The lack of a simple pattern of specificity for acetate or propionate esters vis-à-vis the phylogenetic scheme proposed by Ross (Fig. 2) (*18*) suggests that the interchange of ester groups may have happened more than once during evolutionary history. If such switching back and forth between acetate and propionate esters did actually occur, it indicates that the acid moiety may be a site of biochemical plasticity in pheromone production. Indeed, this possibility is consistent with our observation that the pheromone is stored as the alcohol precursor, which is esterified as the last step before its release from the insect. Such isolation of the esterification step from the other steps in pheromone biosynthesis would make it especially amenable to the changes in the acid moiety that may have contributed to the speciation of the pine sawflies.

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7. For TLC, plates coated with silica gel were developed with a mixture of hexane and ethyl acetate (14 : 1). For preparative GLC a column 6 m by 1 cm, containing 10 percent Dow 11 on Gas-Chrom Q, was used.
8. The system used to measure electroantennograms was similar to that described by W. Roelofs and A. Comeau [*J. Insect Physiol.* **17**, 1969 (1971)].
9. This compound has a retention index of 1.32 compared to the acetate of pentadecan-2-ol, by coinjection on an OV-101 column. After conversion to the corresponding ketone the material had a retention index of 1.23 compared to 2-pentadecanone on the same column. Analytical columns OV-17, XE-60, EGSS-X, NGA, and ECNSS-M were also used.
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12. Proton spectrum, Bruker 90 Mhz, Fourier transform, time averaged; solvent, deuterobenzene.
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14. 3,7-Dimethylpentadecan-2-ol was prepared by the following method: methyl magnesium iodide was reacted with 2,6-dimethyltetradecanal obtained by hydride reduction and subsequent oxidation (*10*) of 2,6-dimethyltetradecanoic acid. The latter was obtained by reacting diethyl methylmalonate with 1-chloro-4-methyldodecane [J. Cason, P. Brewer, E. Pippen, *J. Org. Chem.* **13**, 239 (1948); J. Cason and W. Winans, *ibid.* **15**, 143 (1950)].
15. All compounds isolated from *N. lecontei*, *N. sertifer*, and *D. similis* on the basis of EAG activity have subsequently been found active in the field.
16. Although no contaminants could be detected by GLC of the propionate ester from *D. similis*, hydrolysis and subsequent oxidation to the ketone revealed that a second compound was present. Because only about 1 μg of the ester was available, further purification was not attempted.
17. To test the possibility that the genus specificity might result from different optical isomers at the C-2 position, the active alcohols from *N. lecontei*, *N. sertifer*, and *D. similis* were racemized at the C-2 position by oxidation to the ketones (*10*) and reduction back to the alcohols. After this procedure these compounds (as the appropriate esters) retained their genus specificity and their attractiveness in the field.
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19. Supported by the College of Agriculture and Life Sciences, University of Wisconsin, Madison, by the Wisconsin Department of Natural Resources through the School of Natural Resources, and by the Research Committee of the Graduate School with funds supplied by the Wisconsin Alumni Research Foundation. Drs. A. Tai, R. Howard, and P. Hart gave us valuable help with chemical problems. We depended on Drs. J. Mertins, R. Wilkinson, L. Lyons, J. M. McLeod, P. Barald, M. Kraemer, and D. R. Wallace for supplying insects and making observations in the fields.

16 December 1975

Nonenzymic Joining of Oligoadenylates on a Polyuridylic Acid Template

Abstract. *3′,5′-Linked hexa-adenylic acid with a 2′,3′-cyclic phosphate terminus [$(A)_5A > p$] couples on a polyuridylic acid template in the presence of ethylenediamine to form the dodecamer (24 percent) and octadecamer (5 percent). The bond produced is largely that of the 2′,5′ isomer.*

