equipped with an efficient condenser. The vessel was purged with a flow of dry nitrogen and the solution heated to reflux (100 °C). The solution composition was monitored throughout the reaction by analytical high-pressure LC (25% diisopropyl ether/Skellysolve B on μ-Porasil) at 1-h intervals until no further changes were observed. After 3 h, the solution was cooled, the solvent distilled off under reduced pressure, and the residual oil examined by NMR. Comparison of integrated peak intensities in the olefinic region of the spectrum indicated that a 22:78 equilibrium mixture of model vitamin **11** and previtamin **12** had been established. A similar experiment starting from the previtamin gave an identical result.

Acknowledgment. The U.S. Public Health Service (NIH Grant No. AM-16595) and the Intramural Fund of the University of California, Riverside, provided the financial support for this study. We thank Badische-Anilin and Sodafabrik (Ludwigshafen) and Hoffmann-La Roche (Nutley) for providing several of the starting materials used in this study. Dr. Milton Hammond (Ph.D. Thesis, University of California, Riverside, March 1978) is acknowledged for carrying out several preliminary investigations on vitamin D model systems which led to this investigation.

Registry No. **7**, 74483-09-3; **8**, 74483-10-6; **9**, 78-27-3; **10a**, 10575-77-6; **10b**, 65150-03-0; **10c**, 31814-67-2; **10d**, 5664-20-0; **11**, 74483-11-7; **12**, 74483-12-8; **13**, 74483-13-9; **14**, 74483-14-0; **15**, 74483-15-1; 1,2-diiodoethane, 624-73-7; 3-isobutoxy-2-methylcyclohex-2-en-1-one, 37457-15-1.

Supplementary Material Available: Spectral and analytical data (6 pages). Ordering information is given on any current masthead page.

Studies on Vitamin D (Calciferol) and Its Analogues. 20. Synthesis of 3-Deoxy-3,3-dimethyl-1-hydroxyvitamin D_3 from Vinylallene Intermediates and Related Thermal and Configurational Studies[1]

Antonio Mouriño, Sabina Lewicka-Piekut, Anthony W. Norman, and William H. Okamura*

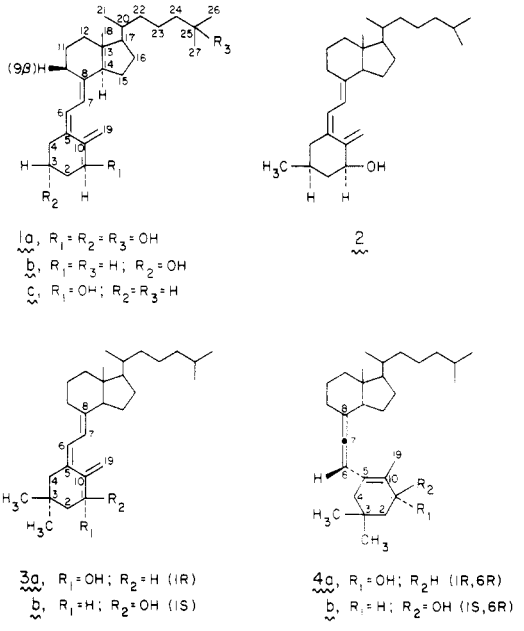
Departments of Chemistry and Biochemistry, University of California, Riverside, California 92521

Received April 11, 1980

The diastereomeric vinylallenols **4a** (1*R*,6*R*) and **4b** (1*S*,6*R*) were synthesized by coupling the known propargylic benzoate **9** with the heterocuprate **8**. The latter was prepared in five steps from 2-methyldimedone (**5**). By use of model systems **10a–c**, **11a–c**, and **12**, comparison of ^{13}C NMR data lends support to the 6*R* configuration assigned to **4a** and **4b**. Comparisons of the specific rotations of **10a,b**, **11a,b**, **4a,b**, and other chiral cyclohexenols previously described lend support to the C_1 configuration assigned to **4a** (1*R*) and **4b** (1*S*). Thermolysis of **4a** (~100 °C, 10 h) affords an ~6.8 to 1 ratio of 7*E* (**3a**) to 7*Z* manifold (**15a + 16a + 17a**) products. Similar thermolysis of **4b** affords a reversed ~1 to 8.3 ratio of 7*E* (**3b**) to 7*Z* manifold (**15b + 16b + 17b**) products. How the reversal in the 7*E* to 7*Z* ratio supports the C_1 configurational assignment is discussed. Neither vitamin (**3a** or **3b**) exhibits any in vivo vitamin D biological activity.

Analogues of 1α,25-dihydroxyvitamin D_3 (**1a**), the hormonally active form of vitamin D_3 (**1b**, cholecalciferol),[2] modified at the 3-position possess biological properties of unusual interest[3] (see Chart I). In earlier papers[4] we reported the synthesis and biological evaluation of 3-deoxy-1α-hydroxyvitamin D_3 (**1c**) in order to evaluate the relative contributions of the various hydroxy groups to the biological properties of **1a**. Interestingly, the 3-unsubstituted analogue **1c** was found to possess a significant ability to elicit intestinal calcium absorption (ICA) but only minimal bone calcium mobilization (BCM). This selectivity in biological action is of potential clinical interest

Chart I



in as much as the natural hormone **1a** is the most active substance known for eliciting both of these classical vitamin D mediated physiologic responses, ICA and BCM. In order to further pursue the notion that 3-substitution might impart interesting biological properties to other

(1) (a) For paper 19, see: Condran, P.; Okamura, W. H. *J. Org. Chem.*, preceding paper in this issue. For earlier vinylallene papers, see also: (b) Hammond, M. L.; Mouriño, A.; Okamura, W. H. *J. Am. Chem. Soc.* **1978**, *100*, 4907; (c) Condran, P.; Hammond, M. L.; Mouriño, A.; Okamura, W. H. *J. Am. Chem. Soc.*, in press.

(2) For general reviews on the subject of vitamin D, see: (a) Norman, A. W. "Vitamin D, the Calcium Homeostatic Steroid Hormone"; Academic Press: New York, 1979; (b) De Luca, H. F.; Paaren, H. E.; Schnoes, H. K. *Top. Curr. Chem.* **1979**, *83*, 1–65; (c) Georghiou, P. E. *Chem. Soc. Rev.* **1977**, *6*, 83; (d) Fieser, L. F.; Fieser, M. "Steroids"; Reinhold: New York, 1959; Chapter 4.

