DORIDOSINE, 1-METHYLISOGUANOSINE, FROM *ANISODORIS NOBILIS;* STRUCTURE, PHARMACOLOGICAL PROPERTIES AND SYNTHESIS

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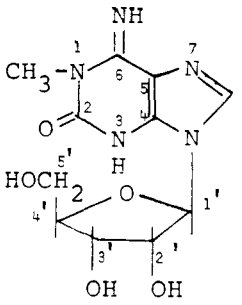
ABSTRACT.—The new *N*-methylnucleoside named doridosine, isolated from the shell-less marine dorid nudibranch *Anisodoris nobilis*, has been identified as 1-methylisoguanosine (**1**) by its spectral properties and by synthesis via methylation of isoguanosine. Doridosine has been shown to be identical to the nucleoside isolated from the Australian sponge *Tedania digitata* by Quinn, Gregson, Cook and Bartlett. They have also proven their product to be 1-methylisoguanosine. Doridosine was shown to cause prolonged reduced arterial pressure and reduced heart rate in the rat. The action is qualitatively similar to that of adenosine but of much greater duration, possibly because doridosine is resistant to attack by adenosine deaminase.

Based on the hypothesis that shell-less marine mollusks, specifically dorid nudibranchs, might contain a toxic substance or substances that protects them from predators, Fuhrman, Fuhrman and DeRiemer (1) prepared various tissue extracts of specimens that were collected along the Monterey Coast of California. Aqueous extracts of these were subjected to a preliminary purification by dialysis followed by lyophilization of the dialysate. The crude concentrates were then screened for general toxicity by injection into mice and crabs. Toxic extracts were studied in more detail in several standard pharmacological preparations. From these studies, it emerged that extracts of the digestive glands of *Anisodoris nobilis* had the unusual property, among extracts from marine animals (2), of producing hypotension and bradycardia in mammals within a few seconds of intravenous injection in anesthetized rats. These extracts caused a marked and prolonged reduction in heart rate and a sharp drop in systolic blood pressure. The activity of the extracts could be followed conveniently by testing on the isolated, spontaneously beating, guinea pig atria (1).

In a preliminary communication (3), we reported that the cardioactive component of the digestive gland of *Anisodoris nobilis* is a new *N*-methylpurine riboside that we named doridosine. Doridosine was tentatively assigned the structure of 1-methylisoguanosine (**1**) based on its spectroscopic properties.

Quinn, Gregson, Cook, and Bartlett at the Roche Institute of Marine Pharmacology, Dee Why, N. S. W. Australia, have recently reported the isolation of a compound with similar properties from the sponge *Tedania digitata* (4, 5). These workers have positively established the structure of their product as 1-methylisoguanosine (**1**) by spectroscopic methods, degradation, and synthesis. We have now exchanged samples with the Australian group and verified that these products from the two very different sources, the nudibranch from California and the sponge from Australia, are indeed identical.

The function and origin of this compound in these marine organisms is unknown. It is known that dorid nudibranchs feed principally on sponges (6, 7), but our preliminary studies have failed to detect any doridosine in several food sources of *Anisodoris nobilis*.



1 Doridosine

(1-Methylisoguanosine)

Doridosine causes reduced arterial pressure and reduced heart rate in mammals in a manner that is qualitatively similar to adenosine but with an unusually long duration of action (1, 3, 8, 9). It also shows skeletal muscle relaxant and hypothermic activity (8, 9). The duration of action may be explained by the observation that doridosine interacts directly with adenosine receptors (21) and that it is resistant to destruction by the enzyme adenosine deaminase (3, 9).

It is the purpose of this paper to describe the spectral and chemical properties of doridosine in detail and give the basis for the structural assignment as 1-methylisoguanosine **1**, and provide further information concerning the pharmacology of this compound.

BIOASSAY OF EXTRACTS.—At all stages, the purification of the active component of extracts of *Anisodoris nobilis* was guided by bioassay on the isolated guinea pig atria. Both crude and highly purified fractions decreased the rate and force of contraction. These effects were dependent upon the dose. The negative inotropic action was less variable than the negative chronotropic action and was used as the criterion of activity. In early stages of purification, we used a log dose-response graph constructed from multiple doses of a single active extract. After crystallization of doridosine, such a graph was constructed from experiments with the purified compound (figure 1). The concentration required to decrease the force of contraction by 50% is 1.4×10^{-5}M.

