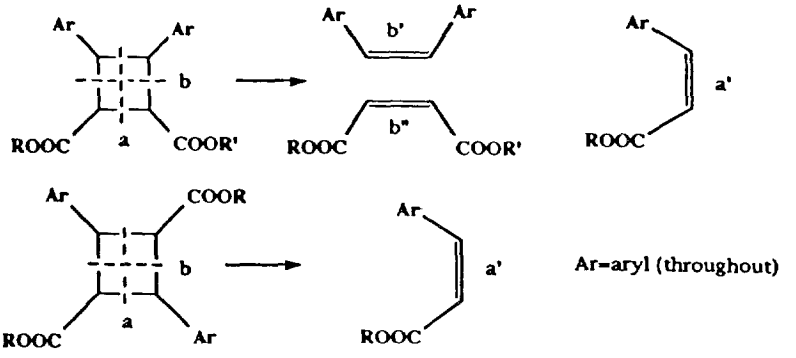
Table 1: ^{13}C NMR spectra of compounds **1** - **5a***

Position#	**1**	**2**	**4**	**3**	**5a**
1	39.8	39.4	39.2	40.9	-
2	27.7	27.4	27.5	27.0	-
3	27.2	27.0	26.0	26.0	-
4	42.3	42.1	42.1	41.3	-
1'	129.3	133.8	135.8, 135.5	-	132.6
2'	111.6	112.0	112.2, 112.1	-	112.0
3'	150.8	148.3	148.2, 148.0	-	148.8
4'	152.1	149.5	149.6, 149.5	-	151.4
5'	112.9	113.4	113.8, 113.5	-	113.2
6'	122.6	120.9	121.0, 120.9	-	120.8
OMe	56.5	56.0	56.0, 55.9	-	55.9
α	141.7	45.5	46.4, 48.2	-	45.6
β	119.4	44.2	45.8, 44.0	-	43.9
C=O	169.1	173.1	179.0, 173.9	-	173.7
C=NH	157.4	157.1	158.0	157.5	-
1"	40.5	40.4	40.2	41.0	-
2"	123.3	120.1	121.1	119.4	-
3"	138.2	137.3	136.4	140.9	-
4"	25.8	25.6	25.8	26.6	-
5"	18.1	18.3	18.2	18.9	-

*75 MHz, TMS as int. stand.. Solvents: **1**: D_2O; **2** and **3**, Me_2CO-d_6/DMSO-d_6, 6:1: **4**: CD_3OD; **5**: Me_2CO-d_6.
relative to compound **1**. Aromatic- and truxinic-rings signals are not equivalent in compound **4**.

