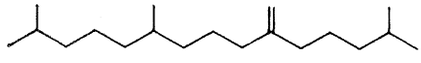
"Zamene," Isomeric C_{19} Monoolefins from Marine Zooplankton, Fishes, and Mammals

Abstract. *Three isomeric C_{19} monoolefins with the carbon skeleton of pristane (2,6,10,14-tetramethylpentadecane) have been isolated from mixed zooplankton of the Gulf of Maine and from liver oils of various marine fishes and mammals. The structures have been elucidated. Zamene (norphytene), the terminal olefin, is a minor component of the mixture. The usage of the older trivial name "zamene" should be restricted to the total mixture of marine C_{19} monoolefins with the pristane skeleton. Conventional chemical nomenclature should be used for individual isomers.*

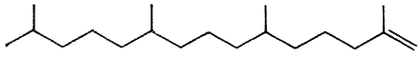
Crude pristane (2,6,10,14-tetramethylpentadecane) from shark liver oils contains unsaturated impurities (*1*). A C_{19}-monoolefin given the trivial name "zamene" was transformed into pristane by hydrogenation (*2*). Christensen and Sörensen (*3*) converted zamene by oxidative degradation to 2,6,10 trimethylpentadecane-14-one, and thus suggested that zamene is identical with norphytene(B).

In our investigation of hydrocarbons from marine sources we have reisolated "zamene" from various planktonic, fish, and mammalian oils. Silica-gel chromatography and subsequent small-scale preparative gas chromatography indicated a mixture of three C_{19} monoolefins. Catalytic hydrogenation of these olefins produced the same compound, identified as pristane by gas chromatographic retention indices and infrared spectra (Table 1). This establishes that the carbon skeleton of the three compounds is identical with that of pristane.

The monoolefins were further characterized by infrared spectroscopy and ozonolysis (*4*).



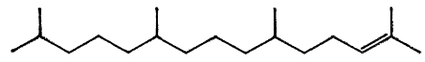
A



B

Compounds A and B have methylenic unsaturation; upon ozonolysis they produce two different C_{18} ketones; that derived from B is identical with 2,6,10-trimethylpentadecan-14-one from the ozonolysis of phytol. This is proof of structure B for compound B. The remaining possible structure A for compound A is supported by the higher ratio of geminal dimethyl to internal methyl (fine structure of 1370 cm^{-1} δ_s CH_3 band; δ_s being symmetrical bending deformation).

Compound C, the principal isomer in all our oils, has a trisubstituted ethylene structure. Ozonolysis produces a C_{16}-aldehyde, which is proof of structure C.



C

The organisms in which we have found the isomeric C_{19} olefins are as follows: mixed zooplankton, Gulf of Maine; *Pomolobus* sp. (alewife); *Centroscymnus coelolepsis*; *Cetorhinus maximus (Gunnerus)* (basking shark); *Lagenorynchus acutus*; *Physeter catodon* (sperm whale). Structure C is the predominant one in all cases, exceeding structures A and B by an approximate factor of ten in the zooplankton extracts and the liver oil of the basking shark. The ratios and relative concentrations of the isomers are the same in distillates from the oils before and after chromatography. This excludes the possibility that isomerization might have taken place in the silica-gel chromatography. In view of this we think that the name "zamene," originally given to the bulk C_{19} unsaturates from basking-shark oil, should no longer be used for the minor isomer B (that is, norphytene). We propose that the usage of "zamene" be restricted to the aggregate of C_{19} monoolefins with pristane skeleton, obtained from marine oils. The use of conventional nomenclature is preferred for dealing with the individual isomers: 2,6,14 trimethyl-10-methylenepentadecane (A); 2,6,10, 14-tetramethyl-1-pentadecene or norphytene (B); and 2,6,10,14-tetramethyl-2-pentadecene (C).

The C_{19} olefins as well as pristane (*5, 6*) and the phytadienes (*4*) originate in the phytol ingested by the zooplankton. A pathway from phytol to norphytene which involves oxidation to an acid and subsequent decarboxylation has been suggested by Sörensen (*7*). Norphytene is also produced in surprisingly good yield in the catalytic dehydration of phytol (*8*). Both of these mechanisms would produce norphytene which might be isomerized to C. It seeems unlikely, though, that isomerization of B can also account for the formation of A. We have been unable to detect phytenes or phytane in zooplankton or fish liver oil in spite of the abundance of the phytadienes (*4*). This suggests different sites for the formation of pristane and of the phytadienes; at one, the loss of a carbon atom from phytol with subsequent hydrogenation occurs; at a different site phytol dehydrates to the phytadienes but no hydrogenation takes place.

The finding of the zamene isomers

Table 1. Gas chromatography and spectra of C_{19} monoolefins and derivatives. Gas chromatograms temperature programmed at 4°C/min, 1.8 m by 0.3 cm steel columns; 3.5 percent RTV 502 (filler free) on Chromosorb G, acid washed, dichloromethylsilane treated; 3.5 percent Carbowax C20M on same kind of column.

Compound	Retention index		Infrared spectra (cm^{-1}) (maxima)					
	RTV 502	C20M	Alkene substitution			δ_s CH_3		
A	17.30	17.37	3065(w)	1645(w)	885(w)	1360	1375(w)	1380
B	17.31	17.54	3070	1645	889	1360(w)	1375(s)	1380(w)
C	17.43	17.64	3020	1670(w)	835			
Pristane from A, B, and C	17.12	16.75						
C_{18} ketone from A	18.0	20.96						
C_{18} ketone from B and phytol	18.36	21.14						
C_{16} aldehyde from C	16.68	19.31						

further broadens the range of related hydrocarbon structures which spread from a common source through the marine environment.

