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# CARACASANAMIDE, A NOVEL HYPOTENSIVE AGENT FROM VERBESINA CARACASANA

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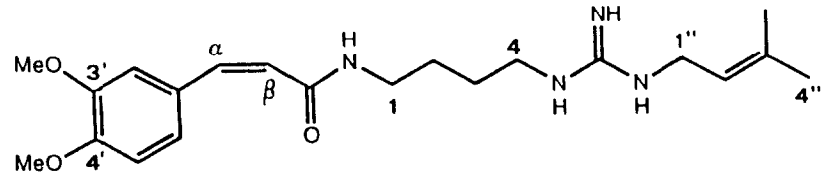
Abstract: The hypotensive agent from Verbesina caracasana is shown to be a novel guanidino-amide which occurs in (Z)- and (E)-forms. The structure of the compound was confirmed by the synthesis of the (E) form.

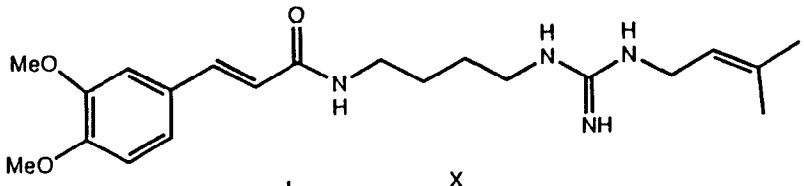
Examination of a crude methanol extract of the Venezuelan plant Verbesina caracasana Fries (Compositae) showed that it had an interesting dose-dependent hypotensive effect, reducing mean blood pressure (BP) after intravenous (i.v.) injection in anesthesized dogs. The maximum effect was obtained with the dose of 2.0 mg/Kg body weight (-58 ±4 with respect to the basal BP values; n=6; mean ± SEM) and did not change under barbiturate or chloralose anesthesia. The extract also induced, when administered by intraperitoneal route in mice, erection of hair and initial stimulation and successive blockade of breathing. Biologically controlled purification, culminating in silica gel chromatography, yielded from the less polar fractions a compound named caracasanamide (G1).[1]

Caracasanamide, $C_{21}H_{32}N_4O_3$, ($M^+$388) gave, on hydrolysis with sodium hydroxide, a mixture of (Z)- and (E)-dimethoxycinnamic acids and examination of $^1$H NMR spectrum of caracasanamide itself showed that it was a mixture of (Z)-(δ 6.78 and 5.98 ppm, J 13Hz) and (E)-(δ 7.44 and 6.83 ppm, J 16 Hz) olefins. The predominating (Z)-isomer was more water soluble than the (E)-form and this provided a basis for the separation. All pharmacological work was carried out with the (Z)-isomer, pure as judged by its $^1$H NMR spectrum.

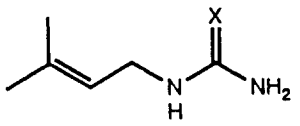
Hydrolysis with barium hydroxide yielded the dimethoxycinnnamic acids (above) and three further products. The first one was the 3-methyl-but-2-enyl urea (3, X=O) with the expected mass fragmentation pattern.[2]



(1)

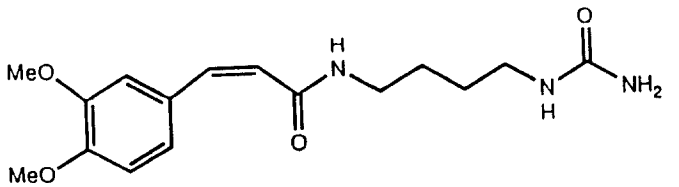


(2)

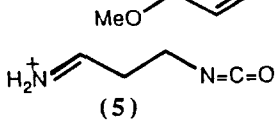


(3)

