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Cyphostemmins A-B, two new antifungal oligostilbenes from *Cyphostemma crotalarioides* (Vitaceae)

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Abstract :

Two new antifungal resveratrol dimers, cyphostemmins A-B (1-2), have been isolated from the roots of Cyphostemma crotalarioides planch *(Vitaceae) together with resveratrol 3 and previously known reseveratrol dimers (4-7). Structures of these new compounds have been established on the basis of their MS and ^{1}H and ^{13}C NMR spectroscopic data.*

keywords: Antifungals / Dimers / Natural products / Polycyclic aromatic compounds

In our on-going programs aimed to the discovery of new pesticides of plant origin, we have extensively studied the composition in secondary metabolites of various indigenous plants from the Sudan, known for their promising biological activity and sometimes even used in traditional agriculture for pest management purposes.

One of our extracts, obtained from *Cyphostemma crotalarioides* [1] exhibited a very promising antifungal activity against *Fusarium nivale*. In a recent report [2], we have described the isolation and the identification of resveratrol and some of its known natural oligomers from the roots of this plant. This type of compounds was known to be produced by numerous plant species [3] and proved to exhibit cancer chemopreventive, antifungal and antibacterial activities [4]. This paper decribes the structure elucidation of two new resveratrol dimers of potential antifungal interest isolated from the ethyl acetate fraction of the methanolic extract of the roots of this plant.

The air-dried, ground roots were macerated with methanol. This extract showed antifungal activity. The dried extract was then dissolved in water and partitioned against ethyl acetate. The last fraction was chromatographed on a reversed phase preparative column packed with silinised silica gel (Kieselgel). For elution, a chromatography gradient started with H_2O 70% / MeOH 30% and ended with MeOH 100%, was used. Fifteen fractions were collected. Each biologically active fraction was further purified by HPLC on a reversed phase silica gel (packing material C-18, 5 µm, pressure 2150 PSI, temperature 40°C) eluted with water/acetonitrile (solvent gradient, flow rate 1.5 ml/min). Manual collection led to the obtention of 8 major homogeneous fractions identified as cyphostemmin A **1** (35 mg/kg), cyphostemmin B **2** (22 mg/kg), resveratrol **3** (85 mg/kg), parthenocissin A [5] **4**

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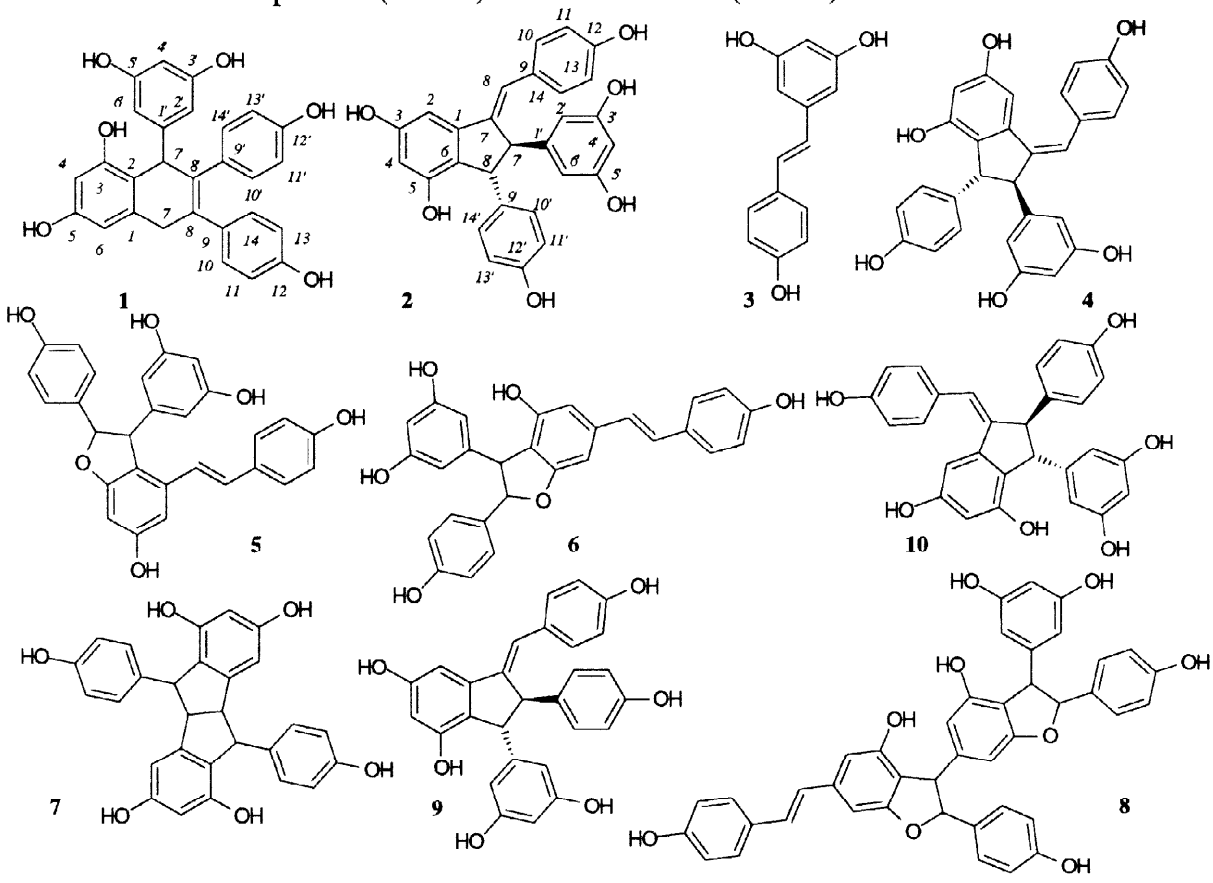
(12 mg/kg), ε-viniferin **5** [6] (90 mg/kg, *trans* -viniferin although, in some chromatography fractions, a substantial amount of the corresponding *cis* isomer was also observed, which has not been earlier described in the literature and has to be related to another cis dehydrodimer of resveratrol recently obtained by incubation of resveratrol in culture filtrates of ***B. cinerea*** [7][1] ; moreover, a slow transformation of this isomer into *trans* -viniferin was observed on standing in solution), gnetin C **6** [8] (90.3 mg/kg), pallidol **7** [9] (10.32 mg/kg) and a trimeric compound, gnetin E **8** [10] (64.50 mg/kg). For economical reasons, we have worked on a small quantity of crude-extract and the amount of material used for the identification was thus about 1 mg for each compound ; therefore, we have not been able to collect the ^{13}C NMR data for all compounds.

