Synthesis of N^1-(4-Hydroxy-1,2-butadien-1-yl)thymine, an Analogue of 3′-Deoxythymidine[1]

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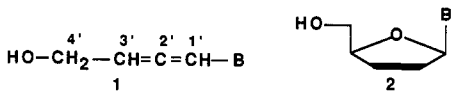
Attempted isomerization of N^1-(4-hydroxy-2-butyn-1-yl)thymine (**3d**) with 0.1 M NaOH at 100 °C led to acetonylthymine (**8**) instead of thymallene (**1d**). In 1 M NaOH compound **3d** afforded oxacyclopentene **9d**, whereas reaction with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) in dimethylformamide (DMF) gave cyclic imino ether **11**. Hydrolysis of **11**, effected by 0.1 M NaOH, furnished β-hydroxy ketone **12**. At 100 °C both **11** and **12** yielded **8**. The results are explained in terms of ionization of thymine base relative to that of alcoholic hydroxy group of **3d** or putative intermediate **1d**. Acetoxybutynol **16** was transformed with potassium *tert*-butoxide (tBuOK) in DMF to butenyne **17**. Protection of **16** with [2-(trimethylsilyl)ethoxy]methyl (SEM) group led to compound **19**. Ammonolysis of **19** gave SEM derivative **20**, which was isomerized with tetrabutylammonium fluoride in tetrahydrofuran to protected thymallene **21**. The latter was deblocked with $SnCl_4$ in CH_2Cl_2 to give thymallene (**1d**). Attempted isomerization of **19** or **20** with tBuOK in DMF led only to elimination of SEM-substituted thymine.

Allenic alcohols **1a–d**, which can be regarded as rigid analogues of 2′,3′-dideoxyribonucleosides, are of interest as antiretroviral agents.[2] Thus, adenallene (**1a**) and cytallene (**1b**) are in vitro inhibitors[3] of replication and cytopathic effect of human immunodeficiency virus (HIV), which is the causative agent of acquired immunodeficiency syndrome (AIDS).. Effectivity of **1a** or **1b** is comparable to that of the corresponding 2′,3′-dideoxyribonucleosides **2a** and **2b**, which are currently undergoing clinical studies. An additional advantage of adenallene (**1a**) over 2′,3′-dideoxyadenosine (**2a**) is its much lower reactivity to the catabolyzing enzyme, adenosine deaminase.[2] 3′-Deoxythymidine (**2d**) has also proved to be an effective in vitro inhibitor of HIV in certain cell lines,[4] and it was therefore of interest to prepare the corresponding allenic analogue **1d**.



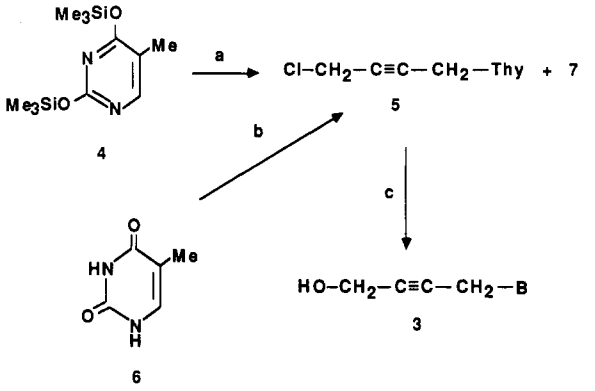
1a, 2a: B = adenine **1c, 2c:** B = guanine

1b, 2b: B = cytosine **1d, 2d:** B = thymine

Results and Discussion

Our previous work has shown[2] that acetylene–allene isomerization is a convenient approach to the synthesis of allenic alcohols derived from nucleic acid bases **1a–c**. The best results were achieved with basic heterocycles in the molecule (compounds **1a** and **1b**) whereas in the case of a guanine base the strongly basic auxilliary group (N^2-(dimethylamino)methylene) was employed in order to obtain guanallene (**1c**) in reasonable yield and purity.[2] These facts indicated that possible difficulties might be expected with attempts to isomerize butynols derived from more acidic nucleic acid bases such as **3d** to the respective allenols, e.g., **1d**.

Scheme I



3a: B = adenine **3c:** B = guanine

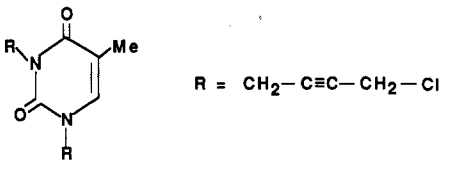
3b: B = cytosine **3d:** B = thymine

a. $Cl–CH_2–C\equiv C–CH_2–Cl, CH_2Cl_2$.

b. $Cl–CH_2–C\equiv C–CH_2–Cl$, K_2CO_3, DMSO.

c. 0.1 M HCl, reflux.

Starting material for our investigations, butynol **3d**, was prepared as follows (Scheme I). Bis(trimethylsilyl)thymine[5] (**4**) was alkylated with a 4-fold excess of 1,4-dichloro-2-butyne to give the (chlorobutynyl)thymine **5** in 47% yield. A direct alkylation of thymine (**6**) employing the method used previously[2] (K_2CO_3–DMSO) afforded **5** (38%) in addition to the dialkylated product **7** (20%). Hydrolysis of **5** in refluxing 0.1 M HCl for 18 h furnished the desired butynol **3d** in 64% yield.



