C. **From 6 and 48% HBr.**—A solution of 1 g (3.22 mmol) of **6** in 10 ml of 48% HBr was refluxed for 30 min, diluted with 10 ml of water, and stored at 4°. The crystals that formed were filtered, washed with water, and dried to give 1 g (90%) of **7** HBr, mp 246–248°, identical in mixture melting point and tlc with the **7** HBr prepared *via* A.

1-(3,4-Dihydroxybenzyl)-6,7-dihydroxyisoquinoline Hydrobromide (8 HBr).—A solution of 2 g (5.9 mmol) of **7** HBr in 20 ml of 48% HBr was refluxed for 8 hr and evaporated *in vacuo*. The residue was crystallized from water to give 1.42 g (74%) of **8** HBr (identical in mixture melting point and tlc with the **8** HBr, obtained from **1** in 80% yield by the same procedure[4]): mp 257–259°; R_f 0.04; nmr δ 4.50 (CH_2), 6.60 ($C_{2'}$, $C_{5'}$, $C_{6'}$), 7.40, 7.65 (C_5, C_8), 8.00, 8.23 (C_3, C_4), 9.00 (broad, OH, NH^+).

Anal. Calcd for $C_{16}H_{13}NO_4 \cdot HBr$: C, 52.75; H, 3.87. Found: C, 52.81; H, 4.01.

Registry No.—**1**, 58-74-2; **2**, 18813-60-0; **3**, 18694-10-5; **6**, 16637-56-2; **6** HCl, 16637-68-6; **6** picrate, 23740-72-9; **7** HCl, 23829-46-1; **7** HBr, 23740-73-0; **8** HBr, 23740-74-1; **9**, 23740-75-2; **10**, 23740-76-3; **11**, 4672-97-3; **12** HCl, 4761-17-5.

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Microbiological Hydroxylation of Allethrone

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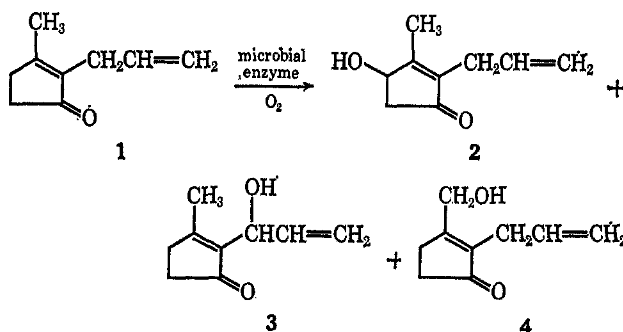
A number of synthetic analogs of the natural pyrethrins[1] exhibit high insecticidal activity. One of these, allethrin,[2] is prepared commercially by esterification of allethrolone (**2**) with chrysanthemic acid. We have recently investigated the microbiological conversion of cinerone into cinerolone[3] and now wish to report a similar conversion of allethrone (**1**) into allethrolone (**2**). Allethrone (**1**) was prepared by treatment of 2-N-pyrrolidino-5-methyl-2-cyclopenten-1-one with allylmagnesium bromide followed by dehydration as described by Dahill.[4]

Incubation of **1** with *Aspergillus niger* NRRL 3228 for 12 days gave a crude product, which was shown by glpc analysis to contain three major components (retention times of 5.5, 6.6, and 11.6 min, respectively). Small amounts of the three pure components were separated by preparative glpc and their mass spectra showed them to be monohydroxylated isomers of allethrone. Based on allethrone consumed, the yield of the mixture was 22%. The major component (73% of the mixture) was identified as allethrolone (**2**) by comparison of its glpc retention time and spectra (ir, nmr, and mass) with those of the authentic compound.[5]

The component with the shortest retention time (5% of the mixture) gave an infrared spectrum with a carbonyl band at 5.95 μ, indicating hydrogen bonding to the ketone. The nmr spectrum revealed three vinyl protons, one ring methyl, and two ring methylenes. The nmr absorption of the side-chain methylene group was absent and was replaced by a broad peak at δ 5.06. These data are consistent with structure **3**, which incorporates a side-chain hydroxyl group.