The purified compounds were then subjected to classical spectroscopic methodologies. First of all, the MS analysis[2] allowed the rapid characterization of resveratrol (M 228), of the various dimeric compounds (M 454) and of the trimer (M 680).



Scheme 1

Structures of the known compounds (**3-8**) were easily inferred from the ^1H NMR spectra (300 MHz, CD_3COCD_3) and compared with the data reported in the literature [5-10].

The fact, **2** was exhibiting ^1H NMR data very similar to these of ampelopsin D **9** [11] and parthenocissin A **4**, prompted us to hypothesize very similar structures for these three

[1] ^1H NMR (300 MHz (CD_3COCD_3, δ (ppm), *J* (Hz)) : 7.17 (d, *9*, 2H) ; 7.13 (d, *9*, 2H) ;6.92 (d, *9*, 2H) ;6.79 (d, *9*, 2H) ; 6.44 (bs, 1H) ; 6.37 (d, *4*, 1H) ; 6.33 (d, *4*, 1H) ; 6.16 (bs, 2H) ; 5.40 (d, *5*, 1H) ; 4.11 (d, *5*, 1H).

[2] Nermag R 30-10 ; FAB-DCI, 95 eV.

products. From the ^1H NMR spectra, some common features were easily identified which were in good agreement with the hypothesis of a resveratrol dimer : two para disubstituted phenolic rings, one meta symetric trisubstituted benzenic ring and one tetrasubstituted aromatic ring. Further resonances were also observed for one proton beared by an sp^2 carbon and two poorly coupled benzylic protons shifted at low field. The ^{13}C NMR data were in agreement with these attributions[3]. Assuming that these compounds were resulting of a non yet described dimerization of resveratrol and according to the informations extracted from the NMR spectra, the only possible structures for cyphostemmin B were those depicted on scheme 1 (**2** or **10**). However, extensive NOE difference experiments (figure 1) allowed us to complete the structure assignments (figure 2) and to refute structure **10**. The head to tail dimerization type was confirmed by the observation in the case of **2** of the following NOE's : 10'→7'→2'→14→8→2. More than the difference in the observed chemical shifts and those reported for ampelopsin D, this constatation gave an additional proof of the difference between cyphostemmin and ampelopsin. Similar NOE experiments performed on **4** allowed us to emphasize similar effects also described in the original publication [5] (2→10→8→7'→6'→10') confirming the structure attribution.

These observations, and especially the 8'→6' NOE, as well as the detection for both **2** and **4** of a small coupling constant (≈ 2 Hz) between the two benzylic protons were also consistent with a trans relationship between the aromatic substituents on the five membered ring in both compounds as depicted on scheme 1 and in agreement with the data of the literature (Only the relative configurations of the stereogenic centersare shown, while the absolute configurations of **1** and **2** remain unknown).