CHEMICAL IDENTIFICATION.—Chemical purification of the active component of the lyophilized crude aqueous extracts of *Anisodoris nobilis* was accomplished as described in the experimental section by repeated gel-permeation chromatography on Bio-Gel P-2 followed by chromatography and re-chromatography on silica gel with *t*-butyl alcohol, ethyl acetate, water, acetic acid (40:10:2:1) as the eluting solvent. This gave five distinct components; only the last and most abundant component to be eluted showed significant activity in the guinea pig atria assay. The active component was crystallized from methyl alcohol and water. It was washed twice with a small amount of pure acetic acid to remove a more soluble impurity; and recrystallization from water, acetic acid, and methanol yielded a highly purified material. The yield of doridosine was about 0.02% based on the wet weight of the digestive glands. The digestive gland, in turn, was about 10% of the wet weight of the whole animal. The other minor components were not investigated because of their small amount and their lack of substantial activity.

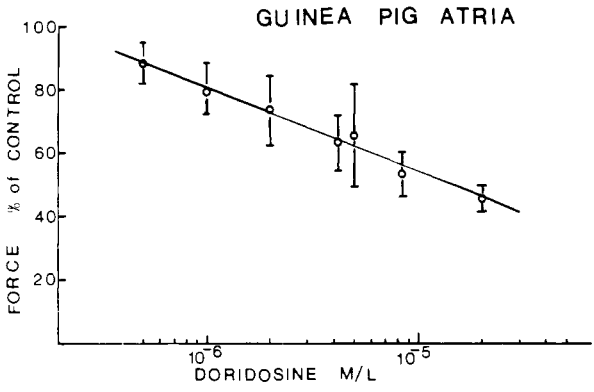
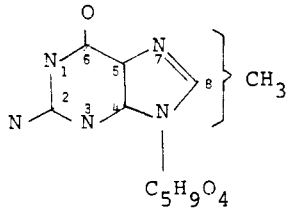


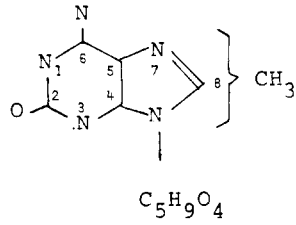
FIG. 1. Concentration-response relationship showing the effect of Doridosine (1-methylisoguanosine) on the force of contraction of the isolated, spontaneously beating guinea pig atria. Each point represents 4 to 12 experiments. The mean and standard deviation are shown.

High resolution mass spectrometry established the parent ion of doridosine as 297.1075 *m/e* corresponding to a molecular formula of $C_{11}H_{15}N_3O_5$. The base peak was at 165.0651 *m/e* corresponding to a formula for this major fragment of $C_6H_7N_5O$. This latter formula agrees with that of a methylated purine nucleus of guanine or isoguanine $C_6H_7N_5O$, *i.e.*, structures **2** or **3** plus a methyl group. Thus this mass spectrum can be interpreted as that of a methyl nucleoside in which the major fragmentation is between the methylated purine nucleus and the pentose moiety. Coupled gas chromatography-mass spectrometry of trimethylsilylated doridosine clearly showed the introduction of five trimethylsilyl groups (*m/e*=657) with two on the purine nucleus (*m/e*=338) and, therefore, three on the carbohydrate moiety. There was some incomplete trimethylsilylation indicating one difficulty replaceable active hydrogen.



2. guanosine skeleton

2a	1-Me
b	N^2-Me
c	3-Me
d	O^6-Me
e	7-Me



3. isoguanosine skeleton

3a	1-Me
b	O^2-Me
c	3-Me
d	N^6-Me
e	7-Me

Doridosine, therefore, has four readily replaceable hydrogens and a fifth that is more difficult to substitute. The mass spectrum of doridosine has the same parent ion and base peaks as N^2-methylguanosine, **2b** (10) but differs in other details. The ultraviolet, long wavelength, absorption band of doridosine at pH

6.5 [λ max, (ε) 292 nm (8500)] undergoes a bathochromic shift when the solution is made either acidic [pH 1.5, λ max (ε) 282 (9000)] or basic [pH 12, λ max (ε) 286 nm (7500)]. Similar behavior is observed for isoguanosine (11, 12); only N^6-methylisoguanosine (13) seems to be reported to show this pattern.

