Gallium reduction was examined in 0.1 *M* sodium salicylate (pH 4) in the presence and absence of 1 *M* KSCN in view of reports[3a,d,e] asserting that the gallium d.c. step is more clearly defined in such media. Admittance curves nearly identical as to position and morphology were obtained for these separate solutions, each giving evidence of two close-lying peaks, which were then made more clearly distinguishable by phase elimination of the 4.5-μa. background current; one such curve is shown as Figure 1B. The current minimum at -0.97 v. *vs.* s.c.e. coincides closely (considering possible differences in junction potential) with the d.c. half-step value of -0.988 v. reported previously.[10] Though a possible stepwise reduction of Ga(III) is not excluded, it would seem more reasonable at this juncture to suspect that the peaks, located at -0.95 and -1.01 v. *vs.* s.c.e., correspond to the reduction of two different Ga(III)–salicylate complex ions. This point is being pursued.

(10) E. Vinogradova and N. Chudinova, *Zavodsk. Lab.*, **22**, 1280 (1956); *Chem. Abstr.*, **51**, 11131*b* (1957).

Edward D. Moorhead
School of Chemistry, Rutgers, The State University
New Brunswick, New Jersey
Received March 29, 1965

Monocarbahexaborane(7)[1]

Sir:

Over the past several years the discovery and characterization of a considerable number of two-carbon carboranes have been reported.[2] In the course of investigating the products of an electric discharge of 1-methylpentaborane we have isolated a material, CB_5H_7, which represents the first known one-carbon carborane.

Using a silent electric discharge apparatus and techniques previously described,[2a,3] 0.15 mole of 1-methylpentaborane yields 13 mg. of a homogeneous compound: R_v (g.l.c.) = 0.65 (relative to B_5H_9, R_v = 1.00); vapor tension, 503 mm. at 26°; mol. wt. (gas density), 74.4, calcd. 73.0. The ^{11}B n.m.r. of the pure compound displays three sets of doublets (δ[4] +19.1, J = 184 c.p.s.; δ +9.7; J = 162 c.p.s.; δ −2.5, J = 174 c.p.s.) with an area ratio of 2:2:1, respectively, which together with the molecular weight data clearly indicates the presence of five borons. Additionally, the coupling constants for the observed doublets signify the attachment of one terminal hydrogen to each boron. The infrared spectrum exhibits medium intensity C–H (2960 cm.$^{-1}$), strong B–H (2640 cm.$^{-1}$), and medium B–H_{bridge}–B symmetric (2167 cm.$^{-1}$) stretching frequencies. However, a maximum of one bridge hydrogen and one hydrogen bonded to carbon is evident from a ^1H n.m.r. area analysis.

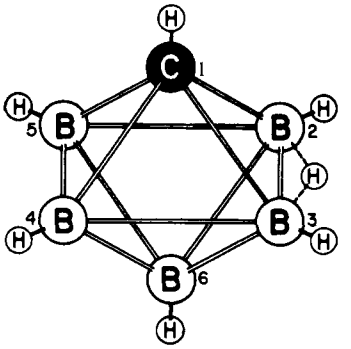

Figure 1. Proposed geometry of CB_5H_7. Lines drawn between central atoms (C, B) are not meant to indicate localized two-electron bonds.

The above evidence requires a molecular formula of CB_5H_7. This is substantiated by a sharp cutoff in the mass spectrum at *m/e* 74 which corresponds to the $^{12}C^{11}B_5{}^1H_7{}^+$ ion.

On the basis of the proposed octahedral structure[5] for the predicted $CB_5H_6^-$, it is expected that the conjugate acid, CB_5H_7, would differ only by a bridge hydrogen linking two adjacent borons. Certainly such a structure is consistent with all available information. From ^{11}B n.m.r. the isomeric possibility depicted in Figure 1 is favored, although placement of the bridge hydrogen between borons 2 and 6 is not excluded if an accidental overlap of resonance lines occurred.

