0014-4754/95/010063-04$1.50 + 0.20

Powerful hepatoprotective and hepatotoxic plant oligostilbenes, isolated from the Oriental medicinal plant *Vitis coignetiae* (Vitaceae)

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Received 14 March 1994; received after revision 14 July 1994; accepted 9 August 1994

Abstract. The methanol extract of the Oriental medicinal plant *Vitis coignetiae* (Vitaceae) showed hepatoprotective activity in the in vitro assay method using primary cultured rat hepatocytes. Activity-guided fractionation of the extract afforded ε-viniferin as an active principle. The protective effect of ε-viniferin against mice carbon tetrachloride-induced hepatic injury in mice was shown by serum enzyme assay as well as by pathological examination. In addition to ε-viniferin, plant oligostilbenes, ampelopsins A, C, F and the mixture of vitisin A and *cis*-vitisin A were also present in the extract. Among them, ampelopsin C and the mixture of vitisin A and *cis*-vitisin A were found to be powerful hepatotoxins.

Key words. Vitaceae; *Vitis coignetiae*; hepatoprotective substance; oligostilbene; hepatotoxin.

Although a number of traditional herbal drugs have been used in Oriental medicine since ancient times to cure hepatic diseases such as hepatitis and liver cirrhosis, the efficacy of only a few of the drugs has been evaluated[1]. In a program to confirm the hepatoprotective actions of Oriental medicinal plants and to identify the active substances involved, methanol extracts of some Vitaceaeous plants were studied. They were found to have a strong heptoprotective activity against injuries of primary cultured rat hepatocytes induced by carbon tetrachloride (CCl_4) and D-galactosamine (D-GalN)[2]. The results prompted us to study the hepatoprotective principle of *Vitis coignetiae* methanol extract in more detail.

Materials and methods

The methanol extract (1 kg) of the plant (10 kg) was separated into fractions soluble in ethyl acetate (300 g), *n*-butanol (200 g) and water solubles (500 g) by solvent partitioning. Since the ethyl acetate-soluble substances exhibited the strongest hepatoprotective activity, they were fractionated further, the fractionation being guided by monitoring the activity. The ethyl acetate-soluble fraction (300 g) was chromatographed over silica gel and the column was eluted with the increasingly polar chloroform-methanol mixtures. The repeated silica gel chromatography of the biologically active compounds (5 g) eluted by chloroform-methanol (92.5:7.5) led to purification of ε-viniferin (2 g)[3] as a main component of the fraction. Identification of ε-viniferin (fig. 1) was carried out by comparison of its physicochemical data ($[\alpha]_D$, EIMS, UV, IR, ^1H and ^{13}C NMR) with those reported by Langcake and Pryce[3]. ε-Viniferin, the main oligostilbene in the active fractions, at a concentration of 0.1 mg/ml, significantly reduced alanine transaminase (ALT) values to 44 and 61% of the control respectively, in CCl_4- and D-GalN-induced cytotoxicity models using primary cultured rat hepatocytes[4,5].

Since experimental liver damage produced by CCl_4 has been extensively studied and the profile of changes in the activity of certain enzymes in serum after the single administration of the hepatic agent has been well established, the hepatoprotective action of ε-viniferin on CCl_4-induced liver lesion was assessed in a mouse experimental model. The treatment group of male ddY mice (6 weeks) was given 3% gum arabic saline solution of ε-viniferin at a dose of 30 mg/kg i.p., and the control group a similar volume of 3% gum arabic saline solution. Two hours after the sample injections, the mice received p.o. 5 ml/kg injections of 0.5% (v/v) CCl_4 in olive oil. Twenty four hours after the CCl_4 injections, blood was drawn from the cervical vein for separation of serum, and ALT values in the serum were assayed using the method of Karmen[6]. In brief, the colorimetric change due to NAD synthesis in the conversion of pyruvic acid, which was produced by ALT from alanine, to lactic acid in the presence of lactic dehydrogenase, was measured by an autoanalyzer.

During the isolation of the hepatoprotective principles from the ethyl acetate solubles, silica gel column chromatography (solvent: benezene-ethyl acetate) followed by HPLC [column: Tosoh TSK gel ODS-120 A (30 cm × 2.15 cm I.D.); solvent: acetonitrile-water