The component with the longest retention time (18% of the mixture) gave a normal cyclopentenone carbonyl band at 5.88 μ in the infrared spectrum. The nmr spectrum exhibited three vinyl protons, two ring methylenes, and a side-chain methylene. The nmr absorption of the ring methyl was absent and a two-proton singlet appeared at δ 4.50 consistent with structure **4**, in which hydroxylation has occurred on the ring methyl group.



Experimental Section[6]

Thirty 500-ml erlenmeyer flasks containing 100-ml quantities of fermentation medium were inoculated with a heavy filamentous growth of *Aspergillus niger* NRRL 3228 and incubated at 28° on a rotary shaker. The shaker was operated at 280 rpm and described a 2-in. circular orbit. After 2 days, 50 mg of allethrone (**1**) dissolved in 2 ml of absolute ethanol was added to each flask. Incubation was continued for 12 days and the contents of the flasks were then pooled and filtered to remove the cells. The cells were washed with 300 ml of distilled water and the washing was added to the filtrate. The filtrate was extracted with three equal volume portions of methylene chloride. After drying (Na_2SO_4), the solvent was removed at reduced pressure and the residual oil was distilled. The low-boiling fraction [bp 35–47° (0.1 mm), 0.49 g] was shown by thin layer chromatography (tlc) to contain only unreacted allethrone. The high-boiling fraction [bp 47–110° (0.1 mm), 0.55 g] on tlc revealed a spot with an identical R_f as authentic allethrone along with a slower moving spot with an identical R_f as authentic allethrolone. The allethrone (0.13 g) was separated from the allethrolone fraction (0.24 g) by preparative tlc on silica gel. Evaporative distillation of the allethrolone fraction at 0.1 mm (bath temperature 120°) gave 0.22 g of a colorless oil, λ_{max}^{EtOH} 229 mμ (ϵ 11,300). Glpc on a 10% EPON column at 200° showed three major peaks: peak 1, structure **3** (5% of the total, retention time of 5.5 min); peak 2, structure **2** (73% of the total, retention time of 6.6 min, identical with the retention time of authentic allethrolone); and peak 3, structure **4** (18% of the total, retention time of 11.6 min). Mass spectra were obtained using a Finnigan mass spectrometer coupled to a gas chromatograph and showed the three components to be isomers of molecular weight 152. The mass spectrum of peak 2

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(6) The ir spectra were determined using a Beckman IR-9 spectrophotometer. The uv spectra were obtained with a Cary 14 spectrophotometer. The nmr spectra were determined using a Varian HA-100 spectrometer with a C-1024 time-averaging computer when necessary. The mass spectra were obtained using a Finnigan mass spectrometer coupled to a Perkin-Elmer gas chromatograph.

was identical with that of authentic allethrolone,[5] while those of the other two peaks exhibited similar fragmentation patterns. The bands at m/e 134 ($M^+ - H_2O$) and 121 ($M^+ - CH_2OH$) were much more intense in peak 3, structure **4**, than in the other two peaks.

Small amounts of the three pure components were separated by preparative glpc using the above column. Data for peak 1, structure **3**, follow: ir (neat) 2.92 (broad), 5.95, and 6.10 μ; nmr ($CDCl_3$, time averaged, 140 scans) δ 2.09 (s, 3, CH_3), 2.42 (m, 2, CH_2CH_2), 2.56 (m, 2, CH_2CH_2), 5.06 (m, 1, CHOH), 5.14 and 5.24 (m, 2, CH_2=), and 6.12 (m, 1, CH=). Peak 2 gave ir and nmr spectra identical with those of authentic allethrolone. Data for peak 3, structure **4**, follow: ir (neat) 2.92, 5.88, and 6.04 μ; nmr ($CDCl_3$) δ 2.36 (m, 2, CH_2CH_2), 2.61 (m, 2, CH_2CH_2), 2.94 (d, 2, J = 6 Hz, $-CH_2CH$=), 4.50 (s, 2, CH_2OH), 4.84 and 4.97 (m, 2, CH_2=), and 5.75 (m, 1, CH=).