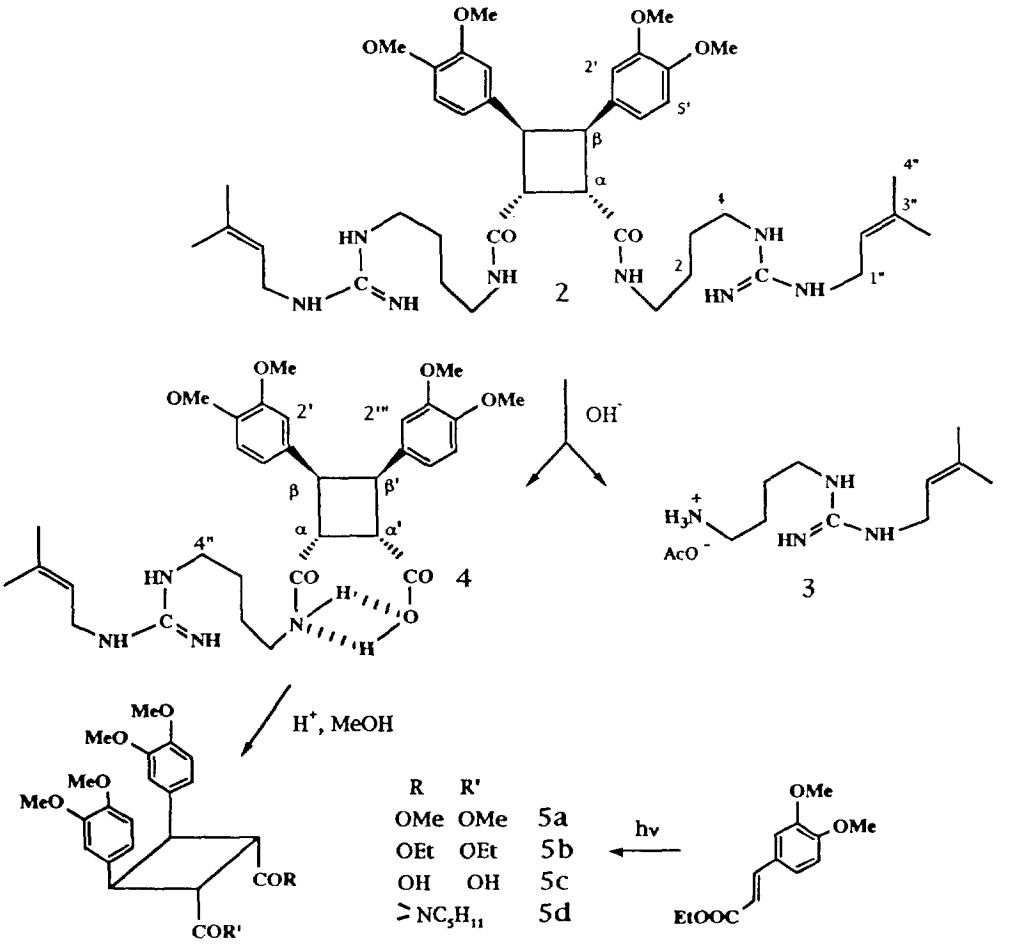
Scheme I

Mass fragmentation of truxinic and truxillic acids



Scheme II

Hydrolyses and partial synthesis of G2



To confirm the assignment, compound **2** was treated with NaOH 2N at room temperature for 4 days. As a result, prenylagmatine **3** (isolated as acetate), prenylurea and a crystalline product (**4**, m.p. 179-80 °C)[7] were obtained from the reaction mixture. Prenylagmatine was identical with one of the hydrolysis products of G1.[2,3] The compound had been also isolated earlier from another *Verbesina* and named galegine.[8]

In the ^{1}H- and ^{13}C NMR spectra of compound **4** the signals of protons and carbons belonging to the truxinic moiety were all doubled, whereas those attributable to the alkyl diaminobutane moiety remained single

Notably, the two distinct carbonyl resonances in the ^{13}C NMR spectrum can be attributed to amido (δ 173.7) and to acid (δ 179.0) groups. The presence of the carboxyl group was also revealed by a large triangle-shaped band between 3500 and 3000 cm^{-1} in the IR spectrum.

On consideration of the FABMS spectrum[7] (MH+ at m/z 597), the product was assigned structure **4,** which was obtained from **2** by the hydrolysis of a prenylguanidobutane unit.

The resistance of compound **4** to a second alkaline hydrolysis, as well as to methylation with CH_2N_2, can be ascribed to the formation of a hydrogen bond between the carboxylic and the amido groups. This arrangment requires that the two carbonyl substituents lie on the same side of the cyclobutane ring.[10]

Hydrolysis of the amido acid **4** was achieved in strongly acidic medium (2N H_2SO_4 in MeOH) to give again prenylagmatine **3**, prenylurea and the product **5a**, which by the signals at δ 3.68 and δ 52.1 in ^{1}H- and ^{13}C- NMR spectra, respectively, was a methyl ester.[9] The compound **5a** showed mass spectral data (peaks at *m/z* 444 and 300) typical of a truxinic acid dimethyl ester. The value of the signal of the two equivalent methoxy groups suggested that the two carbonyl substituents did not face the aryl rings.[10] Since both H-α and H-β showed a mutual NOE effect on the H-2' and H-6' aromatic proton, the H-β protons and the aryl groups must be on the same side of the cyclobutane ring. Therefore, the hydrolyses final product of hydrolysis **5a** was assigned the structure 3',4'-dimethoxy-β-truxinic acid dimethyl ester, which was confirmed by an independant synthesis.

Photochemical dimerization in solid phase[11] of ethyl 3,4-dimethoxy-cinnamate (Scheme II) afforded the β-truxinic ester **5b** (25% after cc and preparative TLC purification) along with a small amount of other stereoisomers[12] and starting material (41%). In order to establish the relative stereochemistry of the cyclobutane substituents, the diester **5b** was hydrolyzed (KOH, EtOH) to the diacid **5c**, which by methylation with CH_2N_2 gave compound **5a**, identical with the hydrolysis product. Moreover, compound **5c** was treated with EtOH and catalytic H_2SO_4 to provide again **5a**, proving that no epimerization occurred during the alkaline hydrolysis.[13] Finally, **5c** was converted (pentylamine, CDI) into the bicyclic imide **5d**, thus confirming a *cis* relationship between the carbethoxy groups.

Since the signal of protons and carbons of the cyclobutane ring did not shift on going from **2** to **5** (Scheme 2) and the same results as for **5a** were obtained in DIF NOE experiments, the skeleton of the two compounds must have identical stereochemistry. Therefore, G2 is β-truxin-[*bis*-3',4'-dimethoxy]-di-[N-(3-methylbut-3-enyl) guanidobutyl]-amide (**2**).

