References and Notes

1. M. Horiguchi and M. Kandatsu, *Nature* **184**, 901 (1959); *Bull. Agr. Chem. Soc. Japan* **24**, 565 (1960).
2. J. S. Kittredge, E. Roberts, D. G. Simonsen, *Biochemistry* **1**, 624 (1962).
3. A. J. de Koning, *Nature* **210**, 113 (1966).
4. L. D. Quin, *Biochemistry* **4**, 324 (1965).
5. T. Hori, O. Itasaka, H. Inoue, K. Yamada, *J. Biochem. Tokyo* **56**, 477 (1964).
6. M. Kandatsu and M. Horiguchi, *Agr. Biol. Chem. Tokyo* **26**, 721 (1962).
7. H. Shimizu, Y. Kakimoto, T. Nakajuma, A. Kanazana, I. Sano, *Nature* **207**, 1197 (1965); M. Kandatsu and M. Horiguchi, *Agr. Biol. Chem. Tokyo* **29**, 781 (1965).
8. G. Rouser, G. Kritchevsky, D. Heller, E. Lieber, *J. Amer. Oil Chemists' Soc.* **40**, 425 (1963).
9. L. D. Quin, *Science* **144**, 1133 (1964); H. Rosenberg, *Nature* **203**, 299 (1964).
10. J. S. Kittredge and R. R. Hughes, *Biochemistry* **3**, 991 (1964).
11. J. S. Kittredge, A. F. Isbell, R. R. Hughes, *ibid.* **6**, 289 (1967).
12. L. D. Zeleznick, T. C. Myers, E. B. Titchener, *Biochim. Biophys. Acta* **78**, 546 (1963).
13. D. R. Harkness, *J. Bacteriol.* **92**, 623 (1966).
14. D. G. Simonsen and E. Roberts, in preparation.
15. A. Waksman and E. Roberts, *Biochem. Biophys. Res. Commun.* **12**, 263 (1963).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. G. Randall, *J. Biol. Chem.* **193**, 265 (1951).
17. A. J. de Koning, *Biochim. Biophys. Acta* **130**, 521 (1966).

18. Aided by contract Nonr 3001(00), (NR 108-458), between ONR and the City of Hope Medical Center; and by NIH grant C-2568. We thank A. F. Isbell, Department of Chemistry, Agricultural and Mechanical College of Texas, for donating the synthetic aminoalkylphosphonic acids that made this work possible.

14 September 1967

Cyclic Uridine-3′,5′-Phosphate: Molecular Structure

Abstract. *The crystal structure of the triethylammonium salt of cyclic uridine-3′,5′-phosphate was solved by use of the tangent formula to refine phase angles based upon the positions of six of the atoms. The two independent uracil rings are planar and in the keto form. The base-sugar torsion angles are in the* anti *range. The sugar puckering is C3′*-endo, *and the ribose conformation about the C5′–C4′ bond is* trans-gauche.

Ribonucleic acid is a polynucleotide in which the monomeric units are bonded together through phosphodiester linkages between the C3′ and C5′ atoms of adjacent sugars. Ribonucleotides containing a cyclic phosphate ester are intermediates in the breakdown of RNA by ribonuclease, acid, or alkali (*1*). Moreover, the preparation of cyclic 3′,5′-ribonucleosides was important in the synthesis of the specific 3′,5′-phosphodiester linkage of a dinucleotide (*2*). No detailed molecular structures of cyclic nucleotides have been reported.

I have studied the crystal structure of cyclic uridine-3′,5′-phosphate (3′,5′-UMP) in order to determine the conformation and stereochemistry of the molecule. Precise stereochemical and structural information about nucleosides and nucleotides is of importance in the development and evaluation of hypotheses regarding the structure and reaction mechanisms of nucleic acids and related species. Recent publications (*3–5*) have compared the results of studies of crystal structure of nucleotides and of a 2′,5′-dinucleoside phosphate (*6*) in order to draw conclusions regarding favorable conformations for these molecules. Sundaralingam analyzed the furanose ring conformations in nucleic acids (*7*) and, on the basis of this work and stereochemical considerations, predicted several structural features for the 3′,5′-cyclic nucleotides; his predictions have proved to be quite accurate for 3′,5′-UMP.