(3) Okamura, W. H.; Norman, A. W.; Wing, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 4194.

(4) (a) Okamura, W. H.; Mitra, M. N.; Wing, R. M.; Norman, A. W. *Biochem. Biophys. Res. Commun.* **1974**, *60*, 179. (b) Okamura, W. H.; Mitra, M. N.; Procsal, D. A.; Norman, A. W. *Ibid.* **1975**, *65*, 24. (c) Lam, H.-Y.; Onisko, B. L.; Schnoes, H. K.; DeLuca, H. F. *Ibid.* **1974**, *59*, 845. (d) Mitra, M. N.; Norman, A. W.; Okamura, W. H. *J. Org. Chem.* **1974**, *39*, 2931. (e) Norman, A. W.; Mitra, M. N.; Okamura, W. H.; Wing, R. M. *Science* **1975**, *188*, 1013. (f) Onisko, B. L.; Lam, H.-Y.; Reeve, L. E.; Schnoes, H. K.; DeLuca, H. F. *Bioorg. Chem.* **1977**, *6*, 203.

0022-3263/80/1945-4015$01.00/0 © 1980 American Chemical Society

Table I. ^{13}C NMR Data for Allenes[a,b]

carbon no.	4a	4b	12	10a	10b	10c	11a	11b	11c
1	70.9	70.9		70.4	70.3	199.6	70.3	70.5	199.5
2-4[c]	45.9, 40.9, 30.8, 30.0, 26.6	45.9, 40.9, 30.8, 30.1, 26.6		32.3, 27.1, 18.2	32.2, 27.1, 18.0	38.0, 28.2, 22.2	32.2, 27.9, 18.1	32.4, 27.7, 18.5	38.0, 28.2, 22.3
5	128.6[d]	128.6[d]		130.1[d]	130.1[d]	150.9	130.0	130.1[d]	150.6
6	93.3	93.4	74.6	93.2	93.2	94.4	93.4	93.4	94.6
7	200.8	200.8	203.4	201.1	201.3	204.4	201.1	201.0	204.3
8	105.5	105.5	102.0	105.8	105.8	106.6	106.0	105.9	106.7
9	31.4	31.3	31.0	31.4	31.4	30.7	31.4	31.4	30.6
10	127.7[d]	127.7[d]		129.7[d]	129.7[d]	130.2	130.0	129.9[d]	130.3
14	52.6	52.6	51.9	52.7	52.7	52.4	52.7	52.8	52.4
18	11.95	11.92	11.92	11.91	11.94	11.97	12.40	12.42	12.45
19	14.5	14.5		16.2	16.3	10.5	16.5	16.2	10.5
20, 22	36.2	36.2	36.2	36.2	36.3	36.2	36.27, 36.19	36.24, 36.16	36.22, 36.14

[a] Brucker WH90D-18 multinuclear FT NMR spectrometer, broad-band proton decoupled mode, $CDCl_3$ with $(CH_3)_4Si$ as internal standard with chemical shifts in δ units. [b] The data (in δ units) for carbons not listed were in close agreement for all of the substances: C-11, 22.8-23.0; C-12, 39.8-40.2; C-13, 44.6-44.9; C-15, 23.4-23.7; C-16, 27.7; C-17, 55.9-56.1; C-21, 18.8-18.9; C-23, 23.9-24.0; C-24, 39.6; C-25, 28.0-28.1; C-26, 22.6; C-27, 22.8-22.9 [c] Data for 4a and 4b include the *gem*-dimethyl groups at C-3. [d] C-5 and C-10 assignments may be reversed.

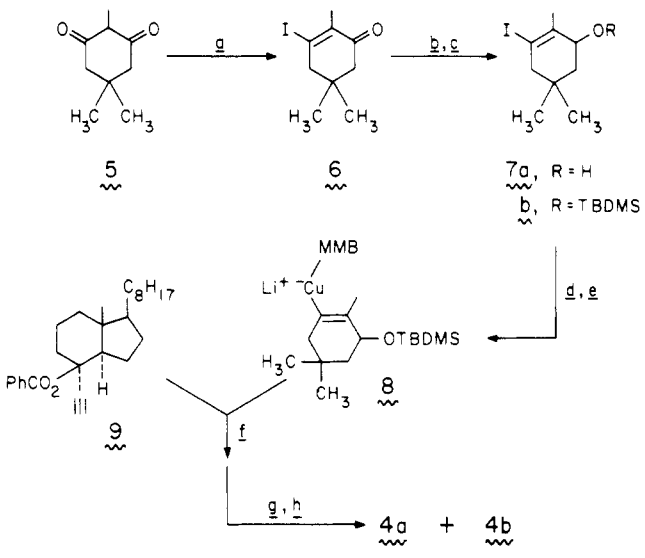
analogues, we synthesized the corresponding 3α-methyl analogue **2**.[5] Curiously, this analogue regained the ability to elicit BCM as well as ICA. Inasmuch as the reported syntheses of **1c**[4] and **2**[5] involved a tedious and time-consuming classical steroid procedure, we were encouraged to develop a more general approach for synthesizing 3-substituted 1-hydroxyvitamin D analogues. Accordingly, we recently reported the development of a new nonphotochemical convergent approach,[1b,c] which entailed as the key step a [1,5] sigmatropic hydrogen shift[6] of a vinylallene[7] intermediate. This new route afforded **1c** or its C_1 epimer in 8.3–16% overall yield (six to eight steps).[1c] By contrast, the earlier reported[4] classical synthesis of **1c** required 11 steps, and the overall yield was only 0.2%. This paper describes the application of the vinylallene route to the preparation of a new 3-substituted analogue, 3-deoxy-3,3-dimethyl-1-hydroxyvitamin D_3 (**3**), and the results of a study of the thermal behavior of vinylallenes **4** and the other valence isomers related to **3**. In addition, the results of absolute configurational studies of vitamin D related allenes are described.