Caracasandiamide (G2), assayed by iv route in anesthesized rats at doses ranging from 50 μg/Kg to 6400 μg/Kg body weight, was shown to reduce chronotropism by central mechanisms. At lower doses, G2 stimulates breathing and increases both inotropism and peripheral vascular resistance, acting on the central respiratory and cardiovascular pathways, without affecting the baroreflex arc. At higher doses, G2 depresses breathing through central neurogenic mechanisms (not involved in the cardiovascular effects) and causes arterial hypotension by reducing peripheral vascular resistance. This last effect seems to involve a reduced activation of the vascular α_1- and α_2-adrenoreceptors and an higher activation of the vascular β2-adrenoreceptors and the muscarinic cholinergic receptors as well as reserpine-like effects.[14]

Similarly to, and more strongly than, G1, caracasandiamide (G2) may be considered a hypotensive and an antihypertensive drug, devoid of the negative side effects, e.g. the reflex tachycardia and decreased cardiac inotropism shown by the majority of the antihypertensive and vasodilator drugs.

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References and Notes

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4. $\alpha_D = 0$; ^{1}H NMR: δ 8.25, 7.95, 7.80, 7.55 (1H each, br s, exchg. D_2O, 4xNH), 6.71 (1H, d, J = 8Hz, H-5'), 6.65 (1H, dd, J = 8 and 2Hz, H-6'), 6.60 (1H, d, J = 2Hz, H-2'), 5.32 (1H, br t, J = 6.5Hz, H-2"), 4.32 (1H, d, J= 6Hz, H-α), 3.95 (1H, d, J = 6Hz, H-β), 3.94 (2H, d, J= 6.5Hz, H_2-1"), 3.68 (3H, s, 4'-OMe), 3.62 (3H, s, 3-OMe, 3.35 (2H, m, H_2-4), 3.26, 3.18 (1H each, m, H_2-1), 1.73, 1.71 (3H each, br s, 2xMe), 1.70, 1.61 (2H each m, 2xCH_2); ^{13}C NMR in Table 1; FAB MS *m z* (rel. int.): 777 $[MH]^+$ (100), 709 $[M - C_5H_8]^+$ (11), 579 (25), 389 $[M/2]^{+2}$ (15), 300 (8), 191 $[Ar\text{-}CH{=}CHCO]^+$ (57).
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7. ^{1}H NMR: δ 7.58 (1H, br s, NHCO), 6.68, 6.66 (1H each, br d, H-5', H-5"'), 6.66, 6.65 (1H each, br dd, J = 8.5 and 2Hz, H-6', H-6"'), 6.57, 6.54 (1H each, br d, J = 2Hz, H-2', H-2"'), 5.29 (1H, br t, J = 6.5Hz, H-2"), 4.34, 4.21 (1H each, br dd, J= 10.5 and 6Hz, H-α, H-α'), 3.85 (2H, br m, H_2-1"), 3.67, 3.66 (3H each,

s, 4'-OMe, 4"'-OMe), 3.63 (2H, br m, H-β, H-β'), 3.59, 3.58 (3H each, 3'-OMe, 3"'-OMe), 3.52 (1H, m, H-1a), 3.18, 3.14 (1H each, m, H_2-4), 2.85 (1H, m, H-1b), 1.84, 1.80 (1H each, m, H_2-2), 1.70, 1.67 (3H each, br s, 2xMe), 1.49, 1.44 (1H each, m, H_2-3); ^{13}C NMR in Table 1. FAB MS *m/z* (rel. int.): 597 $[MH]^+$ (71), 389 $[a']^+$ (40), 300 $[b']^+$ (20), 297 $[b"]^+$ (10), 207 $[a' - NH(CH_2)_4NHC(=NH)NHC_5H_9]^+$ (46), 191 [Ar-CH=CHCO] (100).

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9. ^{1}H NMR: δ 6.72 (1H, d, J = 8Hz, H-5'), 6.68 (1H, dd, J = 8 and 2Hz, H-6'), 6.59 (1H, d, 2Hz, H-2'), 4.24 (1H, d, 6Hz, H-α), 3.91 (1H, d, 6Hz, H-β), 3.69, 3.68 (3H each, s, 4"-OMe, COOMe), 3.61 (3H, s, 3'-OMe); ^{13}C NMR in Table 1; EIMS *m/z* (rel. int.): 444 $[M]^+$ (9), 413 $[M - OMe]^+$ (49), 300 $[b']^+$ (100), 285 $[M - MeOH]^+$ (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).
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