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(5) W. N. Lipscomb, "Boron Hydrides," W. A. Benjamin, Inc., New York, N. Y., 1963, p. 89.

Thomas Onak, Rogers Drake, Gary Dunks
Department of Chemistry
California State College at Los Angeles
Los Angeles, California
Received March 20, 1965

The Selective Photoreduction of Uridine in Polynucleotides

Sir:

Problems dealing with the structure and function of biopolymers require organic reagents and reactions that specifically attack or modify single components of the macromolecule. While selective modifications have been elaborated for proteins,[1] only a relatively small number of such reactions are known for polynucleotides. Compared with the variety of amino acids, the four major base units of DNA and RNA are chemically much less differentiated. This lack of differential reactivity and the restrictions to an aqueous

(1) Based on nomenclature rules adopted for carboranes: R. Adams, *Inorg. Chem.*, **2**, 1087 (1963); also, private communication.

(2) (a) R. E. Williams, C. D. Good, I. Shapiro, and B. Keilin, Abstracts, 140th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1961, p. 14N; *J. Am. Chem. Soc.*, **84**, 3837 (1962); *ibid.*, **85**, 3167 (1963); (b) T. Onak, R. E. Williams, and H. G. Weiss, *ibid.*, **84**, 2380 (1962); (c) T. Onak, F. J. Gerhart, and R. E. Williams, *ibid.*, **85**, 3378 (1963); (d) T. L. Heying, J. W. Ager, S. L. Clark, D. J. Mangold, H. L. Goldstein, M. Hillman, R. J. Polak, and J. W. Szymanski, *Inorg. Chem.*, **2**, 1089 (1963); (e) M. M. Fein, J. Bobinski, N. Mayes, N. Schwartz, and M. S. Cohen, *ibid.*, **2**, 1111 (1963); (f) L. I. Zakharkin, V. E. Stanko, V. A. Bratzev, Yu. A. Chapovsky, and O. Yu. Okhlovystin, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk*, 2238 (1963); (g) D. Grafstein and J. Dvorak, *Inorg. Chem.*, **2**, 1128 (1963); (h) H. Schroeder and G. D. Vickers, *ibid.*, **2**, 1317 (1963); (i) S. Papetti and T. L. Heying, *J. Am. Chem. Soc.*, **86**, 2295 (1964); (j) F. Tebbe, P. M. Garrett, and M. F. Hawthorne, *ibid.*, **86**, 4222 (1964).

(3) T. Onak, R. P. Drake, and G. B. Dunks, *Inorg. Chem.*, **3**, 1686 (1964).

(4) Boron trifluoride was used for the external standard.

(1) *Cf.* B. Witkop, *Advan. Protein Chem.*, **16**, 221 (1961).

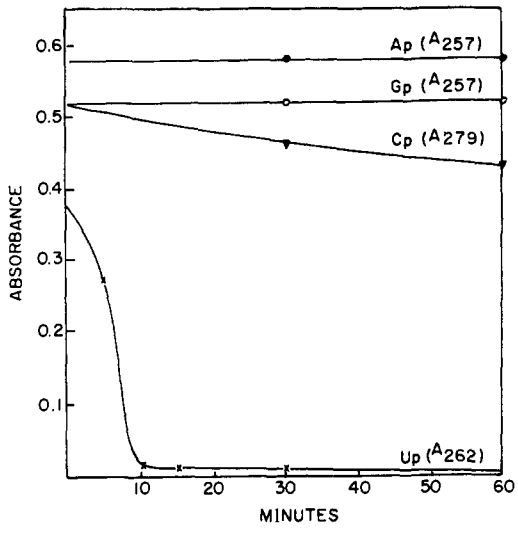


Figure 1. Photoreduction of uridylic acid (Up) to dihydrouridylic acid (H_2Up) compared with the three other major components of RNA.

system, room temperature, and a pH range of 4–10 make the elaboration of selective processes and reagents for nucleic acids a challenge. We wish to report a method for the *selective*[2] reduction of free or bound uridine to its 5,6-dihydro derivative which in the meantime has been discovered as a building stone in alanine transfer RNA.[3]