A direct comparison of the uv spectra of doridosine and an authentic sample of N^6-methylisoguanosine, **3d**, (13) showed that they are not the same. Furthermore, the proton nmr of N^6-methylisoguanosine is not identical to that of doridosine. The decomposition point of doridosine, depending on the rate of heating, is 260–265°, that for N^6-methylisoguanosine is 210°, while that of a mixture of the two is 220–230°.

The proton and carbon-13 nmr spectra of doridosine in both D_2O–CD_3COOD and DMSO-d_6 solvents are shown in table 1. The carbon-13 nmr spectrum of

TABLE 1. Comparison of C-13 and proton nmr chemical shifts[a] of doridosine, guanosine,[b] isoguanosine, N^6-methylisoguanosine and ara A.[c]

Nucleoside	N-CH_3	$C_{5'}$	$C_{2'}$	$C_{3'}$	$C_{4'}$	$C_{1'}$	C_5	C_8	C_6	C_4	C_2
^{13}C											
Doridosine[a]											
DMSO-d_6	30.17	61.90	70.99	72.99	86.06	87.69	108.92	137.92	151.45	151.98	153.67
CD_3COOD-D_2O	32.8	61.9	70.9	70.9	86.0	88.9	110.0	139.0	142.0	150.0	152.0
Guanosine[a, b]											
DMSO-d_6		62.2	71.6	74.9	86.4	87.3	117.6	136.9	157.8	152.4	154.6
CD_3COOD-D_2O		61.1	70.1	74.9	86.0	90.8	121.9	136.4	155.6	149.6[d]	154.8[d]
Isoguanosine[a]											
CD_3COOD-D_2O		61.8	71.5	74.5	86.6	90.3	109–10[e]	138.0	146.0	152.4	152.8
N^6-Me-isoguanosine[a]											
CD_3COOD-D_2O	32.8	63.3	72.9	76.1	88.1	91.5	112–14[e]	138.6	141.2	152.4	154.6
ara-A[c]											
DMSO-d_6[c]		61.0	75.1	76.0	80.1	84.1	118.4	140.8	154.3	149.7	152.8
D_2O[c]		60.95	79.75	74.85	85.25	89.2	118.9	142.85	150.3	145.0	148.0
^1H											
Doridosine[a]											
DMSO-d_6	3.65	3.98	4.62	4.51	4.33	5.99		8.11			
CD_3COOD-D_2O	3.50	3.88	4.51	4.35	4.29	5.87		8.00			
Guanosine[a, b]											
DMSO-d_6		3.57	4.40	4.09	3.87	5.69		7.92			
CD_3COOD-D_2O		3.86	4.66	4.44		6.03		9.10			
Isoguanosine[a]											
CD_3COOD-D_2O		3.90	4.49	4.36	4.32	5.90		8.05			
N^6-Me-isoguanosine[a]											
CD_3COOD-D_2O	3.15	3.88	4.52	4.28		5.88		8.02			

[a] Taken on Varian XL-100 NMR instrument; cf. experimental, chemical shifts, δ, in ppm downfield from TMS.
[b] See: J. B. Strothers, "Carbon-13 NMR Spectroscopy" Academic Press, N.Y.-London, 1972, p. 472.
[c] ara-A, β-D-arabinofuranosyladenosine. In DMSO-d_6, see: E. Wenkert and E. W. Hagaman, *Biochem. Biophys. Res. Comm.*, **51**, 318 (1973); in D_2O, see: E. Breitmaier and W. Voelter, *Tetrahedron*, **29**, 227 (1973).
[d] Assignments are not certain, C-2 and C-4 could be reversed.
[e] Signals neither prominent nor sharp.