Results and Discussion

The vinylallenols **4** were synthesized by the procedure outlined in Scheme I. The overall yield of **4a** and **4b** after chromatographic separation was 50% based on starting 2-methyldimedone[8] (or 46–58% based on commercial vitamin D_3 (**1b**), the precursor of **9**[1b,c]). The cuprate coupling step **8** + **9** occurred with essentially complete stereoselectivity to afford only the C_1 epimeric allenes **4a** and **4b**, which possess the 6*R* configuration. As noted previously for vinylallenes **10** and **11**[1b,c] and model systems **12**[1c] and **13**[7e] (see Chart II), (6*R*)-allenes are characterized by a C_{18} angular methyl group chemical shift of τ 9.35 ± 0.03 and an H_6 triplet splitting of $J \approx 2.9$–3.1 Hz. The corresponding ^1H NMR parameters for the (6*S*)-allenes were τ 9.27 ± 0.03 and $J \approx 3.5$–3.6 Hz. The allene configurations for **4a** and **4b** are therefore based on the similarities of their corresponding parameters (τ 9.34 and $J \approx 3.1$ Hz for **4a**; τ 9.35 and $J \approx 3.0$ Hz for **4b**) with those of the 6*R* series.

Because of the rather modest differences in the ^1H NMR parameters, we have now examined the ^{13}C NMR spectra

Scheme I



[a] Reagents: a, Ph_3PI_2, Et_3N, CH_3CN (72%); b, $NaBH_4$, EtOH (90%); c, *tert*-butyldimethylsilyl chloride (TBDMS-Cl), imidazole, DMF (97%); d, *tert*-BuLi, ether, −78 °C (1.5 h), −30 °C (1 h), −78 °C; e, (3-methoxy-3-methyl-1-butynyl)copper (MMB-CU(I)), ether, −78 °C; f, −78 °C (1 h), −50 to −60 °C (3 h), room temperature (0.5 h), and then quenched; g, $(n\text{-Bu})_4NF$, THF; h, medium-pressure liquid chromatography (41% **4a** and 39% **4b**). See ref 8 of the text for literature citations.

(5) Okamura, W. H.; Mitra, M. N.; Pirio, M. R.; Mouriño, A.; Carey, S. C.; Norman, A. W. *J. Org. Chem.* **1978**, *43*, 574.
(6) Spangler, C. W. *Chem. Rev.* **1976**, *76*, 187.
(7) (a) For a review of enallenes (vinylallenes), see: Eigenburg, I. Z. *Russ. Chem. Rev. (Engl. Transl.)* **1978**, *47*, 900–933; (b) Crowley, K. J. *Proc. Chem. Soc.* **1964**, 17; (c) Mikolajczak, K. L.; Bagby, M. O.; Bates, R. B.; Wolff, I. A. *J. Org. Chem.* **1965**, *30*, 2983; (d) Skattebøl, L. *Tetrahedron* **1969**, *25*, 4933; (e) Bakker, S. A.; Lugtenburg, J.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* **1972**, *91*, 1459; (f) Havinga, E. *Experientia* **1973**, *29*, 1181; (g) van Koeveringe, J. A.; Lugtenburg, J. *Recl. Trav. Chim. Pays-Bas* **1976**, *95*, 80; (h) Minter, D. E.; Fonken, G. J.; Cook, F. T. *Tetrahedron Lett.* **1979**, 711.
(8) (a) Clark, R. D.; Ellis, J. E.; Heathcock, C. H. *Synth. Commun.* **1973**, *3*, 347. (b) Piers, E.; Nagakura, I. *Ibid.* **1975**, *5*, 193. (c) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190. (d) Corey, E. J.; Floyd, D.; Lipshutz, B. H. *J. Org. Chem.* **1978**, *43*, 3418. (e) Corey, E. J.; Beames, D. J. *J. Am. Chem. Soc.* **1972**, *94*, 7210. (f) Rona, P.; Crabbé, P. *Ibid.* **1968**, *90*, 4733; **1969**, *91*, 3289. (g) Luche, J. L.; Barreiro, E.; Dollat, J. M.; Crabbé, P. *Tetrahedron Lett.* **1975**, 4615. (h) Van Dijck, L. A.; Lankwerden, B. J.; Vermeer, J. G. C. M.; Weber, A. J. M. *Recl. Trav. Chim. Pays-Bas* **1971**, *90*, 801. (i) Westmijze, H.; Vermeer, P. *Tetrahedron Lett.* **1979**, 4101. (j) Amos, R. A.; Katzenellenbogen, J. A. *J. Org. Chem.* **1978**, *43*, 555.

Chart II

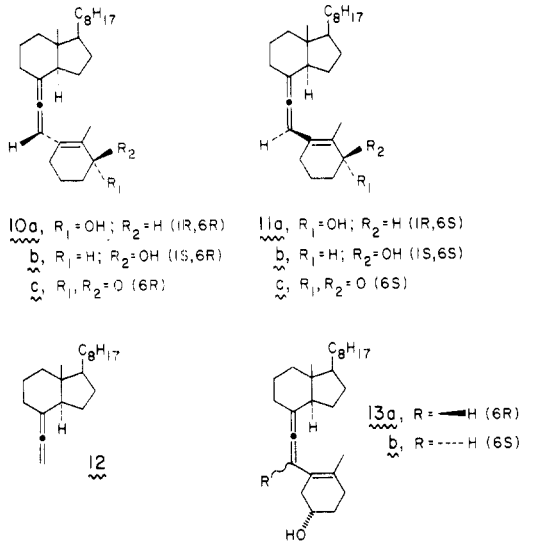


Table II. Summary of Thermal Studies under Standard Conditions[a]

substrate (reaction time, h)	products (% yield; given in order of elution)
4a (9)	3a (68), 15a (~2), 16a (~4), 4a (~4), 17a (~4)
4b (9)	3b (~8), 15b (~11), 17b (35), 4b (~10), 16b (20)
15a, 16a, or 17a (40)	15a (7), 16a (18), 17a (75)[b]
15b, 16b, or 17b (32-40)	15b (9), 17b (26), 16b (65)[c]

[a] Refluxing isooctane (~100 °C) under argon or nitrogen for the time periods indicated. [b] All ±1% average deviation. [c] All ±2% average deviation.

of **10**–**12** as well as **4a** and **4b** in order to further support these empirically derived configurational correlations. Table I summarizes the ^{13}C NMR data for which assignments could be made by analogy with a number of closely related model systems taken primarily from recent vitamin D literature.[9] The essential conclusion is clear. The peak assigned to C-18 seems to also be useful for characterizing the allene configuration. The unsubstituted model **12**[1c] and those isomers (**4** and **10**) with the 6*R* configuration exhibit a peak at δ 11.91–11.97; those with the 6*S* configuration (**11**) exhibit a corresponding signal at δ 12.42–12.45. A slight magnetic nonequivalence induced in the resonances assigned to C-20 and C-22 in the (6*S*)-allenes is also observable. In the 6*R* series, only a single peak assigned to C-20 and C-22 is observed (δ 36.2).