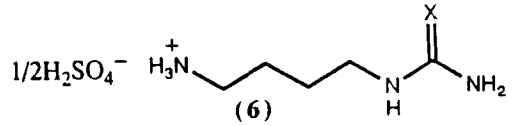
It is noted that 3-methyl-2-butenylguanidine (galegine) (3, X=NH) is a toxic principle occurring in the related plant Verbesina enceloioides.[3] Except for the loss of the dimethylallyl chain, the $^{1}H$- and $^{13}C$-NMR spectra of the second product $C_{16}H_{23}N_3O_4$, (4), isolated by crystallisation as the pure (Z)-isomer, were very similar to (1) and mass spectrometry showed the presence of the dimethoxycinnamoyl ion (m/z 191) and the fragment (5). The third product proved to be 4-amino-1-ureidobutane isolated as its monosulphate (6, X = O). The base, $C_{15}H_{13}N_3$ (M+1: 132, chemical ionisation) showed the expected E.I. mass fragmentation when compared with agmatine (4-amino-1-guanidobutane) (6, X = NH) and had the expected NMR characteristics.



(4)



(5)



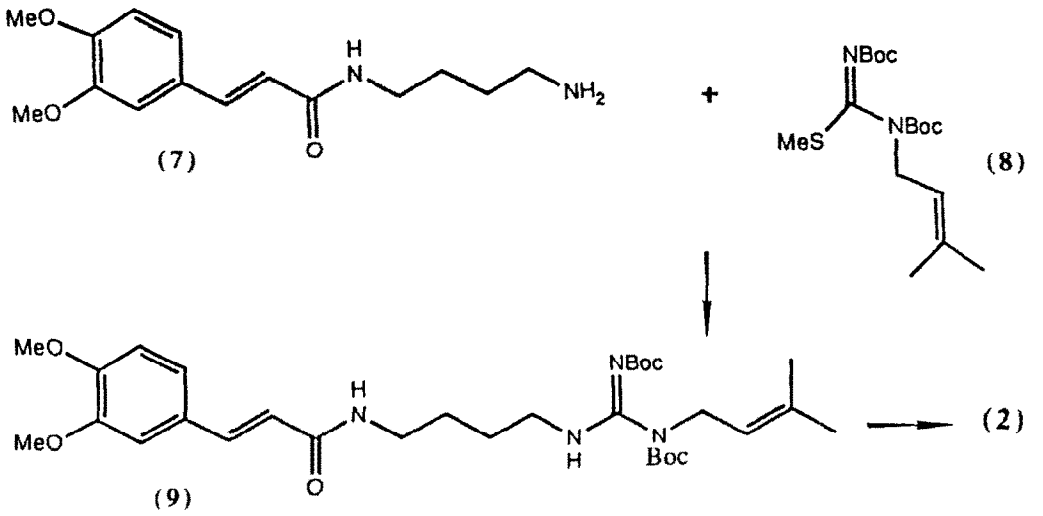
(6)

The presence of the guanidine moiety in (1) can be inferred by consideration of the hydrolysis product, the absorbance at 1655 $cm^{-1}$ in the infra red, and the $^{13}C$ NMR resonance due to the C=NH carbon (δ 155) [cf. guanidine δ 157)]. Taken together with the $^{1}H$- and $^{13}C$-NMR spectra[4] these data establish the structure of (Z)-caracasanamide as (1), occurring with the (E)-form (2).

The structure of (E)-caracasanamide was confirmed by treatment of the aminoamide (7) with the protected isothiourea (8) to give the Boc protected (9) (72% yield). Deprotection by refluxing with methanesulphonic acid in 1,4-dioxan, followed by resin exchange chromatography, gave (E)-caracasanamide[5] (2, 38%) identical with the naturally derived material (Scheme 1).

Scheme 1

Synthesis of (E)-caracasanamide (2).




(Z)-caracasanamide (G1), assayed by i.v. route in anesthesized rats at doses ranging from 50 μg/Kg to 6400 μg/Kg body weight, was shown to decrease blood pressure dose-dependently and to increase cardiac inotropism, respiratory frequency and tidal volume, without changes of heart rate. Higher doses depressed breathing with, in some cases, cardiac arrest.[6] The cardiovascular effects of G1 were dependent on arterial vasodilation caused by local mechanisms, sympathetic hypotone caused by central neurogenic mechanisms and interaction with the cardiac $\beta_1$-adreno-receptors. G1 was more potent than guanethidine in lowering blood pressure and as potent as reserpine and papaverine. Therefore, G1 may be considered a hypotensive drug of low-mild potency, devoid of significant tachycardic effects and with stimulating respiratory effects when given at non toxic doses.[7]

Acknowledgements

We thank Professor L. Crombie for a very helpful discussion of the manuscript.

