H, 6.44. $C_{24}H_{28}O_7$ requires C, 67.27; H, 6.59%), by an unambiguous method.

On the other hand, II has now been obtained from IV by a modification of the above method. The ketone IV with 3-benzyloxy-4-methoxybenzoyl chloride gave 4,6-dibenzyloxy-2-hydroxy-5-methoxy-ω-(3-benzyloxy-4-methoxybenzoyl)-acetophenone (X, m.p. 183–184°. Found: C, 73.69; H, 5.59. $C_{38}H_{34}O_8$ requires C, 73.77; H, 5.54%), which was then converted to 7,3′-dibenzyloxy-6,4′-dimethoxy-5-hydroxyflavone (XI, m.p. 145–146.5°, UV λ_{max} nm (log ε): (EtOH) 243.5 (4.29), 277 (4.24), 339 (4.39); (EtOH-$AlCl_3$) 261 (4.16), 293 (4.25), 364 (4.38). Found: C, 72.84; H, 5.20. $C_{31}H_{26}O_7$ requires: C, 72.93; H, 5.13%) was prepared. The catalytic debenzylation of XI gave II (m.p. 264–266°, IR 3390, 1650, 1615, 1585, 1555, 1518 cm^{-1} (KBr), UV λ_{max} nm (log ε): (EtOH) 245 (4.24), 275 (4.23), 344 (4.40); (EtOH-AcONa) 241 (4.37), 277 (4.36), 356 (4.23). Found: C, 61.56; H, 4.05. $C_{17}H_{14}O_7$ requires: C, 61.82; H, 4.27%) (lit.[2], m.p. 269–272°, IR 3430, 1670, 1630, 1600, 1530, 1514 cm^{-1}, UV λ_{max} nm: 273, 342) (triacetate, m.p. 186.5–187°, UV λ^{max}_{EtOH} nm (log ε): 262 (4.17), 320 (3.93). Found: C, 60.52; H, 4.29. $C_{23}H_{20}O_{10}$ requires: C, 60.52; H, 4.42%) (lit.[2], m.p. 189–190°). The properties of the synthetic samples of I and II were superimposable with those recorded in the literature[1,2] for 6-methoxyluteolin and desmethoxycentaureidin.

Zusammenfassung. Die Synthese von 6-Methoxyluteolin und Desmethoxycentaureidin aus 2,4-Dibenzyloxy-3-Methoxy-6-Oxyacetophenon wird beschrieben.

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19 November 1968.

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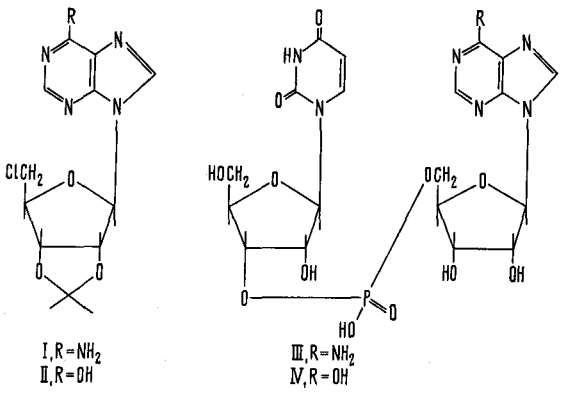
Synthesis of Dinucleoside Phosphates by Reaction of 5′-Chloro-5′-deoxynucleosides with Nucleotide Anions

The use of purine cyclonucleosides in the synthesis of internucleotide bonds was recently reported from this laboratory. The dinucleoside phosphate obtained by treatment of 8,5′-*O*-anhydro-2′,3′-isopropylideneadenosine with uridine-3′-phosphate anion had on OH function at position 8 of the purine moiety[1], while the product obtained from 8,5′-*S*-anhydrognanosine had an SH group, which required treatment with Raney Ni for its removal[2]. In this communication we report the synthesis of uridylyl-(3′-5′)-adenosine and uridylyl-(3′-5′)-inosine by a method which allows the isolation of the products with no substituents. This method involves the treatment of the appropriate 5′-chloro-5′-deoxy-2′,3′-*O*-isopropylidene purine nucleoside with the nucleoside phosphate anion.

5′-Chloro-5′-deoxynucleosides. The required 5′-chloro-5′-deoxynucleosides were conveniently obtained by treating the corresponding 2′,3′-*O*-isopropylidene nucleoside with thionyl chloride as exemplified by the preparation of 5′-chloro-5′-deoxy-2′,3′-*O*-isopropylidene adenosine (I).

A solution of dry 2′,3′-*O*-isopropylidene adenosine (500 mg) in thionyl chloride[3] (1.5 ml) was allowed to stand at room temperature for 12 h in a stoppered flask. Thionyl chloride was removed under reduced pressure and benzene (8 ml) added and evaporated. The residue so obtained was dissolved in an ice-cold mixture of triethylamine (2.5 ml), water (0.5 ml) and alcohol (2.0 ml) and the solution set aside for 30 min before being evaporated to dryness in vacuo. Water was then added to the residue, the whole extracted with benzene (5 × 25 ml) and the benzene extract washed with water (20 ml). Removal of solvent and crystallization of the residue from water yielded the product (316 mg, 60%), m.p. 255–256° (dec., softens at 175°). Rf (B)[4], 0.92; Found: C, 47.71; H, 5.02; N, 22.01; Cl, 10.46. Anal. calcd. for $C_{13}H_{16}N_5O_3Cl$: C, 47.92; H, 4.91; N, 21.50; Cl, 10.90%. λ_{max}, H_2O-260 nm (ε, 13,450); 0.1 *N* HCl-258 nm (ε, 17,500); 0.1 *N* NaOH-261 nm (ε, 16,450). Formic acid treatment of I at room temperature for 48 h yielded the deblocked product, 5′-chloro-5′-deoxyadenosine[5], m.p.



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[3] Thionyl chloride was distilled over quinoline and linseed oil.
[4] Solvent A, isopropanol – ammonia – water (7:1:2) ascending. Solvent B, butanol-acetic acid-water (4:1:5) descending.
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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.

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

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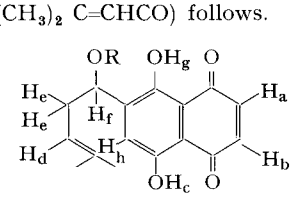
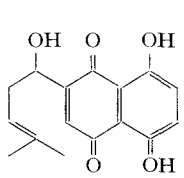
[8] Communication No. 1327 from the Central Drug Research Institute.

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.
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[8] O.R.D. curve indicates not dextro-isomer shikonin [5].