Like the diastereomeric allenes **10** and **11**,[1b,c] thermolysis of **4** leads via competitive [1,5] sigmatropic shifts into the two distinct thermal manifolds (7*E* and 7*Z*) outlined in Scheme II. The 7*E* manifold affords the desired vitamin **3** while the 7*Z* manifold leads to the putative (7*Z*)-vitamin **14**. The putative **14** of the primary process presumably undergoes a [1,7] sigmatropic shift to enter the observed equilibrium manifold consisting of the secondary product **15** (*cis*-isotachysterol[10] analogue) and the tertiary products

Scheme II

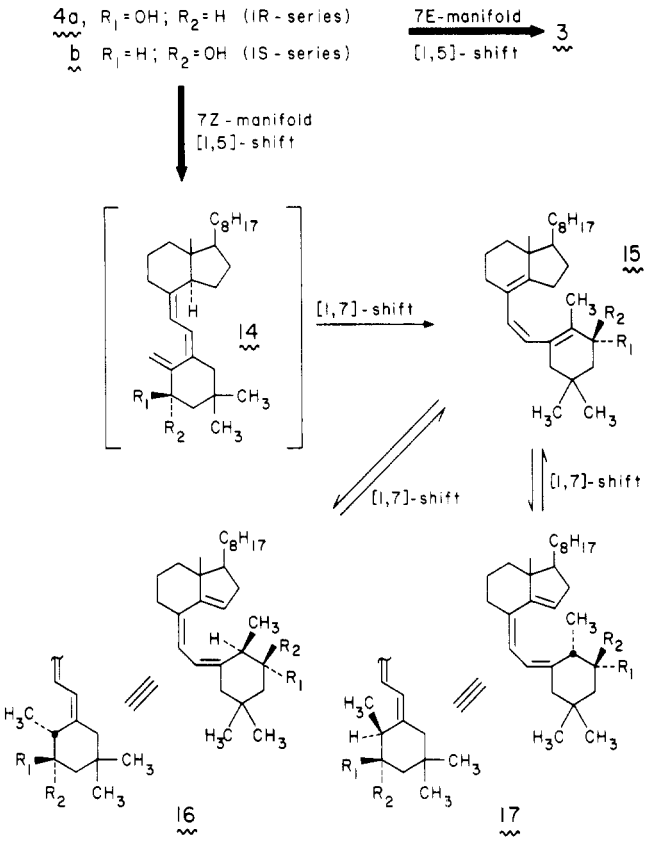


Table III. Specific Rotations (deg) of Vinylallenols[a]

(1*R*,6*R*)-**4a**	−12	(1*S*,6*R*)-**4b**	−52
(1*R*,6*R*)-**10a**	+47	(1*S*,6*R*)-**10b**	−68
(1*R*,6*S*)-**11a**	+210	(1*S*,6*S*)-**11b**	+11

[a] All specific rotations, $[\alpha]^{25}_D$, were determined in duplicate (<±2° average deviation) on separately weighed samples (*c* 1 g/100 mL of $CHCl_3$) with a Perkin-Elmer Model 241 polarimeter.

16 and **17**. The members of this equilibrium manifold are interconvertible by reversible [1,7] sigmatropic hydrogen shifts. Table II summarizes the product distributions from the thermal studies. For **4a**, the ratio of the 7*E* (**3a**) to 7*Z* (**15a** + **16a** + **17a**) products (Scheme II) was ~6.8:1; for **4b**, this ratio (**3b** to **15b** + **16b** + **17b**) was ~1:8.3. The vinylallenes **4** therefore behave in a manner similar to the (6*R*)-allenes **10a** (1*R*) and **10b** (1*S*). For the thermolyses of **10a** and **10b**, the 7*E*/7*Z* ratios were ~2.7:1 and ~1:4.1, respectively. Assuming an *s-cis*-vinylallene conformation during the occurrence of the primary suprafacial [1,5] hydrogen shift process, it was surmised from the studies of **10a,b** as well as **11a,b** that the trajectory of the migrating hydrogen prefers the face opposite to that of the A ring bearing the hydroxyl group.[1b,c] This effect also seems to characterize the isomerization of **4a** and **4b**.

That the thermal behavior of **4a** parallels that of **10a** and **4b** parallels that of **10b** leads to the tentative conclusion that **4a** and **4b** possess the 1*R* and 1*S* configurations, respectively. In order to support this conclusion, we determined the specific rotations of the vinylallenols **4a,b**, **10a,b** and **11a,b** (Table III). In all cases, the substances which are known (**10a** and **11a**) or considered (**4a**) to possess the 1*R* configuration exhibit a more dextrorotatory

(9) (a) Tsukida, K.; Akutsu, K.; Saiki, K. *J. Nutr. Sci. Vitaminol.* **1975**, *21*, 411. (b) Berman, E.; Luz, Z.; Mazur, Y.; Sheves, M. *J. Org. Chem.* **1977**, *42*, 3325. (c) Berman, E.; Friedman, N.; Mazur, Y.; Sheves, M. *J. Am. Chem. Soc.* **1978**, *100*, 5626. (d) Cohen, Z.; Berman, E.; Mazur, Y.; *J. Org. Chem.* **1979**, *44*, 3077. (e) Reischl, W.; Zbiral, E. *Helv. Chim. Acta* **1979**, *62*, 1763. (f) For a general reference, see: Levy, G. C.; Nelson, G. L. "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists"; Wiley-Interscience: New York, 1972.

(10) (a) Verloop, A.; Corts, G. J. B.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* **1960**, *79*, 164. (b) Onisko, B. L.; Schnoes, H. K.; DeLuca, H. F. *J. Org. Chem.* **1978**, *43*, 3441.

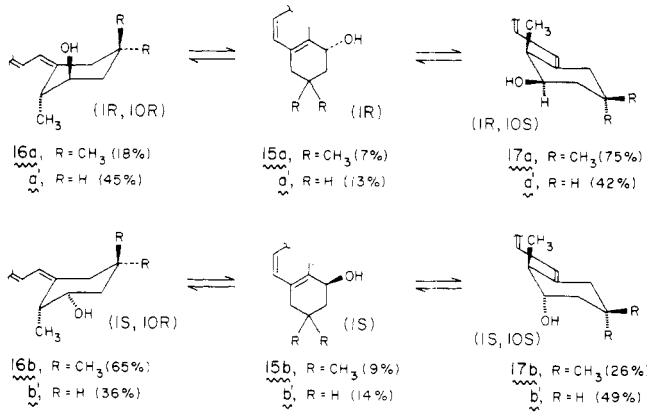
rotation than their 1*S* counterparts (**10b**, **11b**, and **4b**). These configurational assignments completely parallel the empirical correlations developed by Mills[11] for other chiral cyclohexenols. Our attempts to utilize the LIS–NMR method of Yamaguchi[12] have thus far failed presumably because of the lability of the chiral esters derived from the vinylallenols **4**, **10**, and **11**.

