date activates adenylate cyclase (*9*) and, in other systems, inhibits a number of phosphohydrolases (*10*); therefore, its activation of adenylate cyclase probably involves binding to N. We suggest that the ventral photoreceptors of *Limulus* contain a protein similar or perhaps identical to the guanyl nucleotide binding component, N, of hormone-regulated adenylate cyclase. Furthermore, we suggest that GTP-γ-S, fluoride, and vanadate induce bumps by interacting with the proposed guanyl nucleotide binding component. Obviously, such an interpretation is highly speculative.

If the proposed GTP-binding protein participates in the production of light-induced bumps, then F^-, VO_3^-, and GTP-γ-S might modify the cell's light response. We reported earlier that F^- prolongs the response to a dim flash (*4*), and in preliminary experiments, we determined that in some cells VO_3^- and GTP-γ-S also prolong the response to a dim flash. Thus it appears that the proposed GTP-binding protein is normally involved in visual excitation.

According to the above interpretation, GMP-PNP might be expected to have effects similar to those of GTP-γ-S, since GMP-PNP is an analog of GTP known to resist enzymatic hydrolysis. However, GMP-PNP dissociates from the guanyl nucleotide binding site some 50 times faster than GTP-γ-S does (*11*). Thus, similar effects on ventral photoreceptors might require 50 times as much GMP-PNP as GTP-γ-S. This probably explains why, with equal injections, we found GTP-γ-S to be effective and GMP-PNP ineffective. For ventral photoreceptors injected with either GMP-PNP or GTP-γ-S and exposed to bright light, there is an increase in bump frequency in the dark (*12*). For some cells a second bright illumination is necessary before the increase in bump frequency is observed in the dark (*12*). We interpret these findings to imply that light enhances the binding of GMP-PNP and GTP-γ-S to our proposed GTP-binding protein and thereby induces bumps.

The reason for our cautious disclaimer about cyclic nucleotides should now be clear. The effects of F^-, VO_3^-, and GTP-γ-S are only suggestive of the presence of N in the photoreceptor. These effects say nothing about the catalytic moiety C that is being regulated by N. Cyclic nucleotides can be shown to be involved in excitation only by demonstrating a specific effect of introducing the nucleotide to the interior of the photoreceptor. To our knowledge the results of such experiments have not as yet been reported for ventral photoreceptors. Another reason for caution is the finding that in other systems N may regulate effector molecules other than catalytic adenylate cyclase (*6*).

If an N-like protein is present in invertebrate photoreceptors, there must be biochemical evidence for its existence, in addition to the pharmacologic evidence presented above. In the hormonal system described above, N exhibits hormone-activated guanosine triphosphatase activity. By analogy, photoreceptors would be expected to exhibit a light-activated guanosine triphosphatase activity, and such activity has been reported in the photoreceptors of the octopus (*13*), an invertebrate.

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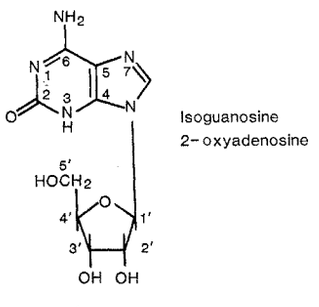
22 September 1980

Isoguanosine: Isolation from an Animal

Abstract. *Isoguanosine (oxyadenosine or crotonoside), previously known to occur in nature only in the croton bean, was isolated from an animal, the marine nudibranch mollusk* Diaulula sandiegensis.

When Emil Fischer synthesized isoguanine (oxyadenine) in 1897, he predicted that it would soon be found in nature (*1*). In fact, isoguanine has been isolated only from butterfly wings (*2*). A report that it occurs in pig blood (*3*) could not be confirmed (*4*). The riboside of isoguanine, isoguanosine (crotonoside), has hitherto been known in nature only as a constituent of the croton bean, *Croton tiglium* L. (*5*). We now report isolation of isoguanosine (**1**) from an animal, the marine nudibranch mollusk *Diaulula sandiegensis*. The biological role of isoguanosine has been neglected, probably because this purine riboside has never before been known to occur in an animal. Nevertheless, isoguanosine has most of the attributes of other purine ribosides. In mammals it produces hypotension, bradycardia, and relaxation of smooth muscle (*6*), but it is more potent and much longer-acting than adenosine. Like adenosine and certain other of its analogs, it stimulates accumulation of adenosine 3′,5′-monophosphate (cyclic AMP) in brain tissue (*7*). It is reported to have negligible antitumor activity (*8*).



Isoguanosine
2-oxyadenosine

1

Approximately 100 specimens of *Diaulula sandiegensis* Cooper, a rather common dorid nudibranch from the Pa-

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Table 1. Proton NMR spectrum of *D. sandiegensis* nucleoside (isoguanosine) in a mixture of D_2O and CD_3COOD. The NMR spectra of isoguanosine and of guanosine were obtained on a Brucker HSX 360 MHz FT instrument by the Stanford Magnetic Resonance Laboratory.