Rammler crystallized the nucleotide as the triethylammonium salt from a mixture of alcohol and water. The cell dimensions of the crystals are: *a*, 15.49 ± 0.03; *b*, 11.67 ± 0.02; *c*, 11.01 ± 0.02; *β*, 98.5 ± 0.2 deg; systematic extinctions indicate that the space group of this optically active molecule is $P2_1$. The measured density, 1.38 g/cm^3, corresponds to two molecules of the 3′,5′-UMP, and two triethylammonium ions in the asymmetric unit, and twice this amount in the unit cell (d_{calc}, 1.38 g/cm^3). The existence of two crystallographically independent molecules in the unit cell provides a useful internal check on the molecular dimensions and the conformation of the nucleotide. The x-ray diffraction data were collected on a G.E. goniostat having a single-crystal orienter using the stationary crystal–stationary counter technique. The preliminary refinement that I report was based upon 2405 nonzero intensities with 2*θ* less than 125 deg.

One phosphate group and the second unique phosphorus were located in the cell by inspection of a sharpened three-dimensional Patterson synthesis. The phase angles predicted on the basis of these six atoms were refined by use of Karle and Hauptman's tangent formula (*8*), and a Fourier map based upon the refined phases and observed amplitudes revealed the rest of the structure quite clearly. Karle (*9*) reports similar successful applications of the tangent formula. The trial structure was refined by use of a block-diagonal least-squares refinement program, with each of the atoms assigned an isotropic temperature parameter. Three cycles of refinement reduced the agreement index *R* to 16 percent; several cycles of anisotropic refinement have further reduced this figure to 8 percent. The molecular structure that I describe is that reflected by the coordinates from the anisotropic refinement. The estimated errors in bond lengths are ± 0.03 Å; in bond angles, ± 2 deg. Additional data are being collected for improvement of the precision of the analysis, but this extension will not alter the conformational features that I describe. The bond distances and angles in the molecules do not differ significantly from the expected values.

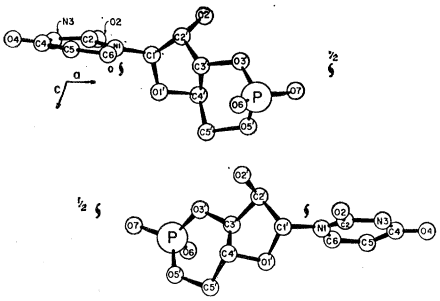

Fig. 1. The molecules viewed down *b*. No hydrogen atoms or triethylammonium ions are shown; C2 of the upper molecule was moved slightly to avoid overlap in the view.

Figure 1 is a schematic representation of the molecule and indicates the numbering system used. The uracil rings are planar within the accuracy of the analysis and are in the keto form. The four carbonyl bonds average 1.19 Å. None of these oxygens are involved in hydrogen bonds, and the distances can be compared with the carbonyl distance of 1.201 Å found in cytidylic acid *b* (*10*). Nitrogen N3 is hydrogen bonded to O6 of a neighboring phosphate group in both unique molecules. The hydrogen bond distances (2.76 and 2.85 Å) are in the expected range, and the P–O6 and P–O7 distances average 1.47 Å. If O6 or O7 were protonated, the P–O distance would be 1.56 Å (*4*). The O7 atoms of the phosphate groups are hydrogen bonded to the nitrogen atom of the triethylammonium ion in both molecules.