doridosine is typical of a purine nucleoside (14). The signal at 139 ppm in the carbon-13 nmr spectrum, corresponding to C-8 in the nucleoside, is a doublet in the non-decoupled spectrum, confirming the proton substitution at C-8 in the purine ring of doridosine. Therefore, we need not consider an oxygen or nitrogen substituent at C-8. The C-13 signal at 30.17 ppm (DMSO-d_6 solvent) is a quartet in the undecoupled spectrum and, therefore, corresponds to the methyl group. For comparison, the published chemical shifts (14) for the *N*-

methyl proton signals (DMSO-d_6 solvent) for the known methyl guanosines and isoguanosines (2-oxyadenosines) are as follows: 1-methylguanosine, **2a**, 3.40 ppm; N^2-methylguanosine, **2b**, 2.95 ppm; 7-methylguanosine, **2e**, 4.1 ppm; N^6-methylisoguanosine, **3d**, 3.15 ppm. Doridosine with N-Me 3.65 ppm cannot be one of these *known* *N*-methylguanosines or *N*-methylisoguanosine nucleosides. The unknown methylguanosines, **2**, and isoguanosines, **3**, that have *not* been reported and which are doridosine candidates are: N^1-methyl- (**3a**) N^3-methyl- (**3c**) and N^7-methyl- (**3e**) isoguanosines, and 3-methylguanosine (**2c**).

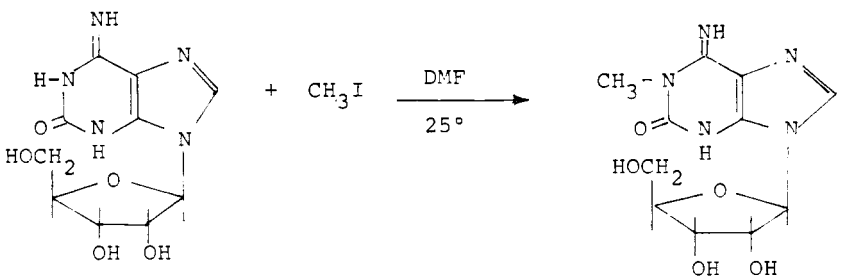
When we consider the proton and C–13 nmr signals assignable to the sugar moiety in doridosine, we find a near perfect correspondence between those observed in doridosine and those for the β-D-riboside moiety of guanosine and isoguanosine (cf. table 1). The chemical shifts are distinctly different from those seen in an arabino nucleoside, such as ara-A [β-D-arabinofuranosyl) adenine (15)]. The assignments based on this correspondence are as given in table 1. Furthermore the optical rotation of doridosine is $[\alpha]^{20}D - 66.2°$ (C = 0.42g/liter, MeOH) (16) very close to that of guanosine, $[\alpha]^{20}D - 60.5°$ (C = 3, 0.1N NaOH), with the known β-D-ribose moiety. Thus, although it is not unequivocally proven, we believe we can safely conclude that the sugar moiety in doridosine has the β-D-ribose configuration. Assuming the β-D-ribosyl sugar moiety in doridosine and knowing that the nmr spectra of the sugar moiety is essentially unperturbed from that in guanosine and isoguanosine in which there is no substituent on the proximate N^3 ring nitrogen, (as is evident in the nmr spectrum of 3-methylguanosine (17)), we can conclude that *doridosine is unsubstituted at* N^3.

Doridosine did not co-chromatograph on two dimensional thin layer chromatography with any of the common methyl-nucleosides encountered in transfer DNA research including 1-methylguanosine (m^1G, **2a**), N^2-methylguanosine (m^2G, **2c**), and N^7-methylguanosine (m^7G, **2e**). The other possible *N*-methylguanosine, 3-methylguanosine (m^3G, **2c**), is apparently unreported; but it can be ruled out based on chemical stability and nmr spectrum, (*vida infra*).

Our evidence indicates that doridosine is either a *N*-methylguanosine or *N*-methylisoguanosine. By direct comparison, doridosine has been shown not to be 1-methyl-, N^2-methyl or 7-methylguanosine or N^6-methylisoguanosine. Both 3-methylguanosine and 3-methylisoguanosine can be eliminated because of the nmr evidence just presented and because of the reported ease of acid or base catalyzed hydrolysis of 3-methyladenosine (17) and 3-methylguanine derivatives (18) that contrasts markedly with the high stability of doridosine under both acid and basic conditions. Furthermore, 7-methylguanosine (an internal salt) is very sensitive to basic conditions. One can only conclude that 7-methylisoguanosine would also be unstable to basic conditions and, therefore, incompatible with the doridosine structure. Thus, the remaining reasonable structural candidate for dorisodine is the previously unreported 1-methylisoguanosine **1**.

