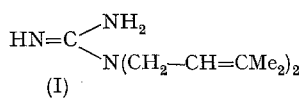
hydrochloride of (I); the dissociates[2] and the spectrum of the free base is obtained showing the molecular ion at m/e 195.

The hydrochloride of (I) is optically inactive in the range 700–300 nm and shows no-selective electronic absorption down to 220 nm; comparison of its NMR-spectra in deuterochloroform and deuterium oxide solutions indicates 3 active hydrogens for the free base. The NMR-spectra also show 2 ethylenic hydrogens and 2 moles of hydrogen are consumed upon catalytic hydrogenation (Pd/C).

The hydrogenated product (II) was characterized as the picrate (mp 164–165 °C), which gave analytical data consistent with the formula $C_{11}H_{25}N_3$ for the free base. This was further supported by the mass spectrum of the hydrochloride prepared from the picrate using Dowex-2 chloride resin; the molecular ion corresponding to the base appears at m/e 199.



(I)

Tetrahydro-pterogynine (II) hydrochloride exhibits colour reactions[3] and infrared bands[4] (at 1665 and 1615 cm^{-1} in KBr) suggesting that it is a N,N-disubstituted guanidine. Hydrolysis with barite gives diisoamylamine (isolated as picrate, mp and mixed mp 94 to 95.5 °C) demonstrating that (II) is N,N-diisoamylguanidine.

Analysis of the NMR-spectra of the hydrochloride of pterogynine established the positions of the ethylenic linkages. The spectrum in deuterochloroform shows 2 peaks at δ 1.67 and 1.75 (12 H), a doublet at 3.95 (4 H), a triplet at 5.15 (2 H) and a broad signal at 7.23 ppm (4 H; absent in D_2O solution). These data are in agreement with the presence of 2 γ,γ-dimethylallyl substituents, and therefore pterogynine possesses structure (I).

Zusammenfassung. Ein neues Alkaloid, Pterogynin, wurde aus der Rinde von *Pterogyne nitens* Tul. (Leguminosae) isoliert und seine Struktur bestimmt. Es handelt sich um das N,N-Di(isopenten-2-yl)-guanidin.

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La Plata (Argentina), 23 May 1969

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The Effect of Oxygen Concentration on the Quantum Yields of the Dye-Sensitized Photoinactivation of Trypsin, α-Chymotrypsin and Lysozyme

Most enzymes are inactivated when illuminated in the presence of photosensitizing dyes and molecular oxygen; oxygen is consumed during the process. This phenomenon is termed 'photodynamic' inactivation[1]. Although the effects of most reaction variables on the rate of the photodynamic inactivation of enzymes have been studied in some detail, only a few cursory reports have appeared concerning the effects of oxygen concentration[2–5]. In general it was found that rates of photodynamic reactions increased when the oxygen concentration was raised from 0–20%; further increase in concentration had little effect. The present paper is concerned with the effects of oxygen concentration on the quantum yields for the photodynamic inactivation of several enzymes as sensitized by a variety of dyes.

Reaction systems were 42 μM in trypsin, or 40 μM in α-chymotrypsin, or 40 μM in egg white lysozyme, in 0.125 M sodium phosphate buffer at pH 8. The dye concentrations were 12.5 μM for methylene blue and eosin Y and 150 μM for riboflavin-5′-phosphate (FMN). 1 ml quantities of the reaction mixtures were illuminated at 15 °C by a 500 watt slide projector equipped with a Baird-Atomic multilayer interference filter (6650 Å for methylene blue, 5170 Å for eosin Y) or by a 1000 watt G.E. A-H6 high pressure mercury arc lamp provided with a similar filter (4370 Å for FMN). The light energy absorbed was measured with a vacuum thermocouple-galvanometer combination calibrated with standard lamps[6].

At intervals during illumination, samples were removed and assayed spectrophotometrically for remaining enzymic activity; inactivation was essentially first order with respect to duration of illumination. Substances used were benzoyl-L-arginine ethyl ester for trypsin, acetyltyrosine ethyl ester for α-chymotrypsin, and lyophilized *Micrococcus lysodeikticus* for lysozyme. Appropriate light and dark controls were run. The oxygen concentration during an experiment was controlled by bubbling gas mixtures (nitrogen; 1%, 2.5%, 5%, 10%, 20%, and 40% oxygen in nitrogen; and 100% oxygen) through the reaction mixture. The quantum yield for the photodynamic inactivation of an enzyme was defined as the initial rate of loss of enzyme activity divided by the initial rate of absorption of photons by the reaction system[6].

Results for trypsin are shown in Figure 1. The quantum yields increased with increasing oxygen concentration up to approximately 20%; above this there was little further increase in yield. The dependence was essentially the same with all 3 dyes although the absolute values were lowest with methylene blue, intermediate with eosin Y, and highest with FMN. The α-chymotrypsin results (Figure 2) were generally similar, although the quantum yields with a given sensitizer were about twice those for

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trypsin. Data for lysozyme are shown in Figure 3. In sharp contrast to the results with trypsin and α-chymotrypsin, the quantum yield with FMN rises very sharply, appears to reach a maximum at less than 1% oxygen, and then decreases at higher concentrations of oxygen.