The torsion angles of the uracil rings about the beta-glycosidic C1′–N1 bonds are −72 and −56 deg, both in the *anti* range, as is found in most nucleic acid derivatives (*5, 11*). The C3′ atoms of the furanose rings are 0.64 and 0.58 Å out of the planes defined by the other four atoms. The displacements are on the same side of the planes as

C5′, in correspondence with the C3′-*endo* conformation, as predicted by Sundaralingam (*12*). The only alternative is C2′-*exo*, as other possibilities place C3′ in an unfavorable position for the formation of a cyclic phosphate through O3′. The ribose conformation about the C5′–C4′ bond is *trans-gauche* in the notation of Shefter and Trueblood (*3*). The ϕoo values are 176 and 174 deg; the ϕoc values, 63 and 60 deg (ϕoo is the dihedral angle between the O5′–C5′–C4′ plane and the C5′–C4′–O1′ plane; ϕoc is the O5′–C5′–C4′ angle with C5′–C4′–C3′). Both angles correspond to views of this portion of the structure down the C5′–C4′ bond.

This is an unusual conformation for nucleotides; all previous studies suggest that the preferred conformation is *gauche-gauche*, with both angles about 60 deg (*3, 13*). It is, however, the only conformation possible for 3′5′-cyclic nucleotides, since the phosphate must be rotated until the C5′–O5′ bond is nearly antiplanar to the C4′–O1′ bond in order to form the 3′ ester linkage. A similar condition holds when one looks down the O3′–C3′ bond. The phosphate must be rotated from its position in, for example, adenosine-3′-phosphate (*12*) so as to make the P–O3′–C3′ and O3–C3′–C4′ dihedral angle about 60 deg (*14*); otherwise, the bond angles about C3′ would have to be greatly distorted to form the cyclic phosphate. The dihedral angles are 60 and 62 deg for the two unique 3′,5′-UMP molecules.

CHARLES L. COULTER
Department of Anatomy, University of Chicago, Chicago, Illinois 60637

References and Notes

1. R. F. Steiner and R. F. Beers, *Polynucleotides* (Elsevier, New York, 1961), p. 41.
2. M. Smith and H. Khorana, *J. Amer. Chem. Soc.* **81**, 2911 (1959).
3. E. Shefter and K. N. Trueblood, *Acta Cryst.* **18**, 1067 (1965).
4. M. Sundaralingam and L. H. Jensen, *J. Mol. Biol.* **13**, 930 (1965).
5. A. E. V. Hashemeyer and A. Rich, *ibid.* **27**, 369 (1967).
6. E. Shefter, M. Barlow, R. Sparks, K. Trueblood, *J. Amer. Chem. Soc.* **86**, 1872 (1964).

M. Sundaralingam, *ibid.* **87**, 599 (1965).

8. J. Karle and H. Hauptman, *Acta Cryst.* **9**, 635 (1956).
9. J. Karle, *ibid.*, in press.
10. M. Sundaralingam and L. H. Jensen, *J. Mol. Biol.* **13**, 914 (1965).
11. J. Donohue and K. N. Trueblood, *ibid.* **2**, 363 (1960).
12. M. Sundaralingam, *Acta Cryst.* **21**, 495 (1966).
13. ———, *Biopolymers*, in press.
14. ———, unpublished.
15. Supported by NSF grant GB-5504 and NIH grant FR-5367. I thank Dr. D. N. Rammler for the crystals, M. Sundaralingam for making available his unpublished results, E. B. Fleischer for use of his diffractometer, and Stuart Hawkinson, Joseph Gatewood, and Karen Harris for technical assistance.

4 December 1967

Brevicomin: Principal Sex Attractant in the Frass of the Female Western Pine Beetle

Abstract. *The principal component of the sex attractant produced by the female western pine beetle* (Dendrotonus brevicomis) *is* exo-*7-ethyl-5-methyl-6,8-dioxabicyclo*[*3.2.1*]*octane; the trivial name* brevicomin *is suggested.*

We report the isolation, identification, and synthesis of the principal component of the sex attractant of the western pine beetle *Dendroctonus brevicomis* (Coleoptera: Scolytidae). The sex attractant in the frass produced by the female *D. brevicomis* boring in ponderosa pine initiates the mass attack that usually kills the tree. The response of both sexes in the laboratory bioassay (*1*) for *Ips confusus* was used to monitor the isolation procedure.