The starting point for this investigation was the observation that uracil and cytosine differ significantly in their electronic absorption spectra and in the π-electron-density or spin-density distribution of their first excited state as computed by the HMO or SCF method.[4]

The 5,6- double bond in both molecules, but more pronounced in uracil, assumes substantial diradical character in the first excited state. Superposition of the electron densities of the two singly occupied orbitals in the first excited state results in a small positive charge increment at C-6 relative to C-5 in uracil compared to an opposite polarization of this double bond in cytosine. This led to the expectation that uracil and cytosine and their derivatives should differ on irradiation in their reactivity toward nucleophilic agents, of which we investigated hydride ion.

When uridine and uridylic acid, either free or bound, were irradiated in the presence of sodium borohydride, rapid selective reduction to the 5,6-dihydro derivatives occurred in a reaction the mechanism and stereochemistry of which are now under investigation. Purine nucleosides and nucleotides are completely stable under these conditions, and cytidine undergoes photoreactions only to a minor extent (Figure 1). The loss of aromaticity upon saturation of the 5,6-double bond in the pyrimidine residues (by hydration, dimerization, and hydrogenation) was followed spectrophotometrically (250–280 mμ at pH 2). Specifically, the reduction to dihydrouridine was measured by the orcinol assay for ribose, which is positive (for the reduced molecule) because of the lability of the β-glycoside linkage, and by the Archibald assay[5] of the β-ureidopropionic acid formed on acid hydrolysis of the glycosidic bond and opening of the dihydrouracil by alkali.

Table I. The Stoichiometry of the Photoreduction of PolyU with $NaBH_4$

Irradiation time, min.	A[a]	B[b]	A + B	C[c]	A + C
0	8.9	0.1	9.0	0.0	8.9
10	5.3	3.3	8.6	3.4	8.7
20	2.0	6.6	8.6	6.9	8.9
30	0.3	8.1	8.4	8.6	8.9

[a] μmoles of unchanged PolyU, O.D.$_{260}$. [b] μmoles of titratable ribose. [c] μmoles of ureidopropionic acid.

Under the following optimal reaction conditions 90–95% of the λ 262 mμ absorption (pH 2) of pU or Up is lost within 15 min., at a substrate concentration of 10^{-3} *M* and an initial concentration of $NaBH_4$ of 10^{-2} *M*, pH 9.0–9.5 and room temperature. The light source is a low-pressure mercury lamp equipped with a Vycor filter. By this method dihydrouridine 5′-phosphate (pH_2U) and dihydrouridylic acid (H_2Up) have been synthesized on a preparative scale. The structures were confirmed by comparison of degradation products with authentic samples of dihydrouracil, ureidopropionic acid, and ribose 3- and 5-phosphate by thin layer chromatography and paper electrophoresis[6] and mass spectroscopy. Mass peaks corresponding to dihydrouracil, 114, have been found for both isomers; a mass peak at 210 for pH_2U was tentatively assigned to pH_2U–(H_3PO_4 + H_2O). The rate for the ring cleavage to ureidopropionic acid derivatives was determined in 0.1 *N* NaOH to be pseudo-first order for both isomers.[2]

The slow loss of A_{279} (pH 2) of Cp (Figure 1) under the conditions of photoreduction is attributed to the addition of water and dimerization[7] rather than to reduction to judge from the minor amount of ribose found by the orcinol assay after 3 hr. irradiation time and the partial recovery of A_{279} after heating the aliquots with acid.[8] Loss of ammonia after 3 hr. of photoreduction was negligible (Nessler assay).

The stoichiometry of the photoreduction of polyuridylic acid with $NaBH_4$ (Table I) and the base composition (total alkaline hydrolysis) of yeast RNA (Baker's yeast according to Crestfield, *et al.*) and transfer RNA (calf liver and *E. coli* B.) (Table II) as a function of exposure to photoreductive conditions demonstrate the selective disappearance of Up residues. The presence of pH_2U after photolysis was proved as outlined above. No fragmentation into smaller subunits was detected during photoreduction of RNA (ultracentrifuge, chromatography).

