of 3-hydroxy acids, which has been suggested by Bohlmann[12] for the biosynthesis of terminally unsaturated polyacetylenes in plants, does not hold for the production of 1-alkenes in insects. This is sufficiently evidenced by the entirely different metabolic fate of the labelled 3-hydroxy-12-phenyl[2,2-$^2H_2$]dodecanoic acid and the successful conversion of *E*-12-phenyl[2,3-$^2H_2$]-dodec-2-enoic acid in *T. confusum*. Instead, the experimental results are framed by an enantiospecific cleavage of the C-H bond of the *pro*-(*S*) C(3)-H of the substrate acid accompanied by fragmentation into an 1-alkene and carbon dioxide via an *anti*-periplanar transition state geometry.

The general course of this fragmentation of fatty acids into 1-alkenes and carbon dioxide is also in line with the biosynthesis of some algal pheromones like e.g. undeca-1,3,5-triene and undeca-1,3,5,8-tetraene from dodeca-3,6-dienoic- or dodeca-3,6,9-trienoic acid[14], respectively. Similarly, the fragmentation of nerolidol into the odoriferous homoterpene 4,8-dimethylnona-1,3,7-triene, and butenone, which appears to be a widespread reaction in flowering plants, fits into this general scheme[15]. Hence, it appears that the oxidative fragmentation of oxygen-containing precursors (acids, secondary and tertiary alcohols) into 1-alkenes, with concomitant oxidation of a C-O single bond to a C = O bond, is a ubiquitous reaction occurring on various substrates and in different forms of life. Further work on the activation of the precursors, namely abstraction of the *pro*-(*S*) C(3)-H atom as a radical or transient insertion of oxygen into the C(3)-H bond followed by immediate fragmentation at the active center of the enzyme, is necessary to understand this type of biotransformation in more detail. Efforts in this direction are under way and will be reported in due course.

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Circulatory and respiratory consequences of massive hemorrhage are reversed by protoveratrines

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Summary. In a rat model of severe hypotension and respiratory depression induced by step-wise bleeding, protoveratrines cause a prompt and sustained improvement of cardiovascular and respiratory functions, both in anesthetized and in conscious animals, seemingly through a magnification of the reflex response originated by the chemoreceptors of aortic and carotid bodies. The restoration of cardiovascular function is attributable to an increase both in total peripheral resistance and cardiac output. The finding could provide the basis for a new approach to the first-aid management of massive blood losses.
Key words. Hemorrhage; hypovolemic shock; hypotension; respiratory depression; protoveratrines.

Veratrum ester alkaloids of the ceveratrum group, when administered in μg/kg doses, exert widespread effects on many body functions[1,2]. Basically, they increase reflex excitability (Bezold-Jarisch reflex and other circulatory and respiratory reflexes, etc.) and sensitize receptors involved in afferent pathways in the apparent absence of normal stimuli (sensation of warmth in the face, mouth, throat, hands, feet and perineum, without reddening of the skin; prickling and tingling sensations). They increase the sodium conductivity of the membrane of excitable cells, increase and extend the negative afterpotential, and cause repetitive discharge in response to single stimuli in nerve and muscle. Stretch- and presso-receptors of the heart and of the carotid sinus are highly sensitive to this so-called 'veratrine response'[1,2]. In hypertensive and normotensive subjects, veratrum alkaloids increase the

train of impulses generated in such areas and the result is a reflex decrease in blood pressure and heart rate (Bezold-Jarisch effect), which justified the now obsolete use of these drugs for the management of hypertensive emergencies.

Conditions of decreased arterial pO_2 and/or increased pCO_2, as well as of decreased systemic blood pressure on the other hand, stimulate the chemoreceptors of aortic and carotid bodies, triggering a reflex response both of respiratory stimulation and, in cases of massive hemorrhage (or in any event of acute hypovolemia), of peripheral vasoconstriction with consequent increase in blood pressure[3]. We set out to discover whether this reflex is also magnified by veratrum alkaloids in a condition of hemorrhage-induced hypotension or even shock.