SYNTHESIS.—Although all the above evidence points to 1-methylisoguanosine as the structure for doridosine, the evidence is exclusive and not conclusive. The limited quantity of material precluded extensive degradation studies; therefore, we undertook the synthesis of 1-methylisoguanosine. Because of the direct one-step process, we chose to attempt the synthesis of 1-methylisoguanosine by direct methylation of isoguanosine, **4**, which is obtainable commercially, in spite of the fact that it would not provide absolute proof of the position of methylation. The conditions chosen were the same as those which convert adenosine to 1-

methyladenosine (20). A heterogeneous mixture of isoguanosine, methyl iodide, and dimethylformamide was allowed to react at room temperature for about one day. As the reaction proceeded, the mixture became homogeneous, and the crude product, obtained as described in the experimental section, was obtained in about 90% yield. Its proton nmr spectrum was indistinguishable from that of doridosine with the exception of some down-field signals indicating impurities. This crude product was purified by thin layer chromatography followed by crystallization to give a purified product which showed mass spectra, proton nmr spectra, C–13 nmr spectra, and optical rotation indistinguishable from natural doridosine.



4 **1**

This synthesis proves the purine nucleus in doridosine is isoguanosine, and the sugar moiety is β-D-ribosyl, i.e., the parent nucleoside is isoguanosine. However, the position of methylation is not established unambiguously by this method of synthesis. Since we have eliminated the N^6-methylisoguanosine structure by direct comparison, and since both 3-methyl- and 7-methyl- isoguanosines structures are highly unlikely because of predicted chemical instability in basic solution, and because of the spectral evidence, we consider this proof of structure satisfactory although not absolute.

Additional research on an unequivocal structural proof became unnecessary with the publication of the work by Quinn, Gregson, Cook and Bartlett on the isolation of 1-methylisoguanosine from the Australian sponge *Tedania digitata*. They proved the structure of their product via degradation (4) and unequivocal synthesis (5); this has been followed by the proof of identity of their sample and ours by direct comparison.

PHARMACOLOGICAL PROPERTIES.—We found that adenosine and doridosine combine with the same receptor (21) in guinea pig intestinal smooth muscle. The Australian group reached the same conclusion using guinea pig brain (9). Adenosine has many actions in mammals and in various cell and organ preparations (22, 23). The principal difference in the action of adenosine and doridosine is the very long duration of action of the latter. This is readily explained. Adenosine is deaminated by adenosine deaminase to inosine, which is practically without pharmacological activity. Doridosine, on the other hand, is resistant to deamina-

tion by this enzyme (3, 9). It is not yet known whether doridosine shares all the pharmacological actions of adenosine. Thus far, doridosine has been shown to produce muscular relaxation, hypothermia, arterial hypotension and bradycardia in mammals (3, 8) and to stimulate adenylate cyclase in guinea pig brain slices (9).

We will consider here only some aspects of the action of doridosine on the cardiovascular system. The hypotension and bradycardia produced by intravenous injection of doridosine into anesthetized rats (3) also occurs in vagotomized rats after administration of atropine. Thus, the bradycardia is not reflex or medullary in origin. Doridosine, 1 x 10^{-4}M, had no effect on the contraction of rat hemidiaphragm *in vitro* stimulated *via* the phrenic nerve. Consequently doridosine does not affect conduction in nerve axons or neuromuscular transmission in skeletal muscle.

The negative inotropic effect of adenosine on atrial muscle has been described by several investigators (24, 25). In ventricular muscle, adenosine inhibits the positive inotropic action of isoproterenol but has little effect in its absence (26). Clearly, doridosine reduces the force of contraction of the atria (fig. 1), and we also found that 1 x 10^{-4}M doridosine has no effect on the force of contraction of electrically driven strips of guinea pig ventricle. It is thus clear that the site of action of doridosine is on the atria.

TABLE 2. Antagonism of doriodsine by theophylline.

