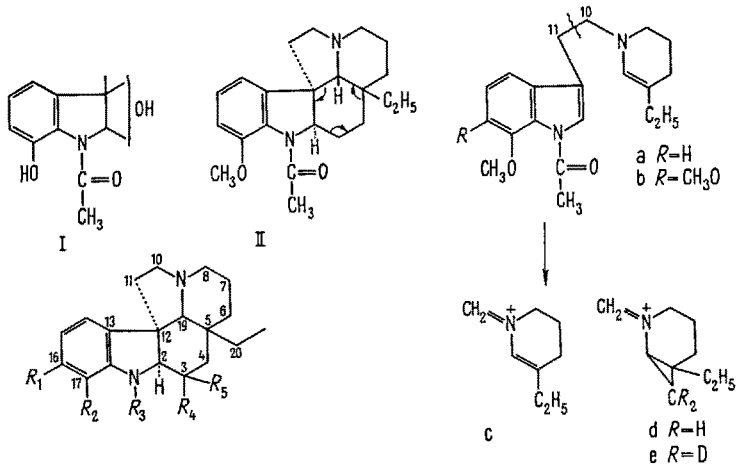
acetyl group was shown by the fact that the m/e 43 peak (CH_3CO^+) was shifted to m/e 46 (CD_3CO^+). The remaining three deuterium atoms must then be present in α-positions to the carbonyl group and such a feature is only possible if the carbonyl function (and hence the hydroxyl substituent in the original alkaloid) is at C-3. Attachment at C-20, though satisfying the deuterium exchange experiment, is excluded by the NMR and mass spectral data.



	R_1	R_2	R_3	R_4	R_5
III	HO	HO	CH_3CO	H	HO
IV	CH_3O	CH_3O	CH_3CO	H	HO
V	CH_3O	CH_3O	CH_3CO	=O	
VI	CH_3O	CH_3O	CD_3CO	=O	(2, 4, 4-d_3)
VII	H	HO	CH_3CO	H	HO
VIII	H	CH_3O	CH_3CO	H	HO
IX	CH_3O	CH_3O	C_2H_5	H	H

The mass spectra of V and VI are extremely interesting, since they demonstrate that a fragmentation process—different from that usually found[9] in aspidospermine (II)-type alkaloids—can occur. The strongest peak in the mass spectrum of the ketone V now occurs at m/e 138 (the 124 peak corresponding to *c* being absent) and is shifted by *two* units to m/e 140 in the deuterated analog VI. The mechanistic implication will be discussed in our detailed paper, but we ascribe the m/e 138 peak to species *d*, and the m/e 140 peak to *e*, both resulting from rupture of the 2–3, 3–4, 10–11, and 12–19 bonds with expulsion of carbon monoxide.

The above mass spectrometric, NMR and chemical data are most compatible with structure III for spegazzinidine[11]. The originally isolated alkaloid spegazzinine[3] can now be assigned structure VII on the following grounds. The NMR spectrum of spegazzinine (VII) closely resembles that of spegazzinidine (III) except for the absence of a signal corresponding to the non-hydrogen-bonded C-16 phenolic group of III and the presence of signals corresponding to *three* aromatic protons. Most importantly, the mass spectrum (Figure 2) of spegazzinine methyl ether (VIII) is virtually identical with that (Figure 1) of spegazzinidine dimethyl ether (IV) except for a 30 mass unit shift (corresponding to the extra methoxyl group of IV) in those peaks (e.g. m/e 400, 383, 382, 371, 356, 343, 301, 289, 245, 204, 190) of IV, in which the aromatic portion of the molecule is still retained. A similar relationship was also observed in the mass spectra of the two parent alkaloids III and VII; as noted earlier[1,4,7,9], this can be considered virtual proof that the two alkaloids possess identical structures and differ only by one substituent in the aromatic ring[12].

Zusammenfassung. Auf Grund von Protonresonanz und massenspektrometrischen Messungen werden die Strukturen III und VII für die Aspidosperma-Alkaloide Spegazzinidin und Spegazzinin vorgeschlagen.

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[11] *Note added in proof.* Chemical verification has now been provided by $LiAlH_4$ reduction of the tosylate of IV which provided the antipode IX ($[\alpha]_D$ −20.6°) of N-ethyldeacetylpyrifolidine ($[\alpha]_D$ + 19.8°), obtained in turn by $LiAlH_4$ reduction of pyrifolidine[7].

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The Reaction Mechanism of Catalase

In his recent review on enzyme models[1], Westheimer has proposed the following scheme for the mechanism of decomposition of hydrogen peroxide by catalase.

$$P\text{–}Fe^{+++} + H_2O_2 \longrightarrow P\text{–}FeO^{+++} + H_2O \quad (1)$$

$$P\text{–}FeO^{+++} + 1e \longrightarrow P\text{–}FeO^{++} \quad (2)$$

$$P\text{–}FeO^{++} + H_2O_2 \longrightarrow P\text{–}FeO_2^{++} + H_2O \quad (3)$$

$$P\text{–}FeO_2^{++} \longrightarrow P\text{–}Fe^{++} + O_2 \quad (4)$$

$$P\text{–}Fe^{++} + H_2O_2 \longrightarrow P\text{–}FeO^{++} + H_2O \quad (5)$$

[1] F. H. Westheimer, *Enzyme Models. The Enzymes*, 2nd Ed. (Edited by P. D. Boyer, H. Lardy, and K. Myrbäck, Academic Press Inc., New York 1959).