Materials and methods

Male and female Wistar rats weighing 240–280 g, were anesthetized (ethylurethane, 1.25 g/kg i.p.) and heparinized (heparin sodium, 600 IU/kg i.v.); indwelling catheters were then implanted in a common carotid artery and in an iliac vein. Systemic blood pressure and pulse pressure (PP) were recorded by means of a pressure transducer (Statham P23 Db) connected to a polygraph (Battaglia-Rangoni, Bologna, Italy). Heart rate was recorded and calculated by the same polygraph. Respiratory rate was recorded by means of three electrodes subcutaneously implanted in the chest and connected to the polygraph through an ARI A380 preamplifier. Blood was intermittently withdrawn from the venous catheter over a period of 25–30 min until mean arterial pressure (MAP) (= diastolic pressure plus one third of PP) fell to, and stabilized at, 40–50 mm Hg (hypovolemic hypotension) or 21–24 mm Hg (hypovolemic shock). This latter condition, if untreated, is invariably incompatible with survival, all animals dying within 20–30 min. The overall volume of blood removed was 1–1.5 ml/100 g b.wt to obtain hypovolemic hypotension, and 2–2.4 ml/100 g b.wt to obtain hypovolemic shock. Protoveratrine AB and protoveratrine B (Sigma Chemical Co., St. Louis, MO, USA), freshly dissolved in saline, were injected i.v. as a bolus. Control rats received equivalent volume of saline (0.1 ml/100 g b.wt).

In a set of experiments (n = 6) complete bilateral vagotomy was performed at the cervical level by tying two silk sutures around each vagal trunk, one above the other, and transecting each trunk between the sutures; the sutures served to ensure complete transection and to facilitate postmortem verification of disconnection. In some rats (n = 6), the carotid body area was surgically exposed, and the region of the bifurcation was infiltrated with a solution of 1 % lidocaine HCl 10–15 min before treatment. In an additional group of rats (n = 7), both bilateral vagotomy and carotid body anesthetization were performed.

In some animals (n = 10; randomly assigned to either saline or protoveratrine AB treatment) cardiac output was measured as ascending aorta flow by placing an electromagnetic flow probe (diameter 2.5 mm; Nycotron, Drammen, Norway) on the ascending aorta immediately above the heart. Late diastolic flow was taken to be zero. Signals from the flowmeter were recorded on the polygraph. These animals were previously intubated and ventilated artificially with room air using a stroke volume of approximately 2 ml/100 g b.wt and a rate of 55 strokes/min.

The effect of protoveratrine AB was also studied in conscious rats (n = 12). 2–3 days before the experiment, heparinized catheters for recording arterial blood pressure and for bleeding and i.v. injections respectively were implanted in a common carotid artery and in an iliac vein under ether anesthesia. The catheters were guided subcutaneously to the neck where they were exteriorized, filled with 0.9 % NaCl and closed with metal plugs. At the time of the experiment, the rat was placed into a small plastic cage (20 × 10 × 10 cm) with a grid lid, and the arterial catheter was connected to the pressure transducer. Stepwise bleeding was performed as previously described through the venous catheter, and when MAP fell to, and stabilized at, 22–26 mm Hg, the rats were randomly given i.v. either protoveratrine AB (n = 6) followed by 0.5 ml saline, or an equal volume of saline (n = 6). The MAP of these animals was recorded for 1 h, or until death if it occurred earlier. Thereafter, the catheters were closed with metal plugs and the animals were placed into single cages with food and water freely available, without any other treatment, and kept under observation for survival time.

Calculations and statistics

Cardiac output was indexed for body weight (cardiac index; CI) and expressed as ml/min/100 g b.wt. Total peripheral resistance index (TPRI; mm Hg/min/100 g b.wt/ml) was calculated from the MAP and CI. All data are given as mean ± SE. MAP and PP of all groups were first compared with each other by means of an analysis of variance (ANOVA), separate comparisons being made for data obtained before bleeding, after bleeding, 15–20 min and 120 min after treatment. In the case of after-treatment data, ANOVA was followed by Dunnett's test for multiple comparisons with a control. For this purpose MAP and PP values of dead rats were considered = 0; when appropriate, a Student's t-test for paired data was also used. Heart rate, respiratory rate, CI, TPRI and venous blood pO_2 were analyzed by Student's t-test, and survival rates by Fisher's exact probability test.