The initial experiments aimed at isomerization of **3d** under catalysis with a strong base did not afford any thymallene (**1d**). Thus, refluxing butynol **3d** in 0.1 M NaOH for 8 h gave acetonylthymine **8** in 38% yield (Scheme II) with properties similar to those described.[6]

(1) Presented at the 15th Nucleic Acid Chemistry Symposium, September 19–21, 1988, Sapporo, Japan. Phadtare, S.; Zemlicka *J. Nucleic Acids Res., Symp. Ser.* **1988**, No. 20, 39, and 197th National Meeting of the American Chemical Society, April 9–14, 1989, Dallas, TX, Abstract MEDI 92.

(2) Phadtare, S.; Zemlicka, J. *J. Am. Chem. Soc.*, in press.

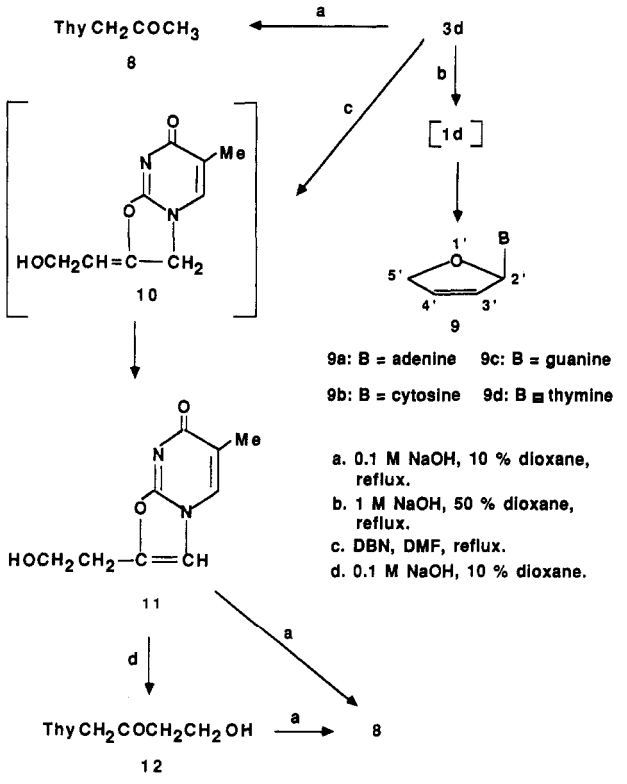
(3) Hayashi, S.; Phadtare, S.; Zemlicka, J.; Matsukura, M.; Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6127.

(4) Herdewijn, P.; Balzarini, J.; DeClercq, E.; Pauwels, R.; Baba, M.; Broder, S.; Vanderhaeghe, H. *J. Med. Chem.* **1987**, *30*, 1270.

(5) Nishimura, T.; Iwai, I. *Chem. Pharm. Bull.* **1964**, *12*, 352.

0022-3263/89/1954-3675$01.50/0

Scheme II

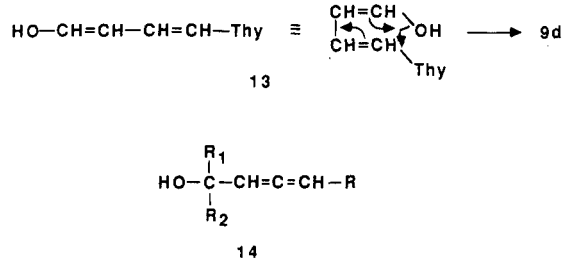


This result indicated that thymallene **1d** was formed but it suffered hydrolysis and subsequent retro-aldol cleavage to give 8. However, reflux of compound **3d** in 1 M NaOH for 1 h afforded oxacyclopentene **9d** in 58% yield, which must have arisen via thymallene (**1d**). Similar transformations of butynols **3a–c** were reported previously.[2]

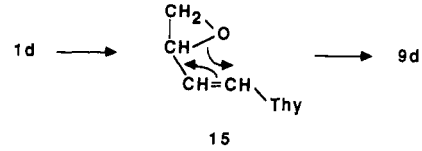
It seems plausible on the basis of steric considerations, namely, the proximity of the thymine 2-carbonyl group to the multiple-bond system, that oxazoles **10** or **11** are intermediates in hydrolysis of **3d** or **1d** in 0.1 M NaOH leading ultimately to acetonylthymine (**8**). One of these compounds, **11**, was prepared independently by reaction of butynol **3d** with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) in dimethylformamide (DMF, 30 min reflux) in 45% yield or by using tBuOK in DMF (1 h reflux) in 28% yield. The structure of **11** was confirmed by the ^1H NMR spectrum, which ruled out the alternate structure **10**. Thus, $H_{1'}$ is a singlet in the heteroaromatic region at δ 8.02 whereas $H_{3'}$ appears as a high-field triplet at δ 2.75. Again, two possible routes can be visualized for the formation of oxazole **11**, cyclization of the intermediary thymallene (**1d**) or butynol **3d**. In the latter case the primary product, oxazole **10** is isomerized to **11**. Both oxazoles, **10** and **11**, can conceivably result from an intramolecular attack of thymine 2-CO group at the cumulated system of double bonds in **1d**.