Position	Chemical shift δ (ppm)	Multiplicity,* relative area	Coupling constants (Hz)
C-5′	3.9	d, 2H	$J_{5'-4'}$ = 1.7
C-4′	4.32	d of d, 1H	$J_{4'-5'}$ = 1.7 $J_{4'-3'}$ = 2.5
C-3′	4.36	d of d, 1H	$J_{3'-4'}$ = 2.5 $J_{3'-2'}$ = 5.2
C-2′	4.49	d of d, 1H	$J_{2'-3'}$ = 5.2 $J_{2'-1'}$ = 6.6
C-1′	5.90	d, 1H	$J_{1'-2'}$ = 6.6
C-8	8.05	s, 1H	

*Singlet, s; doublet, d; doublet of doublets, d of d.

cific Coast of North America, were collected from Monterey Bay and the Channel Islands off Santa Barbara, California. The viscera (27.5 g) consisting mainly of digestive glands, were homogenized with 95 percent ethanol. After centrifugation, the residue was extracted with distilled water. Both extracts were dried at reduced pressure, and the residue was dissolved in a minimum amount of water and fractionated on a column of polyacrylamide gel (Bio-Gel P-2) by elution with water. The fractions were assayed for cardiodepressor activity on isolated guinea pig atria (*9*), and the most active fractions were combined and chromatographed. Crystals formed in the concentrates of the most active fractions, which had been stored for about 2 weeks at 4°C. The total yield was approximately 8 mg, equivalent to about 1 mg of crystals per gram dry weight of tissue.

Mass spectrometry (*10*) on this crystalline material showed a parent ion peak at a mass-to-charge ratio (*m/e*) of 284, which corresponds to an $[M+1]^+$ peak for guanosine, isoguanosine, or some isomer of these. Significant fragment peaks showed at *m/e* equal to 115, 132, and 150. The spectrum was the same as that of an authentic sample of isoguanosine (*11*) and only slightly different from that of guanosine, which could not be ruled out on this evidence alone, but which had no cardiodepressor activity at $1 \times 10^{-4} M$. The proton nuclear magnetic resonance (NMR) spectrum was taken on this same sample with the results given in Table 1. The NMR spectrum is indistinguishable from that of an authentic sample of isoguanosine and quite distinct from that of an authentic sample of guanosine (*12*). The major distinguishing features are the chemical shift of the C-8 proton on the purine nucleus and the pattern of the C-5′ proton signals. That the pentose could be D-ribose but not L-ribose was shown by its optical rotation $[\alpha]_D^{26} - 69° \pm 4°$ (c = 0.5, 0.1N NaOH), which was the same as that of an authentic sample of isoguanosine and corresponds to the value given in the literature (*13*).

The crystals from *D. sandiegensis* melted at 236.5° to 240°C decomposing (isoguanosine, 237° to 240°C decomposing; guanosine, 240°C decomposing); the mixture melting point with authentic isoguanosine was 236° to 242°C decomposing and that with guanosine was 226° to 240°C decomposing. Finally, this compound had the same ultraviolet spectra in acid, neutral, and basic solution as that reported for isoguanosine (*13*). We thus consider the identity of this compound with isoguanosine to be established.

Isoguanosine and its corresponding purine base may appear to be restricted in distribution because they have not been widely sought. We recently isolated 1-methylisoguanosine (doridosine) from a different nudibranch (*14*), and the same compound was found in a sponge (*15*). Sponges contain other purine and pyrimidine nucleosides and free bases (*16*). Since sponges are a principal food of dorid nudibranchs, we considered the possibility that *Diaulula sandiegensis* obtains isoguanosine from a sponge. It is reported (*17*) that at Yaquina Head, Oregon, the purple sponge *Haliclona permollis* is preferred by *Diaulula* as food and that the nudibranch can locate the sponge by chemotaxis. We were not able to isolate isoguanosine from *H. permollis* collected in Monterey Bay, nor were we able to find any cardiac depressor activity in extracts of that sponge. It is, of course, possible that the amount of isoguanosine in the sponge is too small to be detected by our methods and that *Diaulula* concentrates the purine riboside in its digestive gland, or synthesizes it from a pharmacologically inactive precursor.

Aqueous solutions of isoguanosine are reported to form gels of helical structure (*18*), and phosphorylated derivatives have been synthesized (*19*). Isoguanosine can base-pair with cytidine. Isoguanosine-5′-pyrophosphate, in the presence of an oligonucleotide primer, was polymerized by a polynucleotide phosphorylase under conditions analogous to those required for polymerization of guanosine 5′-monophosphate (*20*). Although isoguanosine has not been shown to be incorporated into the nucleic acids of bacteria (*21*), the evidence just cited and the demonstration that it occurs in animals suggest that it might be a constituent of nucleic acids in some animals.

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28 October 1980