was continued for 1 h, hydrogen fluoride was removed in vacuo. To the residue was added 10% sodium carbonate solution (50 ml) and insoluble materials were filtered off. The filtrate was treated with an ion exchange resin, Amberlite IR 120 (H^+ form). The resulting amino acid was eluted with 1% hydrochloric acid and the eluate was evaporated to dryness under reduced pressure. The product was dissolved in water (10 ml) and the pH was adjusted to about 3 with ammonia. After standing overnight in a refrigerator, the resulting crystals were collected and washed with ethanol and then dried. The expected β-fluoroaspartic acid of 3.78 g (25%) was obtained. Structural identification of the amino acid was made by NMR-spectroscopy and elemental analysis as follows. M.p. 164–166 °C (dec.) (recrystallized from H_2O). ^1H NMR (60 MHz, D_2O+CF_3COOD), δ, 4.88 (d,d, 1H, α-CH, $J_{HH}=1.8$ Hz, $J_{HF}=28.8$ Hz), 5.62 (d,d, 1H, β-CH, $J_{HH}=1.8$ Hz, $J_{HF}=46.2$ Hz). Anal. Calculated for $C_4H_6O_4NF$: C, 31.79; H, 4.00; N, 9.27; F, 12.57; found: C, 31.99; H, 4.03; N, 9.52; F, 12.56. Paper electrophoresis (2000 V, buffer pH 3.8, 60 min): mobility = +7.9 cm. Paper chromatography (n-BuOH:CH_3COOH:H_2O = 5:3:1): R_f = 0.09. Although the stereochemistry of the resulting β-fluoroaspartic acid could not be determined from the available data, configuration of this amino acid would be *erythro*-form, judging from the nitrous acid deamination of α-amino acid which had generally been recognized to occur with retention of configuration[17]. In addition, the assumption was supported by finding a small amount of *erythro*-β-hydroxyaspartic acid as a by-product in the above reaction mixture on a paper electrophoresis. Further investigation of the stereochemistry and synthesis of the *threo*-isomer are currently in progress.

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Observation of pentacoordinated phosphorus intermediate in the reactions of nitrones with phosphonate anions

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Summary. The occurrence of a high-field signal in the ^{31}P FT NMR-spectrum of the reaction mixture of nitrones (*1* or *2*) and 2-cyanomethyl-4,5-dimethyl-2-oxo-1,3,2-dioxyphospholane (*7*), is interpreted in terms of a pentacoordinated phosphorus intermediate.

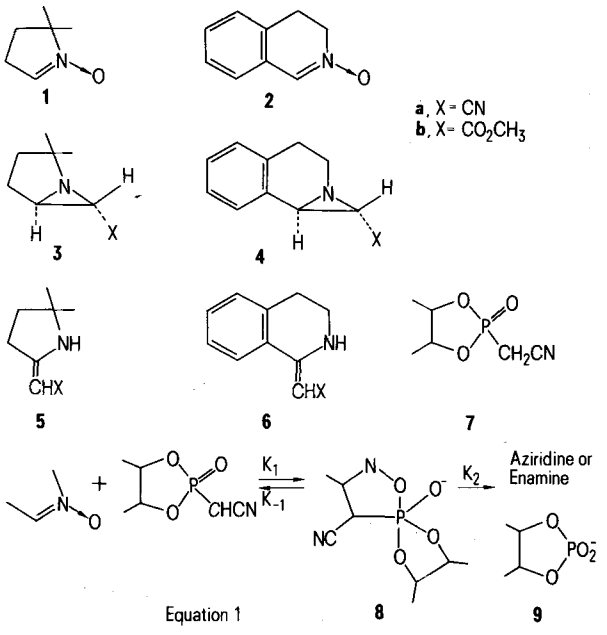
Previously we postulated that the reactions of nitrones (e.g. *1* or *2*) with carbanions of phosphonates[1,2,3] or phosphinoxides[4], that lead to aziridines (e.g. *3* or *4*) or to enamines (e.g. *5* or *6*), proceed via oxazaphospholidine intermediates (such as *8*) containing pentacoordinated phosphorus. In this communication we wish to present evidence that confirms this assumption.

Recently we reported that the reaction of the 5-membered cyclic phosphonate (*7*)[5] with *1* leads exclusively to aziridine *3a*[6], while that of *7* with *2* leads to the enaminonitrile *6a*[7]. In our quest for evidence concerning the existence of intermediate of type *8*, we focused our efforts on the reactions of this phosphonate (*7*), after failing to observe such an intermediate in reactions of open chain phosphonates and phosphinoxides. It is known that maximum stability of pentacoordination is attained when the phosphorus is part of a 'small' ring[8–13].