Mild alkaline hydrolysis of **11** (0.1 M NaOH in 10% dioxane for 6 h at room temperature) gave the known[7] β-hydroxy ketone **12** in 74% yield, which underwent retro-aldol reaction under more vigorous conditions (reflux for 15 min), giving rise to **8** in 55% yield. Acetonylthymine (**8**) was also formed directly from oxazole **11** in 66% yield, respectively. These experiments thus established the sequence of events leading from butynol **3d** to acetonylthymine (**8**) to be as follows: **3d** → **10** → **11** → **12** → **8** or **3d** → **1d** → (**10**) → **11** → **8**.

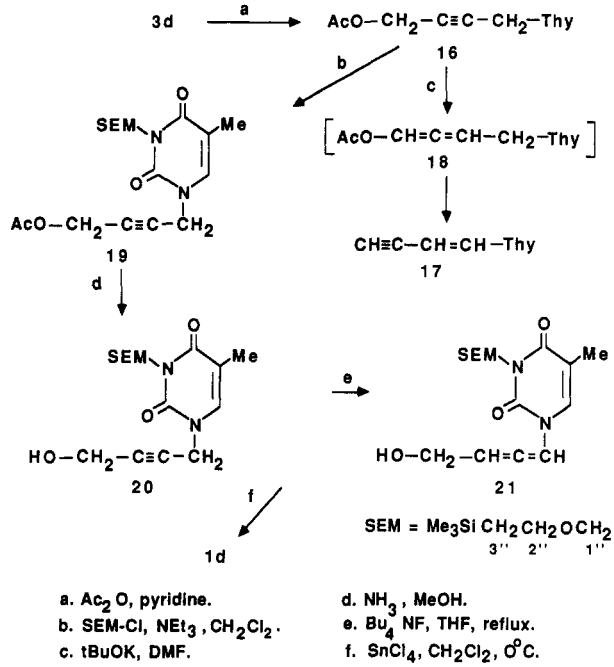
Scheme III



Scheme IV



Scheme V



The dichotomy of the reaction course of hydrolysis of **3d** in 0.1 M and 1 M NaOH can be explained on the basis of ionization of functional groups involved. In 0.1 M NaOH, only the thymine moiety is effectively ionized with a little competition of the hydroxy group. Thus, formation of oxazoles **10** or **11** becomes predominant, leading ultimately to acetonylthymine (**8**) as described above. In 1 M NaOH, ionization of the hydroxy group becomes more important, and cyclization to oxacyclopentene **9d** is favored. It appears that formation of the latter product is favored neither on steric grounds (space-filling models) nor by cyclization rules.[2] Intervention of intermediate[2] **13** (Scheme III) is also unlikely because α,α-disubstituted allenic alcohols **14** that cannot tautomerize to hydroxybutadienes of type **13** are transformed to the respective oxacyclopentenes without any difficulty.[8] Another possibility is the formation of epoxide **15** and a subsequent [1,3]sigmatropic shift to give compound **9d** (Scheme IV). Cyclopropane[9] and aza analogues[10] of **15** have been de-

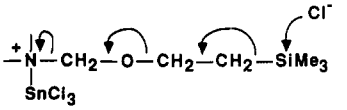
(6) Lee, K.-H.; Wu, Y.-S.; Hall, I. H. *J. Med. Chem.* **1977**, *20*, 911.
(7) Martin, J. C.; Smee, D. F.; Verheyden, J. P. H. *J. Org. Chem.* **1985**, *50*, 755.
(8) Hoff, S.; Brandsma, L.; Arens, J. F. *Recl. Trav. Chim. Pays-Bas* **1969**, *88*, 609.
(9) DeWolfe, R. H. In *Comprehensive Chemical Kinetics*; Bamford, C. H., Tipper, C. F. H., Eds.; Elsevier: New York, 1973; Vol. 9, p 470.

scribed, but, in the absence of detailed studies, the question of mechanism of cyclization of allenes **1a–d** to oxacyclopentenes **9a–d** remains open.

It became clear from these results that synthesis of **1d** would be successful if we could suppress the side reactions by blocking the hydroxy function and/or imido group of thymine of the acetylenic precursor **3d**. Also, employing a less drastic reagent for allenic isomerization appeared desirable. A simple acetylation of butynol **3d** led to the expected acetate **16** in 91% yield (Scheme V). An attempted isomerization of **16** by using tBuOK in DMF at room temperature was not successful. Conjugated butynene **17** was the only product obtained in 35% yield. The structure of the *E* isomer was assigned to compound **17** on the basis of the coupling constant of the olefinic protons $H_{1'}$ and $H_{2'}$ ($J_{1',2'} = J_{2',1'} = 15$ Hz). Its formation is readily explained by isomerization of the acetate **16** to the respective allene **18**, which then undergoes an elimination of the acetoxy group. Similar transformations of simpler allenic acetates are known.[11] An apparent reversal of the direction of allenic isomerization relative to butynols[2] **3a–c** (away from the heterocyclic base) is probably caused by ionization of the thymine moiety of **16** under the reaction conditions. Clearly, the initial carbanion formation occurs at the most distant site from the alredy ionized group, i.e., at the 4′-methylene function of compound **16**.

