Summary. Surveying the various concepts of valency which have been put forward since DALTON for the classification of chemical phenomena, it is found that the principles have been either dualistic (BERZELIUS, BLOMSTRAND, ARRHENIUS, KOSSEL) or unitarian (GERHARD, COUPER, KEKULÉ). The phenomena of inorganic chemistry can be classified only by using dualistic concepts, whereas unitarian systems proved to be superior for the phenomena of organic chemistry. In the conceptions of G. N. LEWIS and N. SIDGWICK, a combination of dualistic and unitarian concepts in one theory was achieved by distinguishing two types of bonds (mobile-immobile, polar-non-polar, ionic-covalent). With the octet rule, ions as well as molecules (uncharged and charged) may be derived and it is readily understood that bonds may vary from extreme polarity to non-polar links.

The coordination theory of WERNER neither fits into the dualistic nor the unitarian class of valency principles. WERNER derives the compounds by using principal and auxiliary valencies ('Haupt- und Nebenvalenzen') and distinguishes addition and insertion compounds ('Anlagerungs- und Einlagerungsverbindungen'). However, he avoids making any statement concerning the nature of the bonds, which makes his system very adaptable but difficult to grasp. Today it is readily understood that WERNER's principal valency characterizes the stoichiometry and his coordination number characterizes the structure of the compound in question without making any statement about the nature of the bonds involved. Because of that WERNER's concepts have survived and are indispensable even today, in spite of the rise of atomic physics which has changed our views on the nature of the chemical bonds so drastically.

SPECIALIA

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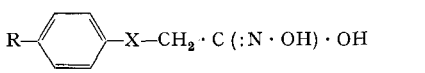
Inhibition of Catalase by (Arylthio)-Acetohydroxamic Acids

There are a number of catalase inhibitors which differ in their mode of action on the enzyme. For example, allylisopropylacetylcarbamide (Sedormid) reduces liver catalase activity by inhibiting the biosynthesis of the enzyme (SCHMID et al.[1]). Hydrocyanic and hydrazoic acids inactivate the enzyme by forming very stable complexes with the iron of the hemin units (DIXON and WEBB[2]). 3-Amino-1,2,4-triazole, a specific inhibitor of catalase, combines irreversibly with the protein moiety of the enzyme (MARGOLIASH et al.[3]). Some inorganic ions are also known to be catalase inhibitors (BEERS and SIZER[4]).

In the present investigation, the effect of some (arylthio)-acetohydroxamic acids on the activity of crystalline liver catalase (Boehringer) was studied. The hydroxamic acids were prepared as described by ZAYED et al.[5], their general formula being:



where R = H, CH_3, OCH_3, Cl, or Br and X = S or SO_2.

The compounds were tested as their K-salts after they had been recrystallized from 90% methanol. The enzyme activity was assayed according to the procedure of FEINSTEIN[6], using sodium perborate as a substrate.

The Table shows the concentration of the hydroxamic acids necessary to produce half inhibition of the enzyme. Substitution in the para position of the benzene ring effected an increased inhibition in the order $CH_3 \rightarrow OCH_3$

Inhibition of catalase by substituted acetohydroxamic acids and some catalase inhibitors

Inhibitor	I_{50} [a]
R = H; X = S	$4.5 \cdot 10^{-3} M$
R = CH_3; X = S	$2.0 \cdot 10^{-3} M$
R = OCH_3; X = S	$8.9 \cdot 10^{-4} M$
R = Cl; X = S	$3.6 \cdot 10^{-4} M$
R = Br; X = S	$1.0 \cdot 10^{-4} M$
R = Cl; X = SO_2	$1.3 \cdot 10^{-3} M$
Dichlorophenol[7]	$4.0 \cdot 10^{-4} M$
Azide[2]	$2.0 \cdot 10^{-5} M$
Cyanide[2]	$5.0 \cdot 10^{-6} M$

[a] Preincubation period (E + I) = 2 min.

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→ Cl → Br; and the transformation S → SO_2 resulted in a decreased inhibition. *p*-Bromophenylthio-acetohydroxamic acid is the most potent inhibitor of the tested group ($pI_{50} = 4.0$); its inhibiting power is of similar order to that of other catalase inhibitors (cf. Table).

When the inhibitor (hydroxamic acid) was added to the enzyme solution, before the addition of substrate, there resulted an initial considerable inhibition which progressed only slowly by increasing the incubation time (2–10 min). In this respect, the arylthio-acetohydroxamic acids behave similarly to sulphide (BEERS and SIZER[8]). The presence of substrate (added simultaneously with the inhibitor to the enzyme solution) did not protect catalase against inhibition. This suggests that the inhibition of catalase does not involve the attachment of the hydroxamic acid to the substrate-binding group.