	Force of contraction as percent of control[a]	
	Doridosine m/l	
	5 x 10^{-6}	2 x 10^{-5}
Without Theophylline	65±16 (10)[b]	45.2±4 (6)
Theophylline 10 μm/l	83 (5)	64 (5)
Theophylline 100 μm/l	95 (2)	82 (4)

[a]Force of contraction of isolated spontaneously bearing guinea pig atria expressed as percent of control.

[b]Figures in parentheses are numbers of determinations.

Evidence that doridosine and adenosine bind to the same receptor is derived not only from the similarities of their actions in various tissues and isolated preparations, but also from the fact that the action of doridosine is blocked by theophylline, a known blocker of adenosine receptors (27). Baird-Lambert *et al.* (8) found that the hypothermic and muscle relaxant effects of doridosine were antagonized by theophylline. Davies *et al.* (9) showed that theophylline antagonized the stimulation of adenylate cyclase by both adenosine and doridosine. We found that theophylline also antagonized the negative inotropic effect of doridosine on guinea pig atria (table 2). The antagonism of the action of doridosine (and adenosine) by theophylline in these varied systems is added evidence for its action on the same receptor as adenosine.

EXPERIMENTAL[1]

ISOLATION.—Specimens of *Anisodoris nobilis* weighing from 2 to 300 g were collected from Monterey Bay near the Hopkins Marine Station of Stanford University in the late spring of 1978. The frozen digestive glands, 128 g, from approximately 1 kg of whole animals were ground and dialyzed (Spectropor #1 tubing; 6000–8000 molecular weight cut off) at 5° against distilled water changed 5–8 times. The water from the dialysates was removed under vacuum and residue treated with methanol. An insoluble portion was discarded and the soluble portion evaporated to dryness and subjected to exclusion chromatography in 3 batches by water elution on a 2 x 27 cm Bio-Gel P2 (Polyacrylamide resin Bio-Rad Laboratories with useful molecular weight limit below 1800). Fractions were tested for activity against the isolated spontaneously beating guinea pig atria as described under pharmacological methods. The active fractions which were eluted after two times the void volume (50 ml) were combined, filtered, and lyophilized to give 6.26 g of a sticky solid. This material was treated with methanol; 1.3 g of a tan solid which was inactive did not dissolve. The solution was evaporated to dryness to give 4.9 g of material which constituted the crude starting material for subsequent studies.

Thin layer chromatography (tlc) of this crude material was studied on several different supports (alumina, silica gel, reverse phase KC18, and cellulose) and in various solvent systems. Silica gel with the solvent mixture t-butanol-ethyl acetate-water-acetic acid (40:20:2:1) gave the best resolution. Five components (A, B_1, B_2, C and D) with Rf values of 0.20, 0.40, 0.43, 0.56 and 0.78 were observed. The slowest moving component (Rf 0.20) was also the most abundant and active in the guinea pig atria assay. The first crystalline material was obtained by preparative tlc, after which the bulk of the 4.9 g of residue was subjected to column chromatography on silica gel (Wöhlm, 0.03–0.06 mm particle size, 2.2 x 22 cm column) with the same solvent system. Approximately 60 20-ml fractions were collected. Each was tested by tlc, and fractions were combined accordingly: component D was found mainly from 90–130 ml, C from 130–190 ml, B_2 from 190–410 ml, B_1 from 410–665 ml, and A from 665–1045 ml.