Our results, except for the FMN-lysozyme system, are generally similar to most of those reported earlier[2-4]. Not enough information is available on the primary mechanisms involved in the photodynamic inactivation of enzymes to permit much speculation on the mechanisms determining the shapes of our curves. The photooxygenation of most organic molecules (substrates) with most sensitizing dyes proceeds by way of the triplet state of the dye[7]. The triplet dye can react with molecular oxygen (triplet state) to produce singlet oxygen (or, possibly, a dye-oxygen complex); these species then oxidize the substrate. Alternatively, the triplet sensitizer can react directly with the substrate, usually by a hydrogen or electron transfer mechanism[7].

BELLIN and YANKUS[8], in studies of the effects of oxygen concentration on the rose bengal-sensitized photooxidation of histidine, found a sharp increase in quantum yield up to approximately 20% oxygen, a plateau to approximately 40% oxygen, and then a decrease in yield at higher oxygen concentrations. The decrease was attributed to the quenching of a dye-oxygen 'photoperoxide' (the species which oxidizes the histidine) by ground state oxygen. Our data (except for the FMN-lysozyme system) show a plateau at higher oxygen concentrations; this suggests a singlet oxygen mechanism

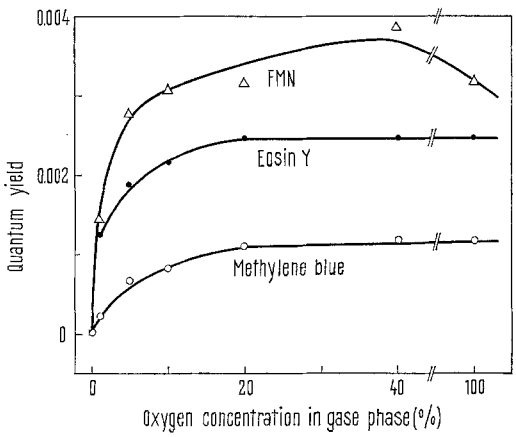

Fig. 1. Quantum yields for the photodynamic inactivation of trypsin as a function of the oxygen concentration in the gas phase during illumination at 15 °C. The reaction systems were 42 μM in enzyme and 0.125 M in sodium phosphate buffer at pH 8. The dye concentrations were 12.5 μM for methylene blue and eosin Y and 150 μM for FMN.

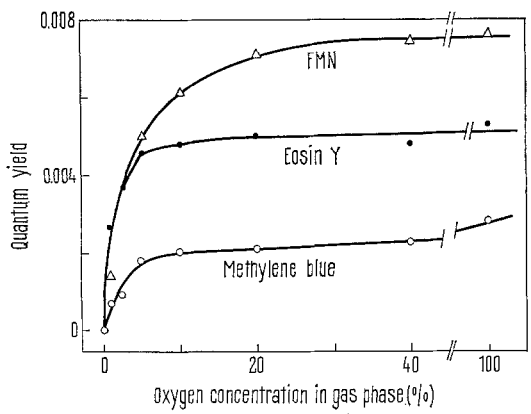

Fig. 2. Quantum yields for the photodynamic inactivation of α-chymotrypsin as a function of the oxygen concentration in the gas phase during illumination. The reaction systems were 40 μM in enzyme; otherwise the reaction conditions were the same as given for Figure 1.

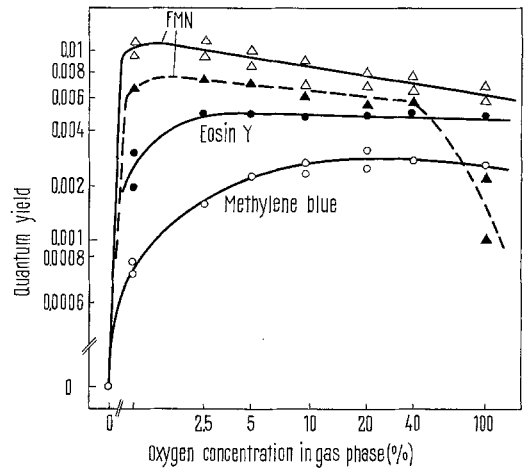

Fig. 3. Quantum yields for the photodynamic inactivation of lysozyme as a function of the oxygen concentration in the gas phase during illumination. The reaction systems were 40 μM in enzyme, and 0.125 M in sodium phosphate buffer at pH 8. The dye concentrations were 12.5 μM for methylene blue and eosin Y, and 100 μM (closed triangles) and 150 μM (open triangles) for FMN. The data are plotted on a log-log scale to separate the experimental points better.

with the plateau region resulting from a quantitative quenching of excited dye by oxygen to give singlet oxygen. Further, it suggests that the excited dye species involved are fairly short-lived since the plateau does not occur until fairly high concentrations of oxygen are reached. The very sharp rise in quantum yield for the FMN-lysozyme system suggests the involvement of a sensitizer-substrate mechanism at low oxygen concentrations; the behavior at higher concentrations might result from competitive quenching of excited dye by molecular oxygen[7].

Résumé. Le travail décrit l'effet de différentes concentrations en oxygène sur la photoinactivation de la trypsine, de l'α-chymotrypsine et du lysozyme. Comme sensibilisateurs on a utilisé le bleu de méthylène, l'éosine Y et le FMN. En général, les rendements quantiques de l'inactivation enzymatique ont augmenté avec l'augmentation des concentrations en oxygène, et atteint un plateau à des concentrations de 20% environ. Ils ne changèrent alors pratiquement plus lorsque les concentrations en oxygène augmentèrent jusqu'à 100%.

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