In this mechanism equations (1) and (2) are chain-initiating, equations (3), (4) and (5), chain-carrying. The main support of the scheme is a good precedent for the real occurrence of reaction (4) in a model compound. An analogous reaction has been postulated by GEORGE[2] to explain the kinetics of the autooxidation of ferrous ion. I wish to point out that good evidence for the occurrence of the reaction (5) with divalent iron porphyrine complexes has been in the literature for some time, but it has been overlooked, possibly because of its very specialized character.

Very small amounts of hemine change characteristically the polarographic electroreduction of oxygen[3]. This change consists in the shift of a part of the wave due to the reduction of hydrogen peroxide to a more positive potential. The height of the new wave, which belongs to a catalyzed reduction of hydrogen peroxide, and its position on the current voltage curve, depends on the concentration of hemine and on the pH of the solution.

The half-wave potential of the catalyzed wave approaches the redox potential of hemine when the height of the catalyzed wave is small in comparison with the uncatalyzed one[4]. It has been shown that there are two interpretations which are in the first approximation polarographically equivalent.

One scheme may be represented by equations (6) and (7),

$$2\,Fe_h^{++} + H_2O_2 + 2H^+ \xrightarrow{k} 2\,Fe_h^{+++} + 2\,H_2O \qquad (6)$$

$$Fe^{+++} + e \longrightarrow Fe^{++} \qquad (7)$$

and it assumes a rapid oxidation of Fe_h^{++} by hydrogen peroxide followed by the reversible reduction of Fe_h^{+++} at the dropping mercury electrode.

The second scheme [equations (8) and (9)] assumes that Fe_h^{++} forms a 'complex' with hydrogen peroxide and this 'complex' is reducible on the dropping mercury electrode at a potential more positive than the redox potential of the Fe_h^{+++}/Fe_h^{++} system.

$$Fe_h^{++} + H_2O_2 \xrightarrow{k} Fe_h^{++}.(H_2O_2) \qquad (8)$$

$$Fe_h^{++}.(H_2O_2) + 2H^+ + 2e \longrightarrow Fe_h^{++} + 2H_2O \qquad (9)$$

Already at this stage, it was clear that the second explanation is the correct one as with many ferrohem complexes the oxidation with hydrogen peroxide does not proceed sufficiently rapidly to explain the polarographic effect.

The phenomenon was re-investigated recently by HANUŠ[5] in the light of the enormous progress which the Prague polarographic school has achieved in the analysis of kinetic and catalytic currents[6]. HANUŠ has shown that the second scheme is the correct one and that the formation of the 'complex' $Fe_h^{++}.(H_2O_2)$ is a reversible reaction. Consequently HANUŠ represented the mechanism of the catalyzed reduction of hydrogen peroxide in the presence of iron porphyrine complexes by the equations (10) and (11). Now it is not possible to calculate the rate constant k_1 for the reaction (10) from the polarographic measurements since this would require the knowledge of the value of the equilibrium constant for this reaction.

$$Fe_h^{++} + H_2O_2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Fe_h^{++}.(H_2O_2) \qquad (10)$$

$$Fe_h^{++}.(H_2O_2) + 2H^+ + 2e \longrightarrow Fe_h^{++} + 2H_2O \qquad (11)$$

It is also impossible to obtain precisely analogous data with catalase itself[7]. However, one can estimate that the reaction (10) for ferrohem must be at least as fast as the decomposition of hydrogen peroxide by catalase, since the assumption of any finite value for k_1/k_2 [in equation (10)] must make k_1 larger than the value of k calculated for the case of an irreversible reaction.

The correspondence of the reaction (10) in the polarographic scheme with reaction (5) in the Westheimer mechanism is now obvious. We can clearly assume that either the complex detected by polarography should be written Fe_hO^{++} instead of $Fe_h^{++}.(H_2O_2)$ and that its observed formation is a model for Westheimer's reaction (5), or that the formation of a complex $P \cdot Fe^{++} \cdot (H_2O_2)$ analogous to the polarographic reaction should be inserted into Westheimer's scheme as the first stage of reaction (5), i.e.

$$P.Fe^{++} + H_2O_2 \rightleftharpoons P.Fe^{++}.(H_2O_2) \qquad (5a)$$

$$P.Fe^{++}.(H_2O_2) \longrightarrow P.FeO^{++} + H_2O \qquad (5b)$$

In both cases, the polarographic work cited seems to constitute a strong support of the equation (5) in Westheimer's scheme[8].

Zusammenfassung. Es werden polarographische Befunde mitgeteilt, welche den von Westheimer vorgeschlagenen Mechanismus der Zersetzung von Wasserstoffperoxyd durch Katalase ergänzen.

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[8] I wish to thank Dr. V. KORYTA from the Polarographic Institute of the Academy of Sciences, Prague, for an exchange of information and views pertinent to this problem.

Blood 'Contamination' of Liver Homogenates and the Liver Cathepsines

HOLZER et al.[1] suggested that the activity of some dehydrogenase enzymes and the concentration of some compounds (α-ketoglutarate, pyruvate) in liver homogenates might be influenced by the presence of blood in the liver. This suggested that the activity of other enzymes might also be influenced by blood. In the present report, which is part of a larger one, the possibility that blood might be a source of error in analysis of liver cathepsine and peptidase activity has been investigated.

Wistar rats, 150–200 g weight, were killed by decapitation, a blood sample was collected, and the liver rapidly excised. 1 ml of whole blood was diluted with 124 Vol of

[1] H. HOLZER, G. SEDLMAYER, and M. KIESE, Biochem. Z. *328*, 176 (1956).