one- to nearly ten-fold, over the whole cell preparation, depending on the substrates used.

Among various substrates examined phenazine methosulfate which served as a fair electron acceptor in the intact cells caused the most rapid hydrogen uptake in cell-free preparation, over 90% of 20 μmoles provided being reduced in 15 min. It seems evident that the enhancement of hydrogenase activity by phenazine methosulfate, methylene blue and other acceptors was due to the elimination of the cell membrane as a barrier to the penetration of substrates into the intact cells. On the other hand, nitrite which was the best hydrogen acceptor in the intact cells failed to bring about hydrogen consumption in the homogenate. It is probable that other enzymes and cofactors participating in nitrite-reducing system were separated during the manipulation.

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1,4-Diamino-2-butanon (2-ketoputrescine) as strong and short acting competitive inhibitor of diamine oxidase

Diamine oxidase (diamine:O_2 oxidoreductase (deaminating), EC 1.4.3.6) a Cu^{2+} and pyridoxal phosphate containing enzyme, is inhibited by metal chelating agents or carbonyl reagents such as cyanide, semicarbazide, hydroxylamine and some hydrazine derivatives[1]. In the course of our study on the oxidative deamination of 2-hydroxyputrescine and 2-hydroxycadaverine[2] we have found that the corresponding ketones, 1,4-diamino-2-butanon and 1,5-diamino-2-pentanon, possess a powerful blocking activity toward plant and animal diamine oxidase. In this paper some results of this action are given.

1,4-Diamino-2-butanon and 1,5-diamino-2-pentanon were synthetized as dihydrochlorides[3] and partially purified pea seedling and pig kidney diamine oxidase was used as in our previous paper[2]. The enzyme activity was determined by modified spectrophotometric method of HOLMSTEDT, LARSSON AND THAM (*cf.* ref. 2), unless otherwise stated. In general, freshly prepared substrate and inhibitor solutions were

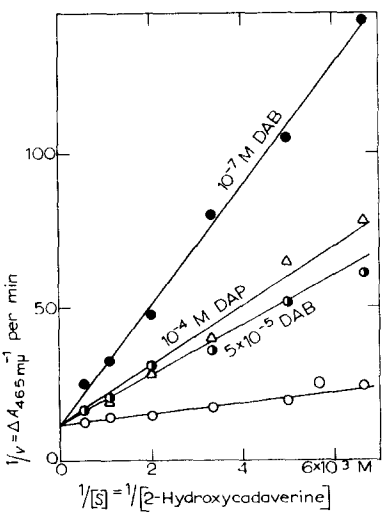
TABLE I

INHIBITORY POWER OF 2-KETODIAMINES

The enzyme activity was assayed in 0.067–0.07 M phosphate buffers at pH optimum for each substrate, temperature and substrate concentrations as indicated. Incubation time was 30 min. The inhibitor concentration giving 50% inhibition has been calculated by interpolating on the inhibition percentage *versus* inhibitor concentration curve established for at least four concentrations corresponding to partial inhibition. DAB, 1,4-diamino-2-butanon; DAP, 1,5-diamino-2-pentanon.

Substrate used (dihydrochloride)	*Oxidation catalyzed by*							
	*Pea seedling diamine oxidase at 28°**				*Pig kidney diamine oxidase at 37°*			
	pH	*Relative rate (%)***	*50% Inhibition concentration (μM)*		*pH*	*Relative rate (%)***	*50% Inhibition concentration (μM)*	
			DAB	*DAP*			*DAB*	*DAP*
0.006 M Putrescine	—	—	—	—	7.2	100	0.5	120
0.01 M Putrescine	7.5	100	2.0	180	—	—	—	—
0.006 M DL-2-Hydroxyputrescine	—	—	—	—	8.9	86	3.6	350
0.006 M Cadaverine	—	—	—	—	7.1	100	0.75	100
0.01 M DL-2-Hydroxycadaverine	7.3	32	—	760	—	—	—	—

* Determined manometrically from the consumption of oxygen.
** Without inhibitor, putrescine = 100%.