The vitamins **3a** and **3b** exhibited spectral properties completely characteristic of other vitamin D systems. For example, their UV spectra revealed a typical vitamin D chromophore (λ_{max} ~264 nm and λ_{min} ~228 nm),[13] and their mass spectra exhibited the characteristic base peak at *m/e* 164 (or *m/e* 146 which corresponds to *m/e* 164 – H_2O) due to the lower portion of the vitamin D skeleton by formal cleavage across the C-7,8 double bond.[14] Besides the usual ^1H NMR signals, **3b** exhibited a signal at τ 5.85 (doublet, $J \approx 10.8$ Hz) attributable to the 1β-H. The magnitude of this coupling implies that **3b** exists as a ~92/8 mixture of the equatorial and axial 1α-OH conformers.[15] Similarly, **3a** exhibits a corresponding signal at τ 5.83 ($J \approx 10.7$ Hz) due to the 1α-H, but now implying a similar bias favoring the 1β-OH equatorial conformer.[15] The attenuated magnitude of $J_{1\beta,2\alpha}$ (~10.8 Hz) observed for **3b** compared to that (~11.5 Hz) of the monomethyl analogue[5] **2** is reasonable. The trans axial C-3 methyl group in the equatorial-OH conformer of **3b** makes it less favorable relative to its axial-OH chair conformer (~92:8) than the favored equatorial-OH conformer of **2** relative to its axial-OH chair conformer (~100% equatorial-OH).

The stereostructures of the 7*Z* manifold products (**15**–**17**) follow from the close similarity of their spectral properties (supplementary material) with those reported for the corresponding isomers derived from the 3,3-didemethyl analogues **11** and **12**[1b,c] and similar isomers derived from the parent vitamin D_3 recently reported by Onisko et al.[10b] Several other features of the thermal results (Table II) deserve comment. First, the distribution of the 7*Z* manifold products from **4b** (~11, 20, and 35% of **15b**, **16b**, and **17b**, respectively) differs from their equilibrium proportions (~9, 65, and 26%, respectively). In terms of the mechanistic hypothesis depicted in Scheme II, it is quite apparent that the process **15b** → **17b** must be faster than **15b** → **16b**. Assuming an antarafacial migration of hydrogen in these [1,7] sigmatropic reactions, this means that the 15α-H of **15** migrates faster to C-10 on the same face of the A ring bearing the OH (i.e., to give **17b** having a trans relationship between OH and CH_3) than the 15β-H of **15** to the face opposite that bearing the same OH (i.e., to give **16b** with a cis C-1 OH and C-10 CH_3). Models imply that what seems to be the more sterically hindered process (**15b** → **17b**) is preferred kinetically. This is quite analogous to what was observed previously in our studies of the 3,3′-didemethyl series.[1c] The origin of this effect is certainly not clear.

Second, the equilibrium distributions of the 7*Z* manifold products observed in this study (3,3-dimethyl series of Scheme III: **15a**, **16a**, and **17a**; **15b**, **16b**, and **17b**) differ significantly from those observed in the previous study[1c]

Scheme III



(3,3-didemethyl series of Scheme III: **15a′**, **16a′**, and **17a′**; **15b′**, **16b′**, and **17b′**). The observed differences can be rationalized on the basis of steric effects. The attenuation of the equilibrium proportion of **15** compared to **16** plus **17** upon incorporation of two methyl groups at C-3 can be attributed to strain of the kind present in 2-methyl-2-cyclohexenol.[16] The enhanced proportion of the equatorial OH conformer in the **16** ⇄ **17** equilibrium upon introduction of *gem*-dimethyl groups at C-3 is attributable to the 1,3-diaxial interaction between the C-1 OH and the cis C-3 CH_3 group in the axial-OH conformer. In the demethyl series (**a′** and **b′** of Scheme III), an axial OH is juxtaposed by a much smaller C-3 H, resulting in an equilibrium value closer to unity for the process **16** ⇄ **17**. Finally, it should be noted that Scheme III depicts only the major chair conformer for each of the eight compounds **16**–**17**. This of course further complicates the analysis.

Biological Activity. By use of classical ICA and BCM bioassay methods, the 1*S* (or 1α) analogue (**1c**) of 3-deoxy-1-hydroxyvitamin D_3 has been shown to elicit ICA activity but negligible BCM activity.[4] In a side-by-side comparison with **1c**, neither **3a** nor **3b** exhibited any activity whatever.[17] Several testable hypotheses can be put forward to account for this lack of biological response. The 1α-OH analogue may suffer an inability to be 25-hydroxylated, a necessary step in biological activation of vitamin D_3,[2a,b,3] or it may be inefficiently transported to appropriate target tissues. Yet another possibility is that its presumed 25-hydroxylated form cannot properly interact with target receptors due to the presence of the additional 3β-methyl group. For the testing of these hypotheses, future studies include the synthesis and evaluation of the C-3 epimer of **2** and the 25-hydroxylated form of the latter as well as that of **3**.

Experimental Section

(1) General Methods. Ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR), and mass spectra and other analytical data are summarized in the supplementary material. Melting points (uncorrected) were obtained with a Thomas-Hoover capillary apparatus. Dry tetrahydrofuran (THF) or dry ether refers

(11) Mills, J. A. *J. Chem. Soc.* **1952**, 4976.

(12) Yamaguchi, S.; Yasuhara, F. *Tetrahedron Lett.* **1977**, 89. In addition, an attempt to directly monitor the chemical shift difference of the methoxyl resonance as a probe of chirality (Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512) was not successful, as expected. The methoxyl resonance for the MTPA esters of **4a,b**, **10a,b**, and **11a,b** differed by less than 0.01 ppm.

(13) For examples, see ref 4 and 5.

(14) Okamura, W. H.; Hammond, M. L.; Jacobs, H. J. C.; Thuijl, J. v. *Tetrahedron Lett.* **1976**, 89.