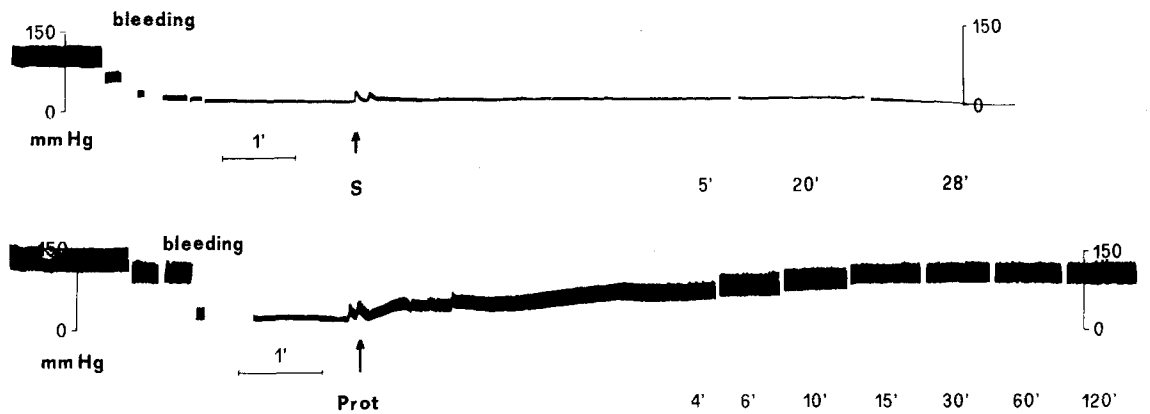
Results and discussion

As shown in table 1 and in the figure, in a model of bleeding-induced hypovolemic shock in anesthetized rats, the i.v. injection of protoveratrine AB 5 min after shock induction caused a prompt (within 30–60 s) and dose-dependent reversal of arterial hypotension and respiratory depression (see below). The effect reached a

Table 1. Protoveratrines improve mean arterial pressure (MAP), pulse pressure (PP) and survival in rats bled to hypovolemic shock (a) or to hypovolemic hypotension (b)

Treatment (μg/kg i.v.)	$\frac{MAP}{PP}$ (mm Hg; $\bar{m} \pm SE$)				No. of deaths 120 min after treatment/No. of treated rats
	Before bleeding	After bleeding	15–20 min after treatment	120 min after treatment	
a)					
Saline	$\frac{99 \pm 5}{39 \pm 4}$	$\frac{24 \pm 2}{10 \pm 1}$	$\frac{26 \pm 3}{11 \pm 2}$	$\frac{0}{0}$	10/10
Protoveratrine AB, (1.25)	$\frac{103 \pm 8}{30 \pm 2}$	$\frac{23 \pm 1}{7 \pm 1}$	$\frac{33 \pm 6}{14 \pm 5}$	$\frac{12 \pm 8}{7 \pm 5}$	6/8
Protoveratrine AB, (2.5)	$\frac{100 \pm 3}{30 \pm 3}$	$\frac{22 \pm 1}{8 \pm 1}$	$\frac{62 \pm 10^*}{28 \pm 3^*}$	$\frac{52 \pm 13^*}{20 \pm 5^*}$	2/8**
Protoveratrine AB, (5)	$\frac{104 \pm 6}{43 \pm 3}$	$\frac{22 \pm 1}{10 \pm 2}$	$\frac{83 \pm 8^*}{34 \pm 5^*}$	$\frac{70 \pm 6^*}{35 \pm 6^*}$	0/10**
Protoveratrine AB, (10)	$\frac{98 \pm 4}{38 \pm 3}$	$\frac{22 \pm 1}{9 \pm 1}$	$\frac{92 \pm 4^*}{42 \pm 4^*}$	$\frac{83 \pm 5^*}{41 \pm 4^*}$	0/8**
Protoveratrine B, (5)	$\frac{103 \pm 8}{37 \pm 4}$	$\frac{22 \pm 1}{8 \pm 1}$	$\frac{79 \pm 6^*}{36 \pm 4^*}$	$\frac{66 \pm 5^*}{35 \pm 5^*}$	0/8**
b)					
Saline	$\frac{90 \pm 5}{45 \pm 4}$	$\frac{50 \pm 3}{30 \pm 3}$	$\frac{65 \pm 4}{37 \pm 3}$	$\frac{58 \pm 5}{37 \pm 4}$	0/10
Protoveratrine AB, (5)	$\frac{87 \pm 4}{51 \pm 3}$	$\frac{44 \pm 2}{28 \pm 2}$	$\frac{87 \pm 2^*}{56 \pm 2^*}$	$\frac{88 \pm 3^*}{55 \pm 4^*}$	0/10
Protoveratrine B, (5)	$\frac{89 \pm 6}{47 \pm 5}$	$\frac{47 \pm 3}{31 \pm 2}$	$\frac{90 \pm 4^*}{48 \pm 5^*}$	$\frac{90 \pm 5^*}{49 \pm 4^*}$	0/10