References and notes

1. During a Poster Session of the 13th IUPAC Symposium on the Chemistry of Natural Products, Pretoria, 1982 the compound G1 was erroneously reported as the ureido-compound rather than the guanidino-compound (Abstract A52).
2. H. Budzikiewicz, C. Djerassi and D. H. Williams, Mass Spectrometry of Organic Compounds, Holden-Day Inc., San Francisco, 1967, pp 503 - 506.
3. P.B. Oelrichs, P.J. Vallely, J.K. MacLeod and I.A.S. Lewis, J. Nat. Prod., 1981, 754.
4. $^{1}$H NMR ($D_2O$): δ 7.15 - 6.95 (3H, m, H-2', H-5', H-6'), 6.78 (1H, d, J 13Hz, H-α), 5.98 (1H, d, J 13Hz, H-β), 5.18 (1H, br t, J 7Hz, H-2"), 3.85, 3.82 (3H each, s, 2xOMe), 3.74 (2H, d, J 7Hz, 1"-$H_2$), 3.5 - 2.9 (4H, m, 1-$H_2$, 4-$H_2$), 1.76 (6H, br s, 2xMe), 1.6 - 1.3 (4H, m, 2-$H_2$, 3-$H_2$); $^{13}$C NMR: δ 169.1 (s, C=O), 157.4 (s, C=NH), 152.1 (s, C-4'), 150.8 (s, C-3'), 141.7 (d, C-β), 138.2 (s, C-3"), 129.3 (s, C-1'), 123.3, 122.6 (each d, C-2", C-6'), 119.4 (d, C-α), 112.9 (d, C-5'), 111.6 (d, C-2'), 56.5 (q,OMe), 42.3 (t, C-1"), 40.5,39.8 (each t, C-1, C-4), 27.7, 27.2 (each t, C-2, C-3), 25.8 (q, t-Me), 18.1 (q, c-Me).
5. $^{1}$H NMR ($CDCl_3$-$CD_3OD$, 3:1): δ 7.44 (1H, d, J 16Hz, H-α), 7.07 (1H, d, J 2Hz, H-2'), 7.04 (1H, d, J 8Hz, H-5'), 6.83 (1H, d, J 16Hz, H-β), 6.81 (1H, dd, J 2 + 8Hz, H-6'), 5.14 (1H, br t, J 7Hz, H-2"), 3.85, 3.82 (3H each, s, 2xOMe), 3.70 (2H, d, J 7Hz, 1"-$H_2$), 3.29, 3.17 (2H each, m, 1-$H_2$, 4-$H_2$), 1.67, 1.62 (3H each, br s, 2xMe), 1.59 (4H, m, 2-$H_2$, 3-$H_2$); $^{13}$C NMR: δ 167.4 (s, C=O), 155.3 (s, C=NH), 150.0 (s, C-4'), 148.6 (C-3'), 140.0 (d, C-β), 137.2 (s, C-3"), 127.6 (s, C-1'), 121.7 (d, C-2"), 118.3, 117.7 (each d, C-6', C-α), 110.6, 109.4 (each d, C-2', C-5'), 55.3 (q, OMe), 40.6 (t, C-1"), 38.9, 38.1 (each t, C-1, C-4), 25.7, 25.3 (each t, C-2, C-3), 24.9 (q, t-Me), 17.2 (q, c-Me).
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7. G. Delle Monache, F. Delle Monache, B. Botta, S. C. de Bonnevaux, R. Espinal, C. De Luca and M. Carmignani, Eur. Pat. n. 0330629 A3., Feb 17, 1989; U. S. Pat. n. 5,059,624, Oct 22, 1991.