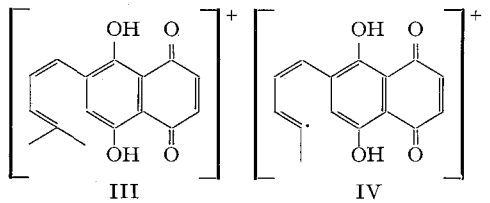
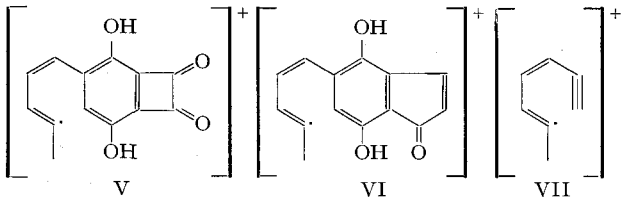
is a common feature of the mass spectra of quinones[9]. Its formation in substantial amounts is evidence that of the 2 tautomeric structures I and II of alkannin[10], the structure I predominates. Mass fragmentation of II should not give the ion m/e 229 but ions with m/e 91 (VII) and 116 which are formed in less significant amounts.



III IV

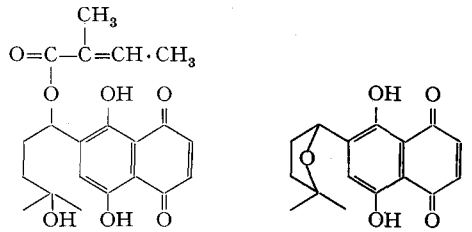


V VI VII

In addition to a UV-maximum at 280 nm, which is present in the spectra of all 4 compounds isolated, A-1 has a maximum at 216.5 nm which along with the strong bands at 1700 and 1150 cm^{-1} in its IR-spectrum indicate that this compound is an α,β-unsaturated ester. Its mass spectrum has a molecular ion peak at m/e 370. This ion readily loses 100 mass units and the subsequent abundant fragments are the same as those observed in the mass spectrum of alkannin except for an additional peak at m/e 100. Hydrolysis of A-1 with *N* KOH in an atmosphere of nitrogen yielded alkannin and an acid ($C_5H_8O_2$; M^+, 100) m.p. 65–66°, which formed an anilide ($C_{11}H_{13}ON$) m.p. 126–127°. This acid was identified as β,β-dimethylacrylic acid (MMP- and IR-, UV- and NMR-spectra). A-1 is, therefore, alkannin β,β-dimethylacrylate (I, R=$(CH_3)_2 \cdot C{=}CH \cdot CO$). This structure is in agreement with its NMR-spectrum[11]: two CH_3 *d* τ 8.42 and 8.30 (*J*, 1.5 cps) [$(CH_3)_2$ C=CH–CH_2–]; two CH_3 *d* τ 8.04 and 7.80 (*J*, 1.5 cps) [$(CH_3)_2$ C=CH–CO–]; *m* (H_eH_e) τ 7.4; *t* (H_d) τ 4.82; *m* (H_i) τ 4.20; *t* (H_f) τ 3.98; *d* (H_h) τ 3.06; *s* (H_aH_b) τ 2.84 and 2*s* (H_cH_g) τ −2.27 and −2.47 (disappeared on D_2O shake) and also the chemical and spectral properties of a tetraacetate ($C_{29}H_{34}O_{10}$) m.p. 192° formed on reductive acetylation of A-1 with zinc dust, acetic anhydride and fused sodium acetate.

A-3 was found to have an acetyl function (IR 1740, 1250 cm^{-1}; NMR *s* (3H) τ 7.85). Its mass spectrum showed no molecular ion peak, the molecule losing 60 mass units to give an abundant peak at m/e 270 and its NMR-spectrum was similar to the NMR-spectrum of A-1. On alkaline hydrolysis, A-3 yielded alkannin and acetic acid and is, therefore, alkannin monoacetate (I, R=Ac).

A-2 ($C_{21}H_{24}O_7$) appears to be a tiglic acid ester of dihydrohydroxyalkannin (VIII) as it yields tiglic acid and the cyclic ether (IX) on alkaline hydrolysis. The structure of A-2 has still to be confirmed.



VIII IX

The antibiotic activity of these alkannin derivatives has been established; A-2 would appear to be the most potent, inhibiting the growths of Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and fungi (*Candida albicans* and *Cryptococcus neoformans*) at a concentration 6.25 µg/ml[12].

Zusammenfassung. Aus den Wurzeln von *Arnebia nobilis* wurden 4 antibiotisch wirksame Stoffe isoliert. Die Struktur von dreien wurde aufgeklärt.

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Central Drug Research Institute, Lucknow (India), 8 October 1968.

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[11] Assigned on the basis of established chemical shifts and a comparison of the spectra of alkannin and its isolated derivatives.

[12] Assay carried out by S. K. Awasthi and Y. S. Bajpai.

[13] On deputation from the Chemistry Department, Lucknow University.

[14] To whom enquiries should be made. The isolation of shikonin monoacetate and β,β-dimethylacrylate from *Lithosperm* erythrorhizon, I. Morimoto, T. Kishi, S. Ikegana and Y. Hirata, Tetrahedron Lett. *4737* (1965), has been brought to our notice since communicating this paper.