a) MAP before bleeding: $p > 0.05$; PP before bleeding: $p > 0.05$; MAP after bleeding: $p > 0.05$; PP after bleeding: $p > 0.05$; MAP 15–20 min after treatment: $p < 0.001$; PP 15–20 min after treatment: $p < 0.001$; MAP 120 min after treatment: $p < 0.001$; PP 120 min after treatment: $p < 0.001$ (ANOVA). *$p < 0.001$ versus the corresponding value of saline-treated rats (Dunnett's test). **$p < 0.005$ versus the corresponding value of saline-treated rats (Fisher's test). b) MAP before bleeding: $p > 0.05$; PP before bleeding: $p > 0.05$; MAP after bleeding: $p > 0.05$; PP after bleeding: $p > 0.05$; MAP 15–20 min after treatment: $p < 0.001$; PP 15–20 min after treatment: $p < 0.001$; MAP 120 min after treatment: $p < 0.001$; PP 120 min after treatment: $p < 0.001$ (ANOVA). *$p < 0.001$ versus the corresponding value of saline-treated rats (Dunnett's test).



Representative recordings showing the effect of the i.v. injection of saline (S) or protoveratrine AB (Prot) on arterial blood pressure and pulse amplitude in rats bled to hypovolemic shock. S: 1 ml/kg; Prot: 10 μg/kg.

maximum within 15–20 min and was sustained, being still highly significant 2 h after treatment. Survival, as assessed 2 h after treatment, was 25 % and 75 % following doses of 1.25 and 2.5 μg/kg respectively, and 100 % following doses of 5 and 10 μg/kg, while all saline-treated rats died within 25 ± 6 min (table 1). The effect of protoveratrine B, tested at the single dose of 5 μg/kg was not different from that of an equal dose of protoveratrine AB (table 1). In our experimental conditions, heart rate was not affected by 10 μg/kg i.v. protoveratrine AB (400 ± 20 beats/min before bleeding; 340 ± 18 after bleeding; 350 ± 21, 15–20 min after treatment). On the

Table 2. Influence of bilateral cervical vagotomy and anesthetization of the carotid-body areas on the effect of protoveratrine AB in rats bled to hypovolemic shock