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Puromycin: Effect on Messenger RNA Synthesis and β-Galactosidase Formation in Escherichia coli 15T⁻

Abstract. *Incubation of* Escherichia coli *15T⁻ in the presence of puromycin inhibits the inducible formation of β-galactosidase to a greater extent than it inhibits protein synthesis. In* E. coli *33.00 β-galactosidase formation is also more sensitive to the presence of puromycin than is protein synthesis. In the presence of glycerol (but not in its absence) puromycin prevents the production of messenger RNA for β-galactosidase, presumably as a result of catabolite repression.*

Puromycin is a potent inhibitor of protein synthesis in microorganisms (*1*, *2*) and mammalian cells (*3*). In *Escherichia coli* 15T⁻, puromycin inhibits protein formation while allowing ribosomal and transfer RNA (*2*) to accumulate. The present investigation was initiated to determine whether puromycin would also allow messenger RNA (mRNA) to be formed in its presence. Thus the ability of puromycin to affect induced synthesis of β-galactosidase was studied. Although formation of this enzyme is inhibited by the antibiotic, evidence is presented indicating that puromycin does not suppress synthesis of mRNA for β-galactosidase.

Puromycin apparently inhibits β-galactosidase formation to a greater extent than it inhibits total protein synthesis. This preferential inhibition of the enzyme synthesis was demonstrated in the following way. Cell crops of *E. coli* 15T⁻ were grown overnight (17 to 19 hours) on a basal salt medium (*4*) containing glycerol (0.1 percent). After harvesting and resuspending the bacteria in fresh medium containing glycerol, two cultures, both containing puromycin (45 μg/ml), were prepared. To one, leucine-C^{14} was added; and to the other, thiomethylgalactoside (TMG) ($5 \times 10^{-4}M$), an inducer of β-galactosidase, was added. Total protein synthesis was determined by measuring the incorporation of leucine-C^{14}. Samples were taken at intervals and added to an equal volume of cold (0°C) 10 percent trichloroacetic acid (TCA). The precipitates were collected on Millipore filters (0.45-μ pore size), washed six times with 5 percent TCA (containing unlabeled leucine), glued to aluminum planchettes, dried, and counted in a Nuclear Chicago gas flow counter (*5*). Beta-galactosidase was measured in cells that had been treated with toluene by the method of Cohn and Torriani (*6*). Although leucine-C^{14} incorporation was inhibited by only 50 percent, the induced synthesis of β-galactosidase was suppressed more than 90 percent (Fig. 1, *a* and *b*). These results suggested that either the induction process or some step in the synthesis of the enzyme was particularly sensitive to the antibiotic.

As an explanation for the results obtained the induction process was ruled out by studying the effect of puromycin upon β-galactosidase formation and protein synthesis in *E. coli* 33.00, which produces the enzyme constitutively. In this case, also, leucine-C^{14} incorporation was inhibited to a lesser extent than was enzyme synthesis (Fig. 1, *c* and *d*). Thus, the selective effect produced by puromycin upon β-galactosidase formation is not due to a direct influence upon the process of induction. In order to examine the situation in more detail, the following experiments were performed.

Suspensions of *E. coli* 15T⁻ were harvested, washed, and resuspended in basal medium lacking Mg ion. The absence of this ion makes the cells more sensitive to puromycin (*2*). Thiomethylgalactoside was then added to cell suspensions, and the incubation was continued at 37°C. At intervals thereafter puromycin (20 μg/ml) was added, and β-galactosidase was measured at 5-minute intervals. After the addition of puromycin, enzyme activity increased for about 10 minutes and then remained constant (Fig. 2*a*). If the incorporation of tryptophan-C^{14} was measured under the same conditions, no significant amount of inhibition of protein synthesis was observed until after 10 minutes in the presence of the antibiotic (Fig. 2*b*). Thus, the effect upon β-galactosidase appears much greater than the effect on overall protein production. A possible explanation for these observations is that the inhibition of production of β-galactosidase which occurs after the addition of puromycin results from cessation of mRNA synthesis specific for this enzyme.

In order to eliminate the possibility that the constant amount of enzyme might only reflect an instability of the enzyme in the presence of puromycin, a suspension of *E. coli* was treated with TMG for 30 minutes, washed free of inducer, and resuspended in basal medium lacking magnesium. Puromycin was added to this suspension, and samples were removed for estimation of enzyme content. Under these conditions, puromycin did not alter the enzyme activity achieved during the treatment with TMG, and even after 60 minutes the degree of enzyme activity was still essentially unaffected by puromycin. Thus, puromycin does not

Table 1. Influence of puromycin upon the synthesis of mRNA specific for β-galactosidase in *E. coli* 15T⁻.

Incubation media*	Glycerol	Enzyme formed (units/ml)
Control	+	7.3
Puromycin	+	1.3
Control	0	7.4
Puromycin	0	7.3

* TMG ($5 \times 10^{-4}M$) was added to the incubation media 5 minutes after the addition of puromycin (22 μg/ml). Incubation continued for 2 minutes; the cells were then washed with cold buffer (0° to 4°C) on a Millipore filter. Cells were resuspended in warm media and then incubated for 18 minutes. Enzyme activity determined according to Cohn and Torriani (*6*).