It became then obvious that protection of a reactive CONH moiety is necessary in order to suppress ionization of the thymine portion. This was achieved with a [2-(trimethylsilyl)ethoxy]methyl (SEM) group, which is reasonably stable over a wide pH range.[12] This function was readily introduced into the molecule of acetate **16** by reaction with SEM chloride in dichloromethane with triethylamine as a base. The fully protected compound **19** was obtained in 67% yield. Attempted isomerization of **19** with tBuOK in DMF led only to elimination of SEM-substituted thymine. Apparently, the latter moiety can function under the reaction conditions employed as an excellent leaving group. Deacetylation of **19** with ammonia in methanol gave alcohol **20** in almost quantitative yield. Nevertheless, attempted isomerization of **20** was also not successful, giving again only the SEM-protected thymine.

Replacement of tBuOK–DMF reagent with a less basic tetrabutylammonium fluoride in tetrahydrofuran (reflux for 6 h) readily afforded the desired thymallene **21** in 60% yield. It is noteworthy that the SEM function is completely stable under the reaction conditions even in the presence of "adventitious water" (ref 12). To the best of our knowledge, this is the first case of acetylene–allene isomerization under fluoride catalysis. The reaction can have considerable significance for preparation of allenes with limited stability toward strong bases. In the last step, the SEM group of **21** was removed by using $SnCl_4$ in CH_2Cl_2 at 0 °C for 1.5 h to give thymallene (**1d**) in 45% yield.[13] The latter reaction, which can be readily interpreted in terms of a push-and-pull mechanism shown in formula **22**, represents a new and extremely mild method for the removal of SEM group. Thus, allenic alcohols **1a–d** derived from all major DNA bases became available.



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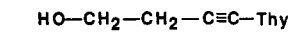

23

The structure of thymallene (**1d**) was confirmed by spectral data. In contrast to allenes[2] **1a–c** the IR spectrum of **1d** exhibited a sharp band of medium intensity at 1965 cm^{-1} belonging to an asymmetric vibration of C=C=C system. The ^1H NMR exhibits the $H_{1'}$ and $H_{3'}$ olefinic protons in the expected region of the spectrum,[2] at δ 7.20 and 6.18, and OH signal as a triplet at δ 5.08. In addition, the ^{13}C NMR showed the line typical for sp-hybridized allenic carbon[2] ($C_{2'}$) at 194 ppm. Compound **1d** is >95% pure as established by ^1H NMR containing <5% of butynol[15] **23**.

Biology. Preliminary tests showed that thymallene (**1d**) did not inhibit replication and cytopathic effect of HIV in $CD4^+$ T-cell clone (ATH8) culture.[16] 3′-Deoxythymidine (**2d**) also showed little activity in the latter assay.[17] Compound **1d** was an inhibitor of growth of murine leukemia L1210 in vitro (IC_{50} 20 μg/mL).[18] In this assay, it is the most active compound of allenols **1a–d**. Detailed biological results will be published elsewhere.

Experimental Section

General Methods. (See ref 2.) The NMR spectra were determined in CD_3SOCD_3 as solvent and $Si(CH_3)_4$ as internal reference unless stated otherwise. For the purpose of assignment of NMR signals, the prime numbering of thymine substituents is used (see formulas **1** and **9**). The electron-impact (EI-MS) and chemical-ionization (CI-MS) mass spectra were recorded as stated previously.[2]

***N*^{1}-(4-Chloro-2-butyn-1-yl)thymine (5). A. Direct Alkylation.** A mixture of thymine (**6**, 1.26 g, 10 mmol), K_2CO_3 (2.76 g, 20 mmol), and 1,4-dichloro-2-butyne (4.88 g, 40 mmol) in DMSO (40 mL) was stirred for 18 h at room temperature. The solution was evaporated, the residue was washed several times with CH_2Cl_2–MeOH (9:1) (total 300 mL), and the crude product was chromatographed on a silica gel column in CH_2Cl_2–MeOH (97:3) to give first the dialkylated product **7** (0.6 g, 20%): mp 118–121 °C, after crystallization from cyclohexane–ethyl acetate (9:1); UV (ethanol) max 267 nm (ϵ 8600), 205 (ϵ 10700); ^1H NMR ($CDCl_3$) δ 7.23 (d, 1, H_6), 4.78, 4.64 (2 t, 2, $H_{1'}$), 4.17, 4.11 (2 t, 2, $H_{4'}$), 1.99 (d, 3, CH_3); EI-MS 298, 300 (M, 2.5, 1.6). Anal. Calcd for $C_{13}H_{12}Cl_2N_2O_2$: C, 52.18; H, 4.04; Cl, 23.70; N, 9.36. Found: C, 52.30; H, 4.27; Cl, 23.40; N, 9.25.

Second fraction afforded compound **5** (0.8 g, 38%), mp 192–195 °C after crystallization from cyclohexane–ethyl acetate (4:1), identical with the product obtained by method B.

