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).
7. 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 ———, unpublished.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 cm³/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 cm³/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 cm³/min). The active compound (~ 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 ~ 5 hz); 6.22 (1 proton, triplet, $J = 6.2$ hz); ~ 8.1 to ~ 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,

60 to 80 mesh, 250°C, H_2 flow rate 50 cm³/min; GLC separation on 5 percent SE 30 on acid-washed Chromosorb W, 60 to 80 mesh, aluminum tubing (6 m by 4.7 mm i.d.), 50° to 150°C at 4°/min] gave *n*-nonane as the major product, identified by mass spectrometry.

From this information, we could write several likely cyclic ketal structures. The following synthetic sequence gave both *exo*- and *endo*-7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane (Fig. 1).

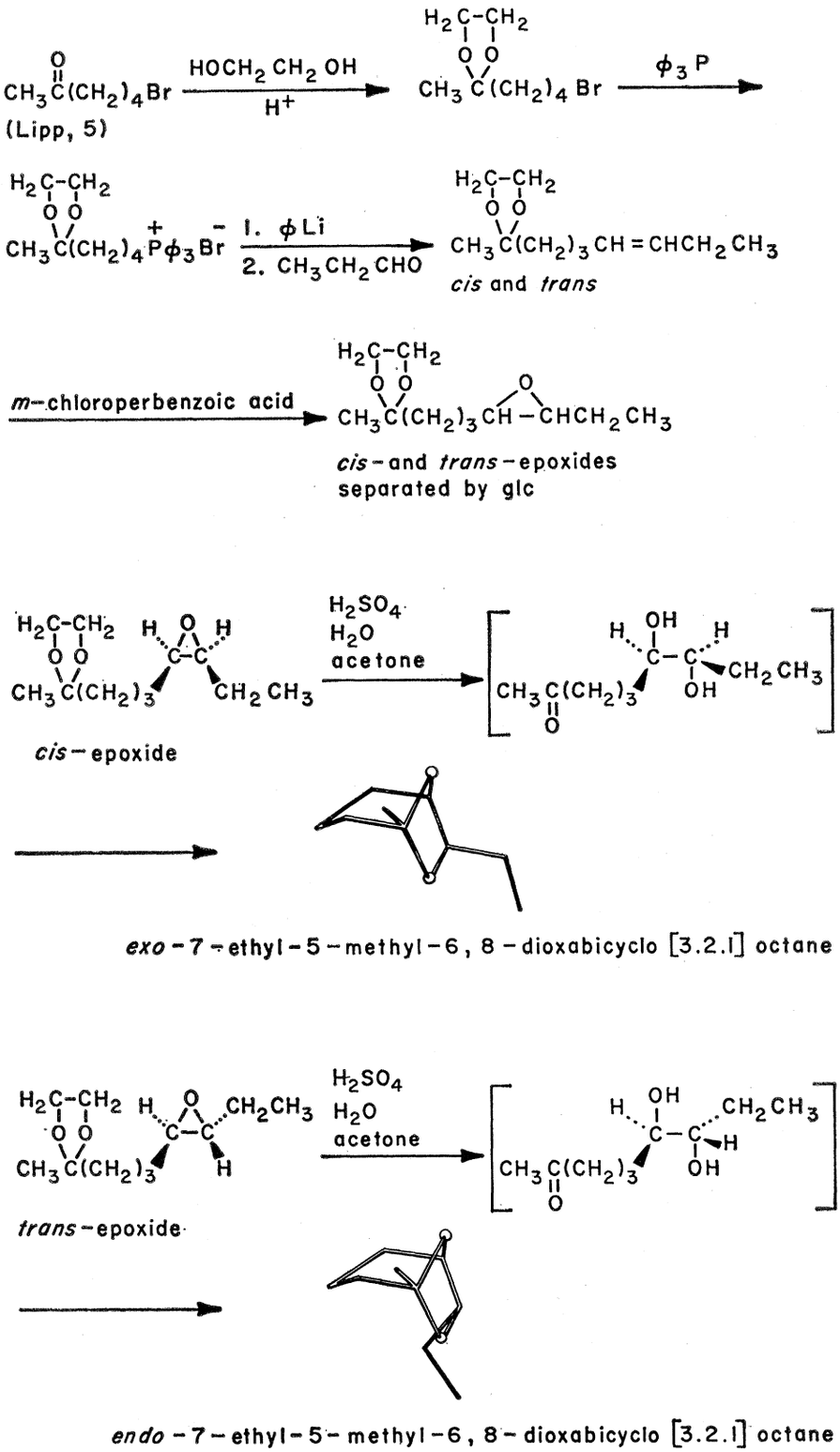

Fig. 1. Synthesis of *exo*- and *endo*-7-ethyl-5-methyl-6,8-dioxabicyclo [3.2.1]octane.

