Tetrahedron Letters,Vol.23,No.38,pp 3855-3858,1982 0040-4039/82/383855-04$03.00/0
Printed in Great Britain

STRUCTURES OF CABENEGRINS A-I AND A-II, POTENT ANTI-SNAKE VENOMS

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Summary: Two potent antidotes against snake venoms have been isolated from the aqueous ethanol extract of a South American plant. The structures have been determined to be 1 and 2.

An aqueous alcoholic extract ("especifico pessoa") of the root of a South American plant called "Cabeca de Negra"[1] is available to plantation workers in the Amazon jungle as an oral antidote against snake and spider venoms. Two potent snake venom antidotes, cabenegrin A-I and A-II, have been isolated from this aqueous ethanol extract and their structures have been determined by spectrosopic studies and synthesis as 1 and 2, new pterocarpans.

The extraction of cabenegrins was carried out according to Scheme I employing mice as the monitoring animal. Thus 20-25 g mice (Swiss Webster white) were intraperitoneally injected with 2.5-fold the lethal dose of Fer de lance (Bothrops atrox) snake venom, the extract fractions were then injected immediately after envenomation, and the mice were checked for survival after 24 h. The crude extract (135 ml) fractionated in this manner finally yielded 44 mg of A-I and ca. 1 mg of A-II; the minimal dosage of the two cabenegrins required for survival was 2.8 mg/kg and 2.0 mg/kg, respectively.

Fraction B shown in Scheme 1, a complex multicomponent mixture, also exhibited activity but the active principle has as yet not been isolated.

Cabenegrin A-I possessed the following constants: m.p. 167-168°, $C_{21}H_{20}O_6$ (EI-MS); IR(KBr), 3550 cm^{-1}(OH), 1600(arom), 1113(ether), 925 ($-O-CH_2-O-$). The 309 nm UV maximum (Fig. 1) suggested A-I to be a pterocarpan. The full structure was fully elucidated by the NMR data shown in 1a and 1b. In particular, the

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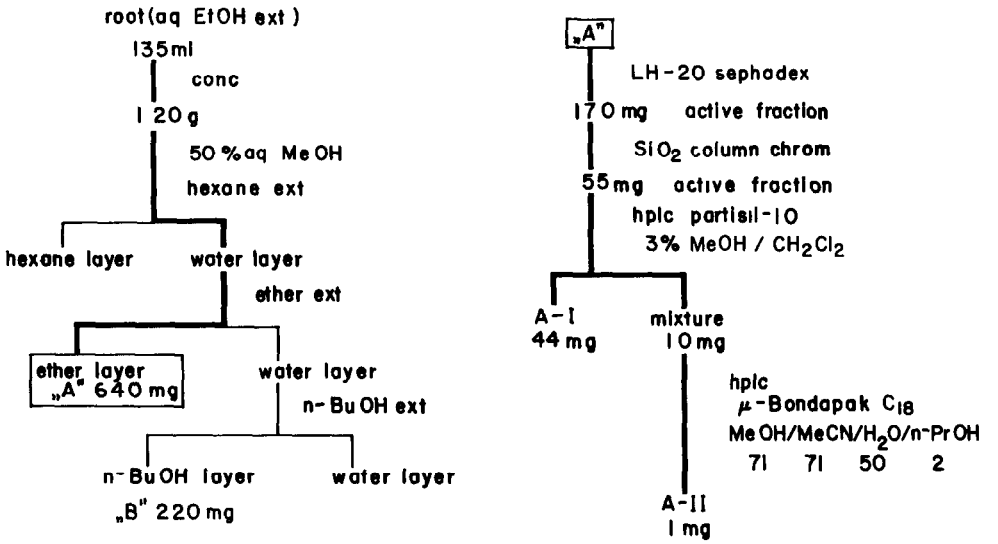


