on day 3 ($p < 0.01$). Thereafter, the serum calcium concentration rises again toward control levels (fig.). Glucagon administration evokes a progressive decrease in the serum inorganic phosphorus level (fig.). This fall outlives the hypocalcemic response of glucagon.

Discussion. The glucagon-induced hypocalcemia in *Rana tigrina* is in consonance with the earlier reports using other vertebrates[1-13,17]. This also derives support from the enhancement of urinary excretion of calcium and other electrolytes in dog[18-21] and man[22] after glucagon administration.

In mammals, glucagon has been reported to stimulate calcitonin release[1,2,7,17]. The hypocalcemia observed in the present study may be attributed to the release of the hypocalcemic factor from the ultimobranchial cells (known to be the source of calcitonin in non-mammals[23,24]) as is evident from the activity of the gland, occurrence of hyperplasia up to day 3 and thereafter, indications of the degeneration of the gland (own, unpublished results).

Glucagon also induces hypophosphatemia in *R. tigrina*. This is in agreement with earlier reports[1,2,17,25-27]. This response can be attributed to the enhanced release of calcitonin caused by glucagon treatment. Talmage et al.[28] have reported that calcitonin lowers the serum calcium by preventing release of calcium from bone whereas it (calcitonin) lowers the serum phosphate by increasing its exit from the circulation rather than by inhibiting its release from bone. Thus, the hypocalcemic and hypophosphatemic effects of calcitonin are independent.

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Ca-antagonistic substance from soft coral of the genus *Sarcophyton*[1]

J. Kobayashi, Y. Ohizumi, H. Nakamura, T. Yamakado, T. Matsuzaki and Y. Hirata

Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooya, Machida-shi, Tokyo 194 (Japan), Research Center, Mitsubishi Chemical Industries Ltd, Kamoshida, Midori-ku, Yokohama 227 (Japan), and Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468 (Japan), April 27, 1982

Summary. A 14-membered ring diterpenoid named cembrane, possessing Ca-antagonistic action on the isolated rabbit aorta, has been isolated from a soft coral of the genus *Sarcophyton*, and its structure established by X-ray and spectroscopic data.

As part of a program to search for biologically active substances from marine organisms[2-7], methanol extracts of numerous soft corals[8] collected in Okinawa water were screened on the isolated rabbit aorta. The screening revealed that the extract of a soft coral of the genus *Sarcophyton*, like Ca-antagonists[9-11], inhibited markedly the contraction of the aorta induced by KCl but did not affect that induced by norepinephrine (NE). In this paper, the isolation and structure of the Ca-antagonistic substance **I** from a soft coral of the genus *Sarcophyton* are described.

Male albino rabbits (2-3 kg) were used. The procedure for preparing the rabbit isolated aorta and the technique of measurement of contractions were carried out as previously described[12]. Soft corals (wet wt 450 g), collected in October 1981 and stored at −20 °C until used, were homogenized in methanol and extracted with the same solvent. The com-

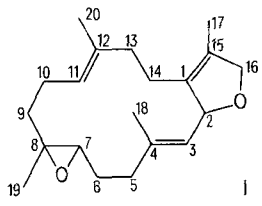

Figure 1. Chemical structure of compound **I**.

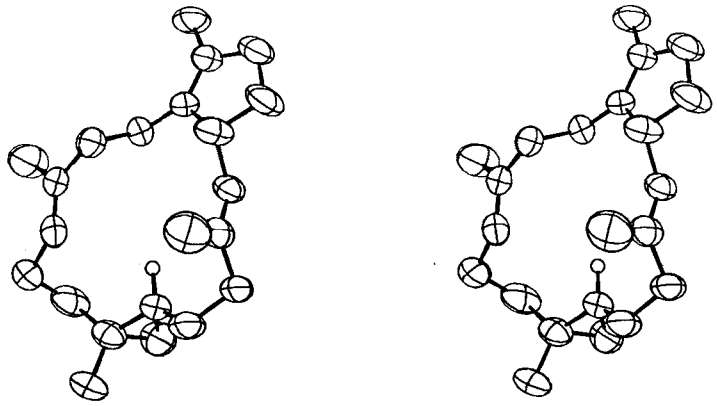

Figure 2. Stereoscopic view of a single molecule of compound **I**.

bined extracts were concentrated in vacuo and the remaining aqueous mixture was extracted with ethyl acetate. The oily residue (7.6 g) from the ethyl acetate extract was chromatographed on a silica gel column using n-hexane/ethyl acetate (3:1) as mobile phase. The eluates exhibiting Ca-antagonistic activity were collected and concentrated in vacuo. The oily residue (3.5 g) was crystallized from a small amount of n-hexane to afford colorless prisms of **I** (2.1 g, 0.46% wet weight of the soft coral), mp. 77–78 °C and $[\alpha]_D + 157°$ (c = 1.0, methanol)[13].