Method B. Alkylation via Silylated Intermediate 4. Compound[5] **4** (1.35 g, 5 mmol) and 1,4-dichloro-2-butyne (2.45 g, 20 mmol) were stirred in CH_2Cl_2 (30 mL) for 3 days at room temperature. The solvent was evaporated, and the residue was chromatographed as in method A to give chlorobutyne **5** (0.5 g, 47%): mp 191–195 °C after crystallization from cyclohexane–ethyl acetate (4:1); UV (ethanol) max 266 nm (ϵ 8700), 207 (ϵ 9000); ^1H NMR δ 11.36 (s, 1, NH), 7.54 (d, 1, H_6), 4.54, 4.46 (2 t, 4, $H_{1'}$ + $H_{4'}$), 1.74 (d, 1, CH_3); EI-MS 212, 214 (M, 6.0, 2.0). Anal. Calcd for $C_9H_9ClN_2O_2$: C, 50.83; H, 4.26; Cl, 16.67; N, 13.17. Found:

(10) Heine, H. W. *Mech. Mol. Migr.* **1971**, *3*, 145.
(11) Brandsma, L.; Montigu, P. P.; Arens, J. F. *Recl. Trav. Chim. Pays-Bas* **1963**, *82*, 1115.
(12) Lipshutz, B. H.; Pegram, J. J. *Tetrahedron Lett.* **1980**, *21*, 3343. To the best of our knowledge, an application of SEM group for protection of reactive amino or imino functions has not yet been reported.
(13) It is recognized that the scope of use of SEM group for protection of reactive CONH moieties could be significantly broader than described herein. Novel blocking functions for this purpose appear to be in constant demand for oligonucleotide synthesis.[14]
(14) Schulz, B. S.; Pfleiderer, W. *Tetrahedron Lett.* **1983**, *24*, 3587 and references cited therein.
(15) Tentative structure (see ref 2).
(16) Hayashi, S.; Matsukura, M.; Mitsuya, H.; Broder, S. Unpublished results.
(17) Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1911.
(18) Kessel, D. Unpublished results.

C, 50.64; H, 4.24; Cl, 16.52; N, 12.98.

N^1-(4-Hydroxy-2-butyn-1-yl)thymine (3d). A solution of compound **5** (1.06 g, 5 mmol) in 0.1 M HCl (80 mL) was refluxed for 15 h. After cooling to 5–10 °C, it was brought to pH 7 with NaOH (pH meter) and evaporated. A solid residue was washed with CH_2Cl_2–MeOH (4:1), and the crude product was chromatographed in CH_2Cl_2–tetrahydrofuran (THF, 4:1) to give butynol **3d** (0.62 g, 64%): mp 168–170 °C after crystallization from the same solvent; UV (pH 7) max 269 nm (ϵ 8500), 209 (ϵ 8600); ^{1}H NMR δ 11.32 (s, 1, NH), 7.53 (d, 1, H_6), 5.18 (t, 1, OH), 4.48 (m, 2, $H_{1'}$), 4.06 (m, 2, $H_{4'}$), 1.74 (d, 3, CH_3); EI-MS 194 (M, 30.1). Anal. Calcd for $C_9H_{10}N_2O_3$: C, 55.66; H, 5.19; N, 14.42. Found: C, 55.49; H, 5.32; N, 14.32.

(±)-N^1-(1-Oxa-3-cyclopenten-2-yl)thymine (9d). Butynol **3d** (0.19 g, 1 mmol) was refluxed in 1 M NaOH in 50% dioxane (15 mL) for 1 h. The reaction mixture was worked up as in the preceding experiment (compound **3d**); the pH was adjusted to 7 with 0.1 M HCl. The crude product was chromatographed on a silica gel column in CH_2Cl_2–MeOH (95:5) to give oxacyclopentene **9d** (0.11 g, 58%): mp 178–180 °C after crystallization from ethyl acetate; UV (pH 7) max 266 nm (ϵ 9200), 207 (ϵ 10 200); ^{1}H NMR δ 11.30 (s, 1, NH), 7.08 (d, 1, H_6), 6.81 (nonet, 1, $H_{2'}$), 6.53 (d of q, 1, $H_{3'}$), 5.86 (m, 1, $H_{4'}$), 4.82, 4.57 (d of oct and d of q, 2, $H_{5'}$); ^{13}C NMR 133.92, 124.09 ($C_{3'}$ + $C_{4'}$), 89.76 ($C_{2'}$), 75.31 ($C_{5'}$); thymine peaks 163.80, 150.59, 135.74, 109.87, 11.99 (CH_3); CI-MS 195 (M + H, 5.3), 167 (M + H – CO, 6.7), 69 ($C_4H_5O^+$, 25.9), 68 (furan, 16.8); thymine peaks 127, 83, 55. Anal. Calcd for $C_9H_{10}N_2O_3$: C, 55.66; H, 5.19; N, 14.42. Found: C, 55.78; H, 5.16; N, 14.32.