Scheme I Extraction of cabenegrins

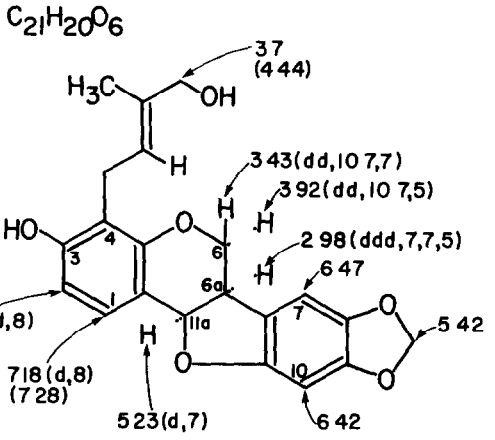
shifts of 6-H and 11a-H are very similar to those reported for other pterocarpans.[2] The negative CD Cotton effect at 238 nm ($\Delta\varepsilon$-9.87) (Fig. 1) showed the absolute configuration to be identical with that of (-)-homo-pterocarpin.[3]

The MS fragments at m/z 176 and 161 (1c and 1d), together with the two aromatic proton singlets at 6.47 and 6.42 ppm (in C_6D_6), defined the location of the methylenedioxy group. Irradiation of 11a-H (5.23 ppm) sharpened the 7.18 ppm peak and hence C-1 is unsubstituted. Since the 2-H signal underwent a low-field shift from 6.22 to 6.73 ppm upon acetylation (Ac_2O-pyridine) (see 1a), the hydroxyl group is attached to C-3[4,5] and the isopropylidene group to C-4.

The side-chain double bond has the olefinic methyl at 13.74 ppm, due to operation of the γ-effect.[6,7] The structure of cabenegrin A-I thus derived has been confirmed by a total synthesis of its racemate.[3]

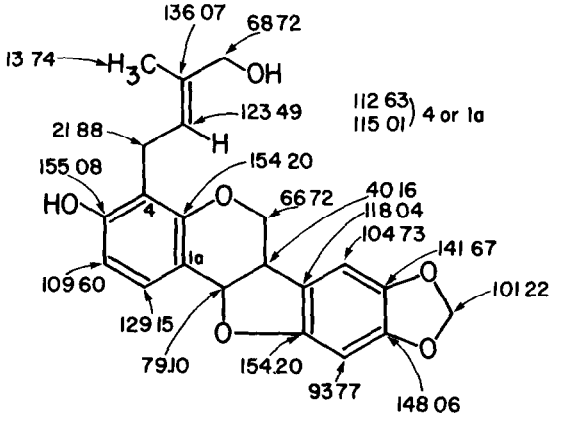
The physical constants summarized below lead to structure 2 for cabenegrin A-II. The ^{1}H-NMR data showed that it is a 3:1 mixture of epimers at C-3'. The structure of cabenegrin A-II has also been confirmed by synthesis of the racemate of a 3'-diastereomeric mixture.[8]

Injection of 2.5 mg/kg i.v. (lethal dose) of Bothrops atrox venom to a male beagle dog (9 kg weight, anesthetized with sodium pentobarbital 35 mg/kg i.v.) led to immediate hypotension followed by respiratory and cardiac arrest. However, when 1.0 mg/kg of A-I (dissolved in 70% EtOH and 30% saline, 10 mg/ml) was injected i.v. 15 min prior to injection of venom, the respiration, blood pressure and cardiac symptoms (electrocardiogram) were restored to normal after 60-90 min (Fig. 2). Conversely, when cabenegrin A-I was injected in the isolated heart preparation (Langendorff) of beagle dogs several minutes after injection of a lethal dose of the venom, the toxic cardiovascular effects were reversed. The effects of A-II were similar to those of A-I.