The chemical structure of **I** was determined (fig. 1) by the following physicochemical data. Mass spectrum: m/z 302 (M^+, 12%) ($C_{20}H_{30}O_2$), 286 (8), 121 (38), 107 (33), 93 (34), 81 (45), 67 (53), 55 (52), 43 (100). The ^{13}C NMR-spectrum (JEOL 25.2 MHz, $CDCl_3$) showed 20 resolved carbons: δ 10.2 (q), 15.1 (q), 15.6 (q), 17.0 (q), 23.5 (t), 25.3 (t), 26.1 (t), 36.7 (t), 37.6 (t), 39.8 (t) 59.8 (s), 61.9 (d) 78.4 (t), 83.7 (d), 123.6 (d), 126.3 (d), 127.8 (s), 133.2 (s), 136.7 (s), 139.2 (s). The first 4 and the last 6 signals were attributed to the 4 methyl carbons and the 6 olefinic carbons, respectively. Compound **I** was suspected to possess 2 ethereal linkage, since neither OH nor CO absorption could be observed in the IR-spectrum (KBr): 2900, 1650, 1440, 1380, 860, 840 cm^{-1}. Furthermore, neither an absorption maximum of UV nor Cotton effect of CD could be detected for **I** in the range of wavelengths above 200 nm. The ^1H NMR-spectrum (Bruker 270 MHz, $CDCl_3$) showed signals for 4 methyl groups at δ 1.27 (3H, s, C-19), 1.60 (3H, s, C-20), 1.65 (3H, s, C-17) and 1.83 (3H, s, C-18), 2 vinyl protons at δ 5.11 (1H, dd, J = 5.5 and 10.0 Hz, C-11) and 5.23 (1H, d, J = 10.0 Hz, C-3) and 4 ethereal protons at δ 2.72 (1H, t, J = 4.5 Hz, C-7), 4.50 (2H, br s, C-16) and 5.54 (1H, m, C-2), respectively. The remainder of the spectrum exhibited well-resolved signals at δ 1.00 (1H, ddd, J = 13.5, 13.5 and 2.5 Hz, C-9), 2.12 (1H, ddd, J = 13.5, 13.0 and 2.5 Hz, C-9) and 2.54 (1H, m, C-5), and overlapping signals at δ 1.6–1.7 (2H, C-6 and 13, hindered by the 2 methyl signals), 1.8–2.0 (4H, C-5, 6, 10 and 14) and 2.2–2.4 (3H, C-10, 13 and 14), respectively. The assignments of the signals were deduced from the double resonance and nuclear Overhauser effect experiments.

The structure of **I** was established by X-ray analysis[14]. Crystals of **I** suitable for X-ray analysis were obtained by recrystallization from n-hexane. Crystal data: orthorhombic, $a = 11{,}640$ (1) Å, $b = 18.748$ (3) Å, $c = 8{,}592$ (1) Å, $v = 1875.0$ Å^3, $z = 4$, $\rho_{calc} = 1.071$ g cm^{-3}, space group $P\,2_12_12_1$. The structure was solved by the direct method and has been refined to an R-factor of 0.091 on 1813 intensities (3σ-level) collected on a Nonius CAD4 diffractometer (Cu K_α). The absolute configuration was not established. Further details of the X-ray work will be published elsewhere. A stereoscopic view of the molecule is shown in figure 2.

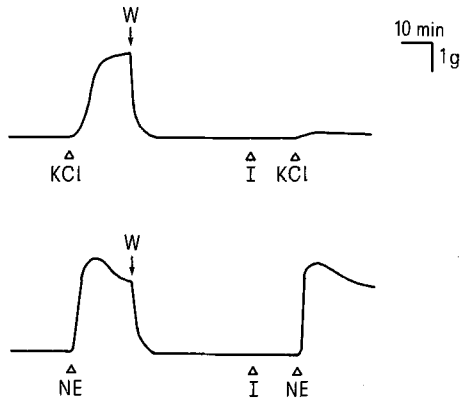

Figure 3. Effects of compound **I** (3×10^{-5} M) on the contracture induced by KCl (4×10^{-2} M) and norepinephrine (NE, 10^{-6} M) in the rabbit isolated aorta. Drugs and **I** were applied at Δ. KCl and NE were added 15 min after administration of **I**. Drugs were washed twice (W) with the fresh medium at arrows.