The spectra of the synthetic *exo*-isomer matched those of the active isolated compound. The spectra of the synthetic *endo*-isomer matched those of an inactive compound isolated from frass to which the *endo*-configuration had been assigned on NMR evidence.

Whereas the *cis*- and *trans*-olefin intermediates showed the same retention time on a Carbowax 20M and on an Apiezon column, the *cis*- and *trans*-epoxides were readily separated on a Carbowax 20M column (8 percent on Chromosorb G, 183 cm by 6.4 mm o.d., 145°C, 100 cm²/min, *cis* retention time, 26 minutes, *trans* retention time 22 minutes). The relative shift positions of the epoxide protons determined the configuration assigned (*3*) (*cis* protons multiplet at $\tau \sim 7.28$, *trans* protons at $\tau \sim 7.50$). The *cis*-epoxide on acid hydrolysis led directly to the *exo*-, and the *trans*-epoxide to the *endo*-bicylic products. The geometry of the bicylic compounds isolated had been tentatively assigned because additional splitting of the 1- and 7-protons was predicted and observed for the *endo*-isomer. In the *exo*-isomer, the 1- and 7-protons are effectively decoupled because they form approximately a 90° dihedral angle.

Thus the structure of the active isolated compound is *exo*-7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane, for which we propose the trivial name *brevicomin*. This ring system is a novel structure for a natural product; it is represented in the literature only by several anhydrosugars.

In the laboratory bioassay, other fractions, though inactive by themselves, are synergistic when added to brevicomin. Male beetles responded to as little as a microgram of synthetic brevicomin. The isolated brevicomin elicited a response from females, but the response varied seasonally. During the autumn months, females failed to respond to either isolated or synthetic brevicomin.

While this paper was in review, the isolation from hop oil of 7,7-dimethyl-6,8-dioxabicyclo[3.2.1]octane was called to our attention (*4*).

ROBERT M. SILVERSTEIN
ROBERT G. BROWNLEE
THOMAS E. BELLAS
Life Sciences Division,
Stanford Research Institute,
Menlo Park, California

DAVID L. WOOD
LLOYD E. BROWNE
Department of Entomology and
Parasitology, University of California,
Berkeley

References and Notes

1. D. L. Wood, L. E. Browne, R. M. Silverstein, J. O. Rodin, *J. Insect Physiol.* **12**, 523 (1966).
2. M. Beroza, *Anal. Chem.* **34**, 1801 (1962); ——— and R. Sarmiento, *ibid.* **35**, 1353 (1963).
3. M. H. Gianni, E. L. Stogoyn, C. M. Orlando, *J. Phys. Chem.* **67**, 1385 (1963); R. J. Aplin and L. Coles, *Chem. Commun.* **1967**, 858 (1967).
4. Y. Naga and M. Kotake, *Tetrahedron Lett.* **1967**, 2459 (1967). We thank Dr. Roger Stevens for this reference.
5. A. Lipp, *Ann. Chem.* **289**, 173 (1896).

6. Supported by Forest Service, U.S. Department of Agriculture under contract No. 13-267 to Stanford Research Institute and grant No. 5 to the University of California, Berkeley. Supplementary support of work at University of California from California Division of Forestry, from T. B. Walker and Surdna foundations, and from several forest industries. We thank W. R. Anderson, Jr., R. F. Muraca, and W. N. Tuttle of SRI for spectral results, and P. Lim of SRI and E. Bunnenberg of Stanford University for the optical rotation determination. Infrared spectra were obtained on a Perkin-Elmer 221, NMR spectra on a Varian HA100, ultraviolet spectra on a Perkin-Elmer 202, mass spectra on CEC 21-103 and 21-110, and optical rotation in a microcell on Japan Spectroscopic Co. ORD/UV-5. Gas chromatography was carried out on a Varian Aerograph A90P3. The Beroza carbon-skeleton determinator was modified to permit introduction of neat microgram samples from a sealed capillary tube that was broken *in situ*; the apparatus was attached to a Varian Aerograph 205-2B.