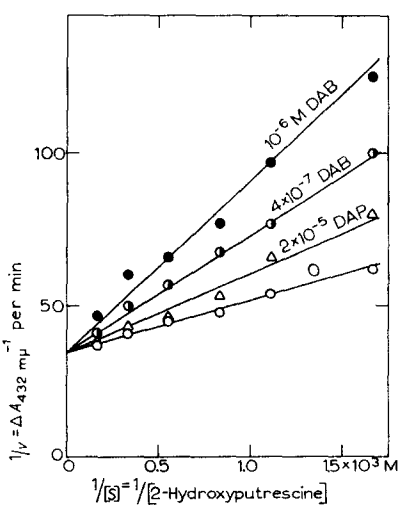


Fig. 1. Competitive inhibition of pea seedling diamine oxidase by 2-ketodiamines—reciprocal plots by the method of Lineweaver and Burk. Assay conditions: DL-2-hydroxycadaverine as substrate; purified enzyme of 0.64 mg of protein; inhibitor concentrations as indicated; 0.07 M phosphate buffer of pH 7.3; total volume 5 ml; temperature 28°. DAB, 1,4-diamino-2-butanon; DAP, 1,5-diamino-2-pentanon.

Fig. 2. Effect of 2-ketodiamines on Lineweaver–Burk plots of pig kidney diamine oxidase. Assay conditions: DL-2-hydroxyputrescine as substrate; partially purified enzyme of 8 mg of protein; inhibitor concentrations as indicated; 0.07 M Na_2HPO_4–NaOH buffer; final pH 8.9; total volume 5 ml; temperature 37°. DAB, 1,4-diamino-2-butanon; DAP, 1,5-diamino-2-pentanon.

present in the reaction mixture simultaneously and the enzyme reaction was started by addition of enzyme.

In Table I the concentrations of both diaminoketones causing the 50% inhibition of diamine oxidase are given. It is obvious, that the inhibition is slightly dependent from the kind of substrate used and is indirectly proportional to the relative rate of their oxidation, *i.e.*, the faster the oxidation, the lower should be the inhibitor concentration. 1,4-Diamino-2-butanon represents a much more effective inhibitor than 1,5-diamino-2-pentanon. This is probably due to the spontaneous ring closure of the latter compound into the 2-aminomethyl-Δ^1-pyrroline (ref. 3). (±)-2-Aminomethylpyrrolidine*, the substance structurally closely related to the pyrroline derivative, has no inhibitory effect on pea seedling diamine oxidase even in concentration $5 \cdot 10^{-3}$ M.

By plotting reciprocal initial velocities *versus* reciprocal substrate concentrations the typical dependence indicating the competitive character of the action of 1,4-diamino-2-butanon or 1,5-diamino-2-pentanon on plant, as well as animal diamine oxidase, has been obtained (Figs. 1 and 2).

It has been further observed that on prolonged incubation of the complete reaction mixture containing pea seedling diamine oxidase and 1,4-diamino-2-butanon as inhibitor, a reactivation of enzyme occurs at neutral pH value. The duration of the initial inhibitory period on the time course curves is approximately 40 min at the temperature of 28°. These results are compatible with the assumption that the dihydrochloride of diaminoketone is destroyed by selfcondensation in the buffered reaction mixture. This explanation has been supported by the fact that the more concentrated colourless solutions of 1,4-diamino-2-butanon (0.05–0.005 M) in 0.1 M phosphate buffers of pH 6.6–7.7 darken rapidly and brown products are formed in a few hours.

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* This compound was prepared together with 2-hydroxycadaverine by catalytic hydrogenation of 1,5-diamino-2-pentanon and separated from the mixture on Dowex 50W-X8 column as described earlier[3].