CO-Binding Pigment (P-450) and Other Electron Transport Components in Hepatoma Bearing Rats

It has been established that liver microsomes contain a family of hydroxylating enzymes[1] which are to a greater or lesser degree coupled to a specific electron flow system. A variety of compounds are metabolized by this oxidation route. These microsomal systems which are responsible for oxidation of (certain) carcinogens and of other foreign metabolites contain 'mixed-function oxidases' in which, commonly, NADPH supplies the necessary reducing equivalents. The type reaction:

$$AH + DH_2 + O_2 \rightarrow AOH + D + H_2O$$

is characteristic of this category of detoxication. One oxygen is incorporated into the carcinogen substrate (AH), the other oxygen is reduced at the expense of a donor (DH_2; usually $NADPH^+ + H^+$) to water. This literature has been viewed by Mason et al.[2] and by King et al.[3] and, with respect to drug oxidation, by Gillette[4].

Kato et al.[5] reported that the oxidation rate of drugs by liver microsomes was significantly lower than normal in rats bearing Walker carcinosarcoma 256. Kato et al.[6] have further noted a lower than normal activity of certain

components of the NADPH electron transport system in liver microsomes of rats bearing Walker carcinosarcoma 256. SUGIMURA et al.[7] have reported microsomal cytochromes, cytochrome b_5, and P-450 and the aromatic hydroxylating activity in 4 Morris hepatomas at equivalent or somewhat lower levels than that found in normal or regenerating liver. In 2 Yoshida ascites hepatomas and embryonal liver, however, these cytochromes were much depressed or deleted.

The present study is an investigation of the activity of several enzyme believed to be directly related to the electron-flow chain and also of the level of the carbon monoxide-binding cytochrome P-450.

Male Buffalo rats (approximately 3 months old, average weight 290 g) with bilateral tumors identified as hepatoma 7777[8] in the hind leg and non-tumor bearing control animals from the same stock, were used. The tumors had been carried by serial transplantation and the animals used in the present study were of the 44th generation. The animals were shipped by air and upon arrival were kept separately in a temperature-controlled room. The rats were sacrificed 1 week after arrival and 3 weeks after inoculation. Control and tumor-bearing animals were used simultaneously. Animals were stunned and decapitated, liver (average wet weight 12 g) was removed and homogenized immediately in 4 vol. of 1.15% KCl solution in a Waring blendor. The slurry was further homogenized in a power-driven Teflon-pestle glass homogenizer. The homogenate was centrifuged 9000 *g* for 20 min. Pellets were rejected and the supernatant was further centrifuged in 10 ml tubes 105,000 *g* for 60 min. Through all manipulations a temperature of 2–4 °C was maintained. Pellets were used immediately or stored at − 30 °C. For use, a pellet was resuspended in 0.1 *M* phosphate buffer pH 7.45. Enzyme activities were measured by spectrophotometric methods using a Cary 15 spectrophotometer. NADPH oxidase was assayed according to the method of GILLETTE et al.[9]. Microsomal ferricyanide reductase and NADPH-cytochrome *c* reductase activities were assayed according to the method of WILLIAMS and KAMIN[10]. P-450 (reduced, carbon-monoxide-bound complex) was assayed by measuring the difference spectrum according to the method of OMURA and SATO[11].

Under the conditions of these experiments, no significant difference appears to exist when normal liver was compared with tumor-bearing animal liver in the activity of the NADPH oxidase or cytochrome *c* reductase. In the liver of 7777-tumor bearing animals, however, NADPH-ferricyanide reductase and the CO-binding cytochrome (P-450) were both markedly reduced. P-450 content of the hepatoma bearing animals showed a mean 41% lower than that of the mean of normal animals of the same strain. The NADPH-ferricyanide reductase was 43% less in the hepatoma bearing animal than in normal Buffalo rat liver.

Our results are similar to those described by KATO and associates and indicate that hepatoma 7777, like the Walker carcinosarcoma 256 which they studied can be correlated with a lower level of liver microsomal hemoprotein and a decreased enzymatic activity of some components of the NADPH-linked electron transport system. The present study does not allow an ontogenetic interpretation of the lower activity of elements of the presumed detoxication system. Presumably microsomal oxidations serve in nature to lower the effect of environmental carcinogens upon the organism by oxidations which yield non-toxic products. The alternative interpretation of the lower levels of certain elements of the NADPH-electron transport system in tumor-bearing rats as a causal relationship in the development of the tumor or secondary consequence cannot at this point be discriminated[12].

Zusammenfassung. Basierend auf früheren Eiweissuntersuchungen bezüglich einer endoplasmatisch-retikulären Elektronenstromkette ergibt sich: NADPH-Oxydase und Zytochrom-*c*-Reduktase sind in der normalen Rattenleber wie in der Hepatoma (Typ 7777) tragenden gleich, während die Ferricyanid-Reduktase und P-450 in der Leber der letzteren herabgesetzt waren.

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Columbia (Missouri 65201, USA), 4 November 1968.

Microsomal electron transport enzyme systems and cytochromes P-450 levels in liver of normal and hepatoma bearing buffalo rats

Type	Animal No.	Activity[a]			P-450[b]
		NADPH-oxidase	NADPH-ferricyanide reductase	NADPH-cytochrome *c* reductase	
Normal	1	4	15	83	15
	2	8	13	64	15
	3	4	15	27	15
	4	10	20	24	13
Mean		6.5	15.7	49.5	14.5
Hepatoma	1	5	8	73	9
bearing	2	11	8	73	8
animal	3	4	10	35	8
	4	5	10	31	9
Mean		6.2	9	53.0	8.5

[a] $\Delta OD \times 10^{-3}$/mg protein · min. [b] $\Delta OD_{450-490} \times 10^{-3}$/mg protein. Reaction mixture contained 2–7 mg of protein. Each point is the average 3 repetitions with a maximum deviation of 1.

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[12] This research was supported by USPHS (NCI) grant No. CA 08023 and No. CA 10729.
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