ca. 190° (dec., softens at 78–85°). Found: C, 41.84; H, 4.62; Anal. calcd. for $C_{10}H_{12}N_5O_3Cl$: C, 42.00; H, 4.20%.

5′-Chloro-5′-deoxy-2′,3′-*O*-isopropylideneionosine was similarly obtained in 49% yield. The product, isolated by extraction with chloroform and crystallization from water, had the physical constants previously reported[6].

Dinucleoside phosphates. Uridylyl-(3′-5′)-adenosine (III) was synthesized by heating 5′-chloro-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (I) with 1.2 equivalents of tri-*n*-butyl ammonium-3′-uridylate in dry DMF for 3 h. The reaction product was isolated after removal of solvent by preparative paper chromatography[4]. Yield 45%; Rf (A), 0.34; Rf (B), 0.16. λ_{max}, H_2O-260 nm; 0.1 *N* HCl-260 nm; 0.1 *N* NaOH-262 nm.

The structure of this product was established by identifying the products of formic acid and enzymatic hydrolyses by paper chromatography. Formic acid hydrolysis gave uracil and adenine, snake venom phosphodiesterase hydrolysis, uridine and adenosine-5′-phosphate and pancreatic ribonuclease hydrolysis uridine-3′-phosphate and adenosine, thus indicating that the isolated product was uridylyl-(3′-5′)-adenosine and not the expected 2′,3′-*O*-isopropylidene adenosine compound. There is no obvious explanation for the loss of the isopropylidene group during the formation of the dinucleoside phosphate. The hydrogen chloride produced during the course of the reaction may be responsible for this deblocking but curiously the same product was obtained even when an extra mole of tri-*n*-butyl amine was added to the reaction mixture.

Besides III, two minor products having λ_{max} at 262 and at 290 nm were also formed. The product with λ_{max} 290 nm is also formed when a DMF solution of I is heated at 100° for 3 h but most of the chloro compound is still present at the end of 3 h. This fact indicates that N_3, 5′-cyclization which is a facile reaction in the case of 2′,3′-*O*-isopropylidene-5′-*O*-*p*-toluene sulfonyl adenosine[7] is much slower with I and hence its ability to react with other nucleophiles. For instance, 2′,3′-*O*-isopropylidene adenosine is obtained when I is treated with 0.1 *N* NaOH at 100° for 3 h. This difference in the behaviour of the tosylate and the corresponding chloro compound may be attributed to the different rates at which these groups are displaced.

Uridylyl-(3′-5′)-inosine (IV) was obtained in 30% yield by refluxing 5′-chloro-5′-deoxy-2′,3′-*O*-isopropylideneinosine (II) with 1.5 equiv. of uridine-3′-phosphate and 2.5 equiv. of tri-*n*-butyl amine in water for 12 h. The structure of the product (IV), Rf (B), 0.35; λ_{max}, H_2O-252.5 nm; 0.1 *N* HCl-252 nm, was established by identifying the products of enzymatic hydrolysis as with the corresponding adenosine compound. In this case too, two products other than IV were formed, one of which was 2′,3′-*O*-isopropylideneionosine. In contrast to the reaction of I, the reaction of II with uridine-3′-phosphate anion in DMF resulted in a mixture of 5 products and dioxane and ethanol proved to be poor solvents for this reaction[8].

Zusammenfassung. Uridylyl-(3′-5′)-adenosin und Uridylyl-(3′-5′)-ionosin wurden durch Umsetzung eines passenden 5′-chloro-5′-deoxy-2′,3′-*O*-isopropylidene-Nukleosids mit Uridin-3′-phosphat synthetisiert.

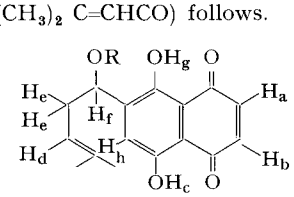
P. C. SRIVASTAVA, K. L. NAGPAL and M. M. DHAR

Central Drug Research Institute, Lucknow (India), 27 November 1968.

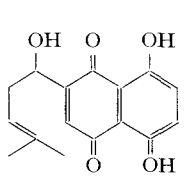
[6] K. KUSASHIO and M. YOSHIKAWA, Bull. chem. Soc. Japan *41*, 142 (1968).
[7] V. M. CLARK, A. R. TODD and J. ZUSSMAN, J. chem. Soc. 2952 (1951).
[8] Communication No. 1327 from the Central Drug Research Institute.