Zusammenfassung. Leberkatalase wird durch (Arylthio)-acetohydroxamsäure gehemmt. I_{50} Werte: $4.5 \cdot 10^{-3}$ to $1.0 \cdot 10^{-4} M$. *p*-Bromoderivat ($pI_{50} = 4.0$) zeigt die stärkste Hemmung. Substitution in der Para-Stellung des Benzol Ringes verursacht eine zunehmende Hemmung in der Reihenfolge: CH_3 → OCH_3 → Cl → Br. Es wird vermutet, dass sich der Hemmstoff nicht mit dem aktiven Zentrum des Ferments verbindet.

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Chemical Investigations of *Alangium lamarckii* III. Isolation of Steroids and Terpenoids from the Leaves

Alangium lamarckii Thw. (N.O. Alangiaceae) is a widely-grown small tree, and is known in different parts of India as *ankola, ankota, ankora, dhera, akarkanta* etc. The bark of the plant has been used in Indian indigenous systems of medicine for the treatment of leprosy, syphilis, various skin diseases and dysentery. It has also been used as a diaphoretic, antipyretic, emetic, laxative and anthelmintic. The leaves are used as a poultice to relieve rheumatic pains[1]. In a recent communication[2], the total tertiary alkaloidal fraction, a mixture of at least 5 alkaloids as revealed by paper chromatography, isolated from the leaves was found to be pharmacologically quite active and showed antispasmodic, hypotensive, anticholinesterase and adrenolytic activities. One of the 5 alkaloids was isolated as a new crystalline phenolic alkaloid, Ankorine[3], $C_{19}H_{29}O_4N$, m.p. 174–176°, which was found to possess hypotensive action of a prolonged duration[4]. Besides, choline chloride[5] was also isolated from the water-soluble quaternary basic fraction, which was pharmacologically found to be cholinergic in nature, after separation of the chloroform-soluble tertiary bases. Preliminary pharmacological studies[6] indicated that *Alangium lamarckii* leaves significantly increased the inflammatory reaction during the first 5 days and then significantly reduced the foot volume from the eleventh day onwards in formalin-induced arthritis in albino rats. Ascorbic acid content of the adrenal gland was found to be significantly raised. These findings suggested that some steroidal principles might be responsible for the anti-inflammatory property of the leaves. This is further corroborated by the fact that the leaves are used in the indigenous systems of medicine in relieving rheumatic pains when applied in the form of a poultice. Attempts were therefore made to isolate the steroids, terpenoids and other principles present in the leaves. For this purpose the petroleum ether extract of the leaves was saponified under different conditions either with strong alkali or acid, and then distilled in steam to remove volatile oils. The non-volatile, non-saponifiable fraction was obtained by extraction with ether. The product was then chromatographed over Brockmann aluminium oxide for chromatography, eluting with petroleum ether, benzene and chloroform in different proportions. Under different conditions of hydrolysis, the following 4 compounds were isolated after repeated chromatography and crystallization.

(1) *Compound A*, needles, m.p. 152–154°, $[\alpha]_D^{20°}$ – 33.8°, – 24.72° ($CHCl_3$). Anal.[7] – Found: C, 84.78, 84.83, 83.96, 83.92; H, 11.42, 11.55, 11.34, 11.44; *M* weight, 310, 302, 365 (Rast); CCH_3, 7.05, 9.40. Episterol[8], $C_{28}H_{46}O$, m.p. 151°, $[\alpha]_D$ – 5°, requires C, 84.35; H, 11.63; *M* weight, 398.65. Campesterol[8], $C_{28}H_{48}O$, m.p. 158°, $[\alpha]_D$ – 33° requires C, 83.93; H, 12.08; *M* weight, 400.66. Compound A has IR-absorption peaks at 2.72, 2.85, 3.35, 6.1, 6.85, 7.28, 7.9, 8.82, 9.2, 9.6, 9.78, 10.25, 10.42, 11.2, 11.88 μ, and UV λ_{max} at 204 nm. With acetic anhydride and pyridine compound A forms an *acetate*, flakes, m.p. 137–138°, $[\alpha]_D^{20°}$ – 38.68°, – 40.45° ($CHCl_3$). Anal. – Found: C, 82.44, 82.27, 82.49, 82.48; H, 10.97, 11.12, 10.86, 11.18; *M* weight, 396, 392, 364 (Rast); CH_3CO, 8.80, 3.84. Episterol acetate, $C_{30}H_{48}O_2$, requires C, 81.76; H, 10.98. Campesterol acetate, $C_{30}H_{50}O_2$, requires C, 81.39; H, 11.38. With benzoyl chloride and pyridine compound A forms a *benzoate*, m.p. 138–140°, $[\alpha]_D^{20°}$ – 14.8°, – 14.24° ($CHCl_3$). Anal. – Found: C, 83.98, 83.81, 83.93, 84.23; H, 9.61, 9.86, 10.28, 10.45; *M* weight, 258, 250, 405 (Rast); C_6H_5CO, 7.15. Episterol benzoate, $C_{35}H_{50}O_2$, requires C, 83.61; H, 10.02. Campesterol benzoate, $C_{35}H_{52}O_2$, requires C, 83.28; H, 10.38. Iodometric titration showed the consumption of 2 moles of perbenzoic acid by compound A, indicating the presence of 2 double bonds.

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