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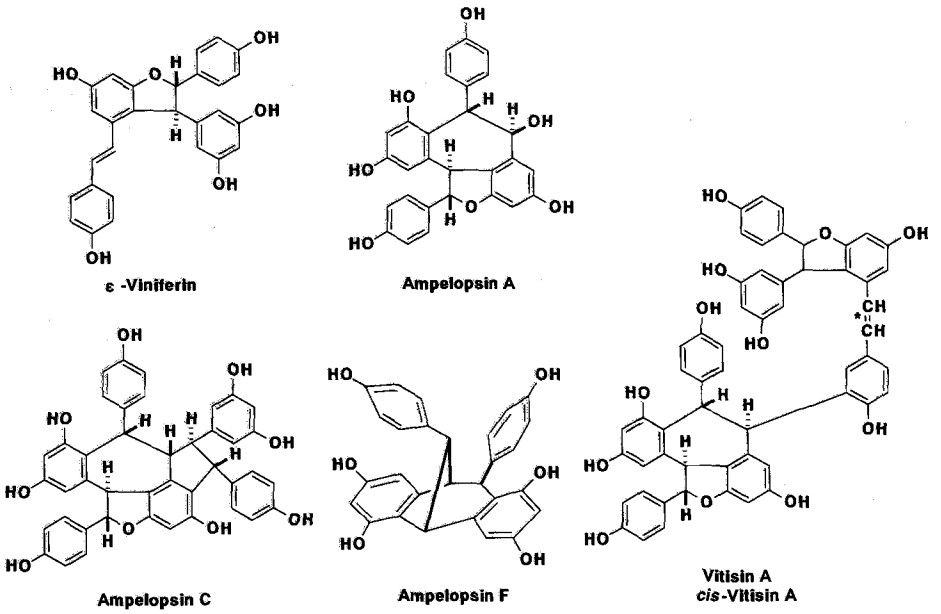

Figure 1. Chemical structures of oligostilbenes isolated from *Vitis coignetiae* (Vitaceae).

(25:75)] of the chloroform-methanol (9:1 and 8:2)-eluting fractions (20 and 8 g, respectively) of the ethyl acetate solubles yielded ampelopsins A (50 mg)[7], C (40 mg)[7] and F (70 mg)[8], as well as a vitisin A/*cis*-vitisin A mixture (100 mg)[9] in sufficient quantities for the assessment of their biological activity. The structures of the oligostilbenes, ampelopsins A, C and F, and the vitisin A/*cis*-vitisin A mixture were elucidated by detailed analysis of their spectroscopic data (fig. 1). Ampelopsin C and the vitisin A/*cis*-vitisin A mixture were administered to mice with or without CCl_4 treatment, at a dose of 30 mg/kg.

In the mice sacrificed at 24 h after the administration of the hepatic agents (CCl_4: 25 µl/kg p.o.), pathological examination of the liver tissues by light microscopy was performed to assess the degree of inflammatory cell infiltration.

Results and discussion

It was found that ε-viniferin injected into CCl_4-treated mice reduced ALT values significantly at a dose of 30 mg/kg (table 1).

Table 1. Effects of plant oligostilbenes pretreatment on the response to CCl_4

Drug	Dose (mg/kg)	No. of mice	ALT[a] (IU/l)	%
Control	-	7	4911 ± 749	100
ε-Viniferin	30	7	1964 ± 225++	40
Ampelopsin A	30	7	5599 ± 725	114
Ampelopsin C	30	4[b]	19152 ± 1339++	390
Ampelopsin F	30	7	4913 ± 752	100
Vitisin A/*cis*-vitisin A	30	5[c]	13308 ± 1111++	271

Statistical analyses were done by means of Student's *t* test.
++Significantly different from the control with $p < 0.01$.
[a]Values are mean ± SE. [b]Three mice died. [c]Two mice died.

Table 2. Effects on ampelopsin C and the vitisin A/*cis*-vitisin A mixture on ALT level

Drug	Dose (mg/kg)	No. of mice	ALT[a] (IU/l)	%
Control	-	7	25 ± 3	100
Ampelopsin C	30	7	3228 ± 614++	12912
Vitisin A/*cis*-vitisin A	30	7	4810 ± 1205++	19240

Statistical analyses were done by means of Student's *t* test.
++Significantly different from the control with $p < 0.01$.
[a]Values are mean ± SE.