Registry No.—**1,** 3569-36-6; **2,** 23680-22-0; **3,** 23680-23-1; **4,** 23680-24-2.

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Conversion of 2′,3′-*O*-Isopropylidene Adenosine into Its 5′,5′-Di-*C*-Methyl Derivative[1]

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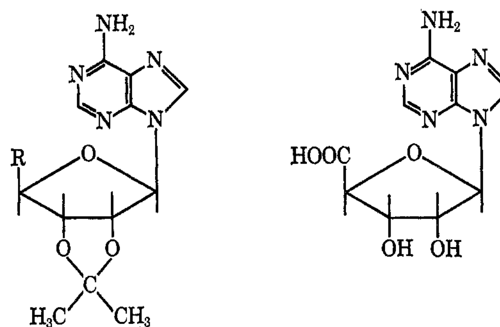
Direct chain extension and other carbon substitutions at the 5′ position of purine ribonucleosides have been restricted to oxidation of nucleosides to the 5′ aldehydes[2] followed by application of the Wittig reaction[3] or to conversion of a 5′-halogeno-5′-deoxy nucleoside into a 5′-cyano compound.[4] Syntheses of other 5′ carbon-substituted purine nucleosides, such as 5′-hydroxymethyl-5′-deoxyadenosine (homoadenosine)[5,6] and 5′,5′-di-*C*-methyladenosine,[7] have been effected by condensation of the respective blocked sugar derivatives with the adenine moiety. We now illustrate the practicability of a new approach which comprises conversion of a nucleoside, *via* its 5′-carboxylic acid, into the 5′-carbomethoxy derivative and application to the latter of the Grignard reaction.

Adenosine has been converted into the 5′-carboxylic acid **6** by oxidation with molecular oxygen in the presence of a platinum catalyst.[8] In our laboratory, this procedure, when applied to 2′,3′-*O*-isopropylidene adenosine (**1**), consistently gave low yields (2–5%) with reduced Adams catalyst from a variety of commerical preparations. Treatment of **1** with chromium trioxide in the presence of pyridine, acetic acid, or water yielded a complex mixture of products. Oxidation with potassium permanganate gave less complex mixtures, and after trials in the pH range of 2–12 and temperatures of 0–80°, a procedure was selected which employed 2 molar equiv of potassium permanganate at room temperature and pH 9–9.5. Although only *ca.* 30% conversion into the carboxylic acid **2** was obtained, the yield based on recovered isopropylidene adenosine was 90%. The product could be isolated directly in pure form and unreacted material could be readily recovered and recycled. When conversion of **1** into **2** was enhanced by the use of stronger oxidizing conditions, additional products were obtained and purification of **2** was rendered more tedious. The purification and properties of one such by-product (as yet of unassigned structure) is detailed in the Experimental Section. Two recently described alkaline potassium permanganate oxidations of **1**[9,10] were found to yield 50–60% homogeneous **2** and three by-products which amounted to 15% of the weight of **1** employed. Acidic treatment of **2** removed the isopropylidene group to furnish 9-(β-D-ribofuranosyluronic acid)adenine (**6**). Attempts to obtain **6** by direct oxidation of adenosine with potassium permanganate, chromium trioxide–acetic acid, or chromium trioxide–pyridine produced a complex mixture of products.



Treatment of the carboxylic acid **2** with diazomethane produced the methyl ester **3** in 90% yield. The only other product detected was trace amounts of the amine **4**, the structure of which was deduced from nmr data and elemental analysis and confirmed by comparison with a specimen prepared by reduction of 5′-azido-5′-deoxy-2′,3′-*O*-isopropylidene adenosine.[11]

(1) This work was supported by USPHS Grants CA-06927, FR-05539, and CA-11196, American Cancer Society Grant IN-49, an appropriation from the Commonwealth of Pennsylvania, and an award from The Pennsylvania Science and Engineering Fund.

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