(15) See ref 5 for leading references to the method of conformational analysis.

(16) (a) Johnson, F. *Chem. Rev.* **1968**, *68*, 375–413. (b) Senda, Y.; Imaizumi, S.; Ochiai, S.; Fujita, K. *Tetrahedron* **1974**, *30*, 539. The 2-methylcyclohex-2-en-1-ol system is expected to possess steric strain in either pseudochair conformation. In one conformer (pseudoequatorial hydroxyl), there is allylic strain between the methyl and hydroxyl. In the other (pseudoaxial hydroxyl), the hydroxyl is 1,3-diaxial to the *cis*-methyl at C_5.

(17) The biological assay method (in vivo in the chick) has been previously described in detail (Hibberd, K.; Norman, A. W. *Biochem. Pharmacol.* **1969**, *18*, 2347). At dose levels up to 303 nmol (~$^1/_8$ mg) of analogue (**3a** or **3b**) per bird and for time periods ranging from 13 to 45 h before bioassay, no ICA or BCM activity could be detected.

to solvent freshly distilled under nitrogen from $LiAlH_4$ or K/benzophenone; lbpe refers to redistilled 30–60 °C low-boiling petroleum ether. Kugelrohr distillation boiling points refer to the external air-bath temperatures. It can be assumed that reactions involving air- and/or moisture-sensitive organometallic reagents or substrates were handled under a blanket of dry nitrogen. Air-sensitive polyolefins or allenes were normally stored in the cold under nitrogen.

(2) Chromatographic Methods. High-pressure liquid chromatography (LC) was carried out on a Waters 6000A solvent delivery system equipped with a U6K injector and a dual detector system (UV at 2537 Å and a refractive index detector). A Whatman M9 10/50 Partisil (10 μm, 9.4 mm i.d. × 50 cm) column was used. Diisopropyl ether (chromatographed over activity I alumina and then distilled from CaH_2), reagent grade isopropyl and isobutyl alcohol, and Skellysolve B (distilled from CaH_2) were used as solvents. Solvents and solvent combinations were normally vacuum filtered through a 0.45-μm Millipore filter immediately before use. Medium-pressure liquid chromatography (LC) was carried out on an apparatus designed by Meyers and co-workers.[18] The absorbant was silica gel 60 (40–600 μm) from E. Merck, and the columns used were either 2.5 × 100 cm or 1.5 × 100 cm. We are grateful to Professor Meyers for providing the details for constructing the medium-pressure LC apparatus. For ordinary column chromatography, Baker analyzed reagent silica gel (60–200 mesh) or Woelm neutral grade III alumina was used. For thin-layer chromatography (TLC), silica gel G (EM reagents, type 60) was used to prepare analytical plates (0.25 mm).

(3) 3-Iodo-2,5,5-trimethylcyclohex-2-en-1-one (6).[8a,b] To a mechanically stirred solution of triphenylphosphine (16.3 g, 62.5 mmol, recrystallized from EtOAc–CH_3OH) in acetonitrile (250 mL, freshly distilled from P_2O_5) was added iodine (15.67 g, 62 mmol), and then the mixture was allowed to stir for 3 h (room temperature, N_2). Triethylamine (8.53 mL, 62 mmol, freshly distilled from $LiAlH_4$) and then 2,5,5-trimethylcyclohexane-1,3-dione (**5**; 8.65 g, 51.5 mmol) were added successively to the yellow suspension of the triphenylphosphonium diiodide. After the dark brown mixture was refluxed for 4 h, the mixture was cooled and then concentrated under vacuum. The resulting residue in ether was filtered (short silica gel column), concentrated, and then cooled (−10 to 0 °C, 12 h) to afford a crystalline solid. After filtration, the ether filtrate was concentrated to a syrup which was Kugelrohr distilled (60–80 °C, 0.0003 mm). The iodo enone **6** was obtained in 71.5% yield (10.2 g).

(4) 3-Iodo-2,5,5-trimethylcyclohex-2-en-1-ol (7a). Sodium borohydride (1.5 g, 40 mmol) was added in portions to a solution of iodo enone **6** (7.5 g, 28 mmol) in 95% ethanol (75 mL). The mixture was magnetically stirred for 3 h at room temperature (N_2), ice cooled, and then quenched with sufficient 1 M HCl(aq) until a clear solution was obtained. After a conventional workup (water, ether; Na_2SO_4 drying; concentration), a pale yellow oil was obtained which was crystallized (hexanes) to afford the desired iodo enol **7a**: 6.75 g (90%); mp 64–66 °C.

(5) 1-(*tert*-Butyldimethylsiloxy)-3-iodo-2,5,5-trimethylcyclohex-2-ene (7b).[8c] The iodo enol **7a** (1.7 g, 6.4 mmol) was added to a solution of *tert*-butyldimethylsilyl chloride (1.43 g, 9.5 mmol) and imidazole (1.3 g, 20 mmol) in dry *N,N*-dimethylformamide (6 mL). After being stirred for 3 h (room temperature, N_2), the mixture was poured into water (100 mL) and then worked up [ether extraction (2 × 50 mL), back-washed with 1 M HCl (1 × 50 mL), saturated aqueous $NaHCO_3$ (1 × 50 mL), and water (2 × 50 mL), dried (Na_2SO_4), filtered, and then concentrated under vacuum] to afford an oil. Kugelrohr distillation (98–100 °C, 0.1 mm) gave the silyl ether in 97% yield (2.36 g).

(6) (1*R*,6*R*)- and (1*S*,6*R*)-1-Hydroxy-3,3-dimethyl-9,10-secocholesta-5(10),6,7-trienes 4a,b.[8c-j] To an ice-cooled solution (argon atmosphere) of 3-methoxy-3-methyl-1-butyne (MMB; 750 mg, 7.66 mmol) in dry ether (7 mL) was added *n*-butyllithium/hexane (7.46 mmol, 1.61 M; syringe). After the mixture was stirred for 20 min, half of the resulting suspension was added (double-ended needle) to cuprous iodide (802 mg, 4.21 mmol; purified by Soxhlet extraction with THF followed by drying) contained in another vessel (stirring, 0 °C). The suspension (MMB-Cu) was stirred for 1 h (room temperature) and then cooled to −78 °C before reaction with the vinyllithium.