Chemical Constituents of the Antibiotic Fraction of *Arnebia nobilis*[1]

Ethanol extracts of the roots of *Arnebia nobilis* Rachinger (N.O. Boraginaceae)[2] showed bactericidal and fungicidal activity when put through a wide screen of biological tests[3]. The antibiotic activity was found to be associated with the hexane soluble fraction of this material. This fraction on silica gel chromatography yielded 4 dark red crystalline products, provisionally designated as A-1 ($C_{21}H_{22}O_6$)[4] m.p. 116–117°, A-2 ($C_{21}H_{24}O_7$) m.p. 92–94°, A-3 ($C_{18}H_{18}O_6$) m.p. 104–105° and A-4 ($C_{16}H_{16}O_5$) m.p. 146°. The data leading to the characterization of A-4 as the naphthaquinone alkanin (I, R=H) originally isolated from *Alkanna tinctoria*[5] and subsequently from *Onosma echiodes*[6] and *Arnebia hispidissima*[7] and of A-3 and A-1 as alkannin monoacetate (I, R=Ac) and as alkannin β,β-dimethylacrylate (I, R=$(CH_3)_2$ C=CHCO) follows.



I A-1 (R = $(CH_3)_2C{=}CH_2C$–)

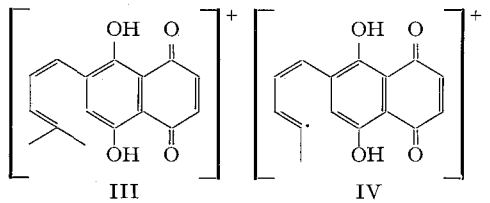


II

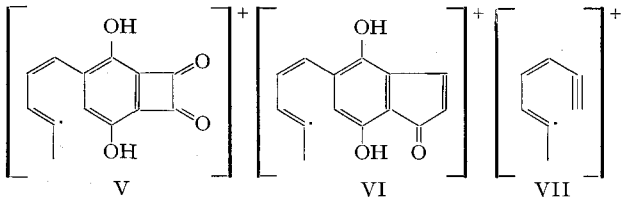
A-4 was identified as alkannin[8] as it formed a triacetate m.p. 132°, a dibenzoate m.p. 175–178° and a monomethyl ether m.p. 99° on treatment with methanolic hydrochloric acid. Its mass spectrum had a M^+ peak at 288 and significant peaks at m/e 270, 255, 229 and 227 assignable to the ions III, IV, V and VI respectively. The formation of an ion such as V by the loss of HC ≡ CH

[1] Communication No. 1305 from the Central Drug Research Institute.
[2] Brought to our notice by S. H. CAPTAIN of Industrial Perfumes Ltd., Bombay, after a significant clinical trial on himself.
[3] Extracts of plants put through 61 biological tests to locate antibiotic, anticancer, antifertility, hypoglycaemic and pharmacological activities. For test procedures and data on 285 plants see Indian J. exp. Biol. *6*, 232 (1968).
[4] Satisfactory analytical data on all reported compounds obtained and UV (MeOH), IR (KBr) and NMR (60 Mcs in $CDCl_3$ with TMS as internal standard) routinely determined.
[5] H. BROCKMAN, Justus Liebigs Annln Chem. *521*, 1 (1936).
[6] R. H. THOMSON, in *Naturally Occurring Quinones* (Butterworths, London 1957), p. 111.
[7] A. C. JAIN and S. K. MATHUR, Bull. natn. Inst. Sci. India *28*, 52 (1965).
[8] O.R.D. curve indicates not dextro-isomer shikonin [5].

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

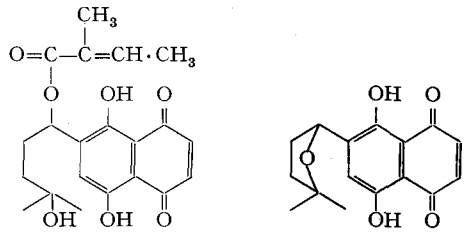


V VI VII

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.

Y. N. Shukla[13], J. S. Tandon,
D. S. Bhakuni and M. M. Dhar[14]

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

[9] J. H. Beynon, G. R. Lester and A. E. Williams, J. chem. Phys. *63*, 1861 (1959).

[10] K. W. Bentley, in *The Chemistry of Natural Products*, vol. 4 (Interscience Publishers Inc., New York 1960), p. 204.

[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