27 November 1967

Corticotropin Release: Inhibition by Intrahypothalamic Implantation of Atropine

Abstract. *Anterior hypothalamic implantations of crystalline atropine markedly inhibit the adrenocortical responses evoked by surgical stress, ether anesthesia, or intravenous injection of arginine vasopressin. Similar implants in nearby regions of the brain or sham implantations in the same region were ineffective. The data suggest that the hypothalamic control of pituitary corticotropin may have a cholinergic component.*

Although there is general agreement that the hypothalamus controls pituitary secretion of corticotropin (ACTH) by way of a neurohumoral agent, corticotropin-releasing factor (CRF), relatively little is known about the factors that regulate the production and release of CRF itself. Histochemical studies have demonstrated the presence of acetylcholinesterase (*1*) and monoamine oxidase (*2*) in the hypothalamus, which suggest the presence of cholinergic and monoaminergic transmission. Moreover, fluorescence techniques have shown that norepinephrine and other biologically active monoamines may be found in the hypothalamus (*3*).

The functional significance of such substances in the hypothalamus remains unknown, but, since some of them undoubtedly act as synaptic transmitters peripherally, one may logically expect them to play a similar role in the central nervous system; if so, one or more of these substances may play a significant role in mediation of the neural control of production or release (or both) of CRF.

We have investigated this possibility by implanting some of these substances or their blocking agents in various hypothalamic areas and by then observing the effect on the adrenocortical stress response. We now report results with the anticholinergic agent atropine. For all experiments we used female albino rats weighing 110 to 120 g. Small pellets (200 to 250 μg) of crystalline atropine were implanted stereotaxically under pentobarbital anesthesia; sham operations were similar except that the implantation cannula contained no atropine. The responses of the adrenocortical system to various stimuli were then assayed by determining the concentration of corticosterone in plasma or corticosteroid production by adrenal glands incubated in vitro in a manner described (*4*). The basal levels for these assays in animals receiving only pentobarbital anesthesia are 5.0 ± 1.8 μg/100 ml and 7.9 ± 0.8 μg/100 mg hour^{-1}, respectively.

The stress inherent in the implantation procedure results in a rather prolonged release of ACTH, as is indicated by either of the assay methods (Fig. 1). In contrast, implantation of atropine in the region of the anterior hypothalamus quickly terminates this response. Activity of the adrenocortical system is very low 30 minutes after implantation and can be considered basal at 60 minutes.

The effective site of the atropine implants is on the midline just rostral to the paraventricular nuclei (Fig. 2). Similar implants in nearby regions had no effect, so the possibility is eliminated that the anterior hypothalamic implants were effective because of systemic distribution of the atropine by the circulation. Other drugs (for example, norepinephrine, epinephrine, and *d*-amphetamine) implanted in the effective site did not inhibit release of ACTH.

This inhibitive effect of atropine was observed even when other noxious stimuli were combined with the stress of implantation; for instance, when ether was substituted for the pentobarbital, corticoid production was again basal within 60 minutes of implantation. Similarly, when 50 milliunits of arginine vasopressin was administered intravenously immediately before the implantation of atropine, a very marked (but not quite complete) inhibition of arginine vasopressin–induced release of ACTH was observed.

Thus it was apparent that atropine in the anterior hypothalamus could terminate an adrenocortical response that had been evoked by any of several different stimuli. Our data suggest the presence of a cholinergic step in the mediation of the adrenocortical stress response. This suggestion is consistent with recent observations (*5*, *6*) of release of ACTH after cholinergic stimulation of various hypothalamic areas. Krieger and Krieger (*6*) have also prevented the normal diurnal rise in corti-

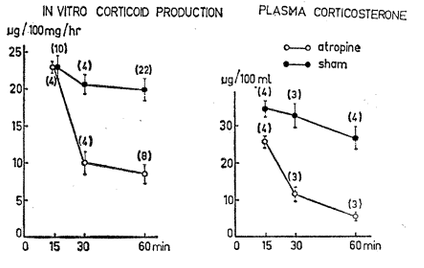

Fig. 1. Time courses of adrenocortical activities following implantations (of atropine or sham) in the anterior hypothalamus; implantation at zero time.

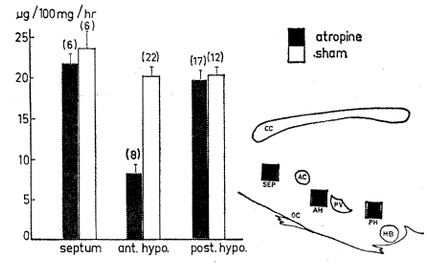

Fig. 2. Effective site of implantation. The histogram indicates corticoid productions in vitro 60 minutes after implantations were made in the three brain areas depicted in the accompanying midsagittal section of the rat brain. Abbreviations: *CC*, corpus callosum; *SEP*, septum; *AC*, anterior commissure; *OC*, optic chiasm; *AH*, anterior hypothalamus; *PV*, paraventricular nucleus; *PH*, posterior hypothalamus; *MB*, mammillary body.