N^1-(2-Oxoprop-1-yl)thymine (8). A. From Butynol 3d. Compound **3d** (0.19 g, 1 mmol) was refluxed in 0.1 M NaOH in 10% dioxane (15 mL) for 5 h. The reaction mixture was worked up as in the preceding experiment (compound **9d**) to give ketone **8** (70 mg, 38%): mp 183–185 °C after crystallization from ethyl acetate (lit.[6] mp 197 °C). UV (pH 7) max 267 nm (ϵ 8100), 209 (ϵ 8500); ^{1}H NMR δ 11.30 (s, 1, NH), 7.32 (d, 1, H_6), 4.56 (s, 2, $H_{1'}$), 2.12 (s, 3, $H_{3'}$), 1.72 (d, 3, 5-CH_3); ^{13}C NMR 202.24 ($C_{2'}$), 56.03 ($C_{1'}$), 26.81 ($C_{3'}$); thymine peaks 164.34, 150.91, 141.76, 108.22, 11.87 (5-CH_3); EI-MS 182 (M, 30.8). Anal. Calcd for $C_8H_{10}N_2O_3$: C, 52.73; H, 5.53; N, 15.38. Found: C, 52.73; H, 5.65; N, 15.18.

B. From Oxazole 11. Compound **11** (20 mg, 0.1 mmol) was refluxed in 0.1 M NaOH in 10% dioxane (5 mL) for 20 min. The solution was worked up as described in method A, and the crude product was chromatographed in CH_2Cl_2–MeOH (9:1) to give ketone **8** (12 mg, 66%), mp. 188–190 °C, identical (mixed melting point, IR, and TLC) with the sample prepared by method A.

C. From β-Hydroxy Ketone 12. Method B was followed with ketone **12** instead of oxazole **11** (30 min reflux) to give compound **8** (10 mg, 55%), mp 189–192 °C, indistinguishable from samples prepared by methods A and B.

Oxazole 11. A mixture of butynol **3d** (0.19 g, 1 mmol) and DBN (0.12 g, 1 mmol) was refluxed in DMF (12 mL) under N_2 for 30 min. After cooling, DMF was evaporated, and the residue was chromatographed on a silica gel column in CH_2Cl_2–MeOH (9:1) to give compound **11** (87 mg, 45%): mp 271–275 °C after crystallization from ethyl acetate–MeOH (3:2); UV (pH 7) max 273 nm (ϵ 9200), 246 (ϵ 9800), 206 (ϵ 9600); ^{1}H NMR δ 8.02 (s, 1, $H_{1'}$), 7.54 (s, 1, H_6), 4.85 (t, 1, OH), 3.65 (q, 2, $H_{4'}$), 2.75 (t, 2, $H_{3'}$), 1.87 (s, 3, CH_3); EI-MS 194 (M, 100.0). Anal. Calcd for $C_9H_{10}N_2O_3$: C, 55.66; H, 5.19; N, 14.42. Found: C, 55.73; H, 5.27; N, 14.49.

N^1-(4-Hydroxy-2-oxobut-1-yl)thymine (12). A solution of oxazole **11** (0.19 g, 1 mmol) in 0.1 M NaOH in 10% dioxane (15 mL) was stirred for 6 h at room temperature. Dowex 50 (WX4, 200–400 mesh, H^+ form) was added to bring the pH to 7 (pH meter). The resin was filtered off, it was washed with water (2 × 10 mL), and the filtrate was evaporated. The resultant solid was chromatographed on a silica gel column in CH_2Cl_2–MeOH (9:1) to give ketone **12** (0.16 g, 74%), mp 173–176 °C after crystallization from ether–MeOH (9:1) (lit.[7] mp 184–185 °C). UV, NMR, and EI-MS data correspond to those described.[7] Anal. Calcd for $C_9H_{12}N_2O_4$: C, 50.93; H, 5.70; N, 13.20. Found: C, 51.09; H, 5.75; N, 13.04.

N^1-(4-Acetoxy-2-butyn-1-yl)thymine (16). Butynol **3d** (1.94 g, 10 mmol) was made anhydrous by co-evaporation with dry pyridine (3 × 20 mL) in vacuo at room temperature (oil pump and dry ice condenser). It was then dissolved in pyridine (30 mL), acetic anhydride (4.08 g, 40 mmol) and 4-(dimethylamino)pyridine (0.61 g, 5 mmol) were added, and the mixture was stirred at room temperature for 1.5 h. The clear solution was evaporated to a syrup, which was dissolved in CH_2Cl_2–THF (9:1). The solution was filtered through a short silica gel column, which was then eluted with the same solvent mixture. The appropriate fraction was evaporated to give 0.22 g (91%) of acetate **16**: mp 129–131 °C after crystallization from cyclohexane–THF (4:1); UV (ethanol) max 267 nm (ϵ 8100), 211 (ϵ 5600); ^{1}H NMR δ 11.36 (s, 1, NH), 7.55 (s, 1, H_6), 4.70, 4.52 (2 s, 2, $H_{1'}$ + $H_{4'}$), 2.02 (s, 3, CH_3CO), 1.74 (s, 3, 5-CH_3); ^{13}C NMR δ 169.73 (CO, Ac), 81.17, 79.01 ($C_{2'}$ + $C_{3'}$), 51.74, 36.53 ($C_{1'}$ + $C_{4'}$), 20.44 (CH_3, Ac); thymine peaks 164.16, 150.41, 140.15, 109.42, 11.93 (CH_3); EI-MS 236 (M, 38.7). Anal. Calcd for $C_{11}H_{12}N_2O_4$: C, 55.92; H, 5.12; N, 11.86. Found: C, 56.06; H, 5.22; N, 11.83.

