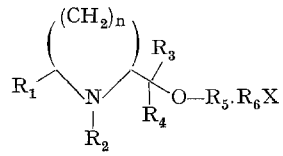
The eyes were kept open for 30 sec and after a further 60 sec the corneal reflex was tested by touching the centre of the cornea with a horsehair capable of exercising a force of 200 mg before flexing. The stimulation was effected once every minute for 5 min, the procedure then being repeated on the other eye. Each concentration was tested on 10 animals giving a total of 100 responses. The median anaesthetic concentrations (EC_{50}) reported in the Table have been calculated according to the method of FINNEY[10] on the basis of the percentage of positive responses (i.e. the absence of corneal reflex).



No.[a]	Form	n	R_1	R_2	R_3	R_4	R_5	R_6	X	mp	Corneal anaesthesia[e] EC_{50} (mg/ml)
1	DL	3	H	CH_3	CH_3	CH_3	Diphenylacetyl	H	Cl	168°	0.85
2[b]	D	3	H	CH_3	CH_3	CH_3	Diphenylacetyl	H	Cl	164°	0.66
3[c]	L	3	H	CH_3	CH_3	CH_3	Diphenylacetyl	H	Cl	164°	1.1
4	DL	3	H	CH_3	CH_3	CH_3	Diphenylacetyl	CH_3	I	172°	i.a.
5	DL	3	H	$CH_3CH_2C_6H_5$	CH_3	CH_3	Diphenylacetyl	H	Cl	166°	i.a.
6	DL	3	H	CH_3	CH_3	CH_3	Phenylacetyl	H	Cl	135°	6.4
7	DL	3	H	CH_3	CH_3	CH_3	*p*-aminobenzoyl	–	–	159°	i.a.
8[d]	DL	3	H	CH_3	CH_3	CH_3	Phenylcyclopentyl-acetyl	H	Cl	–	4.9
9	DL	3	H	CH_3	CH_3	CH_3	Diphenylglycolyl	H	Cl	166°	1.0
10	DL	3	H	CH_3	$-(CH_2)_4-$		Diphenylacetyl	H	Cl	165°	10.0
11	DL	3	H	CH_3	$-(CH_2)_5-$		Diphenylacetyl	H	Cl	164°	i.a.
12	DL	3	H	CH_3	CH_3	H	Diphenylacetyl	H	Cl	193°	1.9
13	DL	3	H	CH_3	H	H	Diphenylacetyl	H	Cl	200°	1.7
14[f]	L	2	H	CH_3	CH_3	CH_3	Diphenylacetyl	$C_4H_6O_6$		135°	i.a.
15	DL *cis*	3	CH_3	CH_3	CH_3	CH_3	Diphenylacetyl	H	Cl	197°	1.25
16	DL *trans*	3	CH_3	CH_3	CH_3	CH_3	Diphenylacetyl	H	Cl	175°	1.85
Procaine, HCl											48.6
Lidocaine, HCl											23.0

[a] All the compounds gave satisfactory elemental analysis (C, H, N). [b] $[\alpha]_D^{20} + 10.5$ ($c = 1$, H_2O). [c] $[\alpha]_D^{20} - 11$ ($c = 1$, H_2O). [d] Amorphous. [e] i.a., inactive at the maximum permissible concentration. [f] Tartrate. $[\alpha]_D^{20} + 23$ ($c = 1$, EtOH).

Structure-activity relationship. From the data in the Table the following observations can be made: (a) Good anaesthetic activity has been found only in compounds having 2 phenyl groups in the acyl residue (cf. 1 and 9 with 6, 7 and 8): particular mention should be made of the fact that the *p*-aminobenzoate (No. 7) is practically inactive. (b) On quaternization (No. 4) the activity is lost. (c) Optimum activity has been found for $R_3 = R_4 = CH_3$ (Nos. 1, 9, 15 and 16); when R_3 and R_4 form part of a cyclic structure (Nos. 10 and 11) the activity is strongly reduced. (d) The anaesthetic activity seems to be strongly dependent on the size of the ring (Nos. 3 and 14), but is relatively independent of subtle steric effects as shown by the fact that there is only a limited difference in activity between D and L forms (Nos. 2 and 3) and between the *cis* and *trans* forms of the 6-methyl derivatives (Nos. 15 and 16).

Riassunto. Vengono brevemente discussi i rapporti fra struttura e attività anestetica di un gruppo di difenilacetati di amino alcoli ciclici riportati nella tabella

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[10] D. J. FINNEY, *Probit Analysis* (Cambridge University Press, London 1947).

A New Guanidine Alkaloid[1]

The crude mixture of alkaloids obtained from the alcoholic extract of the bark of *Pterogyne nitens* Tul. (Leguminosae) was subjected to column chromatography on alumina followed by purification through the picrate.

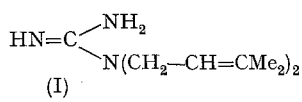
A new alkaloid named pterogynine (I) was isolated as the picrate salt (mp 157–158 °C) and further characterized as perchlorate (mp 101–102 °C) and hydrochloride (mp 142–143 °C). There were difficulties in determining the molecular formula because of the explosive decomposition of the salts during some of the combustion analyses. The analytical data of the 3 salts and proton-counting in the NMR-spectrum (in $CDCl_3$) of the hydrochloride led to the probable molecular composition $C_{11}H_{21}N_3$ for the free base. This was ascertained by the mass spectrum of the

[1] Part XIV of *Studies on Plants*; preceding part, R. A. CORRAL, O. O. ORAZI and I. A. BENAGES, Tetrahedron Letters *545* (1968).

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

[2] Several mass spectra of guanidine salts have recently been published by J. H. BEYNON, J. A. HOPKINSON and A. E. WILLIAMS, Org. Mass Spectrom. *1*, 169 (1968).
[3] I. SMITH, *Chromatographic and Electrophoretic Techniques* (Heinemann-Medical Books, London 1960), vol. 1, p. 225.
[4] T. GOTO, K. NAKANISHI and M. OHASHI, Bull. chem. Soc. Japan *30*, 723 (1957).

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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