Experimental condition	Treatment (μg/kg i.v.)	$\frac{MAP}{PP}$ (mm Hg; $\bar{m} \pm S E$)				No. of deaths 120 min after treatment/No. of treated rats
		Before bleeding	After bleeding	15–20 min after treatment	120 min after treatment	
Sham-operated	Saline	$\frac{101 \pm 5}{49 \pm 4}$	$\frac{24 \pm 2}{10 \pm 1}$	$\frac{27 \pm 3^*}{12 \pm 3^*}$	$\frac{0^*}{0^*}$	6/6***
Sham-operated	Protoveratrine AB (10)	$\frac{99 \pm 4}{50 \pm 5}$	$\frac{25 \pm 1}{9 \pm 1}$	$\frac{95 \pm 3}{49 \pm 4}$	$\frac{85 \pm 4}{48 \pm 3}$	0/8
Bilateral vagotomy	Protoveratrine AB (10)	$\frac{89 \pm 5}{52 \pm 4}$	$\frac{22 \pm 1}{13 \pm 2}$	$\frac{81 \pm 4}{44 \pm 5}$	$\frac{73 \pm 5}{44 \pm 4}$	0/6
Bilateral vagotomy and carotid-body anesthetization	Protoveratrine AB (10)	$\frac{108 \pm 4}{48 \pm 4}$	$\frac{23 \pm 1}{10 \pm 1}$	$\frac{33 \pm 6^*}{15 \pm 4^*}$	$\frac{13 \pm 8^*}{8 \pm 5^*}$	5/7**
Carotid-body anesthetization	Protoveratrine AB (10)	$\frac{98 \pm 5}{49 \pm 3}$	$\frac{22 \pm 1}{12 \pm 1}$	$\frac{61 \pm 8^*}{35 \pm 4^*}$	$\frac{31 \pm 10^*}{23 \pm 9^*}$	2/6

MAP before bleeding: $p > 0.05$; PP before bleeding: $p > 0.05$; MAP after bleeding: $p > 0.05$; PP after bleeding: $p > 0.05$; MAP 15–20 min after treatment: $p < 0.001$; PP 15–20 min after treatment: $p < 0.001$; MAP 120 min after treatment: $p < 0.001$; PP 120 min after treatment: $p < 0.001$ (ANOVA). *$p < 0.01$, at least, versus the corresponding value of sham-operated and protoveratrine AB-treated rats (Dunnett's test). **$p < 0.01$ and ***$p < 0.005$ versus the corresponding value of sham-operated and protoveratrine AB-treated rats (Fisher's test).

other hand, the bleeding-induced respiratory depression was reversed (120 ± 5 breaths/min before bleeding; 44 ± 3 after bleeding; 100 ± 4, 15–20 min after treatment; $p < 0.001$ compared with saline-treated rats; Student's t-test); as was the reduction in venous blood pO_2 (52 ± 4 mm Hg before bleeding; 22 ± 2 after bleeding; 40 ± 4, 15–20 min after treatment; $p < 0.001$ compared with saline-treated rats; Student's t-test). CI (28.3 ± 4.18 ml/min/100 g b.wt before starting bleeding) fell to 18.3 ± 2.60 during shock, and raised to 27.3 ± 3.72 within 15–20 min after i.v. injection of protoveratrine AB, 10 μg/kg ($p < 0.001$ compared with saline-treated rats; Student's t-test). The same treatment induced a likewise almost complete restoration of TPRI: 3.50 ± 0.41 mm Hg/min/100 g b.wt/ml before starting bleeding, 1.37 ± 0.11 during shock, 3.33 ± 0.51 within 15–20 min after protoveratrine injection ($p < 0.001$ compared with saline-treated rats; Student's t-test).

In a condition of bleeding-induced hypotension, again in anesthetized rats, with MAP values of 40–50 mm Hg, a significant reversal of hypotension was observed even following saline injection ($p < 0.01$ versus value after bleeding; Student's t-test for paired data); however, the effect of protoveratrines AB and B, i.v. injected at the single dose-level of 5 μg/kg, was significantly greater, MAP and PP being completely restored within 15–20 min after treatment and the effect remaining stable throughout the 2 h of observation (table 1).

Bilateral vagotomy at the cervical level had no influence on the protoveratrine-induced shock reversal, which was, however, almost completely prevented by the concurrent bilateral anesthetization of the carotid body areas and bilateral vagotomy, and only partly prevented by the carotid bodies anesthetization alone (table 2), suggesting that both aortic and carotid body chemoreceptors are involved.

In conscious rats step-wise bled to hypovolemic shock, the i.v. injection of protoveratrine AB at the dose of 10 μg/kg had an effect quite similar to that observed in anesthetized animals, with prompt and sustained reversal of the hypotension; and while all control (saline-treated) rats died within 35 min after treatment, all protoveratrine treated ones were still surviving 15 days after the experiment ($p < 0.005$; Fisher's test).