(*E*)-N^1-(1-Buten-3-yn-1-yl)thymine (17). Acetate **16** (0.47 g, 2 mmol) was made anhydrous by co-evaporation with DMF (see the preceding experiment). A solution of **16** and freshly sublimed tBuOK (0.25 g, 2.2 mmol) in DMF (15 mL) was stirred for 16 h at room temperature under N_2. TLC in CH_2Cl_2–ether (4:1) showed a faster moving component in addition to **16**. The reaction was quenched by adding 1% AcOH (5 mL) to the reaction mixture cooled in an ice bath. The volatile materials were evaporated, and the crude product was chromatographed on a silica gel column by using CH_2Cl_2–ether (4:1). The appropriate fractions were evaporated to give butenyne **17** (0.12 g, 35%): mp 230 °C dec after crystallization from cyclohexane–THF (4:1) and starting material **16** (0.19 g, 50%); UV (compound **17**, ethanol) max 295 nm (ϵ 12 400), 249 (ϵ 6600), 206 (ϵ 5100); ^{1}H NMR δ 11.58 (s, 1, NH), 7.84 (s, 1, H_6), 7.33 (d, 1, $H_{1'}$, $J_{1',2'}$ = 14.9 Hz), 6.00 (dd, 1, $H_{2'}$, $J_{2',1'}$ = 14.9 Hz), 3.95 (d, 1, $H_{4'}$), 1.79 (s, 3, CH_3); ^{13}C NMR δ 163.36 (C_2), 148.83 (C_4), 134.72, 134.59 (C_6 + $C_{1'}$), 111.36 (C_5), 94.86 ($C_{2'}$), 82.00, 80.25 ($C_{3'}$ + $C_{4'}$), 11.97 (CH_3); EI-MS 177 (M + H, 26.2), 176 (M, 100.0). Anal. Calcd for $C_9H_8N_2O_2$: C, 61.35; H, 4.57; N, 15.90. Found: C, 61.40; H, 4.41; N, 15.80.

N^1-(4-Acetoxy-2-butyn-1-yl)-N^3-[[2-(trimethylsilyl)ethoxy]methyl]thymine (19). A mixture of compound **16** (0.31 g, 1.3 mmol), triethylamine (0.66 g, 6.5 mmol), and SEM-Cl (0.65 g, 3.9 mmol) in CH_2Cl_2 (30 mL) was refluxed for 24 h under N_2. The solvent was evaporated, and the crude product was chromatographed on a silica gel column in cyclohexane–THF (4:1) to give syrupy compound **19** (0.32 g, 67%): UV (ethanol) max 269 nm (ϵ 7500), 212 (3400). ^{1}H NMR[19] ($CDCl_3$) δ 7.23 (s, 1, H_6), 5.42 (s, 2, $H_{1''}$), 4.71, 4.60 (2 t, 4, $H_{1'}$ + $H_{4'}$), 3.67 (t, 2, $H_{2''}$), 2.12 (s, 3, CH_3CO), 1.98 (s, 3, 5-CH_3), 1.00 (t, 2, $H_{3''}$), 0.01 (s, 9, Me_3Si). Anal. Calcd for $C_{17}H_{26}N_2O_5Si$: C, 55.71; H, 7.15; N, 7.64. Found: C, 55.86; H, 7.24; N, 7.65.

N^1-(4-Hydroxy-2-butyn-1-yl)-N^2-[[2-(trimethylsilyl)ethoxy]methyl]thymine (20). A solution of compound **19** (0.37 g, 1 mmol) in methanol saturated with ammonia at 0 °C (20 mL) was stirred for 16 h at room temperature. Evaporation afforded a TLC [CH_2Cl_2–THF (4:1)] uniform compound **20** (0.32 g, 99%) as a syrup: UV (ethanol) max 270 nm (ϵ 7000), 211 (3800); ^{1}H NMR[19] ($CDCl_3$) δ 7.24 (s, 1, H_6), 5.42 (s, 2, $H_{1''}$), 4.59, 4.32 (2 t, 4, $H_{1'}$ + $H_{4'}$), 3.69 (t, 2, $H_{2''}$), 1.97 (s, 3, 5-CH_3), 0.98 (t, 2, $H_{3''}$), 0.01 (s, 3, Me_3Si). Anal. Calcd for $C_{15}H_{24}N_2O_4Si$: C, 55.52; H, 7.45; N, 8.63. Found: C, 55.55; H, 7.51; N, 8.46.

(±)-N^1-(4-Hydroxy-1,2-butadien-1-yl)-N^3-[[2-(trimethylsilyl)ethoxy]methyl]thymine (21). A solution of compound **20** (0.81 g, 2.5 mmol) and 1 M NBu_4F in THF (2.5 mL, 2.5 mmol) in THF (30 mL) was refluxed under N_2 for 18 h. TLC [CH_2Cl_2–MeOH (95:5), double development] showed the presence of compound **21** moving faster than starting material **20** (minor component). The mixture was evaporated, and the residue was chromatographed on a silica gel column in CH_2Cl_2–MeOH (97:3) to afford compound **21** (0.52 g, 60%) as a syrup. For analysis a sample of **21** was rechromatographed on a 20 × 20 cm, 2 mm thick layer of silica gel in the above-mentioned solvent mixture (double development): UV (ethanol) max 288 nm (ϵ 6400), 226 (ϵ 6900); IR (NaCl) 1970 cm^{-1} (weak, C=C=C); ^{1}H NMR[19] ($CDCl_3$) δ 7.37 (qt, 1, $H_{1'}$), 7.24 (s, 1, H_6), 6.18 (q, 1, $H_{3'}$), 5.41 (s, 2, $H_{1''}$), 4.29

(19) For numbering of the SEM group see Scheme V.