Ia

δ's in C_6D_6(Hz)
(δ's of acetate)

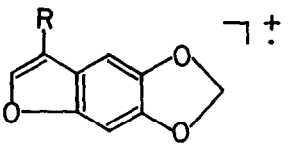


Ib

δ's in $CDCl_3$

EI-MS (18eV)

368 (M, 50%)
350 ($M-H_2O$, 100%)
335 ($M-H_2O$-Me, 95%)



Ic R Me m/z 176 (65%)
Id R H m/z 161 (90%)

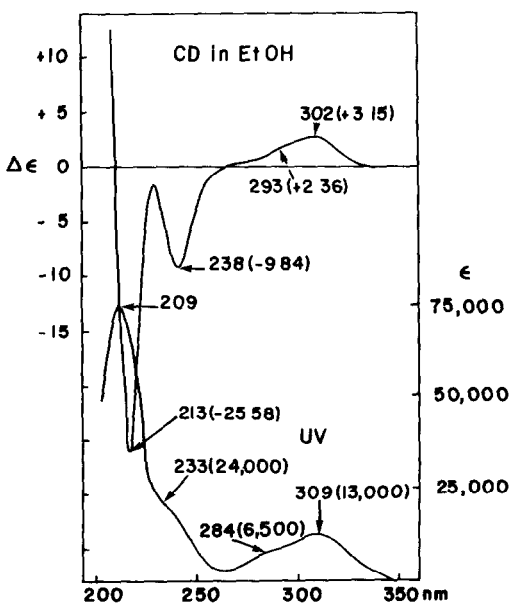
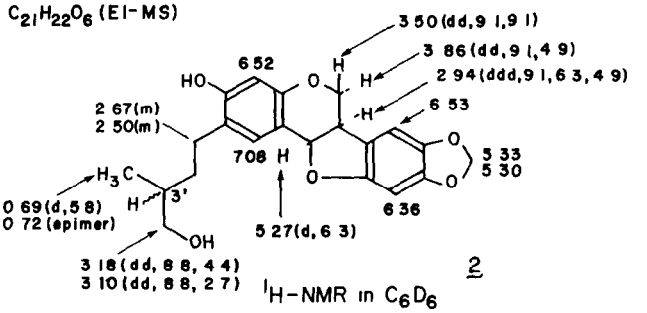


Fig. 1. UV and CD of cabenegrin A-I (EtOH)

Cabenegrin A-II
$C_{21}H_{22}O_6$ (EI-MS)



2

^{1}H-NMR in C_6D_6

UV (MeOH)
204nm(ε 16,000)
230nm(ε 8,000)
292nm(ε 9,400)
308nm(ε 11,800)

CD (MeOH)
237 nm (Δε -6.68)
280 nm (Δε -0.46)
299 nm (Δε +1.72)

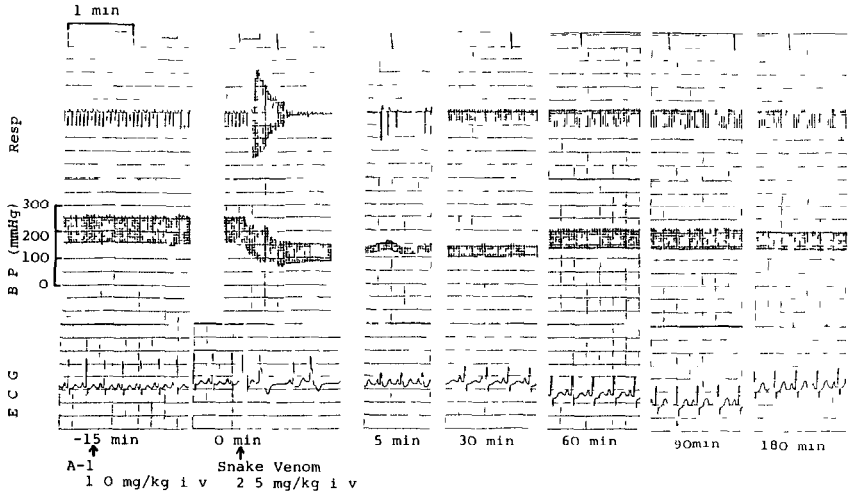


Fig. 2 Effect of cabenegrin A-I on respiratory and cardiovascular responses to snake venom in anesthetized dog.

Acknowledgements: We are grateful to Dr. K. Aisaka who carried out part of the bioassays.

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1. We have so far not been able to identify the plant; there are about ten plants called by this name in South America.
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(Received in USA 22 June 1982)