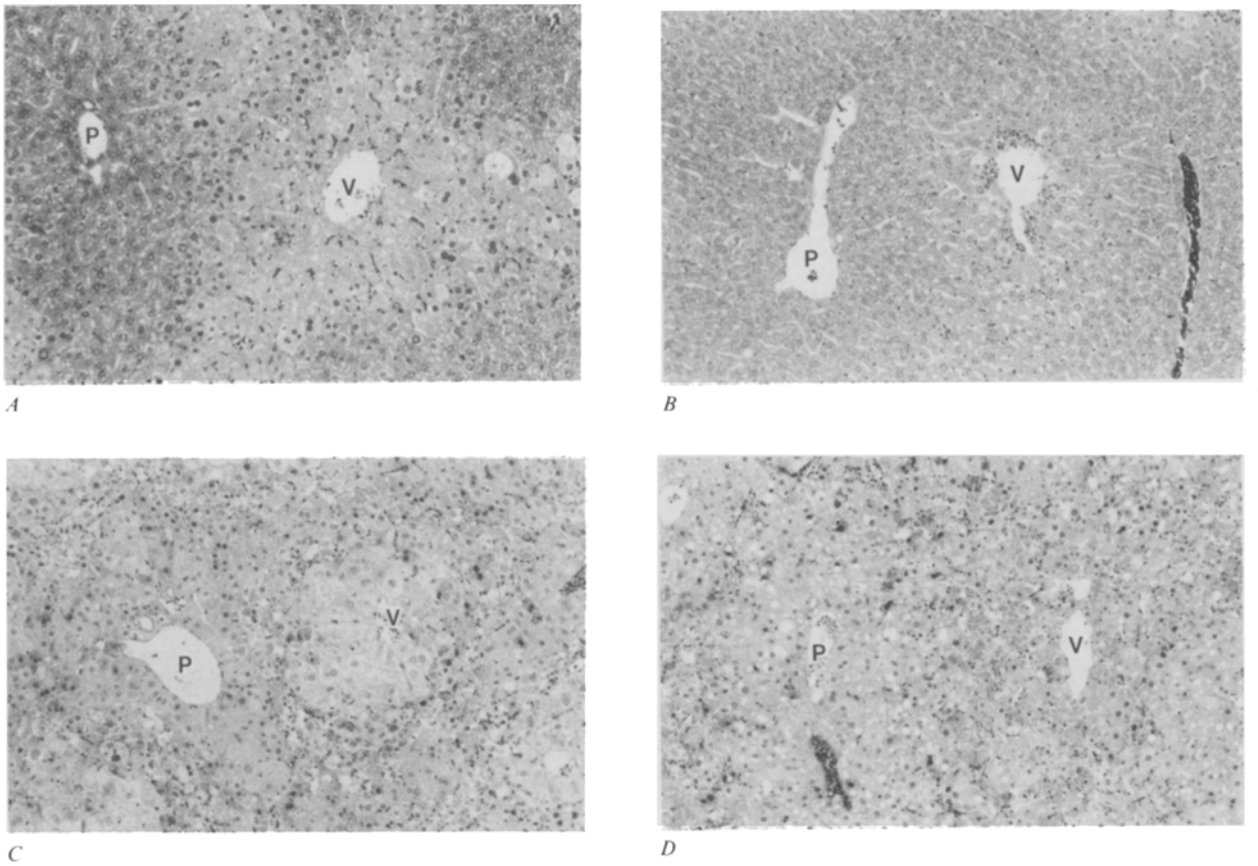

Figure 2. Pathological effects of oligostilbenes isolated from *Vitis coignetiae* (Vitaceae) on mouse liver. *A* Severe necrosis of the liver in mice treated with saline and CCl_4. There is zonal coagulative necrosis in the centrilobular area, and pyknotic nuclei in hepatocytes in the midlobular area. *B* Slight changes in the mice treated with ε-viniferin and CCl_4. There are a few degenerated hepatocytes, and focal infiltration of inflammatory cells around a central vein. *C* Swelling of the hepatocytes in centrilobular and midlobular hepatic necrosis, with collapse developed in the mice treated with ampelopsin C. *D* Centrilobular coagulative necrosis and fatty change in the periportal area developed in the mice treated with vitisin A/*cis*-vitisin A mixture.
V: central vein, P: portal tract. H&E; ×300 (*A*–*D*).

Ampelopsin C and the vitisin A/*cis*-vitisin A mixture increased ALT values approximately 4.1 and 5.7 times from the control value, respectively (table 1), when given at 30 mg/kg to CCl_4-treated mice. The administration of ampelopsin C and the vitisin A/*cis*-vitisin A mixture to mice at 30 mg/kg, without the injection of CCl_4, also showed that they were strong hepatotoxins (table 2).

The administration of CCl_4 to mice pretreated with 3% gum arabic saline solution produced intense centrilobular necrosis (fig. 2a). Pretreatment with ε-viniferin (30 mg/kg i.p.) resulted in a remarkable degree of protection against the coagulative necrosis in the mid- and centrilobular area caused by CCl_4 (fig. 2b). The area of centrilobular necrosis was measured by a Ludex Image Analyzer (Nikon) on the printed picture magnified 160 times. The necrosis area was decreased by ε-viniferin from 9.5 ± 4.4 (CCl_4 alone) to 3.6 ± 1.1 cm^2 (CCl_4 + ε-viniferin) or a 62% decrease. The difference was shown to be significant by means of Student's *t*-test ($p < 0.01$).

After the administration of ampelopsin C (30 mg/kg i.p.) (fig. 2c) and the vitisin A/*cis*-vitisin A mixture (30 mg/kg i.p.) (fig. 2d), severe pathological changes were observed in the mouse liver tissues, as in the CCl_4-injected mouse.

We succeeded in isolating the hepatoprotective substance ε-viniferin from an Oriental crude drug used in the treatment of liver diseases. It has been reported that an important aspect of the necrogenic activity of CCl_4 in liver is a so-called activation stage, in which CCl_4 is metabolized to a powerful destructive free radical form[10,11]. It is possible that the hepatoprotective mechanism of ε-viniferin is related to the metabolic activation stage of CCl_4. Detailed investigations of the pharmacological properties of ε-viniferin are under way.

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