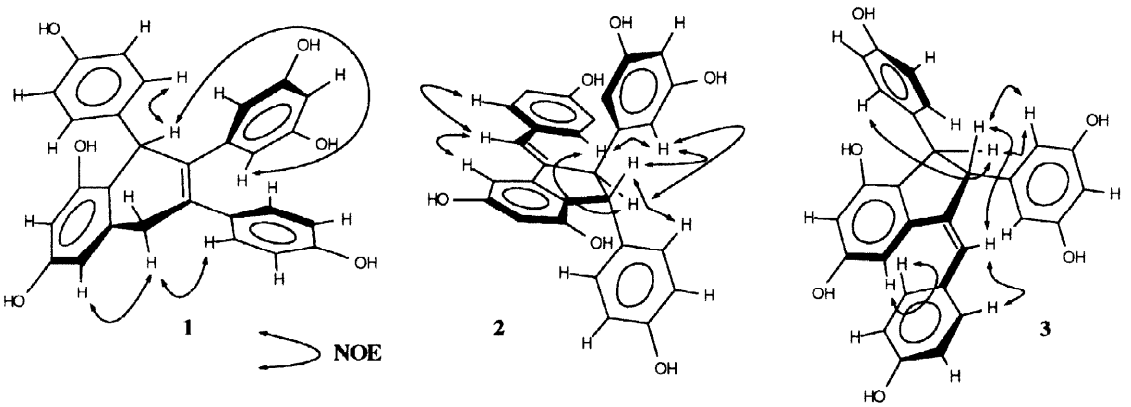


Figure 1

Structure of cyphostemmin A exhibited, as well as cyphostemmin B, two para disubstituted phenolic rings, one meta symetric trisubstituted benzenic ring and one tetrasubstituted aromatic ring, but also a unique feature consisting in a benzylic CH_2 where the two protons were magnetically unequivalent, only one beeing poorely coupled with another benzylic proton (J ≈ 1 Hz). According to the observed NOE's (6→7→10 and 2'→7'→10'), the only acceptable structure was **1**. The cyclohexenic structure of

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[3] **cyphostemmine A** : ^{13}C NMR (75 MHz (CD_3COCD_3, δ (ppm)) : 158.4 (3C), 155.9 (2C), 153.2, 149.5, 148.0, 138.3, 136.5, 130.7 (2C), 129.7 (4CH), 124.3, 115.2 (2CH), 114.8 (2CH), 107.6 (2CH), 101.8 (CH), 100.3 (CH), 100.1 (CH), 54.6 (CH), 32.1 (CH_2).

cyphostemmine B : ^{13}C NMR (75 MHz (CD_3COCD_3, δ (ppm)) : 159.2 (3C), 156.5, 155.5, 155.1, 148.2, 146.8, 142.0, 137.1, 130.8 (2CH), 129.1, 128.2 (2CH), 123.9, 122.4 (CH), 115.3 (4CH), 105.5 (2CH), 103.0 (CH), 101.8 (CH), 97.7 (CH), 59.9 (CH), 56.8 (CH).

cyphostemmin A is confirmed by the observation of a characteristic ^5J coupling constant between one proton of the allylic CH_2 and the benzylic isolated proton. Although we were not able to perform heteronuclear correlations for this compound, which would have allowed the unambiguous attributions of the aromatic resonances, structure **1** is the only one compatible with the hypothesis of a resveratrol dimer.

The production by the same plant of these new resveratrol head to tail dehydrodimers as well of five other resveratrol oligomers has prompted us to undergo the study of the enzymatic oxydation system of *Cyphostemma crotalarioides* responsible for this "combinatorial" oligomerization of resveratrol.

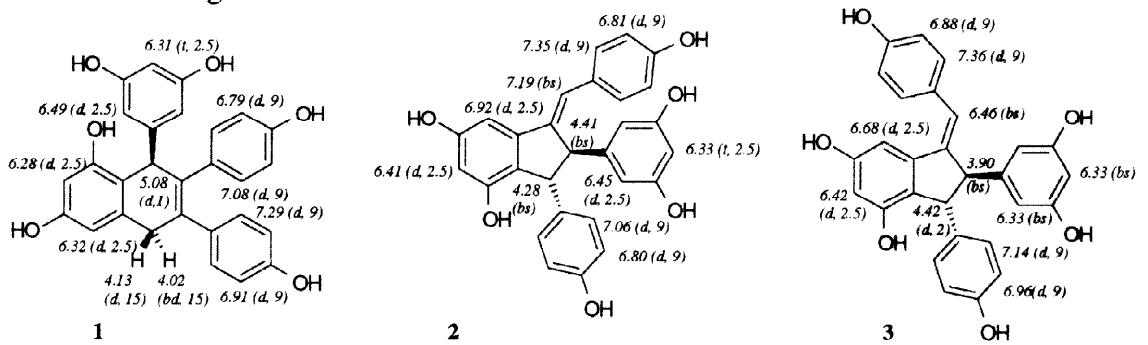

Figure 2 : ^1H NMR data of cyphostemmins and parthenocissin A (δ (ppm), J (Hz))

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