The residues from the active fractions (665–1045 ml) were combined and crystallized several times, first from t-butanol-methanol-water, then methanol-water and methanol-water-acetic acid to give 34 mg of doridosine. To remove persistent impurity the material was washed with CD_3COOD (0.25 and 0.10 ml); the purified product was used for the proton nmr in CD_3COOD: D_2O solvent. Lyophilization of this material gave 28 mg, mp 260–263° $[\alpha]_D^{20}$ −66.2° (c=0.42 g/100 ml, CH_3OH); uv, λ max (ε) at pH 6.5, 292 (8,500), 248 (6,500); at pH 12,286 (7,500), 254 (6,000); at pH 1.5, 282 (9000). The high resolution mass spectrum showed the parent ion peak at 297.1076 *m/e* (calculated for $C_{11}H_{15}N_5O_5$ is 297.1073); the base peak at 165.0652 *m/e* (calculated for $C_6H_7N_5O$, purine base + H is 165.0650); and a major peak at 194.0676 *m/e* (calculated for $C_7H_8N_5O_2$, purine base + CH_2O, 194.0678). Trimethylsilyation of this sample according to the report of McCloskey and coworkers (28) with (*N,O*-bistrimethylsilyl)-trifluoroacetamide/trimethylsilyl chloride (BSTFA/TMCS) reagent followed by gc-ms gave the parent ion peak at 657 *m/e* (nucleoside + 5 TMS), significant peaks at 585 *m/e* (nucleoside + 4 TMS), 642 *m/e* (nucleoside−CH_3+5 TMS), 338 *m/e* (purine base + 2 TMS), 294 (purine base−CH_3+2 TMS). From these results, we conclude that two trimethylsilyl (TMS) groups were introduced into the purine moiety and three in the carbohydrate moiety.

To obtain the infrared spectrum, the sample was dissolved in KBr-H_2O, lyophilized and pressed into a KBr window; significant bands were observed at: 3430 (broad, OH), *ca* 3100 (broad shoulder, NH), 2975 (shoulder, CH), 2935 (strong, CH), 2850 (medium, CH), 1690, 1634, 1578 cm^{-1} (C=O and C=N).

METHYLATION OF ISOGUANOSINE.—A mixture of isoguanosine, (Vega Biochemicals) 4, 55 mg, and methyl iodide, 56 μl, in dimethylformamide, 700 μl, was stirred in a closed system under nitrogen at 25° for 29 hrs. The initial heterogeneous mixture slowly became homogeneous; a small amount of insoluble material was removed by centrifugation. Acetone, 5 ml, and anhydrous ether, 4 ml, were added to the supernate; an immediate precipitate formed. The mixture was stored at 4° overnight and centrifuged; the solid was separated and dried, (49 mg). This solid was dissolved in water-methanol, the pH adjusted to 8 with 10% ammonia solution and the solution lyophilized. The residue was twice exchanged with 99.99% D_2O and then lyophilized each time, and its nmr taken in $CD_3COOD+D_2O$. The proton nmr

[1]The nuclear magnetic resonance (nmr) spectra were taken on a Varian XL-100 instrument. A 1.7 mm capillary was used with microprobe attachment for carbon-13 spectra. The spectra with solvents used are recorded in table 1. When D_2O solvent was used the samples were twice equilibrated with 99.7% D_2O then lyophilized each time before being dissolved in "100%" D_2O for the spectral determination. The high resolution mass spectra were taken on a CEC 110 instrument with direct heated inlet; the GC–MS of the trimethylsilyl derivatives (28) were taken on an LKB 9000 (Swedish) instrument. The ultraviolet spectra were determined on a Cary M–14 spectrophotometer; the optical rotations were determined on a Rudolph Autopol II polarimeter which measures to 0.001°; the infrared spectra were taken on a Nicolet 7199 Fourier transform IR instrument.

perfectly duplicated all the peaks in doridosine except for three additional downfield signals attributed to impurities. Several recrystallizations from methanol gave material indistinguishable from doridosine.

Methylation of isoguanosine with methyl *p*-toluene-sulfonate and powdered potassium carbonate suspended in dimethylacetamide at 80° for 0.5 hr also gave doridosine, but the separation from the reagent proved more difficult.

PHARMACOLOGICAL METHODS.—Isolated guinea pig atria were suspended in Krebs bicarbonate-Ringer solution (29) equilibrated with 5% CO_2-95% O_2 at 30° in a 10 ml bath. Contraction was recorded on a Grass model 5 polygraph with a Grass model 5P1 preamplifier and FT.03 force transducer. Force, or rate after addition of doridosine, is expressed as a percent of the force or rate immediately before addition to the bath (Percent of Control).

For cardiovascular experiments on animals, very large (600 g) Long-Evans rats were anesthetized with sodium pentobarbital and the arterial pressure recorded in the conventional way from a cannula in one carotid artery with a Statham model P 23AC transducer. The rat phrenic nerve diaphragm preparation was that described by Bülbring (30).

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