(2) Chemical and catalytic reductions would not differentiate between uridine [W. E. Cohn and D. G. Doherty, *J. Am. Chem. Soc.*, **78**, 2863 (1956)] and cytidine [M. Green and S. S. Cohen, *J. Biol. Chem.*, **228**, 601 (1958)].

(3) J. R. Penswick and R. W. Holley, *Proc. Natl. Acad. Sci. U. S.*, **53**, 543 (1965); R. W. Holley, *et al.*, *Science*, **147**, 1462 (1965); *cf.* D. O. Carr and S. Grisolia, *J. Biol. Chem.*, **239**, 160 (1964).

(4) M. Mantione and B. Pullman, *Biochim. Biophys. Acta*, **91**, 387 (1964); J. Baudet, G. Berthier, and B. Pullman, *Compt. rend.*, **254**, 762 (1962).

(5) R. M. Archibald, *J. Biol. Chem.*, **156**, 121 (1944).

(6) For the detection of dihydrouracil and ureidopropionic acid a modified Ehrlich reagent was used as described by R. M. Fink, R. E. Cline, Ch. McGaughey, and K. Fink, *Anal. Chem.*, **28**, 4 (1956).

(7) D. Shugar in "The Nucleic Acids," Vol. III, E. Chargaff and J. N. Davidson, Ed., Academic Press Inc., New York, N. Y., pp. 40–104.

(8) R. L. Sinsheimer, *Radiation Res.*, **1**, 505 (1954).

Table II. The Base Composition of RNA[a] and Transfer RNA from Calf Liver and *E. coli* B. after Exposure to Light in the Presence of $NaBH_4$

Materials	Irradiation time, min.	% Up	% Cp	% Ap	% Gp	% X [100−(Up+Cp+Ap+Gp)]
RNA (Baker's yeast)	0	24.9	22.3	25.0	27.7	0
	60	14.3	21.8	25.1	27.5	11.3
	120	9.0	21.4	25.2	27.5	16.9
	180	6.5	20.5	25.0	27.4	20.6
	240	5.6	21.1	25.3	27.3	20.7
Calf-liver transfer RNA	0	18.1	26.6	17.6	37.7	0
	270	10.4	26.3	17.0	36.9	9.4
E. coli B. transfer RNA	0	19.4	27.0	20.2	33.4	0
	270	11.7	25.8	20.1	33.7	8.7

[a] Baker's yeast according to Crestfield, *et al.*, Sigma.

For the sequence determination of polynucleotides the dihydrouridine units offer a handle for changing the pattern of enzymatic cleavage. The reductive modification of uridine-containing nucleotides, including coding triplets, may now help to answer a number of questions in biogenetics.

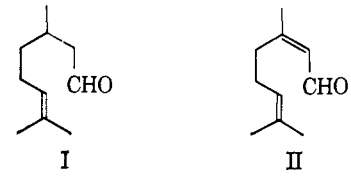
Peter Cerutti, Keiko Ikeda, Bernhard Witkop
National Institute of Arthritis and Metabolic Diseases
National Institutes of Health, Bethesda, Maryland 20014
Received April 14, 1965

Biosynthesis of Arthropod Secretions. I. Monoterpene Synthesis in an Ant (*Acanthomyops claviger*)[1,2]

Sir:

We wish to report the first experimental evidence for monoterpene synthesis in an arthropod. It is well known that many arthropods are able to accumulate repellent defensive secretions.[3] These secretions may contain from one to a large number of relatively simple components, including aliphatic hydrocarbons, carbonyl compounds, carboxylic acids, terpenes, phenols, and quinones.[3] In spite of extensive biological and chemical studies of this group of natural products, little is known of their origin. The possibility that insects and other arthropods which possess these secretions acquire and concentrate the preformed toxic components from their diet has been supported recently by Brower and Brower.[4] On the other hand, preliminary investigations of Gordon, Waterhouse, and Gilby indicate that green vegetable bugs [*Nezara viridula* (Fabr.) Pentatomidae] are able to incorporate acetate into a very complex mixture of aliphatic compounds.[5]