It has been proposed that in vascular smooth muscle the KCl-induced contraction is due to an increase in Ca influx, while the NE-induced contraction is attributed to the facilitation of release of intracellularly stored Ca[15–17]. Ca-antagonists such as verapamil and D-600 had a specific inhibitory effect on the KCl-induced contraction of vascular smooth muscles[9–11]. Compound **I** (3×10^{-5} M) as well as their Ca-antagonists nearly abolished the KCl (4×10^{-2} M)-induced contraction of the aorta but did not affect that induced by NE (10^{-6} M) (fig. 3). These observations suggest that compound **I** selectively inhibits Ca influx into the muscle cells of the aorta. Further clarification of the pharmacological properties of **I** is in progress.

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Superoxide dismutase activity in rabbit reticulocytes[1]

Y. Kobayashi, Y. Yoshimitsu, S. Okahata and T. Usui

Department of Pediatrics, Hiroshima University School of Medicine, Hiroshima 734 (Japan), March 22, 1982

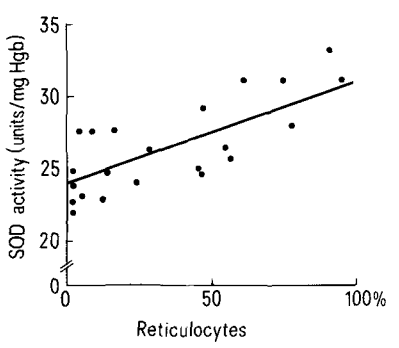
Summary. Reticulocytosis was induced in rabbits by bleeding anemia and erythrocyte superoxide dismutase activity was determined. Reticulocytes were found to contain about 1.3 times as much activity as mature erythrocytes.

Superoxide dismutase (SOD, EC 1.15.1.1) is ubiquitously present in oxygen-metabolizing cells, and serves as the first line of defense mechanism against oxygen toxicity[2,3]. The role of this enzyme is the subject of current intensive research, and clinical implications of SOD levels have been evaluated in physiological as well as pathological conditions. One example is the process of aging[4,5], and SOD activity was measured in whole organisms[6,7], several organs[4,8,9], and in certain types of cells[10,11]. Because of their relative ease of sampling and limited life span, erythrocytes are a suitable model for such a study. In the report of Bartosz et al.[10] bovine erythrocyte SOD was shown to decrease during aging. The purpose of this article is to extend further the above observation and to demonstrate that reticulocytes, the youngest erythrocytes in the circulatory system, have the highest SOD activity.

Materials and methods. Reticulocytosis was induced in 3 male rabbits weighing 2.1–2.5 kg by successive daily bleeding through ear vessels of about 10 ml/kg. When the peripheral reticulocyte count exceeded 30%, usually attainable on the 6th–8th days, heparinized blood was centrifuged and the plasma and buffy coat removed. After washing and diluting with saline, the erythrocyte suspension was then applied to a differential centrifugation in a gum acacia solution according to the method of Kimura et al.[12], and specimens with various reticulocyte concentrations were prepared. Daily bleeding was usually terminated on the 8th day and was followed by an i.v. injection of saccharated ferric oxide, 20 mg/kg. SOD activity was measured by the ferricytochrome c reduction inhibition method of McCord and Fridovich[2] at pH 10[13]. As proposed by McCord and Fridovich[2], 1 unit of activity was defined as the amount of enzyme activity which inhibits the rate of reduction of ferricytochrome c by 50%. SOD activity was then calculated on logit paper[14] and expressed as units/g hemoglobin, as the relationship between cell count and hemoglobin content was virtually parallel.

Results. The figure shows SOD activities of erythrocytes as a function of the reticulocyte population. The results for the 3 rabbits were combined as they were comparable. As shown in the figure, SOD activity increases as the reticulocyte population increases. The relationship is calculated in the following equation; $y = 0.07\ x + 24.08$, where y is an SOD activity, and x is a percentage of reticulocytes ($r = 0.71$, $p < 0.01$). Enzyme activity of reticulocytes was completely inhibited by 1 mM cyanide.

Discussion. The present study indicates that the erythrocyte SOD activity increases correspondingly with an increase in the reticulocyte population, and that reticulocytes have about 1.29 times as much activity as mature erythrocytes. This finding may be interpreted as a counterpart of observations of Bartosz et al.[10] that the activity of this enzyme decreases during erythrocyte aging. Furthermore, this increase is found to be accompanied by elevations of erythrocyte copper and zinc[15], both of which are constituent metals of the enzyme. Although the differences between the animal species studied and of methods of determination of



Rabbit erythrocyte SOD activity in relation to reticulocyte population.