The discovery of a nonenzymic system that could carry out the faithful replication of a polynucleotide under plausible prebiotic conditions would have a major effect on contemporary thought concerning the origin of life. Some experiments to find such a system have already been made. The coupling of thymidine oligomers on a polyadenylic acid template (*1*), adenosine derivatives on polyuridylic acid [poly(U)] (*2*), and 2′-*O*-methylinosine oligomers on polycytidylic acid (*3*) have been achieved by means of water-soluble carbodiimides, but the use of this class of coupling reagent would appear to exclude these experiments as models of prebiotic polymerization (*4*). Nucleoside phosphoramidates (*5*) and imidazolides (*4*) have also been investigated and have appeared promising as activated precursors to oligonucleotides. Nucleoside 2′,3′-monophosphates (cyclic nucleoside monophosphates) qualify as plausible prebiotic compounds (*6*), and a driving force for polymerization has been sought in the large negative standard enthalpy of hydrolysis (*7*) and high reactivity (*8*) of these five-membered ring cyclic phosphates. Adenosine 2′,3′-monophosphate forms a triple helix with poly(U) at low temperatures in the presence of various helix stabilizing compounds, which in the case of short chain diaminoalkanes also catalyze the formation of the internucleotide link (*6*). Later, it was shown that the poly(U) can be replaced by poly(vinyluracil) (*9*). The yield of dimer did not exceed about 23 percent maximum and only traces of the trimer were detected; about 97 percent of the internucleotide bonds were the unnatural 2′,5′ isomer (*6*). However, it was considered on the basis of equilibrium calculations that, even if all hydrolysis were suppressed (a competing reaction), the yield of all oligomers higher than the nonamer would total less than 8 percent in a solution that was 1*M* in adenosine base residues, and this appeared to place a severe restriction on the use of cyclic phosphates as activated monomers for template directed polymer formation (*6*).

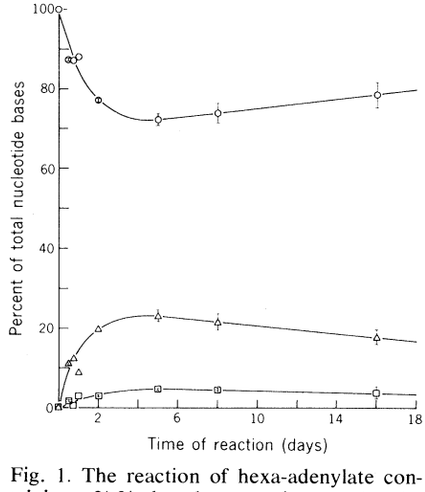

Fig. 1. The reaction of hexa-adenylate containing a 2′,3′-phosphate terminus to give the dodecamer and octadecamer on a poly(U) template. Experimental points: ○, hexamer; △, dodecamer; and □, octadecamer. The curves have no theoretical significance. The reaction mixture was analyzed by means of a diethylaminoethyl-Sephadex column with a triethylammonium bicarbonate gradient. The eluate was monitored at 260 nm with a Zeiss PMQ-H spectrophotometer.

We now report that, contrary to previous expectations, 2′,3′-monophosphates can be used to drive the coupling of two oligoadenylates, if both oligomers contain only the natural 3′,5′ internuleotide linkage. The new bond formed in the coupling reaction is roughly 95 percent 2′,5′ linkage (*10*), as found previously for the coupling of two monomer units (*6*). This joining reaction is made possible by the large relative kinetic stability of the 3′,5′ bonds, when the oligomers are bound in a right-handed helix. Under these conditions, the rate of cleavage of the 2′,5′ bond is about equal to the rate of its formation; that of a 3′,5′ bond is several hundred times slower (*11*). If it were not for this stabilization, the equilibrium calculations of Renz *et al.* (*6*) would apply to both types of bond, and the rate of degradation of the hexamer to shorter oligomers would greatly exceed the rate of coupling. Thus hexaadenylic acid with a terminal 2′,3′-phosphate (Miles Laboratories) in the presence of a poly(U) template gave 24 percent of the dodecamer and 5 percent of the octadecamer in 5 days [1*M* aqueous ethylenediamine hydrochloride (*p*H 8, 2°C); the solution was 0.025*M* in adenosine units and 0.05*M* in uridine units]. The products were determined by chromatography on diethylaminoethyl-Sephadex with a triethylammonium bicarbonate buffer, and high-pressure liquid chromatography on RPC-5 (*12*) with a gradient of 0.2 to 0.6*M* KCl (0.01*M* tris-HCl, *p*H 8.1).