In conditions of severe or extreme hypotension, the impulses originating from the stretch- and presso-receptors of the heart and of the carotid sinus are greatly reduced[1,2], while those originating from the chemoreceptors of the carotid bodies are enhanced by the hemorrhage-induced decreased systemic blood pressure[4,5]. The present data indicate that protoveratrines cause a prompt and sustained reversal of the respiratory and cardiovascular depression seemingly through the magnification of this latter reflex. Moreover our results indicate that the rise in blood pressure is due both to an increase in total peripheral resistance and to an increase in cardiac output. Particularly impressive and important, in our opinion, is the fact that a complete shock reversal has been obtained also in conscious animals, with 100% survival without any other treatment. Of course, our present data must be extended and confirmed in other animal species before speculating a possible use of protoveratrines in the first-aid management of hemorrhagic shock in humans.

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Increase in the survival time of mice exposed to ionizing radiation by a new class of free radical scavengers

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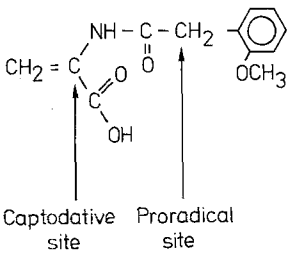
Summary. N-acyl dehydroalanines react with and scavenge mainly superoxide radical ($O_2^{\cdot-}$) and hydroxyl radical ($HO^{\cdot}$). The ortho-methoxyphenylacetyl dehydroalanine derivative, indexed as AD-20, protects mice against damage resulting from total body X-irradiation, as measured by the increase in their survival time. AD-20 increases the LD_{50} at 30 days from 6.1 to 7.3 Gy in animals exposed to a wide range of X-rays (6 to 10 Gy). The dose reduction factor (D R F) of AD-20 is 1.20. We postulate that such radioprotective effect may result from its free radical scavenging activity.
Key words. Oxygen-derived free radical scavenger; N-acyl dehydroalanines; ionizing radiation toxicity; radioprotective effect.

Ionizing radiation, through production of oxygen radical species, can result in DNA damage, especially by forming thymidine hydroperoxide [1]. Moreover, ionizing radiation may overwhelm the balance between the enzymatic protective defense and the production of endogenous oxidizing species such as superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($HO^{\cdot}$) and singlet oxygen ($^1O_2$), leading to a metabolic amplification of the initial physico-chemical damage.

Recently a new way to modulate the reactivity of free radicals has been proposed [2]. Olefins which are substituted at the geminal carbon atom by both an electron-donating and an electron-withdrawing group (captodative substitution), have been shown to inactivate free radicals by forming stabilized free radical adducts, which do not polymerize but disappear mostly by dimerization or by reacting with another free radical [3]. Among these molecules, we have reported that the N-acyl dehydroalanines (indexed as AD compounds) react with and scavenge $O_2^{\cdot-}$ and $HO^{\cdot}$ [4,5]. They inhibit both in vitro and in vivo processes mediated by free radicals [6–8]. AD compounds inhibit lipid peroxidation initiated when rat liver microsomal suspensions are exposed to gamma rays [9], showing that they may give protection against the deleterious effects of ionizing radiation.

In the present work we examined the capacity of AD 20 (see structure of (ortho-methoxyphenylacetyl)-dehydroalanine) to inhibit in vivo radiation damage. The parameters observed in order to analyse the radioprotective effect of AD-20 were the percentage of survivors and the survival time of animals exposed to a total body X-ray irradiation.



Structure of AD-20.

Materials and methods

Animals. Female NMRI mice, weighing approximately 25 g, were obtained from Animalerie Centrale – UCL. They were housed in groups of 10 in plastic cages ($40 \times 25 \times 15\ cm^3$) and fed with standard food (AO4, UAR, France) and tap water ad libitum.

Irradiation procedures. Mice were exposed in groups of 10 to 250 kV X-rays (Phillips, RT 200/250) while restrained in perforated lucite cages placed on a rotatory disk at 60 cm from the source. The disk rotated in a horizontal plane during exposure. The filtration was 1.0 mm of copper, and the dose rate was 0.6 Gy/min. Animals were observed during a period of 30 days, and mortality was recorded daily. Mice were not left to die spontaneously; when they had become paralysed and