To a solution (−78 °C, stirred) of iodosilyl ether **7b** (1.45 g, 3.83 mmol) in dry ether (7 mL) was added *tert*-butyllithium/pentane (4.76 mL, 7.66 mmol, 1.61 M) by syringe. The reaction mixture was stirred (−78 °C, 1.5 h; −30 °C, 1 h; recooled to −78 °C) and then transferred (double-ended needle) to the −78 °C cooled suspension of MMB-Cu described above. After 1 h (−78 °C) of stirring, a solution of des-*A,B*-8α-ethynyl-8β-cholestanol benzoate (**9**; 1.1 g, 2.8 mmol) in ether (4 and 2 mL for rinsing) was added to the cuprate suspension (at −78 °C). The mixture was stirred at −78 °C (1 h), at −50 to −60 °C (3 h), and then at room temperature (~0.5 h). After addition of water (20 mL), the mixture was stirred (10 min), filtered, extracted (saturated aqueous NH_4Cl and 1:1 ether–pentane), and then refiltered. The organic phase was washed (water), dried ($MgSO_4$), filtered, and then concentrated to afford a yellow syrup (thoroughly dried under high vacuum to remove volatiles). A solution of tetra-*n*-butylammonium fluoride (0.5 M in THF, 32 mL, 16 mmol) was added to the yellow syrup, and the resulting solution was stirred for 5 h at room temperature. After concentration (≲30 °C), the residue was extracted with ether–pentane and water. The separated organic phase was washed (saturated aqueous $NaHCO_3$), dried ($MgSO_4$), and then concentrated to afford a vinylallenol mixture. Medium-pressure LC on the Meyers system (15% ether–lbpe) afforded clean separation into a less polar fraction A (473 mg, 41%) and a more polar fraction B (452 mg, 39%). A and B were assigned as the 1*R*,6*R* (**4a**) and 1*S*,6*R* (**4b**) isomers, respectively, and both were obtained as white foams.

(7) Thermolysis of Vinylallenol 4a: (1*R*)-3-Deoxy-1-hydroxy-3,3-dimethylvitamin D_3 (3a), (1*R*)-(6*Z*)-1-Hydroxy-3,3-dimethyl-9,10-secocholesta-5(10),6,8-triene (15a), and (1*R*,10*R*)-(5*Z*,7*Z*)- and (1*R*,10*S*)-(5*Z*,7*Z*)-1-Hydroxy-3,3-dimethyl-9,10-secocholesta-5,7,14-trienes 16a and 17a. An isooctane (25 mL; freshly distilled from $LiAlH_4$ under nitrogen) solution of vinylallenol **4a** (isomer A, 100 mg, 0.24 mmol) was heated (100 °C) under argon for 9 h. Removal of the solvent (vacuum) followed by preparative high-pressure LC (Partisil, 2 mL/min of 40% diisopropyl ether/skellysolve B) afforded excellent resolution of five components eluted in the following order: 1A (68 mg, 68%), 2A, 3A, 4A, and 5A. By combining the minor components from a second identical thermolysis experiment, the following amounts of 2A–5A were obtained: 2A (4 mg, ~2%; UV calcd), 3A (8 mg, ~4%), 4A (8 mg, ~4%), and 5A (7 mg, ~4%). About ~82% of the theoretical amount could be accounted for, and the five fractions 1A–5A were found to correspond to **3a**, **15a**, **16a**, starting material (**4a**), and **17a**.

The relative amounts of 1A–5A correspond to 83, 2, 5, 5, and 5%, respectively, calculated from the absolute yields given above. On the basis of the integration of the uncalibrated high pressure LC refractive index detector trace, the relative proportions of 1A–5A were determined to be 85, 3, 6, 6, and 1%, respectively. It seems clear from this as well as other high-pressure LC analyses that more 3A than 5A is formed.

(8) Thermolysis of Vinylallenol 4b: (1*S*)-3-Deoxy-1-hydroxy-3,3-dimethylvitamin D_3 (3b), (1*S*)-(6*Z*)-1-Hydroxy-3,3-dimethyl-9,10-secocholesta-5(10),6,8-triene (15b), and (1*S*,10*S*)-(5*Z*,7*Z*)- and (1*S*,10*R*)-(5*Z*,7*Z*)-1-Hydroxy-3,3-dimethyl-9,10-secocholesta-5,7,14-trienes 17b and 16b. A solution of vinylallenol **4b** (isomer B, 100 mg, 0.24 mmol) in isooctane (25 mL; freshly distilled from $LiAlH_4$ under argon) was heated at 100 °C under argon for 9 h. After removal of the solvent under high vacuum, the resulting products were cleanly separated by high-pressure LC (Partisil, 2 mL/min of 40% diisopropyl ether/skellysolve B) to afford in the order of elution 1B (8.3 mg, ~8%), 2B (10.6 mg, ~11%), 3B (34.6 mg, ~35%), 4B (10 mg, ~10%), and 5B (20 mg, 20%), corresponding to **3b**, **15b**, **17b**, starting material (**4b**), and **16b**, respectively (84% total yield). The relative proportions of 1B–5B calculated from these data correspond to 10, 13, 42, 12, and 24%, respectively. High-pressure LC analysis (refractive index detector; not calibrated) indicated the presence of 14, 17, 36, 13, and 21%, respectively, of 1B–5B.

(9) Thermal Equilibration of 15a, 16a, and 17a. The following extinction coefficients were assumed for **15a**, **16a**, and **17a** in isooctane as solvent: ϵ_{254nm} 14 000, ϵ_{272nm} 20 000 and ϵ_{273nm} 20 000,

(18) Meyers, A. I.; Slade, J.; Smith, R. K.; Mihelich, E. D.; Hershenson, F. M.; Liang, C. D. *J. Org. Chem.* **1979**, *44*, 2247.

respectively. In parallel experiments, **15a** (3.2 mg), **16a** (2.6 mg), and **17a** (2.2 mg), each dissolved in isooctane (2 mL), were heated at reflux (~100 °C; oil bath temperature 110 °C) under N_2 for 40 h. Analysis of the product mixture from each thermolysis experiment by high-pressure LC (Partisil; 40% diisopropyl ether/skellysolve B) revealed the presence of the same equilibrium mixture (integration of the refractive index detector trace) of **15a**, **16a**, and **17a**: 8, 17, and 75% from **15a**; 7, 19, and 74% from **16a**; 7, 17, and 76% from **17a** (average: 7, 18, and 75% of **15a**, **16a**, and **17a**, respectively, with an average deviation of ±1%). In the case of the **17a** thermolysis, the products were collected preparatively by high-pressure LC, and then the absolute yields of products were estimated by UV calculations. The results were as follows: **15a** (0.12 mg), **16a** (0.75 mg), and **17a** (1.1 mg) for a mass balance of ~91%. The relative amounts of the three products correspond to 8, 17, and 75%, respectively, which agree well with the uncorrected refractive index detector trace integration.