Mixing curves at 260 and 280 nm show that under our conditions the hexamer forms the expected triple helix (*13*) with two poly(U) strands (measured at 2°C in a cell having a 0.025-mm pathlength); the melting temperature of the triple helix is about 70°C. If the reactants are mixed in the ratio of one U to one A, dodecamer is still formed, but more slowly (16 percent after 5 days); if the poly(U) is omitted the yield of dodecamer is about 1 percent. When the cyclic phosphate ring was opened prior to mixing it with poly(U), no dodecamer was detected. The decrease in concentration of dodecamer at later times (Fig. 1) is due to the relatively rapid cleavage of the 2′,5′ bond to reform the hexamer cyclic phosphate, and gradual hydrolysis of the cyclic phosphate terminus to give "inactive" 2′- and 3′-phosphate monoesters. The cleavage reaction of the 2′,5′ and 3′,5′ linkages has been described (*11*).

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17 November 1975; revised 5 January 1976

Human Glyceraldehyde-3-Phosphate Dehydrogenase in Man-Rodent Somatic Cell Hybrids

Abstract. *The human enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forms heteropolymers with the rodent enzyme in man-rodent somatic cell hybrids. A gene specifying GAPDH is syntenic with the genes specifying the glycolytic enzymes triosephosphate isomerase (TPI) and lactate dehydrogenase B (LDH-B). The synteny of GAPDH, TPI, and LDH-B is the first evidence for the syntenic association of human genes that specify enzymes of the Embden-Meyerhof glycolytic pathway.*

The selective loss of human chromosomes by somatic cell hybrids formed between cultured aneuploid rodent cells and human fibroblasts or white blood cells (*1, 2*) affords a unique opportunity to determine the organization of loci in the human genome. In this report we describe the expression of human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (E.C. 1.2.1.12) in man-mouse and man-hamster somatic cell hybrids and the syntenic association of a gene specifying this enzyme with the gene which specifies lactate dehydrogenase B (LDH-B), an enzyme catalytically and structurally similar to GAPDH (*3*), and with the gene which specifies triosephosphate isomerase (TPI), the enzyme responsible for the reversible isomerization of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. This is the first example of synteny of human genes which determine enzymes that catalyze sequential metabolic steps.

Rodent parental cells used for the preparation of hybrids included the mouse cell RAG and the hamster cell E36, both of which are deficient in hypoxanthine-guanine phosphoribosyl transferase (*4*). Human parental cells included white blood cells from a normal male and white blood cells from two unrelated females carrying different translocations involving chromosomes X and 19. Parental cells were treated in suspension with β-propiolactone inactivated Sendai virus (*2*), and hybrid colonies were selected in Dulbecco's modified Eagle's medium supplemented with 5 percent fetal calf serum, $1 \times 10^{-4}M$ hypoxanthine, $4 \times 10^{-7}M$ aminopterin, and $1.6 \times 10^{-5}M$ thymidine (HAT medium) (*5*). For electrophoretic analysis, cells from primary hybrid clones grown in HAT medium were harvested by trypsinization 9 to 12 weeks after hybridization.

Sonicates (4×10^7 cell/ml) in 0.1*M* tris-HCl, *p*H 7.0, containing $1 \times 10^{-3}M$ β-mercaptoethanol and $1 \times 10^{-4}M$ nicotinamide adenine dinucleotide (NAD) were analyzed for GAPDH on 9 percent starch gels. The hybrid clones were also analyzed by starch gel, agarose, acrylamide, or Cellogel electrophoresis for the presence of human and rodent forms of a number of additional enzymes which included: adenosine deaminase (E.C. 3.5.4.2), adenylate kinase-1 and -2 (AK-1, AK-2) (E.C. 2.7.4.3), aspartate aminotransferase-1 (E.C. 2.6.1.1), esterase D, α-galactosidase (E.C. 3.2.1.22), β-glucuronidase (β-GCR) (E.C. 3.2.1.31), glucose-6-phosphate dehydrogenase (G6PD) (E.C. 1.1.1.49), glucose phosphate isomerase (E.C. 5.3.1.9), glutathione reductase (GR) (E.C. 1.6.4.2), hexosaminidase A and B (E.C. 3.2.1.30), isocitrate dehydrogenase-1 (IDH-1) (E.C. 1.1.1.42), lactate dehydrogenase A and B (E.C. 1.1.1.27), lysosomal acid phosphatase ACP-2 (ACP-2) (E.C. 3.1.3.2), malate oxidoreductase-1