A total of 1.6 kg of frass produced by unmated female beetles was collected continuously from beetle-infested logs. The frass dropped directly into bottles containing benzene; after an 8-hour collection period, each bottle was removed and stored at −40°C. Batches of 150 g of benzene-soaked frass were stirred in 300 ml of benzene, under nitrogen, at 70°C for 1 hour. The benzene slurry was extracted in a Waring Blendor for 10 minutes and filtered. The solid material was again extracted in the Waring Blendor with two 300-ml portions of benzene. The combined filtrate was concentrated to about 150 ml at about 160 mm-Hg; the benzene distillate was used for subsequent extractions. The concentrate was distilled in a short-path (2 cm) apparatus at 100°C and 0.03 mm-Hg for 1 hour onto a condenser cooled with dry ice. Distillate equivalent to 300 g of frass was concentrated to 15 ml under reduced pressure (160 mm-Hg) and chromatographed on a silica gel column [Gallard-Schlesinger, 90 to 200 mesh, 60 g; water-cooled, glass column (42 by 1.8 cm); 20°C].

The column was eluted with 200 ml of benzene. The next 200 ml was added from a reservoir of 100 ml of benzene to which 100 ml of ether was added continuously, with stirring, at the same rate as the reservoir contents were added to the column. After this gradient elution, the column was eluted successively with 200 ml of ether, 200 ml of acetone, and 200 ml of methanol. The active fraction, which was eluted in the volume between 300 and 450 ml, was concentrated under reduced pressure (160 mm-Hg) to about 2 ml and fractionated in 0.5-ml portions by gas-liquid chromatography (GLC) [4 percent SE 30 on Chromosorb G, 45 to 60 mesh, glass column (91 cm by 11 mm inside diameter)]. Injections were made directly on a glass "pre-column" (35 by 11 mm i.d., Chromosorb W-HMDS, 30 to 60 mesh). The temperature was programmed 100° to 150°C at 6°/min, 150°C for 45 minutes, backflushed at 200°C for 18 minutes; the He flow rate was 50 cm^3/min. The active fraction (82 mg), which eluted between 9 and 45 minutes, was fractionated in three portions on a GLC glass column (4 percent Carbowax 20M on Chromosorb G, 60 to 80 mesh, 3 m by 4 mm i.d., 50° to 150°C at 2°/min; raised at full power to 200°C; total time 80 minutes; He flow rate 25 cm^3/min). The active material (5.7 mg), which was eluted between 22 and 31 minutes, was fractionated on a GLC column (4 percent FFAP on Chromosorb G, 60 to 80 mesh, 6 m by 4 mm i.d. glass, 150°C, He, 25 cm^3/min). The active compound ($\sim$ 2 mg) had a retention time of 17 minutes.

The molecular weight of the active compound by high-resolution mass spectrometry was 156.11572 (calculated for $C_9H_{16}O_2$: 156.11502). The infrared spectrum showed intense peaks between 8 and 11.7 μm, but neither hydroxyl nor carbonyl groups were present. The NMR spectrum (CCl_4, τ) showed: 6.02 (1 proton, width at ½ height $\sim$ 5 hz); 6.22 (1 proton, triplet, $J = 6.2$ hz); $\sim$ 8.1 to $\sim$ 8.9 (8 protons); 8.70 (3 protons, singlet); 9.13 (3 protons, slightly distorted triplet). A solution (1 μg/ml) in hexane showed no absorption in the ultraviolet. A 0.05 percent hexane solution showed no optical rotation between 350 nm and 250 nm. Treatment of 50-μg samples with the following reagents gave only starting material: diborane in tetrahydrofuran at room temperature for 4 hours, lithium aluminum hydride in tetrahydrofuran at room temperature for 4 hours, and lithium aluminum hydride in tetrahydrofuran at reflux for 1 hour. Catalytic hydrogenolysis of a 50-μg sample (no solvent) in a modified Beroza carbon-skeleton determinator (*2*) [1 percent neutral palladium on Gas-Chrom P,