(10) Thermal Equilibration of 15b, 17b, and 16b. A solution of 1*S*,10*S* isomer **17b** (64 mg, 0.15 mmol) in isooctane (16 mL, freshly distilled from $LiAlH_4$ under argon) was heated (100 °C) under argon for 40 h. NMR analysis of the residue after solvent removal revealed the following composition: **15b** (9%), **17b** (27%), and **16b** (64%). The ratio of **15b** to **17b**/**16b** was obtained by integrating the τ 4.16 ($H_{6,7}$ of **15b**) and 4.55 (H_{15} of **17b** and **16b**) signals; the ratio of **17b**/**16b** to **16b** was obtained by integrating the τ 6.1–6.4 (H_1 of **17b** and **16b**) and ~6.8 (H_{10} of **16b**) signals.

Similar thermolysis of **16b** (59 mg in 15 mL of isooctane, 40 h reflux, ~100 °C) revealed the following composition of products (analyzed by NMR as for **17b**): **15b** (7%), **17b** (26%), and **16b** (67%).

A solution of **15b** (~10 mg) in isooctane (3 mL) was heated as above for 32 h. Concentration and then preparative high-pressure LC (Partisil M9 10/50 column; 40% diisopropyl ether/skellysolve B; refractive index detector) afforded **15b** (0.6 mg, 10%; assuming ϵ_{253} ~14000), **17b** (1.4 mg, ~23%; assuming ϵ_{274} ~20000), and **16b** (4 mg, ~67%; assuming ϵ_{273} ~20000). Direct integration of the high-pressure LC trace (refractive index detector without correction) afforded the values 14, 25, and 62%, respectively. The average of the two determinations were 12% **15b**, 24% **17b**, and 65% **16b**.

The overall average equilibrium product distributions for the three separate thermolyses were 9 ± 2% **15b**, 26 ± 1% **17b**, and 65 ± 1% **16b** (average deviations are given).

Acknowledgment. The U.S. Public Health Service (NIH Grants No. AM-16595 and AM-09012) and the Intramural Fund of the University of California at Riverside provided the financial support for this study. We thank Dr. M. Rappoldt of Philips-Duphar (Weesp, The Netherlands) for generous gifts of vitamin D_3. A.M. was a postdoctoral fellow supported by a grant from the Program of the United States-Spanish Joint Committee for Scientific and Technological Cooperation. Some technical assistance from Dr. Alan W. Messing is also acknowledged. The reaction conditions for the cuprate coupling method described in Scheme I were developed during the course of vitamin A studies by Mr. Christopher G. Knudsen of this laboratory. Professor David Kearns kindly provided the 300-MHz spectral data.

Registry No. 3a, 74398-18-8; **3b**, 74398-19-9; **4a**, 74398-20-2; **4b**, 74398-21-3; **5**, 1125-11-7; **6**, 74398-22-4; **7a**, 74398-23-5; **7b**, 74398-24-6; **9**, 74398-25-7; **10a**, 67632-48-8; **10b**, 67670-78-4; **10c**, 74398-26-8; **11a**, 67670-79-5; **11b**, 67670-80-8; **11c**, 74398-27-9; **12**, 74398-28-0; **15a**, 74431-69-9; **15b**, 74398-29-1; **16a**, 74398-30-4; **16b**, 74398-31-5; **17a**, 74431-20-2; **17b**, 74431-21-3.

Supplementary Material Available: Spectral and analytical data (7 pages). Ordering information is given on any current masthead page.

1-Methylisoguanosine, a Pharmacologically Active Agent from a Marine Sponge

Alan F. Cook* and Robert T. Bartlett

Department of Chemical Research, Hoffmann-La Roche Inc., Nutley, New Jersey 07110

Richard P. Gregson and Ronald J. Quinn*

Roche Research Institute of Marine Pharmacology, Dee Why, N.S.W. 2099, Australia

Received April 7, 1980

Aqueous ethanolic extracts of the marine sponge *Tedania digitata* possessed a number of pharmacological properties. The active constituent, a new N-methylated purine nucleoside, was isolated by ion-exchange chromatography and the structure elucidated by spectral methods, particularly ^{13}C NMR and mass spectroscopy, combined with chemical degradations. Mild acid hydrolysis of the glycosyl bond gave D-ribose while strong acid hydrolysis gave 1-methylxanthine (**3**), establishing the position of methylation of the purine nucleus. Synthesis by two routes confirmed the structure as 1-methylisoguanosine (**1**). Synthesis from 5-amino-4-carbamoyl-1β-D-ribofuranosylimidazole (**5**) proceeded in high overall yield, employing cyclization of the protected 5-amino-4-cyanoimidazole (**7**) with methylisocyanate to form the correctly substituted pyrimidine ring of **1**.

Methylated nucleosides have been isolated from a variety of natural sources. Transfer ribonucleic acid (tRNA) has been shown to contain a number of methylated components, over 20 of which have been isolated and identified.[1] Messenger RNA has been shown to possess several methylated species, associated primarily with the 5′-terminal region.[2] Bacterial sources have provided methylated nucleosides such as puromycin[3] and 5,6-dihydro-5-azathymidine,[4] and 2-methoxyadenosine (spongosine) has been isolated from a sponge.[5] Recently a new methylated purine nucleoside, 1-methylisoguanosine (**1**), has been isolated from the marine sponge *Tedania digitata*[6] and

(1) S. Nishimura, *Prog. Nucleic Acid. Res. Mol. Biol.*, **12**, 49–85 (1972).
(2) F. M. Rottman, R. C. Desrosiers, and K. Friderici, *Prog. Nucleic Acid Res. Mol. Biol.*, **19**, 21–38 (1976).
(3) R. J. Suhadolnik, *Prog. Nucleic Acid Res. Mol. Biol.*, **22**, 193–291 (1979).
(4) C. De Boer and B. Bannister, U.S. Patents 3 907 463 and 3 907 779 (1975).
(5) W. Bergmann and D. C. Burke, *J. Org. Chem.*, **21**, 226–228 (1956).

0022-3263/80/1945-4020$01.00/0 © 1980 American Chemical Society