(20) Note added in proof: "3'-Deoxythymidine" but not "2',3'-dideoxythymidine" (see ref 3, 17 and elsewhere) is the correct nomenclature of compound **2d** (*Biochim. Biophys. Acta* **1971**, *247*, 1).

(br s, 2, $H_{4'}$), 3.68 (t, 2, $H_{2''}$), 1.95 (s, 3, 5-CH_3), 0.94 (t, 2, $H_{3''}$), 0.01 (s, 9, Me_3Si); ^{13}C NMR δ 194.22 ($C_{2'}$), 107.26, 99.91 ($C_{1'}$ + $C_{3'}$), 70.59, 67.72 ($C_{1''}$ + $C_{2''}$), 60.20 ($C_{4'}$), 18.25 ($C_{3''}$), −1.39 (CH_3, Me_3Si); thymine peaks 163.23, 149.91, 134.79, 111.57, 13.16 (CH_3). Anal. Calcd for $C_{15}H_{24}N_2O_4Si$: C, 55.52; H, 7.45; N, 8.63. Found: C, 55.74; H, 7.45; N, 8.52.

(±)-N^1-(4-Hydroxy-1,2-butadien-1-yl)thymine (Thymallene, 1d). $SnCl_4$ (0.26 g, 1 mmol) was added with stirring and external ice cooling to a solution of compound **21** (0.32 g, 1 mmol) in CH_2Cl_2 (15 mL). The stirring continued for 2 h whereupon 4% NaOH in methanol (2.5 mL) was added. After 15 min, the reaction mixture was filtered through silica gel, which was then washed with CH_2Cl_2–MeOH (3 × 15 mL). The filtrate was evaporated, and the crude product was chromatographed on a silica gel column in CH_2Cl_2–MeOH (95:5). The appropriate fraction was evaporated to give thymallene (**1d**, 87 mg, 45%): mp 173–175 °C after crystallization from ethyl acetate–ether (4:1); UV (pH 7) max 287 nm (ε 8000), 225 (8000); IR (KBr) 1965 cm^{-1} (C=C=C); ^1H NMR δ 7.36 (d, 1, H_6), 7.19 (qt, 1, $H_{1'}$), 6.18 (q, 1, $H_{3'}$), 5.08 (t, 1, OH), 4.07 (t, 2, $H_{4'}$), 1.79 (d, 3, CH_3); ^{13}C NMR 194.11 ($C_{2'}$), 108.40, 98.35 ($C_{1'}$ + $C_{3'}$), 59.56 ($C_{4'}$); thymine peaks 164.18, 149.58, 136.51, 111.22, 12.54 (CH_3); EI-MS 194 (M, 26.0). Anal. Calcd for $C_9H_{10}N_2O_3$: C, 55.66; H, 5.19; N, 14.42. Found: C, 55.70; H, 5.35; N, 14.65.

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Diosgenin-Bearing, Molluscicidal Saponins from *Allium vineale*: An NMR Approach for the Structural Assignment of Oligosaccharide Units

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An investigation into the molluscicidal substituents of field garlic, *Allium vineale*, has resulted in the isolation and identification of seven new saponins with up to six sugars. The isolations were accomplished by using a combination of countercurrent and adsorption chromatography. The aglycon of five of these natural products was shown to be diosgenin while the remaining two saponins bore nuatigenin and isonuatigenin aglycons, respectively. Extensive one- and two-dimensional NMR experiments were utilized to assign the structures.

Introduction

A great variety of *Allium* species (Liliaceae) have long been used in traditional medicines throughout the world for a great variety of medicinal purposes.[1] While the sulfur-bearing natural products of *Allium* spp. are well known for their biological activities,[2] interest in the saponin content increased during World War II as part of the search for alternative sources of diosgenin.[3] The important biological activities of saponins[4] are of increasing interest as recently illustrated by their potential value as antifungal agents.[4,5] A serious difficulty in assigning the structures of saponins is identifying the oligosaccharide unit.[4,6] In this paper we describe the isolation and structure assignment of nine saponins from field garlic, *Allium vineale*, seven of which were previously unreported.[7] Five of the new saponins have diosgenin as the aglycon, while the remaining two new natural products bear nuatigenin and isonuatigenin, respectively. The general approach to identifying the oligosaccharide units relies almost entirely on one- and two-dimensional NMR methods, supported by analyses of the mass spectral fragmentation patterns using negative ion FAB.

Results and Discussion

Isolation. Whole plants were divided into three parts (bulbs, stems and leaves, and flowers), extracted, and screened for activity. The methanol extract of the bulbs, which showed molluscicidal activity against the South American snail *Biomphalaria pfeifferei*,[8] was partitioned between water and ethyl acetate, and the active, aqueous fraction subsequently partitioned between water and 1-

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