As experimental technique we have chosen ^{31}P FT NMR-spectroscopy (using a Bruker WP-60 instrument at 24.2 MHz). By this technique it is possible to observe species, even if they exist only as transient intermediates, provided that their steady-state concentration is sufficiently high. The steady-state concentration of *8* is proportional to $K_1/K_{-1}+K_2$ (see equation 1). Initial attempts to observe *8* were made by monitoring the reaction of *1* with *7* using sodium hydride in tetrahydrofuran at temperatures ranging from 0 °C to −60 °C, collecting spectra from 1000 scans at intervals of 10 °C (a fresh sample was used at each temperature). In these spectra we could observe signals at −30, −50 and −15 ppm (downfield from 85% H_3PO_4 as external standard) resulting from the phosphonate *7*[5], its anion and the cyclic phosphate *9*[14] respectively. The presence of the latter indicated that at all these temperatures the reaction proceeded. Following this we have considered that as the 1st step of the reaction is expected to be the rate-determining one, its activation energy will be higher than those of the 2nd step and of the reverse reaction (if there is such). Therefore the value of K_1 will be much more temperature-

dependent than those of K_{-1} and of K_2. Consequently, raising the temperature will result in an increase of the value of $K_1/K_{-1}+K_2$ and with it the steady-state concentration of *8*.

Indeed, when examining the reaction of *1* with *7* in tetrahydrofuran at 25 °C after 1000 scans, we could observe a signal at +36 ppm (upfield from 85% H_3PO_4) indicating the existence of a pentacoordinated phosphorus derivative, in addition to signals arising from starting materials and product. The same phenomenon could be observed in the following solvents: N,N-dimethylformamide (+34.7 ppm), chlorobenzene (+35.8 ppm) and 1,2-dimethoxyethane (DME) (+38.0 ppm). Thin layer chromatographic examination of the reaction mixtures showed the presence of aziridine *3a* as the sole product of the reactions. Monitoring the reaction of 3,4-dihydroisoquinoline N-oxide (*2*) with *7* in DME using sodium hydride at room temperature, we could also observe a signal at +36.3 ppm. Since, in contrast to the reaction of *1* with *7*, the latter reaction leads only to enaminonitrile (*6a*), our observations described in this communication supply evidence in support of the assumption we have expressed previously, that both types of products, namely aziridines and enamines are formed from a common oxazaphospholidine intermediate.



Equation 1

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Catalase and dehydroascorbate reductase in human polymorphonuclear leukocytes (PMN): Possible functional relationship

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Summary. In leukocytes (PMN) of individuals with Swiss type acatalasemia, the rate of dehydroascorbate reduction is 4 times normal. This observation suggests that the protective function served by catalase in human PMN is supported by dehydroascorbate reductase.

Catalase (E.C.1.11.1.6.) and dehydroascorbate reductase (E.C.1.6.5.4.) may constitute parts of cellular mechanisms which protect against and repair oxidant injury to biomolecules[2-4]. In tissues, H_2O_2 degradation is catalyzed by catalase and glutathione peroxidase. Their relative contribution varies, depending on differences in enzyme concentration among tissues and species, rates of peroxide generation and experimental conditions[5,6]. Only the most profoundly deficient variants of human hereditary acatalasemia (Japanese type) predispose to disease. About half of such individuals develop ulcerative *gingivitis* and *stomatitis*, presumably because of local growth of catalase-negative bacteria producing tissue-damaging amounts of H_2O_2[7]. Acatalasemic red cells accumulate more methemoglobin than do normal erythrocytes during exposure to ionizing irradiation[2]. H_2O_2, one of the oxidant products of radiation-activated water which mediate radiation tissue injury, oxidizes hemoglobin to methemoglobin. Thus, in the human red cell, catalase together with glutathione peroxidase are necessary for efficient removal of hydrogen peroxide.

Reduced ascorbate (AH_2) reacts readily with a variety of oxidants and free radicals. Among human leukocytes, radioresistance correlates with capacity to take up and reduce dehydroascorbate (A)[3,4]. Dehydroascorbate reductase activity, measured in normal granulocyte and lymphocyte homogenates, is directly proportional to measured dehydroascorbate uptake and reduction[8]. Thus, capacity to maintain ascorbate in reduced form may be a determinant of cell capacity to inactivate oxidants and free radicals.

The present study was designed to evaluate the capacity of selected enzymes to promote inactivation of oxidants and free radicals in leukocytes. Granulocytes were isolated from normal subjects and from 1 heterozygous and 2 homozygous Swiss acatalasemic individuals. The data given in the table show that activities of glutathione peroxidase, glutathione reductase and superoxide dismutase were normal.