We have chosen for initial study the terpenoid secretion of the mandibular glands of an ant [*Acanthomyops claviger* (Roger)]; the chief components of this secretion have recently been identified as citronellal (I) and citral (II), in a ratio of about 9:1.[6] Not only are these terpenes of defensive importance, but, along with a number of other isoprenoid substances, they also take on a role in chemical communication,[7] which makes this group of compounds especially significant.



I II

Groups of 1000–1500 worker ants were freshly collected near Ithaca, N. Y., and maintained in laboratory colonies for the duration of each experiment. In separate experiments, these ants were fed portions of the potential precursors (sodium 1-^{14}C-acetate, sodium 2-^{14}C-acetate, 2-^{14}C-mevalonic lactone) in aqueous glucose solutions. After 7–10 days, the ants were frozen and extracted with methylene chloride. A few milligrams of cold I and II were added to the extract as carrier. Thin layer chromatography [silica gel G developed with hexane–ethyl acetate (92:8)] separated I and II from each other and from the other lipids. The two aldehydes were detected by spraying the plates with a solution of 2,4-dinitrophenylhydrazine in tetrahydrofuran.[8] The eluted terpenes were further treated with the same reagent in order to complete the formation of their 2,4-dinitrophenylhydrazones. The separated derivatives were subjected to four successive thin layer chromatographic purifications [silica gel G (benzene), aluminum oxide G (petroleum ether–diethyl ether, 24:1), silica gel G (chloroform), and silica gel G (diethyl ether)] and brought to constant specific activity. The results of these experiments are summarized in Table I.[9]

Table I

Compds. fed to *A. claviger*: Compd.	Amount, mg.	Activity, d.p.m.	Activity of terpene aldehyde, d.p.m./mmole: Citronellal	Citral
Acetate-1-^{14}C	0.18	2×10^7	3.3×10^6	3.3×10^6
Acetate-2-^{14}C	0.048	3×10^7	3.3×10^6	2.7×10^6
Mevalonate-2-^{14}C	2.32	1×10^7	1.4×10^6	1×10^6
Mevalonate-1-^{14}C	6.2	1×10^7	0	0

It is apparent from these results that *these ants are able to use the labeled acetate and mevalonate for terpene biosynthesis*, and that they need not rely on preformed terpenes in their food. The utilization of these specific precursors further suggests that the normal "mevalonic acid" pathway of terpene biosynthesis is

(1) Partial support of this work by the National Institutes of Health (Grant AI 2908) is acknowledged with pleasure.
(2) Presented in part at the Symposium on Bio-Organic Chemistry, University of California at Santa Barbara, Jan. 21, 1965, and at the 149th National Meeting of the American Chemical Society, April 1965, Detroit, Mich.
(3) L. M. Roth and T. Eisner, *Ann. Rev. Entomol.*, 7, 107 (1962); H. Schildknecht, *Angew. Chem.*, **75**, 762 (1963).
(4) L. P. Brower and J. van Z. Brower, *Zoologica*, **49**, 137 (1964).
(5) H. T. Gordon, D. F. Waterhouse, and A. R. Gilby, *Nature*, **197**, 818 (1963).
(6) M. S. Chadha, T. Eisner, A. Monro, and J. Meinwald, *J. Insect Physiol.*, **8**, 175 (1962).
(7) For a general review of the importance of isoprenoid compounds in arthropods, see H. A. Schneiderman and L. I. Gilbert, *Science*, **143**, 325 (1964).
(8) H. J. Shine, *J. Org. Chem.*, **24**, 252, 1790 (1959).
(9) The per cent incorporation obtained in these experiments ranged from 0.07 to 0.10% for citronellal.